Chapter 1

1.1 Introduction

An emerging field of nanomaterials is currently receiving a lot of attention in the bioimaging and cancer diagnostics domain. Due to their diverse range of features and broad applicability, these nanomaterials have become essential components in various technological advances such as therapeutics, bioimaging, nanomedicine, and the study of biological systems at a mechanistic level. [1–4] However, the available fluorescent materials and magnetic resonance (MR) imaging agents suffer from heavy metal toxicity along with non-informative image acquisitions from the region of interest (ROI). [5] Hence, to tackle the above problems, a new class of imaging agents needs to be developed to enable fast, informative, bright, and clear images with a high signal-to-noise ratio. [6,7] In line with this, the current thesis plans to investigate the development of a new synthetic route that can allow the development of a dual imaging agent and provide better images along with biocompatibility and thermal stability. As the material is reduced from bulk to nanosized range, the thermal stability becomes a key to the existence of the material in nanosized. [8] Further, despite the wide popularity of the synthesis of a number of nanomaterials, its operational electromagnetic spectrum is narrow, less biocompatible, non-water soluble, and thermodynamically unstable.

It is important to assert that diffusion of nanomaterials in a cell is one of the major parameters for homogenous distribution, which may either occur through non-endocytic entrance or direct membrane penetration, which is contingent upon their smaller size (specifically less than 10 nm, as endocytosis is limited in this range) along with the surface chemistry. [9] For instance, the presence of an arginine terminal is known to have unique interactions with the cell membrane. [9]

Additionally, this interaction of nanomaterials with cells is transitory in nature, in which the distortion of the cell membrane through chemical and physical stimulation may happen, resulting in membrane manipulation. Therefore, the development of small-sized nanomaterials is crucial for their uniform biodistribution within the cell and maximal contrast enhancement in MR imaging. [10] However, this synthesis may require a delicate balance of thermodynamic stability and nucleation rates. Such balances are tricky, which makes the synthesis of small-size nanomaterials with great reproducibility a challenging task to achieve. [11] Not only this, but also the nucleation process in the aqueous phase is difficult to control due to its random nature. Hence, understanding the fundamental physical and chemical processes of nanomaterials that drive nucleation is of paramount interest therefore, the process of nucleation, wherein new particles are formed from molecular precursors, is of utmost importance in synthesizing nanomaterials. [12,13] To achieve the synthesis of nanomaterials with certain qualities such as size, shape, composition, and morphology and to forecast their behavior precisely, a more profound understanding of thermodynamic parameters, nucleation rate, and interfacial energy is required. [14,15] In the context of homogeneous nucleation, it is generally observed that the free energy tends to vary as a function of the size of nanomaterials, suggesting a decrease in colloidal stability. [16–18] However, once the nanomaterial of the critical radius is evolved as a new solid phase, the free energy of the system starts to decrease, allowing for the growth of clusters until the concentration of the solution decreases below the supersaturation. Additionally, the thermodynamic instability of ultrasmall systems in suspension and their spontaneous evolution can be attributed to the Ostwald ripening process. [19–21]

The process of dissolution-crystallization commonly characterizes particle growth and coalescence events. [22,23] Such difficulties have been fundamentally handled using

thermodynamic and kinetic control. The kinetic control is the most popular method to control the size of the nanomaterials and is used widely. During nanoclusters synthesis, the use of protein and polymeric templates are the most popular method. [24–26] Size restriction by polymer templates not only imparts colloidal stability of the nanomaterials but also imparts charge or charge neutrality over the nanomaterials. [27] Due to which the diffusion rate of the nanomaterials into the tissues and cells is governed. Availability of colloidally stable nanomaterials in the vicinity of the cell or tissue in ample quantity may ensure diffusion of nanomaterials in large quantities, and hence, their biodistribution inside the cells or tissue can be anticipated to be high. [28–30] It is very well known that to obtain a good image from the cells and tissues, it is necessary that a significant number of photons should be emitted and captured by the device. [31] In general, the number of photons emitted by a large number of nanomaterials will be higher than that by a single nanomaterial. Therefore, the larger the diffusion of nanomaterials within the cells, the better and brighter the image will be. [32] Also, smaller nanomaterials are liable for a small residence time within the cells, and hence, it is important to select an adopted material so that molecular weight is large so that the residence time of the nanomaterials can be increased. [33,34]The increased residence time would allow the acquisition of bright and higher S/N ratio images. [35] Further, to obtain good images of these tissues/cells, the surface of such materials also decides their biodistribution in the cytoplasm of the cells. Their biodistribution generally depends on the nanomaterials' size because the smaller nanomaterials are generally not engulfed by macrophages of the immune systems and are not treated as foreign bodies, which shows less toxicity towards the cell. [29,30,36,37] However, in order to optimize cellular communication and elicit a biological response, it is imperative for nanomaterials to possess the capability to navigate through the extracellular matrix (ECM). ECM’s porous mesh-like structure effectively regulates the migration of

nanomaterials across the extracellular matrix (ECM). [9] The very good distribution of nanomaterials inside the cell /tissue gives better fluorescent imaging with efficient contrast. In line with this, the particle size from 1 to 6 nm changes the fluorescence maxima from a lower wavelength to a higher wavelength. Since electronic transitions are band gap dependent, the size of nanomaterial becomes very important. [38,39] The separation between the conduction and valence band increases as the bulk material is reduced to the nano level; also, colloidal solutions containing nanomaterial of different sizes will emit different colors under blue light, and as the size decreases, the emission colors will shift from red to blue. [35,40] Bioimaging probes under continuous excitation tend to fade in brightness and also photobleached; besides, they display broad fluorescence emission bands. Additionally, it is important to note that the magnetic features of nanoparticles are also size-dependent. [10] For instance, large-sized nanoparticles display T2-weighted images, whereas smaller ones tend to display T1-weighted images. It has been observed that T2-weighted contrast increases as a function of size (4 to 12 nm) in magnetic resonance imaging (MRI), where the T1-weighted contrast characteristic was shown to rise as the size drops notably; a size of 3 nm demonstrated improved contrast enhancement. The primary role of surface sites in T1 contrast agents is to induce relaxation. [41] Nanomaterials with a higher surface-to-volume ratio possess a greater abundance of surface sites, hence providing more opportunities for relaxation induction. Consequently, the degree of relaxation is more pronounced in smaller nanomaterials, rendering them more effective as T1 contrast agents than in bigger nanomaterials. The magnetic contrast is enhanced by an increase in the magnitude of mass magnetization, which is directly proportional to the size of the object. [42]

Hence, to synthesize desired size nanomaterials, the prior prediction of thermal stability is required for process optimization and development of customizable nanomaterial with physical and chemical properties. This requires computing thermodynamic parameters, nucleation rate, and interfacial energy along with the type of precursor to be used, reducing agent required, and capping agent so that the desired nanomaterial can be obtained. [43–45] The estimated parameters present notable benefits across various applications, encompassing biomedical disciplines, energy storage, catalysis, electronics, etc. [46–48]

1.2 Cancer: A Group of Disease

One of the worst diseases that is frequently seen around the world is cancer. According to IARC (International Agency for Research on Cancer) data, approximately 19.3 million new cases have been reported worldwide in 2023. [49,50] The World Health Organisation (WHO) defines cancer as "the rapid development of abnormal cells that grow outside of their normal boundaries and can invade surrounding body regions and spread to other organs." The estimated annual new cases of cancer till 2020 were 18.1 million worldwide, of which 9.3 million are involved in male cases and 8.8 million involved women cases, [51] which makes cancer the leading cause of highest death worldwide next to cardiovascular diseases. Therefore, early detection of cancer is essential to prevent death. [50]

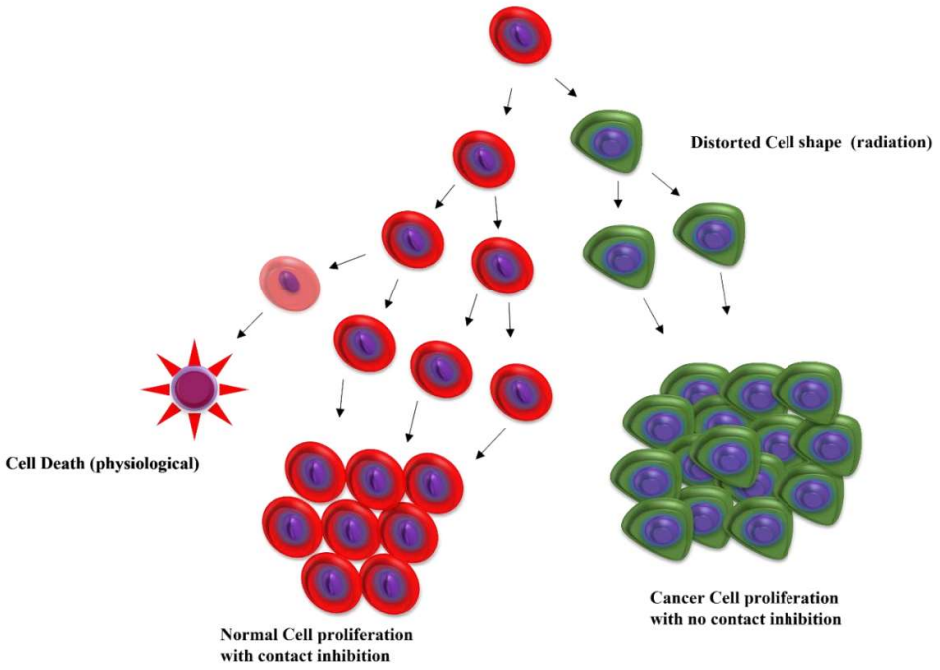


Figure 1.1: Cancer cell proliferation mechanism from a normal cell.

