Chapter 1

Introduction and Literature review

Nanotechnology emerges as a highly promising field in the 21st century, offering the capability to translate nanoscience theory into practical applications. This involves the observation, measurement, manipulation, assembly, control, and manufacturing of materials on a nanometer scale. The National Nanotechnology Initiative (NNI) in the United States defines nanotechnology as the exploration of science, engineering, and technology at the nanoscale (1 to 100 nm), leveraging unique phenomena to create innovative applications across diverse domains, from chemistry, physics, and biology to medicine, engineering, and electronics (National Nanotechnology Initiative (NNI) 2019). This definition underscores two critical aspects of nanotechnology. Firstly, it revolves around scale – the ability to control the shape and size of structures at the nanometer level. Secondly, it emphasizes novelty, where nanotechnology harnesses the distinctive properties arising from the nanoscale (Allhoff 2007). The implementation of nanotechnology involves two primary manufacturing approaches: top-down and bottom-up, each varying in terms of quality, speed, and cost.

The top-down strategy involves the reduction of bulk materials to attain nano-sized particles. Accomplishing this involves advanced techniques such as precision engineering and lithography, which have been refined by the industry over the past decades. Precision engineering plays a pivotal role in the microelectronics sector, supported by advancements like nanostructures based on diamond or cubic boron nitride,

size-controlling sensors, numerical control, and advanced servo-drive technologies. Lithography entails shaping a surface by exposure to light, ions, or electrons, followed by material deposition to achieve the desired outcome (Iqbal et al., 2012).

Conversely, the bottom-up approach entails constructing nanostructures from the ground up, atom by atom or molecule by molecule, through physical and chemical methods operating within the nanoscale range (1 nm to 100 nm). This approach relies on controlled manipulation and self-assembly of atoms and molecules. Chemical synthesis produces raw materials that can be utilized either directly in their disordered form or as building blocks for more intricate ordered materials. Self-assembly represents a bottom-up strategy in which atoms or molecules arrange themselves into organized nanostructures through chemical-physical interactions. Positional assembly stands out as a technique allowing the precise positioning of individual atoms, molecules, or clusters (Iqbal et al., 2012).

The fundamental concepts of top-down and bottom-up approaches, along with the diverse techniques employed to synthesize nanoparticles through these methods, are summarized in Figure 1.1

1.1 History of Nanotechnology

In the fourth century AD, the Romans utilized nanoparticles and structures, presenting a captivating demonstration of early nanotechnology. The Lycurgus cup, a remarkable artifact housed in the British Museum, stands as a prominent accomplishment in the ancient glass-making craft. This cup, renowned as the earliest instance of dichroic glass, showcases an intriguing optical phenomenon where it exhibits dual colours. When illuminated directly, the glass takes on a green hue, while when light passes through it, it transforms into a reddish-purple shade. (Figure 1.2) (© British Museum, Identification number 1958, 1202.1).

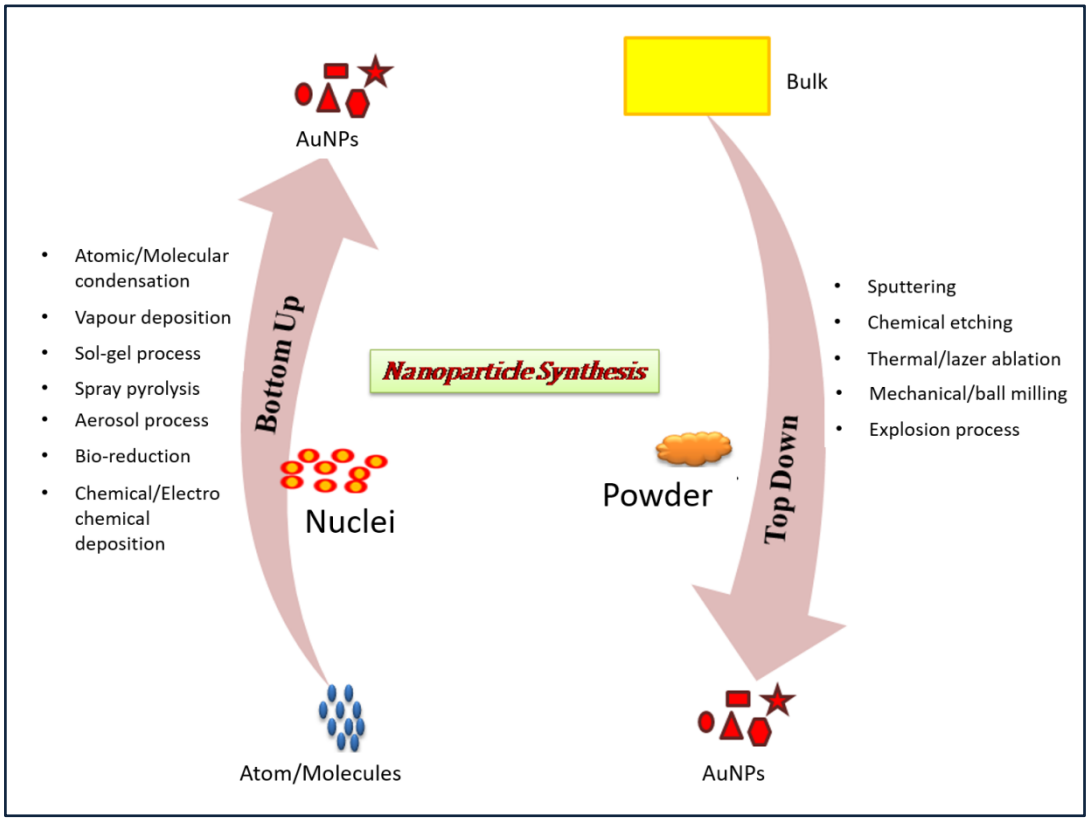

Figure 1.1 *The concept of top down and bottom up technology: different methods for nanoparticles synthesis*

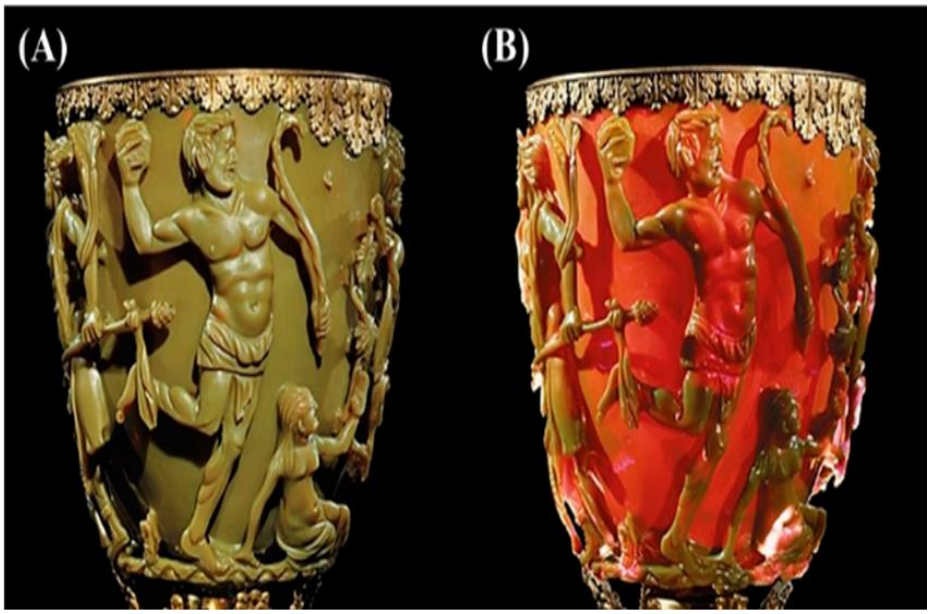

Figure 1.2 *The Lycurgus cup. The glass appears green in reflected light (**A**) and red-purple in transmitted light (**B**). (© British Museum Identification number 1958, 1202.1)*

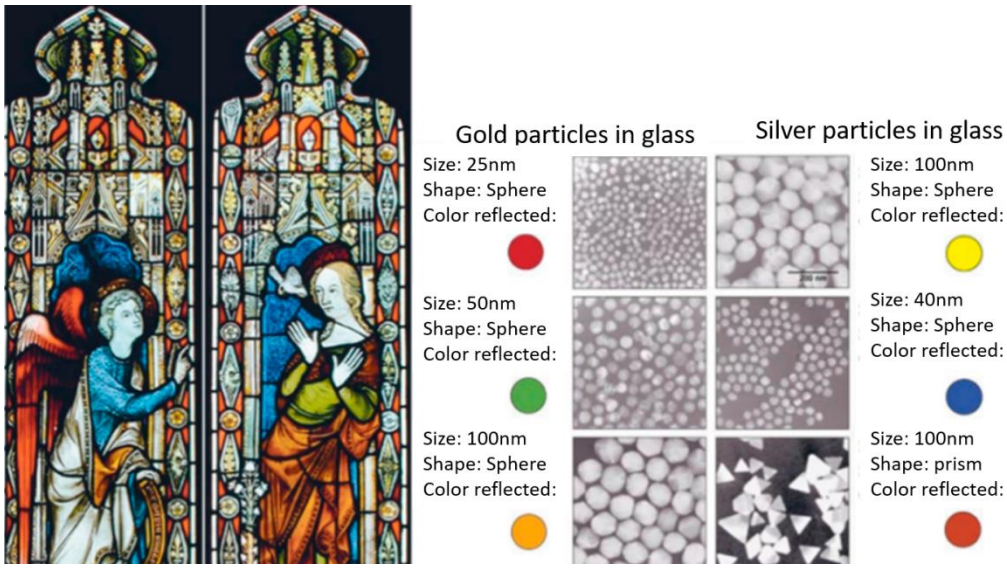

Figure 1.3 *Effect of nanoparticles on the colours of the stained glass windows. (The New York Times, February 22, 2005)*

In 1990, researchers utilized transmission electron microscopy (TEM) to analyze a cup, aiming to elucidate the phenomenon of dichroism (Barber et al., 1990). The observed dichroism, characterized by the presence of two distinct colours, was attributed to the existence of nanoparticles measuring 50–100 nm in diameter. Subsequent X-ray analysis revealed that these nanoparticles constituted an alloy of silver and gold (Ag-Au) in a ratio of approximately 7:3, along with a minor composition of around 10% copper (Cu), dispersed within a glass matrix (Freestone et al., 2004) (Freestone et al., 2007; Wagner et al., 2000). The absorption of light at a wavelength of ~520 nm resulted in a red colour, attributed to the presence of gold (Au) nanoparticles. The reddish-purple hue was ascribed to the absorption by larger particles, while the green colour emerged from the light scattering effect by colloidal dispersions of silver (Ag) nanoparticles exceeding 40 nm in size. The Lycurgus cup is renowned as one of the earliest examples of synthetic nanomaterial (Mansoori et al., 2005). A comparable phenomenon is observed in stained glass windows from the late medieval period, radiating luminous red and yellow colours due to the integration of gold (Au) and silver (Ag) nanoparticles into the glass. Figure 1.3

provides an illustrative instance of the influence of nanoparticles with varying sizes on stained glass windows.