The normal cell turns into cancer cells primarily due to gene mutation via DNA damage, or it may be inherited from parents. Mostly excessive exposure to harmful substances like alcohol, cigarette smoke, UV rays, X-ray radiation, and other carcinogens are common agents generally encountered in daily life, resulting in gene degeneration. [49,52] A cancer cell behaves differently from a normal cell in terms of continuous chemical signals between each other in contrast, normal cells follow signals that tell them when to stop further dividing. On the other hand, cancer cells stop normal signalling and divide uncontrollably (Figure 1.1). Any cell in the body might become cancerous. [53] In order to overcome the preceding limitations and optimize the effectiveness of cancer therapy, it is imperative to incorporate a diagnostic protocol as a first measure.

The optical Imaging techniques utilized in the diagnostic process not only offer comprehensive physiological and morphological information about tumor tissues but also function as a valuable tool in guiding the entirety of the cancer treatment procedure. In a similar way, the utilization of imaging techniques can offer valuable insights into the efficacy of specific drug bio-distribution at the tumor site.

1.3 Multimodal Cancer Imaging

The early and accurate detection of cancer for its effective management is urgently required. To cater to this need, a number of alternate guided molecular imaging agents for better clinical diagnosis are being widely investigated. [54] The major thrust is to develop molecular agents that can easily provide a number of critical information from the images quickly with greater ease. For instance, well-resolved images with a clear and distinct contrast between the diseased and healthy tissues, the distinction between differentiating and non-differentiating tissues, changes in the condition of tissues within the small time period, and good quality images from organs shielded by the rib cage, the cranium of other organs. [55] Among a number of techniques available, optical imaging provides in-depth, real-time monitoring from imaging probes with wide spectral windows of absorbance or emission. However, the amount of information which can be obtained from these probes is dependent on tissue thickness, radiation wavelength used for excitation, and emission wavelength from the molecular probe, due to which one needs to look for alternative techniques such as magnetic resonance imaging. At the same time, the Magnetic Resonance Imaging (MRI) is one of the greatest non-invasive imaging technologies. [7,53,56] Recently, dual modalities like Magnetic Resonance and Fluorescence Imaging are much required for modern medical diagnosis purposes. [57] Although contrast-enhanced MRI is

well known for its great spatial resolution, it suffers from low intensity; this is where multimodal imaging can be helpful.

The aim behind multimodal imaging is to compensate for the shortcomings of one imaging modality with the strengths of the others. For example, MRI imaging is most often helpful in cases where tissue depth is larger or organs are shielded by the rib caging or cranium or located at the posterior end of the body. [58] Under such hindrance, fluorescence imaging may miserably fail, and MRI imaging, despite of low intensity, may provide necessary information. However, in the case of shallow imaging fluorescence can become the major agent. The utilization of these dual modalities in the field of biomedical provides extended and complete information for better diagnosis. (Figure 1.2) [56,59] The MRI helps to plan the treatment, whereas fluorescence imaging can be used during surgery to track real-time procedures. Considering this, the origin of multimodal imaging is advantageous. Surprisingly, the demonstrated nanomaterials provide an admirable and decisive scheme for integrating with various imaging modalities for novel properties and synergistic applications. Thereupon, over the course of years, significant efforts have been carried out to build a synthetic method such as complementary magnetic nanoparticles, heterostructure NPs, and co-encapsulation with magnetic fluorescent components like Silica, lipid, and other co-polymers. Despite significant interest in the application of Gd_2O_3 NPs, currently available synthesis methods are complex, require high temperatures (180 °C), and are time-consuming. [5,60] Hence, the substantial advancements in synthesis technology have allowed for cancer diagnosis.

Imaging modality*	Radiation	Depth	Materials	Advantage	Disadvantages	Ref.
MRI	Radio wave (1-100 MHz)	Limitless	Magnetic (SPINOs, gadolinium oxide, manganese oxide)	Nonionization radiation, noninvasive method	Low spatial resolution	[61,62]
Optical Fluorescence imaging	Visible light and NIR (~400 - 900 nm)	~ 1 cm, it may also go to an inch from NIR	Organic dye, quantum dost, lanthanide nanomaterials)	Noninvasive, no harmful (non ionization radiation)	Low sensitivity, high post-processing time	[63,64]
CT	X- rays (80-140 keV)	Limitless	Iodine, gadolinium, gold nanoparticle, bismuth sulfide nanoplate	Noninvasive, high-resolution	High ionization radiation, limited up to hard tissue	[65,66]
PET	High energy γ radiation (511 keV)	Limitless	Radio isotopes (11C, 15O, 18F, 131I, 99mTc, 111In)	High sensitivity, Noninvasive	Limited to digestive organ and bone	[67,68]
SPECT	Gamma rays (20–300 keV)	Limitless	Radio isotopes (123I, 99mTc, 133Xe, 201Tl, and 18F)	Reduce structural noise	Inferior spatial resolution	[69,70]
US	High frequency sound (2–15 MHz)	mm to cm	Microbubbles, Piezoelectric nanomaterials	Real-time, low cost, no harmful	Limited spatial resolution, limited soft tissue resolutions	[71,72]

Table 1.1: Comparative details of available imaging modalities.

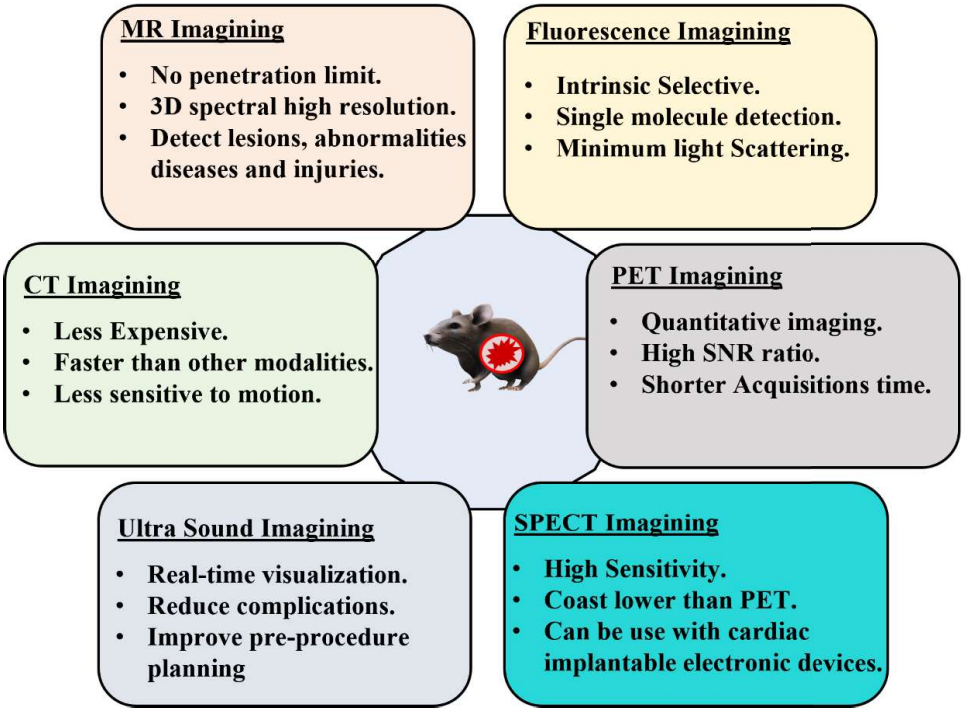


Figure 1.2 Possible imaging modalities with their advantage used in optical imaging.