1.2 Metal based Nanoparticles and Biomedical applications

Nanomaterial have permeated various sectors, including energy, the environment, the food industry, medicine, and beyond (Mir et al., 2013; Khan et al., 2015; Sultana et al., 2015; Umar et al., 2016). They continue to captivate both scientific and commercial interest (Xue et al., 2019). The distinctive properties of Nanomaterial exhibit size-dependent characteristics, leading to important chemical and physical characteristics. Recent developments have resulted in the improved modelling and designing of a wide range of medical and biological instruments and applications (Rathore et al., 2019; Yaqoob et al., 2019). Nanotechnology has achieved significant commercial utilization in the contemporary world. The small-scale dimensions (1–100 nm) of Nanomaterial confer upon them unique traits. These encompass heightened electrical, mechanical, and thermal stability, extensive surface area, as well as remarkable optical and magnetic features (Khoshnevisan et al., 2019; Yaqoob et al., 2020a). These enhanced attributes have facilitated the integration of nanomaterial into various domains, including electronics, magnetic, optics, and electrical devices. Additionally, engineered nanomaterial, exemplified by modifications in substances like titanium oxide, silver oxide, copper, zinc, and others, are also present. Many of these engineered materials find utility in commercial production zones, such as sunscreen and stain-resistant clothing manufacturing. Moreover, they serve investigative purposes in pharmaceuticals, diagnostic kits, imaging techniques, magnetic resonance imaging (MRI), drug delivery, and an array of medical equipment and procedures (DaRocha et al., 2019; Ferreira et al., 2019; Silva and Rocha, 2019). Nanomaterial with significant roles across various medical science applications are commonly found in several fundamental configurations,

illustrated in Figure 1.4. The initial classification pertains to Nanomaterial in their pure metallic nanoparticles form, often referred to as metal nanoparticles. Examples include silver, copper, gold, titanium, platinum, zinc, magnesium, iron, and alginate nanoparticles. Additionally, metal oxide nanoparticles (e.g., titanium dioxide, silver oxide, zinc oxide) constitute another variant. An alternative category comprises doped Nanomaterial involving metals, metal oxides, or a combination thereof, forming a distinct class within the realm of Nanomaterial. (Dar et al., 2011; Umar et al., 2013).

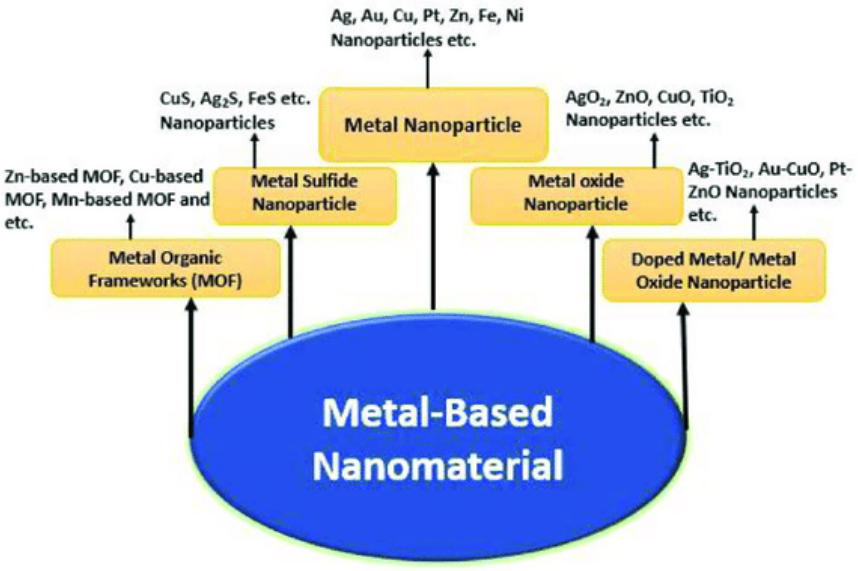

Figure 1.4 *Various types of metal-based Nanomaterial.*

Nanomedicine, rooted in the nano concept, is gaining increasing attention for its advancements in diagnostic accuracy and diverse treatment methodologies, employing nanoparticles within various diagnostic tools (Banerjee, 2018; Gurav et al., 2019; Ouyang et al., 2019).

Nanoparticles of noble metals (like Ag, Au, and Pt) have garnered significant interest because of their distinctive photothermal and optical properties (Yaqoob et al., 2020b).

These metallic nanoparticles display a phenomenon called localized surface plasmon resonance, characterized by the oscillation of electrons (Khan et al., 2017). The effective incorporation of these noble metal nanoparticles into biological systems without causing toxicity has had a substantial influence on both medical and biological research. Among the noble metallic nanoparticles, gold nanoparticles have garnered substantial interest due to their distinctively low toxicity, straightforward preparation, and favourable interaction with biological molecules (Elahi et al., 2018). Silver nanoparticles have demonstrated exceptional antimicrobial efficacy against virulent viruses, microbes, germs, and other nucleic acid-containing microorganisms. These nanoparticles are undeniably the most extensively employed material, finding utility even in textile industries as antimicrobial agents (Hasan, 2015). Noble metal nanoparticles are recognized as highly specific and versatile agents, encompassing a range of biomedical applications. These applications encompass highly sensitive investigative assays, augmentation of radiotherapy, gene delivery, thermal ablation, and drug delivery. Within the scientific community, these metallic nanoparticles are also considered non-harmful for gene and drug delivery purposes. Additionally, they offer the potential for simultaneous diagnostic and therapeutic utilization (Yamada et al., 2015). Ceramics, including metal oxide nanoparticles, are emerging as antimicrobial agents with diagnostic applications, contributions to drug delivery devices, and roles in various pharmaceutical and medical treatments.

1.2.1 Applications

1.2.1.1 Antimicrobial Agent

Numerous antimicrobial agents have been reported to exhibit toxicity towards all living organisms. To address this challenge, the synthesis of different inorganic and metal-based antibacterial agents with robust thermal resistance, sustainability, and enhanced

stability is essential. These agents should be produced under stringent processing conditions. (Rajawat and Qureshi, 2012; Hossain et al., 2015; Vijayakumari et al., 2019). Currently, key metallic nanoparticles such as Pt, Ag, Au, TiO2, and ZnO are extensively employed as antibacterial agents. These metallic nanoparticles find prominent use in biomedical applications due to their enduring stability and exceptional biocompatibility. Scientific investigations have demonstrated that metal-based nanoparticles possess biocidal activity against both gram-negative and gram-positive bacteria (Franci et al., 2015; Chiriac et al., 2016; Rajeshkumar et al., 2016; Wang L. et al., 2017; Ovais et al., 2019). This antimicrobial effect is attributed to their small nano-size and high surface-to-volume ratio, which facilitates penetration of bacterial membranes. The antibacterial mechanisms of metallic nanoparticles encompass metal ion release, oxidative stress, and nonoxidative-based stress, as depicted in Figure 1.5. Essentially, these nanoparticles become effective when they interact with the microbe's cell walls.

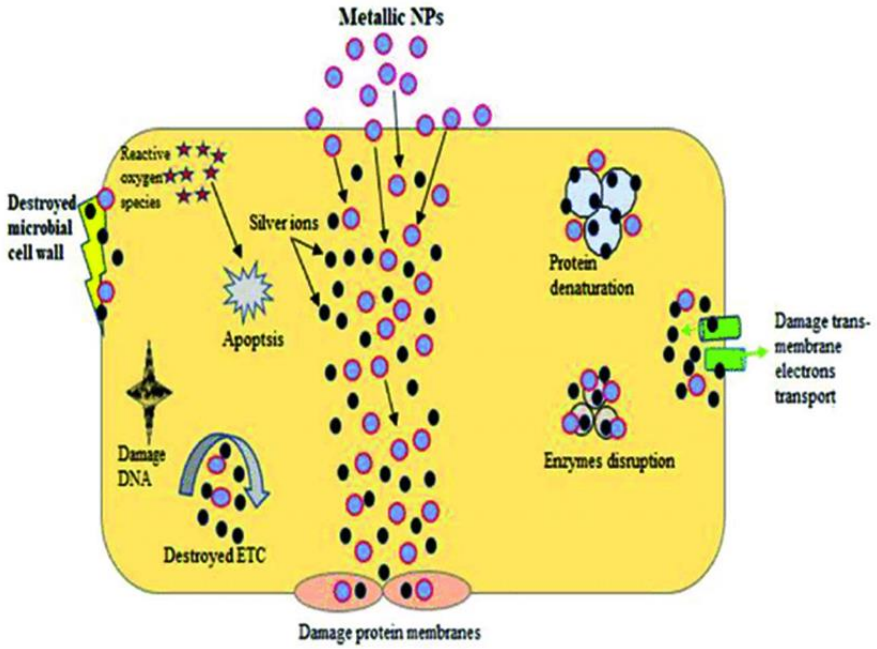

Figure 1.5 *General mechanisms of antimicrobial activities of metal-based nanoparticles*

Various approaches, such as van der Waals forces, electrostatic attraction, receptor/ligand interactions, and hydrophobic interactions, are employed to bring nanoparticles into contact with microbes. Upon successful contact, metallic nanoparticles can traverse inner membranes, interact with metabolic pathways, and induce alterations in membrane structure. Once inside the microbial cellular machinery, these nanoparticles hinder enzyme functions, deactivate proteins, disrupt electrolyte balance, induce oxidative stress, and modulate gene expression patterns (Vijayakumari et al., 2019).

The three primary antimicrobial mechanisms of metallic nanoparticles are oxidative stress, nonoxidative stress, and the release of dissolved metal ions. Oxidative stress induced by reactive oxygen species (ROS) is a potent means by which metallic nanoparticles combat microbes. Under normal circumstances, there is a balance between the production and clearance of ROS within microbial cells. However, excessive ROS production disrupts the intracellular redox state, leading to oxidative damage to the cell membranes. Metallic nanoparticles generate ROS ions, including hydroxyl radicals, which impede microbial growth (Sangaonkar and Pawar, 2018). Additionally, another prevalent mechanism involves the gradual release of metallic ions from metal oxides in aqueous environments. These ions are subsequently absorbed by cell membranes, leading to direct interactions with nucleic acids and proteins. The interactions of metallic nanoparticles exert widespread effects, encompassing changes in cell physiology and abnormal enzyme activities, ultimately disrupting normal physiological processes. Notably, Ag and Pd nanoparticles have demonstrated antimicrobial effects attributed to the release of metallic ions into solution (Shaikh et al., 2019). Zhao and Ashraf, 2015 investigated the antimicrobial mechanism of Ag nanoparticles, revealing their ability to infiltrate biofilms and inhibit their growth by modulating gene expression. Consequently, most metal-based nanoparticles exhibit antimicrobial properties through diverse

mechanisms. This multifaceted action limits the likelihood of microbial resistance. Developing resistance to metal-based nanoparticles would necessitate simultaneous mutations in multiple genes within bacterial cells, an impractical scenario.

1.2.1.2 Bioimaging Application

According to Conde et al. 2012, noble metals such as gold, silver, and platinum nanoparticles possess the ability to instantly respond and track due to their light absorption properties in biological tissue at near-infrared wavelengths. These common noble metallic nanoparticles have found applications in live imaging therapies due to their strong near-infrared radiation absorption, making them effective contrasting agents. However, noble metallic Nanomaterial can go beyond this by synergistically combining multiple imaging modalities, offering more valuable information compared to individual bioimaging techniques.

The emerging trend of three-dimensional imaging could potentially be achieved through computed tomography, where a series of cross-sectional images are woven together by a computer to create clear three-dimensional results. Nanoparticles composites, like the FeO/Si core and Au shell composite, serve as dual contrast agents for computed tomography and MRI in vivo. This combination exhibited notable computed tomography attenuation and improved magnetic resonance results for hepatoma (Gunko, 2016). Chauhan et al. 2019, demonstrated that Gd-Au nanocomposites hold promise as dynamic multimodal imaging agents for contrast. Furthermore, Zhang W.H. et al. 2016, explored the use of Si nanoparticles loaded with dye for fluorescence imaging, both in vivo and in vitro. Si nanoparticles exhibit excellent biocompatibility, low toxicity, strong hydrophilicity, and potential optical transparency, making them ideal substrates for generating fluorescent probes—an essential element in cell bioimaging.