1.4 Fluorescence Bio-Imaging (Optical)

The bioimaging technology has seen numerous scientific breakthroughs, such as that has been recognized with the Nobel Prize in the 21st century, including the use of green fluorescent protein in optical bioimaging in 2008 and super-resolution fluorescence microscopy in 2014. [1,73] Recent years have seen a huge push for theragnostic coupled with the growth of nanotechnology and nanomaterials. Fluorescence bioimaging has a wide range of potential applications because it may produce visualization results for various biological parameters or events. [74]

Accessibility to interactions between light and tissue and the corresponding photophysical and photochemical processes at the molecular level (e.g., fluorescence, luminescence,

multiphoton absorption, and second-harmonic generation) is one of the fundamental advantages of fluorescence imaging use in biomedical research. [75,76]

1.4.1 Size Effect of Nanomaterial for Bioimaging

The utilization of nanomaterials for *in-vivo* targeted purposes frequently entails the administration of these materials by intravenous injection. [77] The successful implementation of these applications necessitates the development of a suitable material size that can optimize the bioavailability at specific locations. [78] The significance of this feature lies in the necessity for these nanomaterials to be transported from the site of injection to the desired location through the bloodstream in order to achieve optimal functionality. The phenomenon of targeting is contingent upon the size of particles for two distinct reasons. The colloidal stability of nanomaterials decreased as their size increased. [79] Generally, inorganic nanomaterials larger than 100 nm and organic nanomaterials larger than 500 nm tend to have diminished dispersion stability. Therefore, bigger nanomaterials exhibiting low colloidal stability tend to precipitate in close proximity to the injection site, impeding their further migration. Furthermore, it has been observed that the renal excretion of administered nanoparticles occurs quickly when their size is below 5 nm. The underlying cause can be attributed to the size restriction imposed by the glomerular filter pore in the kidney, which normally falls within the range of 2−8 nm. [80] Furthermore, the size effect is influenced by a combination of factors, including surface chemistry, biochemical stability, and particle shape. For instance, nanoparticles that are structured like rods or wires exhibit superior *in-vivo* targeting capabilities compared to spheres of comparable dimensions. This is attributed to their ability to prolong their presence in the bloodstream by aligning themselves in the direction of blood flow. Charged nanoparticles elicit an immune response that facilitates prompt elimination from the

bloodstream through the reticuloendothelial system, encompassing organs such as the liver and spleen. Therefore, it is common practice to surface-modified nanomaterials with polymer in order to reduce their clearance. One notable side effect worth mentioning is the differential behavior observed in nanomaterials of varying sizes.

1.5 Fluorescence -imaging-based cancer therapy

1.5.1 Fluorescence imaging in drug delivery (Theranostic)

The physicochemical properties of recent nanoparticles have contributed to significant advances in cancer theragnostic in recent years, enabling both diagnostic and therapeutic functions to be simultaneously achieved. [81] The field of nanomedicine is particularly interesting for theranostics, which aims to provide image-guided cancer therapy by integrating imaging and therapy. At least there are three components to make up theranostic nanoparticles in which, the first one is the biological payload, the second is a carrier, and the final one is the surface modifiers. The biological payload includes therapeutic and imaging agents. Therefore, they can simultaneously deliver therapeutic agents to the tumor site and monitor their biodistribution in vivo in real time. [82–84] The optical carriers like fluorophores are commonly employed in optical imaging to track molecular processes, for example, fluorescent dyes, bioluminescent proteins, and fluorescent proteins. They are, however, readily photobleached by sunlight. [85,86] To address this shortcoming of fluorophores, Scientific communities have developed fluorescent nanoparticles (such as quantum dots, copper and gold nanoparticles, and upconversion nanoparticles). [87–89] (Figure 1.3)

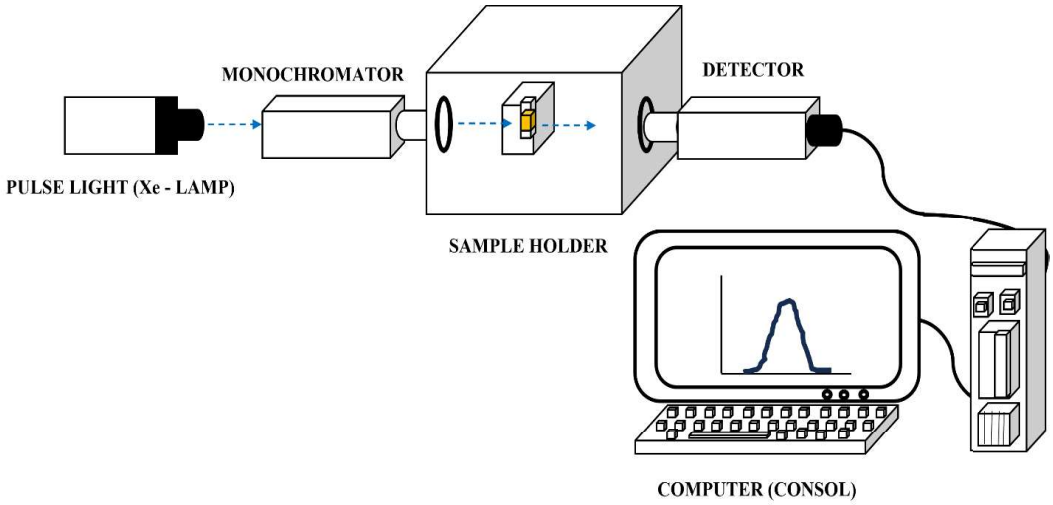


Figure 1.3: Basic fluorescence detection setup for nanomaterials.

Gold nanoparticles are the most popular type of nanoparticle employed in biomedical applications because of their many useful features, including biocompatibility and easy modification. Their surface plasmon resonance (SPR) is also modifiable because of their size, shape, and structure. Due to the longitudinal surface plasmon resonance, rod-shaped gold nanorods can be employed as direct NIR absorption imaging probes. [90]

1.5.2 Fluorescence imaging-guided surgery

The most important goal of cancer surgery is the radical resection of the tumor with minimal collateral injury. To attain this objective, fluorescence imaging techniques are required, leading to "image-guided surgery". [91,92]

Fluorescence imaging is particularly well designed for targeted surgery because the fluorescence signals can provide real-time guidance to distinguish positive tumor margins and local malignant masses from normal tissues during surgery. (Figure 1.4) Therefore, it increases the chance of long-term patient survival. [6] Image-guided surgery is appropriate for tumors that are difficult to distinguish from adjacent normal tissues (such as breast

cancers) and tumors that are adjacent to complex structures with essential physiological functions (such as brain tumors). This fluorescence imaging procedure is sophisticated. For proper interpretation of the presented intraoperative fluorescent image of the tumor, the surgeon must understand that the target-specific fluorescent agent had to reach and remain within the tumor, but due to absorbing materials and scattering events from the tissue, that particular portion of the signal gets reduced. Additionally, to improve the signal, the autofluorescence component must be subtracted. [93] Despite of three decades of intensive research, complete removal of errors associated with imaging (scattering, autofluorescence, astigmatism, etc.) is difficult to remove.

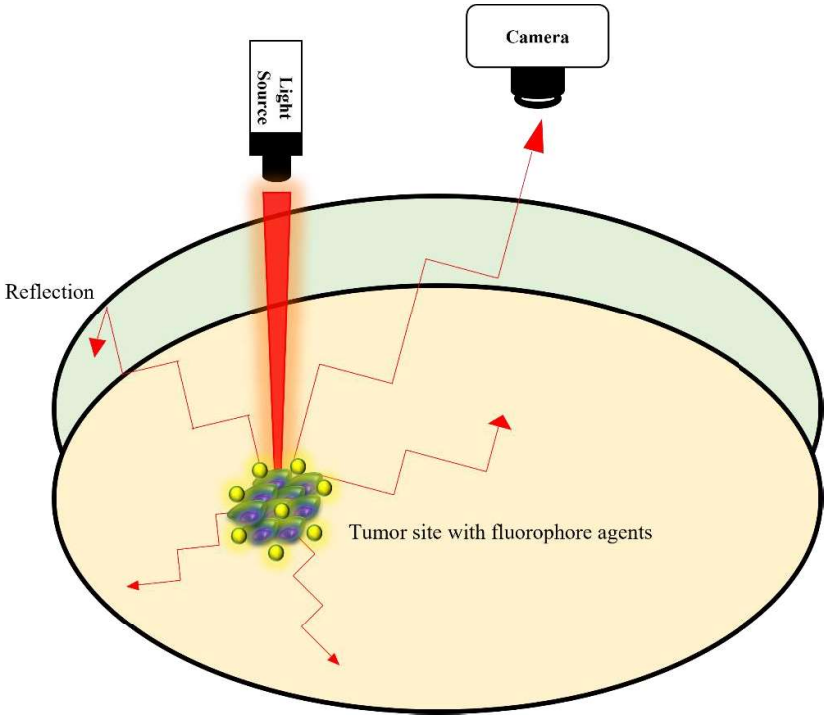


Figure 1.4: Basic fluorescence mechanism in tissue tagged through fluorophore agents.