1.2.1.3 Drug Delivery

Utilizing nanoparticles for drug encapsulation has emerged as a promising and effective strategy for drug delivery. Moreover, the incorporation of diverse polymer-based nanoparticles has enhanced the bioviability of this approach (Nasimi and Haidari, 2013; Lu and Thum, 2019; Vissers et al., 2019). Metallic nanoparticles therapies have demonstrated the ability to reduce therapeutic dosages, thereby improving the efficacy of cancer treatment. Injectable nanomedicine plays a vital role in targeting cancer cells (Couvreur, 2013; Sanku et al., 2019; Sharma et al., 2019). Conversely, these metal nanoparticles can readily bind with various agents like antibodies, peptides, and RNA/DNA to target diverse cell types. They are environmentally compatible, allowing for prolonged in vivo circulation for gene and drug delivery applications. Additionally, they can convert light into heat energy, enabling the precise targeting of cancer cells through thermal ablation. Antisense RNA serves as a valuable nucleic acid that directs RNA within cancer cells (Young et al., 2016). Furthermore, challenges related to cell permeability and drug solubility hinder the use of nanoparticles as therapeutic agents. Nanotechnology offers a solution by improving delivery systems. Generally, drug delivery methods employ multiple nanoparticles, each with its own limitations. However, altering factors such as size, shape, layers, coating substances, solvents, surface potential, and fabrication techniques can address these limitations effectively.

1.2.1.4 Tissue Engineering

Nanoparticles have garnered significant attention in recent times within the field of tissue engineering. These tiny particles, infused with engineering and biological principles, serve as practical substitutes for various damaged tissue types. Specifically, nanoparticles that possess attributes like low toxicity, customizable properties, contrasting agent capabilities, targeted delivery potential, and precise behavioural control are proving to be

ideal candidates for tissue development and regeneration (Corchero and Villaverde, 2009). Researchers have produced nanoparticles using diverse materials and composites to enhance their efficiency and role in tissue repair. Among these, metallic nanoparticles such as Titanium (Ti), Gold (Au), Silver (Ag), and others have demonstrated remarkable potential. Gold nanoparticles, for instance, find application in regenerative medicine, particularly in tissue replacement due to the emergence of tumor cells. (Lizundia et al., 2018; Bapat et al., 2019; Fathi-Achachelouei et al., 2019). Bioactive glass ceramic-based nanoparticles (BGC nanoparticles) have emerged as a promising material frequently employed in bone tissue regeneration. Li et al. 2018 have explored multifunctional poly-citrate-siloxane elastomer-based BGC nanoparticles, utilizing them effectively for bone tissue regeneration. Similarly, titanium nanoparticles have been utilized for bone repair, finding broad use in dentistry and orthopaedics due to their improved fracture resistance, enhanced ductility, and favourable weight-to-strength ratio. However, they do exhibit lower bioactivity, hindering cell adhesion and growth. In light of this, apatite coatings are preferred as bioactive materials, although they come with their own limitations such as uneven thickness, poor adhesion, and reduced mechanical stability. A stable, absorbent structure is crucial for providing the necessary nutrients during cell growth. Natural bone, a valuable nanocomposite material composed mainly of collagen and hydroxyapatite crystallites in an organic medium, possesses exceptional mechanical toughness that safeguards against damage. The nanoscale mechanism responsible for this amalgamation remains a topic of ongoing research (Scott et al., 2013; Walmsley et al., 2015; Azizian et al., 2018; De-Witte et al., 2018). To encapsulate the overarching concept of bone tissue engineering, a visual representation of the general mechanism is presented in Figure 1.6, offering a concise summary.

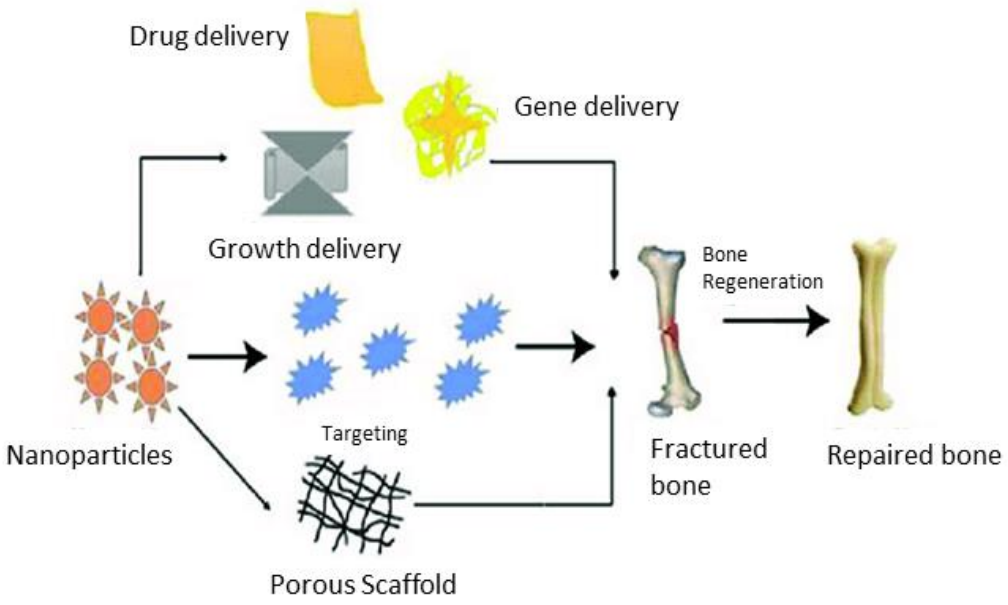

Figure 1.6 *A schematic mechanism of the bone tissue engineering process by using nanoparticles and drug molecules.*