1.6 Magnetic Resonance Imaging (MR- Imaging)

MR imaging is one of the most extraordinary innovations for medical diagnosis since the discovery of the X-rays in the early 20th century. [94] In 1973, Lauterbur was the one who developed the very first image utilizing nuclear magnetic resonance. [95] The MRI is a very advanced and powerful imaging technology due to its wide range of therapeutic applications in fields as diverse as cardiac, gastrointestinal, vascular, and neuronal medicine and the detection of tumors in other organs. [96–98] This accomplishment is attributable to the inherent characteristics of anatomical MRI, which include excellent soft tissue contrasts based on multiple contrast parameters, the ability to image in oblique orientations, and the capacity to provide two-dimensional (2D) and three-dimensional (3D) data. [53] The radiofrequency (RF) range is used in MRI because of a phenomenon known as nuclear magnetic resonance (NMR), in which atomic nuclei absorb and reemit electromagnetic waves at characteristic or "resonant" frequencies. [99] MRI's versatility stems from the abundance of data it provides about the biochemistry and macroscopic structural characteristics of tissues. White matter tracts, lesions, and arteries can all be visualized in exquisite detail thanks to the flexibility of the elicitation and analysis methods used to produce the signal. [100] While MRI has mostly been used for depicting anatomy in the past, the fields are beginning to converge as scientists discover ways to detect functional qualities and gather spatially localized spectra. [101]

1.6.1 Working Principle of MRI

Magnetic resonance imaging (MRI) uses the body's natural magnetic properties to create detailed images of any part of the body. The abundant water molecules in the cell's tissues provide a basis for the MRI signal, where protons of the water molecules behave like a

magnetic dipole under a strong magnetic field. [102] (Figure 1.5) Although without a magnetic field, the proton orientation is truly randomized and, therefore, zero magnetization. However, in the presence of a magnetic field, protons can either orient themselves parallel or antiparallel to the magnetic field in addition to spinning up and spinning down, which is dependent on the intensity of the magnetic field. [103] The proton will generally spin or wobble at the resonant frequency. This frequency is referred to as the Larmor frequency. [104]

The process of creating a clinical image using MRI can be explained using three fundamental concepts. First, a signal is generated from the MR properties, then an image is generated from these signals, and finally, tissue contrast is generated. [57,105] The inherent susceptibility of hydrogen atoms to a magnetic field is utilized in the signal generation process, as this property allows them to align themselves along a dominant magnetic field. By administering a dominant external magnetic field, MRI creates images of hydrogen atoms in free water, proteins, lipids, etc. in the investigated tissues. As a result of the deployment of this external magnetic field, protons (hydrogen atoms) rotate in a cone-shaped manner, a phenomenon known as precession. [106] (Figure 1.6) Due to the precession of atoms, a secondary weak magnetic field is generated. By introducing a pulsed radiofrequency (usually in ms) to the existing strong magnetic field can be perturbed, resulting in perturbed magnetization of tissues. This perturbation is reversed to its original unaltered state. This magnetization recovery occurs via two distinct relaxation processes, longitudinal (T_1) and transverse (T_2) magnetization. [107] During these relaxation processes, protons emit radio waves that are converted into signals as part of the second phase of MR imaging.

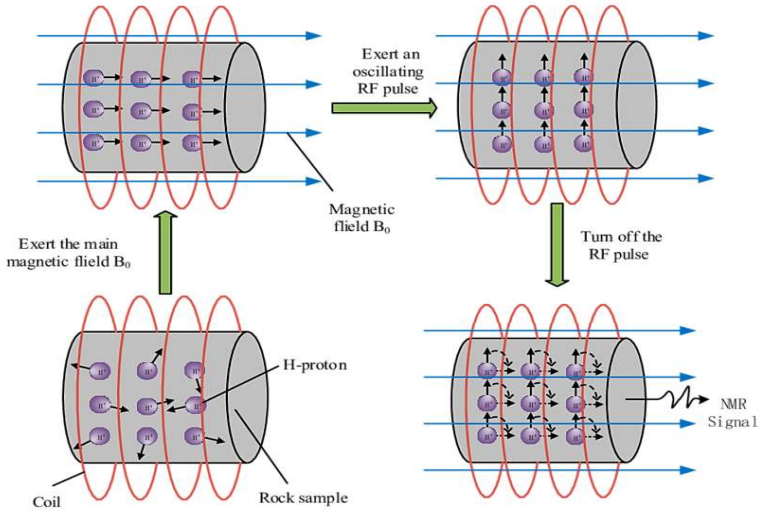


Figure 1.5 Schematic diagram of the NMR technique test mechanism. [108]

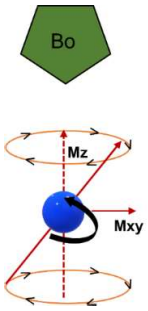


Figure 1.6 The net magnetization (M) is a vector that can be split into longitudinal (Mz) and transverse (Mxy) components relative to the principal magnetic field (Bo).

1.6.2 T1-Weighted Relaxation Process (Longitudinal Relaxation)

The T1 relaxation is the time constant and also referred to as spin-lattice relaxation and thermal relaxation, which measures the exponential relaxation process of spin (Figure 1.7) recovering to its net magnetization zero on the ground state in the direction of the magnetic field (B_o). [109] This spin recovery of nuclei is associated with the decay of energy from a high-energy state to a low-energy state.

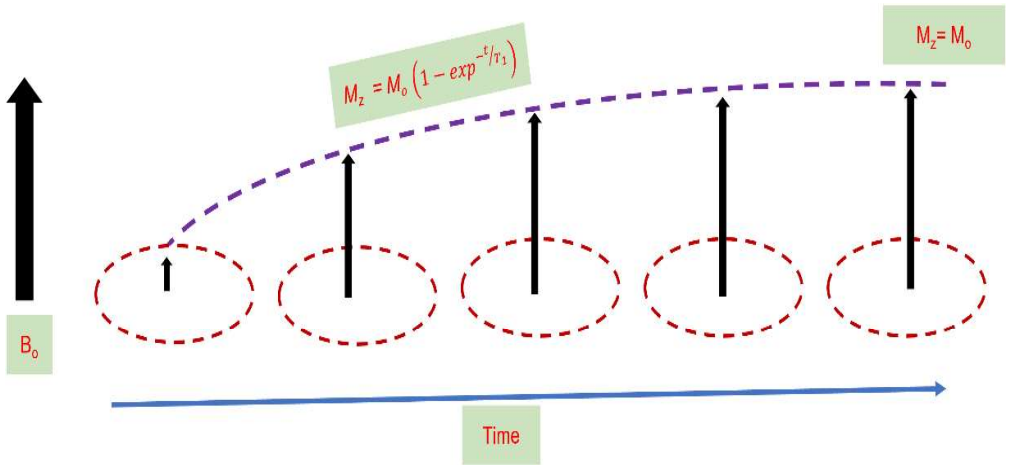


Figure 1.7 The T1 relaxation process is the return of the net magnetization (M) to its initial maximal value (Mo).

Felix Bloch, the Swiss-American scientist, proposed the model for the T1 time constant as simple exponentials of first-order kinetics. [107] The T1 can be calculated as the time needed for the z-axis of Magnetization (M) to attain (1-$^1/_e$) or nearly 63% of its maximum value (M_o). Eq. (1) T1 values in biological materials typically range from a few tenths of a second to several milliseconds. [110–112]

$$M_z = Mo\left(1 - exp^{-t/T_1}\right) \dots\dots\dots\dots\dots\dots 1$$

1.6.3 T2-Weighted Relaxation Process (Transvers Relaxations)

T2 Relaxation is also known as spin-spin relaxations. (Figure 1.8) In this process the transverse component of magnetization (M_{xy}) is decay by approximately 37% of initial value of magnetization. [113] T2 is time constant and follow first order kinetics with simple exponential decay shows in Eq.2

$$M_{xy} = Mo\left(exp^{-t/T_2}\right) \dots\dots\dots\dots\dots\dots 2$$

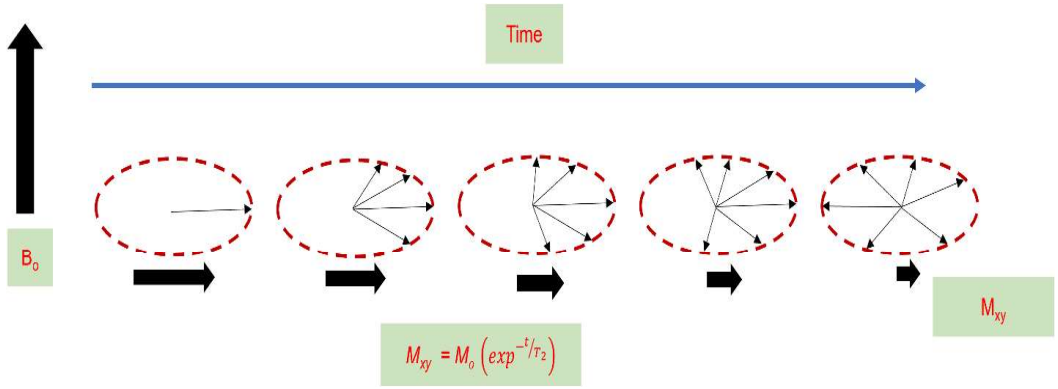


Figure 1.8: Transverse relaxation processes (T2) A diagram illustrating the process of transverse relaxation following the application of a 90° RF pulse at equilibrium.