1.2.2 Clinical aspects

In the present landscape of medical nanotechnology, numerous products employing nanotechnology are currently being utilized in clinical practice, as detailed in Table 1.1. Notably, these nanomedicines are primarily designed for drugs characterized by low aqueous solubility and high toxicity. These nano-formulations often demonstrate the capability to reduce toxicity while simultaneously enhancing the pharmacokinetic properties of the respective drugs. Although the FDA has regulated only a few nanomedicines, there are numerous ongoing initiatives involving clinical trials, suggesting that many new drugs based on nanotechnology will soon enter the market. The ongoing studies encompass various areas, with 18 directed towards chemotherapeutics, 15 intended for antimicrobial agents, 28 for diverse medical applications and psychological diseases, autoimmune conditions, and others, along with 30 focused on nucleic acid-based therapies (Caster et al., 2016). Table 1.1 provides a

classification of FDA-approved nanomedicines based on the type of carrier/material used in the formulation preparation.

Table 1.1 *Some examples of FDA-approved nanomedicine (Since 2000)* (Bobo el al., 2016)

Name	**Material Description**	**Nanoparticles advantage**	**Indication**	**Year of approval**
Polymer Nanoparticles – synthetic polymer particles combined with drugs or biologics				
Cimzia®/certolizumab pegol (UCB)	PEGylated antibody fragment (Certolizumab)	Improved circulation time and greater stability in vivo.	Crohn's disease	2008
			Rheumatoid arthritis	2009
			Psoriatic Arthritis	2013
			Ankylosing Spondylitis	2013
Eligard® (Tolmar)	Leuprolide acetate and polymer(PLGH (poly (DL-Lactide-co-glycolide))	Controlled delivery of payload with longer circulation time	Prostate Cancer	2002
Mircera®/Methoxypolyethylene glycol-epoetinbeta (Hoffman-La Roche)	Chemically synthesized ESA (erythropoiesis-stimulating agent)	Improved stability of aptamer as a result of PEGylation	Anemia associated withchronic kidney disease	2007
PegIntron® (Merck)	PEGylated IFN alpha-2b protein	Improved stability of protein through PEGylation	Hepatitis C	2001
Pegasys® (Genentech)	PEGylated IFN alpha-2a protein	Improved stability of protein through PEGylation	Hepatitis B; Hepatitis C	2002

ADYNOVATE (Baxalta)	Polymer-protein conjugate(PEGylated factor VIII)	Improved stability of protein through PEGylation	Hemophilia	2015
Liposome formulations combined with drugs or biologics				
Marqibo® (Onco TCS)	Liposomal Vincristine	Increased delivery to tumour site; lower systemic toxicity arising from side-effects	Acute Lymphoblastic Leukemia	2012
Visudyne® (Bausch andLomb)	Liposomal Verteporfin	Increased delivery to site of diseased vessels; photosensitive release	Macular degeneration, wet agerelated; myopia; ocular histoplasmosis	2000
Doxil®/Caelyx™ (Janssen)	Liposomal doxorubicin	Improved delivery to site of disease; decrease in systemic toxicity of free drug.	Ovarian cancer	2005
			multiple myeloma	2008
Micellar nanoparticles combined with drugs or biologics				
Estrasorb™ (Novavax)	Micellar Estradiol	Controlled delivery of therapeutic	Menopausal therapy	2003
Protein nanoparticles combined with drugs or biologics				
Abraxane®/ABI-007(Celgene)	Albumin-bound paclitaxel nanoparticles	Improved solubility; improved delivery to tumor	Breast cancer	2005
			NSCLC	2012
			Pancreatic	2013

			cancer	
Nanocrystals				
Megace ES® (ParPharmaceuticals)	Megestrol acetate	Reduced dosing	Anti-anorexic	2001
Ritalin LA® (Novartis)	Metyhlphenidate HCl	Increased drug loading and bioavailability	Psychostimulant	2002
Vitoss® (Stryker)	Calcium phosphate	Mimics bone structure allowing cell adhesion and growth	Bone substitute	2003
NanOss® (Rti Surgical)	Hydroxyapatite	Mimics bone structure allowing cell adhesion and growth	Bone substitute	2005
Inorganic and metallic nanoparticles				
Nanotherm® (MagForce)	Iron oxide	Allows cell uptake and introduces superparamagnetism	Glioblastoma	2010
Venofer® (LuitpoldPharmaceuticals)	Iron sucrose	Allows increased dose	iron deficiency in chronic kidney disease (CKD)	2000
GastroMARK™; umirem®(AMAG pharmaceuticals)	SPION coated with silicone	Superparamagnetic character	Imaging agent	2001
Feridex®/Endorem®(AMAG pharmaceuticals)	SPION coated with dextran	Superparamagnetic character	Imaging agent	2008

1.3 Surface modification strategies to improve properties of metal-based nanoparticles

The surface characteristics of newly synthesized materials often fall short in terms of their suitability for biological compatibility, toxicity, adhesion properties, and wettability. Therefore, it is crucial to enhance these properties to a satisfactory level before applying them practically or using potential processing techniques like coating with specific materials. The field of metal-based nanoparticles is rapidly advancing in research, showing promise for various applications. Despite the remarkable physicochemical properties of many types of nanomaterial, they may lack the necessary surface specificity for particular uses. Thus, upgrading surface properties becomes essential. Modifying the surface of metal-based nanoparticles offers several advantages. Firstly, this modification helps prevent the nanoparticles from clumping together, enhancing stability. Secondly, it promotes self-organization, and thirdly, it promotes compatibility with other materials (Viswanathan et al., 2019). For instance, attaching metal nanoparticles to suitable functional groups can render them water-soluble. Another example is altering the surface of inorganic (nano-) fillers with organic compounds. This modification resolves compatibility and homogeneity issues between these fillers and organic compounds, ultimately improving the mechanical properties of organic/inorganic composites (Rahman et al., 2002). Even materials used in medical kits require surface modification prior to medical treatment. Surface modification yields clinical benefits such as effective antimicrobial action, high bioactivity, favourable cell growth and tissue interaction, and increased durability (Izman et al., 2012). Effective surface modification of metallic nanoparticles helps enhance the material's properties, including formability, relatively low modulus, and robust mechanical strength. Various approaches to surface modification, including chemical, mechanical, oxidation, sol–gel, physical vapour

deposition, and ion implantation, are widely employed and summarized in Figure 1.7, commonly employed to enhance biomedical applications using metal-derived nanoparticles (Kumar et al., 2020).