The net magnetization (M) in the transverse plane does not magically adhere all the spins into phase. [102] Those same spins that initially exhibited a statistical "preference" for the z-direction prior to the RF pulse subsequently rotates into the transverse plane. The initial "ordering" of spins in the z-direction of the Boltzmann distribution has been preserved and transformed by rotation into what can be termed "phase coherence" in the XY plane. [114,115]

1.7 MRI Contrast Agents

The development of contrast agents for magnetic resonance imaging has gained huge attention for advancement in MRI technology. [3,116] These agents have been used worldwide to increase the contrast difference between abnormal and normal tissue with a more distinct image of enlarged, detectable organs and organ systems. (Figure 1.9) [57] A contrast-enhanced MRI is conducted in a hospital or imaging facility. A healthcare provider will typically inject contrast material into a vein in your arm and then position you in an MRI machine during the procedure. When contrast is introduced to the bloodstream of an individual, the targeted tissues will no longer appear as shades of grey, unlike in standard.

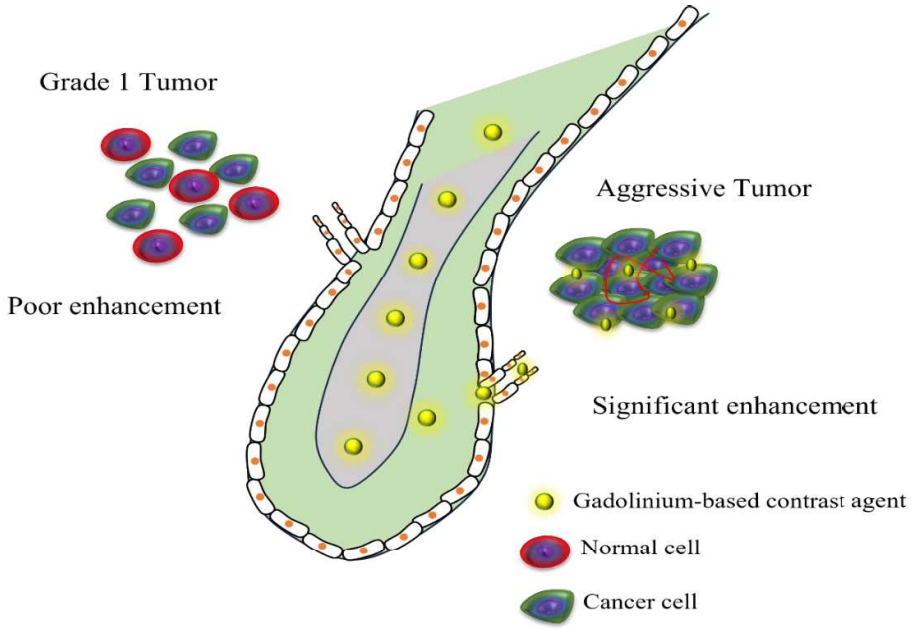


Figure 1.9 The diagram shows that the contrast agents bind to the aggressive tumor and give significant single.

MRI. [117] They will appear to illuminate, providing a healthcare provider with a clearer picture of what's happening inside your body.

The infected tissues, like tumors and cancer cells, have very higher vascularity than healthy ones, which indicates the affinity of the contrast agent and gives enhanced MRI images. [118] In earlier 1981, the first contrast agent was developed for MRI investigation in the gastrointestinal tract (GI Tract) of humans as ferric chloride. [96] In 1987, a group of researchers from Germany, Hans Weinmann et al., first discovered gadolinium as a potential candidate for MRI contrast agents. [119,120] Figure 1.10 shows the brain image of a person with glioblastoma with an aggressive tumor on the left side of the hemispheres before and after the gadolinium-based contrast agent, and it is very clear that after administration of contrast, the BBB (blood-brain barrier) of tumor significantly visualized. While shortening T_1 relaxation enhances signal intensity in T_1-weighted MRI images. (Figure 1.10)

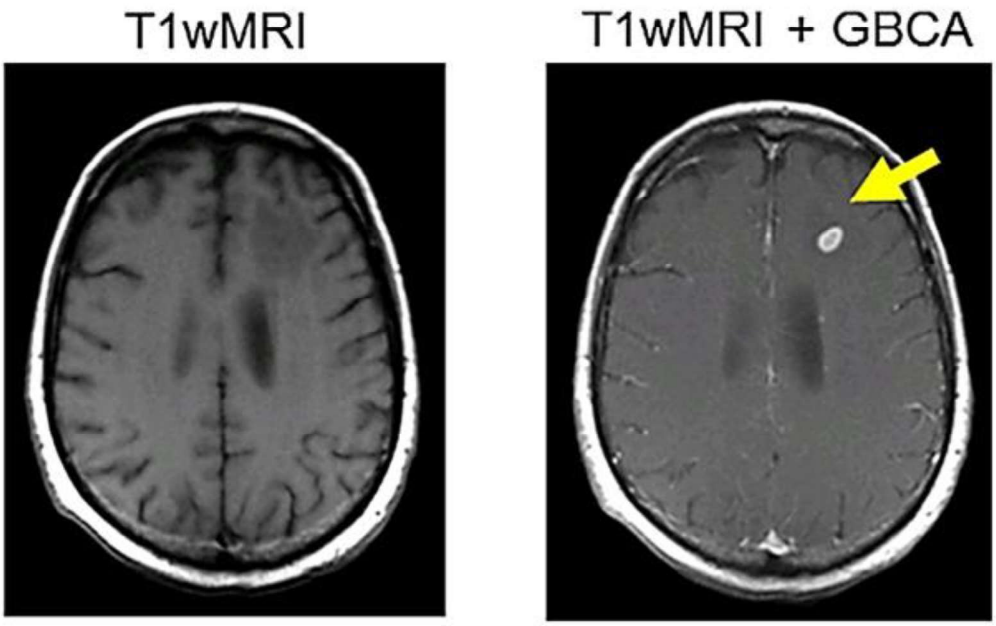


Figure 1.10: Pre- and post-gadolinium-based contrast agent (GBCA) T1 weighted (T1wMRI) image from malignant tumor patient for improved visualization for a medical expert. [121]

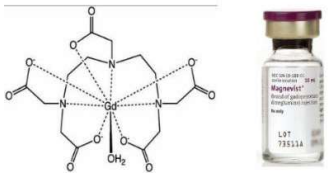
However, the commercially available intravascular contrast compounds like Magnetevist, Omniscan, and others are not suited to any cell type and accumulate in tumorous tissues based on cancer vasculature. [121]

Generally, these contrast agents are divided on the basis of their chemical composition into positive and negative contrast to reduce the T1 and T2 relaxation periods. T1-weighted images provide a positive contrast, whereas T2-weighted images provide a negative contrast. Superparamagnetic iron oxide nanoparticles (SPIONs), which provide negative contrast, and gadolinium-based paramagnetic agents, which provide positive contrast, have been introduced for clinical use until now. [121,122]

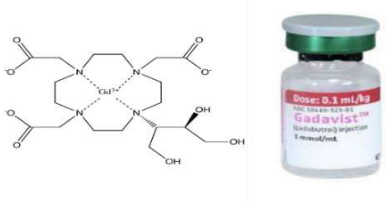
SPIONs have a powerful effect on T2 relaxation due to their superparamagnetic properties, and their minuscule size facilitates their transport across the cell membranes. [123] The SPIONs can be used to label cells *in-vitro* by incubating the cells for better visualization using MRI. Due to their magnetic properties and biocompatibility, [124] SPIONs are also utilized for drug/gene delivery, [125] magnetic separation, magnetic hyperthermia for cancer treatment, [126] and numerous other biomedical applications. But there is some disadvantage of SPIONs, like the reduction of signal amplitude from MRI responsible for low resolution and dark images that can be misled to healthcare professional to correct diagnosis. The blooming artifacts are one of the problems created by SPIONs results, producing difficulty in cell specification and characterization. [127] Apart from the above discussion, The SPIONs are very specific to the liver and spleen, limiting their application for complete body imaging; hence, the positive contrast agents are suitable for use as contrast on many organs, such as gadolinium-based contrast agents. [128]

1.8 Gadolinium as Positive Contrast Agent

Currently, there is a lot of interest in researching Gd_2O_3 in the biological field is developing due to their excellent physicochemical properties related to their high optical response, [129] high thermal and chemical stability, [32] and their capacity to be produced in different shapes, particle sizes, and textures [130]. These attributes make them superior compared to other particles. Additionally, Gd_2O_3 nanoclusters have attracted huge scientific attention due to their anomalous capacity to enhance imaging techniques such as Magnetic Resonance Imaging (MRI), Optical imaging, and computed tomography imaging (CT). [131,132] Among several metals, Gd-based agents are captivating in terms of large magnetic moments (7 unpaired electrons) and applicability toward different organs (liver, spleen, and lungs). [133] The gadolinium-based contrast agents are chelated to remove the

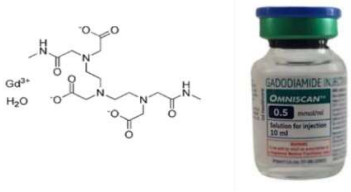


Magnevist Structure



Gadavist (gadobutrol) formula

Dotarem formula



Omniscan (gadodiamide)

Figure 1.11: Commercially available gadolinium-based contrast agents with their structure [136–138].

high toxicity of naked Gd^{3+} ions. In 1988 the first commercially available Gd-based contrast agent was named as a trademark "*Magnevist"* (Berlex, USA) that was proved to be clinically useful. [134,135] Some other Gd-based contrasts are also available in the market, which are widely used for clinical purposes. (Figure 1.11) However, gadolinium oxide-based nanomaterials are more demanding due to their higher molecular weight, which prevents quick renal clearance.

However, this positive aspect may lead to perturbation of the biocompatibility issue, and we know that biocompatibility is a critical aspect when considering the use of gadolinium oxide nanoparticles in real-time applications. [139] Hence, despite having significant interest and applicability of Gd_2O_3 nanomaterial in the biomedical field, their production in the micro or nanoscale is a constant challenge. Moreover, their complex synthesis

procedure, biocompatibility issue, and lack of stability assessment limit their efficacy and commercialization due to costly reactants and tardy optimization. Furthermore, the thermal stability of materials plays a crucial role in the limitation of the application of the materials.

Hence, it would be interesting to find out if it is possible to use it at high temperatures. The available synthesis procedure utilizes a high reacting temperature (180º C), an organic solvent medium, and a time-consuming multistep. [140] The surface modification of gadolinium oxide nanoparticles with biocompatible coatings and the appropriate size control can mitigate potential risks, reducing the likelihood of adverse reactions in the body. Nevertheless, ongoing research is necessary to comprehensively assess their biocompatibility, thus promoting the development of safe and efficient gadolinium oxide nanoparticles for clinical MRI applications.

1.9 Gadolinium Oxide as a Dual Modalities

Rare-earth oxides are cutting-edge materials that are frequently used as host lattices in the development of sensors and luminous materials. [141,142] They are known for their good chemical and thermal stability, low phonon energy (300-600 cm^{-1}), and ease of doping with lanthanide ions. [143] Gadolinium oxide (Gd_2O_3) is regarded as one of the most promising materials for the development of magnetic resonance and fluorescence imaging contrast agents (Figure 1.12). This is owing to gadolinium's trivalent state inside the matrix, [144] which creates a highly stable 4f-shell with seven unpaired spins, making it substantially paramagnetic. [145] Similarly, the intrinsic optical features of Gd_2O_3 allow it to create crisp wavelength absorptions and photostability, making it excellent for imaging applications. Doping the matrix with additional lanthanide ions can improve the optical characteristics of Gd-based materials. As a result of the selection of good dopants, photoluminescent materials with strong Stokes shifts, crisp emission spectra (in the visible or NIR areas),

extended lifetimes, minimal photobleaching, and multiphoton absorption are conceivable. [40] Furthermore, the excitation and emission wavelengths can be tuned as desired. Because of these features, gadolinium-oxide nanomaterials are considered good materials for photoluminescence applications in both the technological and biomedical fields.

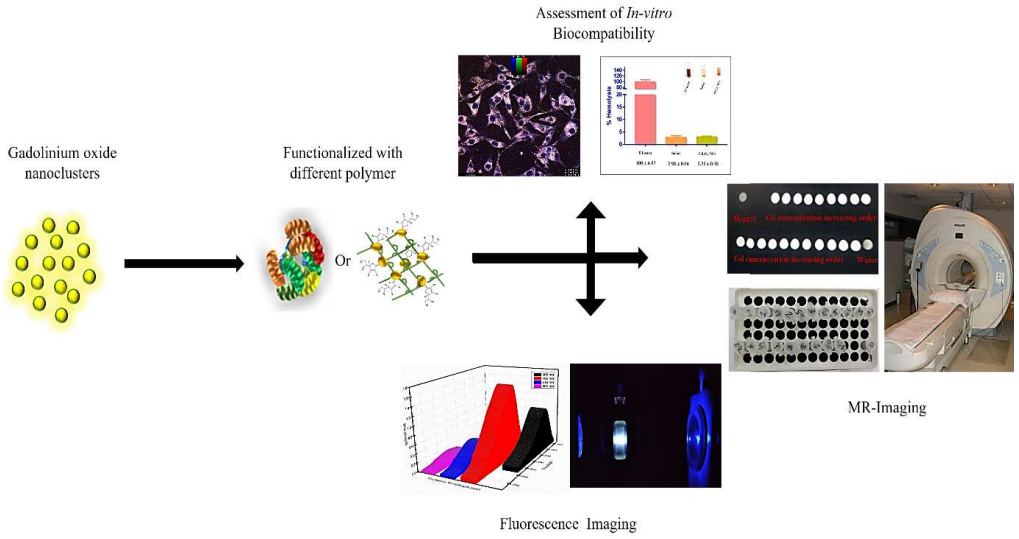


Figure 1.12: Synthesis mechanism of biocompatible gadolinium oxide nanomaterials with different polymers in possible fluorescent imaging and MR imaging

1.9.1 Various Synthesis Routes of Gd_2O_3 Nanomaterial

In the synthesis procedure of gadolinium oxide nanomaterials, the morphology, size, capping agent and stabilizing agent can be modulated with the change in pH, stirring rpm and concentration which plays very crucial and utmost important parameter. It is well known that on the increasing concentration of capping agents can change or diminish the optoelectronics properties of these nanomaterials. [146] To synthesize the gadolinium oxide metallic nanoclusters, numerous capping agents such as thiolates, polymers, peptides, protein templates, amino acids, etc. are utilized. [33,147,148] The synthesis of these nanoclusters can be performed using either the top-down or bottom-up approach. (Figure

1.13) The top-down approach was carried out with the etching of chemicals to reduce the size, whereas the bottom-up approach is entirely different with the use of chemical and environmentally friendly reducing agents to reduce the size .[149,150] Other techniques, such as the chemical reduction method, hydrothermal approach [151] and microwave synthesis [152] are also included in the bottom-up method, whereas the sonochemical method, [148] ball-milling approach [153] and thermal decomposition methods involve in the top-down approach. [32] The mechanisms involved in the synthesis of metallic clusters are described in detail below:

1.9.2 Chemical Reduction Method

The chemical reduction method is quite time-efficient, straightforward, and has great potential for large-scale production. Numerous works have been done on the chemical reduction synthesis of gadolinium oxide nanomaterials with various types of chemical reducing agents used for the synthesis purpose of metallic nanoclusters. [154] To synthesize nanoparticles, precursor salts, reducing agents, and stabilizers are utilized, and in most circumstances, a catalyst and heat are employed (Figure 1.14). Sodium borohydride

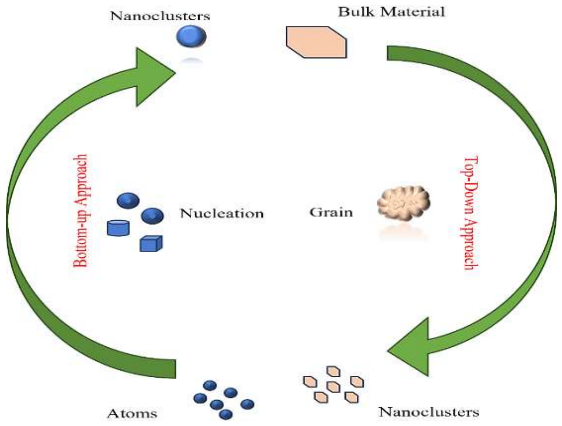


Figure 1.13: Top-down and bottom-up approach for the synthesis of nanomaterials.