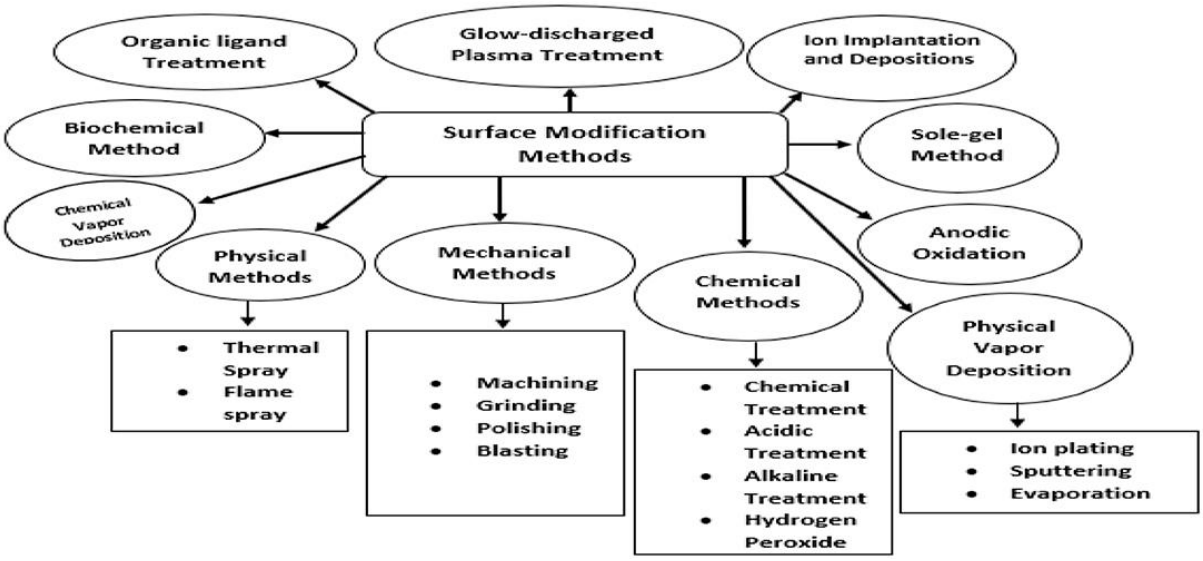

Figure 1.7 *Summary of surface modification methods of metal-based nanoparticles*

Moreover, organic ligands are considered effective for surface modification, yielding improved outcomes. These organic groups prevent nanoparticles aggregation, and functional groups on the nanoparticles surface enable precise interactions with other molecules. The mechanisms underlying these methods have been extensively detailed in previous literature by various research groups, including Kango et al. (2013), Asri et al. (2017), Qi et al. (2019), Mozetič (2019), Oun et al. (2020), and Liu et al. (2020), respectively.

1.4 Gold nanoparticles

Gold nanoparticles (AuNPs) have a rich history in chemistry, dating back to ancient Roman times where they were used to stain glasses for decorative purposes. The modern era of AuNPs synthesis began over 150 years ago with the work of Michael Faraday,

who was possibly the first to observe that colloidal gold solutions have properties that differ from bulk gold (Hayat 2012; Edwards and Thomas 2007). Reliable and high-yielding methods for the synthesis of AuNPs, including those with spherical and non-spherical shapes, have been developed over the last half century (Daniel et al., 2004). The resulting AuNPs have unique properties, such as size- and shape-dependent optical and electronic features, a high surface area to volume ratio, and surfaces that can be readily modified with ligands containing functional groups such as thiols, phosphines, and amines, which exhibit affinity for gold surfaces (Daniel et al., 2004). By using these functional groups to anchor the ligands, additional moieties such as oligonucleotides, proteins, and antibodies can be used to impart even greater functionality. The realization of such gold nanoconjugates has enabled a broad range of investigations, including programmed assembly and crystallization of materials (Mirkin et al., 1996; Park et al., 2008), arrangement of nanoparticles into dimers and trimers onto DNA templates (Alivisatos et al., 1996), bioelectronics (Park et al., 2002; Wang et al., 2003; Xiao et al., 2003), and detection methods (He et al., 2002; Liu et al., 2003). The application of gold nanoconjugates for biodetection and biodiagnostics has been reviewed elsewhere (Penn et al., 2003; Katz et al., 2004; Rosi et al., 2005). In recent years, gold nanoconjugates and their properties have led to new and exciting developments with enormous potential in biology and medicine. These investigations represent a new direction that greatly deviates from the more established use of gold nanoconjugates as labels for electron microscopy (Faulk et al., 1971). Our recent studies, as well as those of several other research groups, have shown that gold nanoconjugates, when functionalized with appropriate surface moieties, can readily enter living cells. These developments have forged a new frontier in nanoparticles research, including the broader use of gold

nanoconjugates in cellular biology and the promise for their eventual use as therapeutic agents.

1.4.1 Surface Functionalization of Gold nanoparticles

The metal nanoparticles most commonly investigated encompass gold, silver, titanium oxide, and iron nanoparticles (El-Ansary et al., 2009). Among these, gold stands out as inert and significantly less detrimental, finding extensive use in various applications, notably for drug and gene delivery (Connor et al., 2005; Ghosh et al., 2008; Pissuwan et al., 2009).