($NaBH_4$), Sodium citrate, hydrazine, alcohols, formaldehyde ethylene glycol, and citrate ions are used as reducing agents and utilized in the synthesis of nanomaterials. [155–158] For nanoparticle synthesis, chemical reduction methods can be divided into two subcategories: the first one requires the simultaneous reduction of two metal salts, while the second one includes the sequential reduction of two metal salts. The second technique is commonly used to prepare bimetallic nanoparticles with a core-shell configuration. Organic synthesis, self-assembly, and colloidal aggregation are all components of the traditional wet chemical method, as seen above. [159] Various chemical reactions, nucleation, and growth processes are linked to molecular components as starting materials to facilitate the production of complex nanoclusters.

1.9.3 Hydrothermal Method

The hydrothermal procedure is one of the ubiquitous techniques for devolving nanomaterials, which follows the solution reaction-based strategy. In hydrothermal synthesis, a precursor solution is heated at elevated temperature and pressure in an impenetrable vessel. [151] The nanomaterial formation can occur at temperatures ranging from ambient temperature to extremely high temperatures.

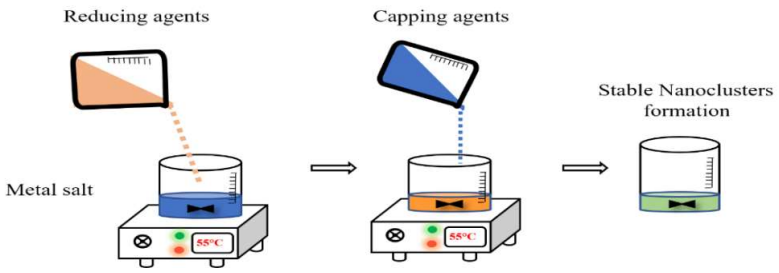


Figure 1.14: The mechanism for synthesis of nanomaterials using the chemical reduction method.

Depending on the vapor pressure of the primary salt in the reaction, low-pressure or high-pressure conditions can be used to control the morphology of the materials to be prepared. [160] (Figure 1.15) This technique is strongly suggested for the fabrication of anisotropic structures of any nanomaterials. There are numerous advantages of hydrothermal synthesis over alternative methods. Hydrothermal synthesis can generate nanomaterials, which are unstable at high temperatures and result in minimal material loss due to high vapor pressure and facilitated large production. The several articles have been published on the hydrothermal synthesis of nanoparticles, nanorods, nanotubes, hollow nanospheres, and graphene nanosheets while under these conditions, nanorods and nanowires are the most frequently synthesized low-dimensional nanostructures, although spherical nanoparticles are also possible. [161]

1.9.4 Microwave-Assisted Synthesis

Microwave-assisted irradiation can be used to accelerate the formation of nanoparticles by providing rapid heating and efficient energy transfer to the reaction mixture. Therefore, microwave-assisted irradiation technology is widely used for the synthesis of nanomaterial to reduce synthesis time and heat. [152] (Figure 1.16)

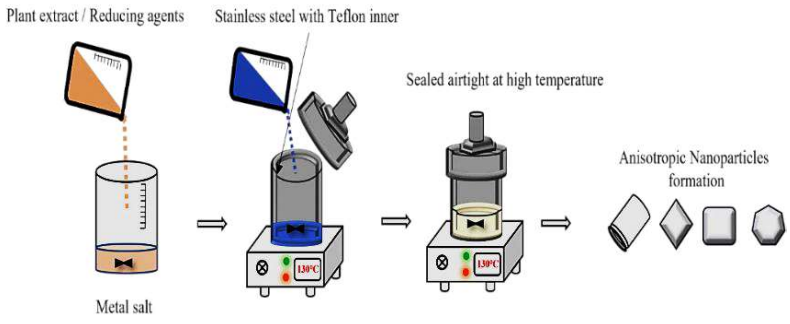


Figure 1.15: Hydrothermal approaches to the synthesis of nanomaterials with different shapes.

The high dielectric constant containing solvent interacts with radiation, which comes from microwave radiation energy. Microwave-assisted synthesis allows scientists to selectively, quantitatively, and precisely produce catalytic materials, nanomaterials, and desired organic compounds. Researchers can now develop advanced nanomaterial design and development by changing microwave parameters like temperature, pressure, and ramping temperature and solvents. [162,163]

1.9.5 Biomineralization

Biomineralization is an efficient natural approach to synthesizing nanomaterials. [164] The biological synthesis of nanoparticles is a fascinating alternative method for environmentally intended production. This approach includes the use of low-toxic compounds, as well as reduced temperatures and pressures throughout the synthesis process. [165] Researchers have utilized biological resources, including plants, [166] microbial organisms (fungus, bacteria) [167,168] and their byproducts, to develop several biogenic approaches for the synthesis of nanomaterials. (Figure 1.17)

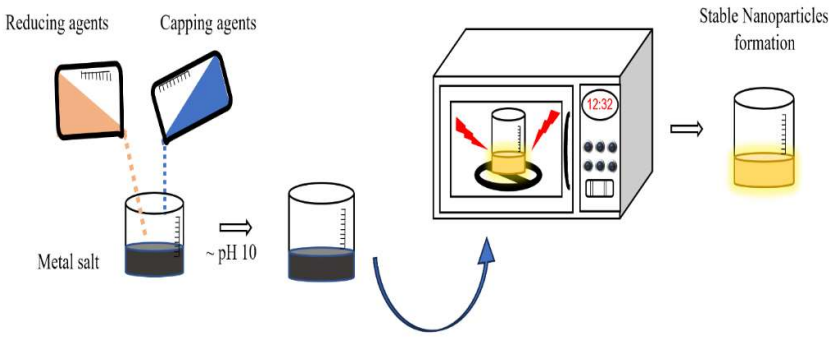


Figure 1.16: Microwave-assisted synthesis of nanomaterials.

The application of biological elements, such as proteins, peptides, dendrimers, polymers, or cells, for the purpose of controlling the initiation and development of metal clusters with certain fluorescence characteristics. [169–171] The production of metal nanoparticles (NPs) by microorganisms can occur either intracellularly or extracellularly. The intracellular production of nanoparticles necessitates supplementary procedures, such as the use of ultrasound or the reaction with suitable detergents, in order to facilitate the liberation of the synthesized nanoparticles. Simultaneously, extracellular biosynthesis presents a cost-effective alternative that entails simpler processing. [172] Consequently, it is imperative to investigate its potential applications in the context of large-scale manufacturing of nanoparticles. Due to this rationale, numerous investigations have prioritized extracellular approaches in the production of metal nanoparticles. Moreover, within the natural ecosystem, microorganisms engage in the production of nanomaterials as an integral component of their metabolic processes, so rendering them suitable for a diverse range of practical applications. Microorganisms exhibit rapid rates of reproduction. [173]

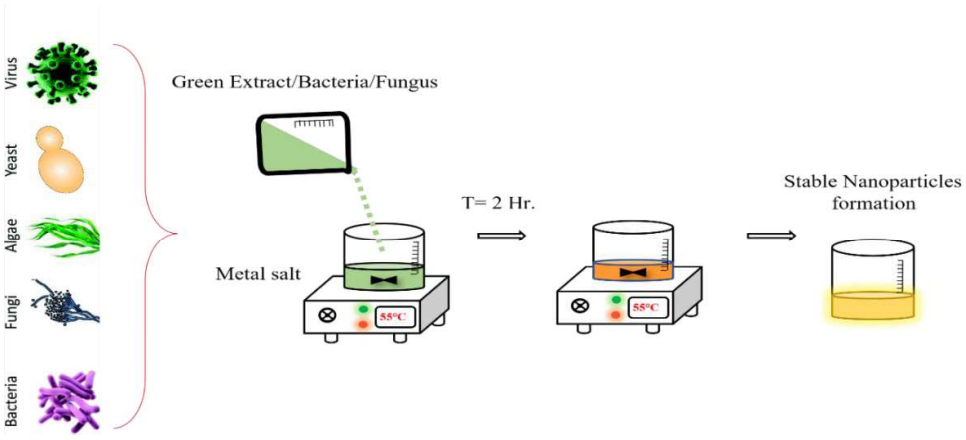


Figure 1.17: Biomineralization Encourage Synthesis of nanomaterials using different plant extracts or microorganisms like fungi, bacteria, and yeast.

1.9.6 Sol-Gel Method

The sol-gel method is a wet chemical technology that involves a number of hydrolysis steps with the condensation, gelation, and crystallization of precursor materials to form nanoparticles. [174,175] It typically utilizes metal salts as precursors and stabilizing agents to control particle size and morphology. The sol-gel process is used for the production of diverse nanostructures, with a particular emphasis on metal oxide nanoparticles. [176] In this approach, the molecular precursor, often a metal alkoxide, is solubilized in a solvent such as water or alcohol and subsequently transformed into a gel. The hydrolysis or alcoholysis is facilitated by heating and stirring. [177] Given that the gel derived from the hydrolysis/alcoholysis process exhibits a moist or damp state, it becomes necessary to employ suitable techniques for its dehydration, taking into account the specific characteristics and intended use of the gel. [178]

1.10 Functionalization of Gd_2O_3 nanomaterial

Functionalizing gadolinium oxide (Gd_2O_3) nanomaterials involves modifying their surface by using specific capping agents for the purpose of imparting desired properties or functions.

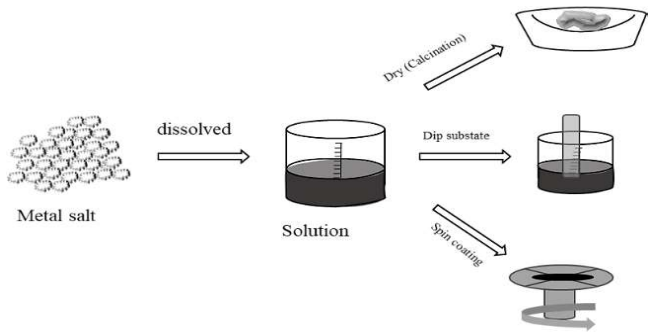


Figure 1.18: Sol-gel approach for synthesizing nanomaterials with different disposition mechanisms.

As stabilizers, capping agents prevent nanoparticle aggregation and provide a substrate for subsequent functionalization.

A capping agents selection depends on the intended application for better diagnosis and therapeutic. The following are common capping agents used in functionalization: -

1.10.1 Citric acid

Citric acid is a widely used capping agent for gadolinium oxide nanoparticles. It forms a stable surface coating and can provide functional groups for further conjugation with biomolecules or other ligands. Citric acid is a widely recognized molecule with a short-chain structure characterized by the presence of three carboxyl functional groups. [179,180]

In the context of nanoparticles, one or two of these carboxyl groups can be absorbed onto the surface of the particles, while at least one remains free for convenient functionalization. This property is advantageous as it aids in preventing the undesirable aggregates of gadolinium oxide nanoparticles. [181]

1.10.2 Polyethylene glycol (PEG)

PEG is a hydrophilic polymer that can provide stability and enhance the biocompatibility of gadolinium oxide nanoparticles. PEGylation helps to reduce nonspecific interactions with biological components and increases circulation time in vivo. [182] In contrast, polyethylene glycol (PEG) possesses numerous advantageous characteristics. These include notable hydrophilicity, flexibility, the ability to resist phagocytosis by macrophages, immunological recognition resistance, non-binding affinity with proteins, and biocompatibility. [183]

1.10.3 Polyvinylpyrrolidone (PVP)

Polyvinylpyrrolidone (PVP) is a water-soluble polymer that lacks an electrical charge and exhibits non-toxic properties that can stabilize nanoparticles and prevent aggregation. It finds utility in a wide range of medical applications. [184]. It has been used as a capping agent to improve the dispersion of gadolinium oxide nanoparticles in various solutions. [185] Polyvinylpyrrolidone (PVP) has the potential to fulfill multiple roles in various applications, including acting as a surface stabilizer, growth additive, nanoparticle dispersion, and reducing agent. [186]

1.10.4 Silica (SiO_2)

Silica coating on gadolinium oxide nanoparticles can improve their stability, biocompatibility and provide a platform for additional functionalization with other molecules or targeting ligands. Furthermore, the augmentation of T1 contrast in gadolinium can be achieved by doping the gadolinium oxide particles with mesoporous silica, which is attributed to the geometric confinement provided by the silica material.[187] Mesoporous or nonporous (solid) silica nanoparticles may be used for biomedical applications. Both varieties are composed of amorphous silica. Sol–gel, Stőber's process, and reverse microemulsion technique are synthetic methods for solid silica nanoparticles. [188] Sol–gel synthesis of mesoporous silica nanoparticles is possible using a surface-templated template. Silica nanoparticles neither absorb light nor disrupt a magnetic field, making them an excellent choice for MRI contrast agents. [189] The use of silica nanoparticles in drug delivery systems and optical contrast agents in imaging is widespread.

1.10.5 Antibodies or peptides:

Antibodies or peptides can be directly conjugated onto the surface of gadolinium oxide nanoparticles to enable targeted imaging or therapy. [190] These immunoglobulins, often known as antibodies which, are a class of glycoproteins that play an essential role in the unique defense systems of vertebrate animals. [191]

Hence, Antibody-conjugated, drug-loaded nanoparticles may specifically target and release substantial amounts of a drug in the cytoplasm of malignant cells while minimizing detrimental side effects. The efficacy of the delivery is contingent on the ability of each antibody to reach its target in sufficient quantities and on the small number of nanoparticles captured by the cell. [192]

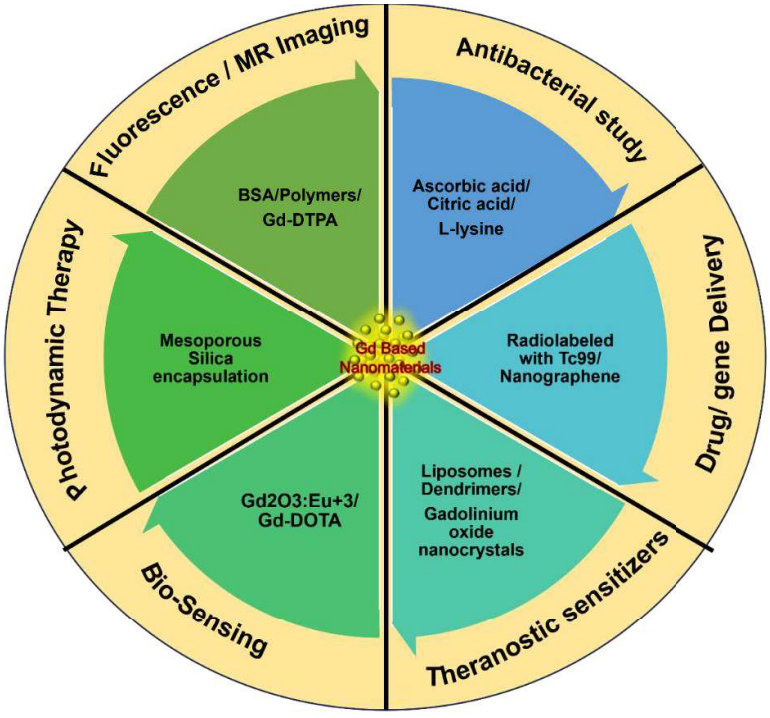


Figure 1.19. All plausible applications for gadolinium-based nanomaterials functionalized with different ligands, proteins, acids and polymers.

1.11 Problem statement

Lanthanide-based nanomaterials have proven to be effective tools in various biomedical applications, including magnetic resonance imaging, bioimaging, biosensing, antibacterial activity, drug transport, and nanomedicine. However, the traditionally available nanomaterials frequently exhibit cytotoxicity, colloidal instability, limited emission wavelength, non-specific targeting, and poor biodegradability, limiting their widespread use in therapeutic settings [193,194]. Additionally, the existing methods for synthesizing gadolinium oxide nanoparticles often result in a wide size distribution and irregular shapes, which limit their potential applications. [40] Also, the methods used for the synthesis are expensive and require harsh reaction conditions, making them inconvenient for large-scale production. It is important to note that so far, methods developing multimodal imaging agents based on Gd_2O_3 nanomaterials are known to be developed by doping other elements, enabling MRI and fluorescence synergistically, but the external dopant may pose a serious toxic risk and cause defects and structural deformation due to its incompatible atomic size and valence properties. Also, the diffusion of the dopants may alter the functionality and effectiveness of the prepared nanomaterial. [195] Additionally, one of the major challenges for the luminescent material is the problem of autofluorescence offered by the indigenous fluorescence of tissues, which arises in the region of 300-400 nm, resulting in a decrease in the S/N ratio of the image. [31,35] This problem can be tackled if the emission from the luminescent nanomaterial is beyond 400 nm. It will not only allow improved image quality due to the removal of autofluorescence from indigenous fluorophores, but also, a greater number of photons will be able to reach the detector of the microscope due to better tissue penetrability.[93] Therefore, the current thesis undertakes the challenge of developing a Gd_2O_3-based nanomaterial capable of generating broad luminescent emission and MRI

signals. Subsequently, develop and optimize new synthetic methods to produce thermodynamically stable uniformed size nanoparticles. The current thesis further undertakes to select a different capping agent with paramagnetic properties to stabilize the size and luminescence properties. Further, developed nanoparticles will be studied *in-vivo* for various biological and physical properties, and MRI contrast induced by the nanomaterial will be compared with the commercially available MRI contrast agents.