However, a major obstacle in utilizing these nanoparticles for targeted delivery to specific tissues is their small "nano" size, which allows them to readily enter numerous cells.

Scientists have been devising strategies to achieve targeted distribution by linking these nanoparticles with diverse biomolecules and ligands, aiming to circumvent this concern. In order to increase the applicability and lessen the toxicity of the NPs, a procedure known as surface functionalization involves adding appropriate materials to the surface of the NPs (Jinhao et al., 2009). Surface functionalization of Nanomaterial involves the use of a wide variety of materials, including tiny organic compounds, dyes, polymers, inorganic materials, and biomolecules (Sarmento et al., 2018). Functional nano-materials, particularly gold (Au) have gained significant attention in the field of biomedical applications due to their unique properties and promising potential. Functional AuNPs possess distinct properties such as high surface area, tunable optical properties, excellent biocompatibility, and ease of surface functionalization. These characteristics make them attractive for various biomedical applications, including diagnostics, therapeutics, imaging, and drug delivery. Surface functionalization of AuNPs is key element for their stability, functionality, and biocompatibility. It is simple to functionalize AuNPs with

various biological molecules or chemical functional groups to build an excellent vehicle for a variety of uses, including drug delivery, cancer therapy, and diagnostic procedures (De Long, et al. 2010). The ultimate goal is to maintain the unique properties of AuNPs and attached molecules i.e., potent plasmon absorption bands and biorecognition features, respectively. Functionalized AuNPs can often enter the target tissues and carry out a therapeutic or detective function. Depending on the ligand's type and the methods used for functionalization, there are two types of interactions that can occur between AuNPs and ligands: physical and chemical (Suk, et al. 2016). In chemical interaction, covalent bonding is used to modulate of AuNPs due to its potency and excellent stability in physiological environment. The use of covalent modifications is simply, however the biocompatible and available ligands for this method are restricted (Aminabad et al., 2019).

Ligand exchange is an essential step to conjugate ligands on the surface of AuNPs and to produce NPs with desirable surface properties. During this process, firstly the reactive group of the functional compound binds to AuNPs surface, while the other head group of the compound serves as a binding site for biomolecules like peptides or antibodies (Pellegrino et al., 2005). Trialkoxysilane molecule is commonly used in this method due to the number of coupling sites that have the capacity for interaction with vinyl and amino groups (Liu et al., 2008). A physical interaction includes the noncovalent interactions, like electrostatic interaction between positively charged molecules and negatively charged AuNPs. Another frequently used method for functionalizing the surface of AuNPs is self-assembly monolayer. This technique relies on composing a certain monolayer on the gold particle, spontaneously (Abdelrahman et al. 2006).

The proper candidates for AuNPs self-assembly among diverse compounds are amides, thiols, and carboxylic acids. Different types of molecules can be chosen depending on the

size of NPs that are needed, the application area, and the functionalization method that is being employed (Ajnai et al. 2014). Thiol-containing ligands, such as 3-mercaptopropionate, a-mercaptoacetate, and mercaptoalkanoic acid, are more suitable ligands for functionalizing AuNPs with sulfur-gold interaction (Locatelli 2014). One of the most popular techniques for functionalizing AuNPs is polymeric coating. A biopolymer called polyethylene glycol (PEG) is frequently utilized to modify the surface of AuNPs. Since PEGylated AuNPs reject one another due to steric effects, this polymer gives AuNPs their colloidal stability (Kanaras et al. 2002). PEG is used to coat AuNPs either by itself or in combination with other biomolecules like peptides. Due to their capacity to connect cell membranes and access target cells, these functionalized AuNPs can function as appropriate drug delivery systems. Amino acid functionalization of AuNPs is a practical technique to improve the specificity and efficacy of NPs-based delivery systems. These specific AuNPs have a greater affinity for DNA and can deliver genes without cytotoxicity (Ghosh et al. 2008).

1.4.2 Biomedical Applications of *f*AuNPs

On the basis of the functional moieties and their abilities, *f*AuNPs have established their route from diagnostics to therapeutic in biomedical. AuNPs have unique optical, physical, and chemical properties due to their shape and size; also, they can be employed in the research, particularly in the field of diagnostics, biosensing, therapy, and drug/gene delivery.

Gold nanoparticles (AuNPs) play a pivotal role in various applications, including the administration of nucleic acids, proteins, gene therapy, in vivo delivery, and targeting. Table 1.2 presents some notable applications of these functionalized AuNPs.

Table 1.2 *Some examples of Functional AuNPs and their biomedical applications*

S. No.	Functional Group	Ligands /Carrier Molecule	Key Feature	Application	Ref
1	Polyethylene Glycol (PEG)	PEG with ligands such as a dye attached through thiol group	Adherence to the cell membrane	Cellular and intracellular targeting, bio distribution studies	Lipka et al., 2010; Cho et al., 2010; Takae et al., 2005; Ishii et al., 2004; Khalil et al., 2004
2	Amine Group	PEG	siRNA carrier	Useful in RNAi technology	Lee et al., 2008
3	Carboxyl Group	Proteins	-	Various depending on the protein	Wangoo et al., 2008
4	Peptide	Cell surface receptors, amyloid inhibitory peptide + sweet arrow peptide, antibody, octrotide peptide	Cytoplasmic and nuclear translocation, adjuvant, targeting carcinoma cells analogue of somatostatin	Cellular and intracellular targeting, macrophage and pro-inflammatory cytokine elicitation bioimaging imaging of cancer cells	Sun et al., 2008; Tkachenko et al., 2004; Bastis et al., 2009; Rayavarrapu et al.,

					2007; Surujpaul et al., 2008
5	DNA	Aptamer, PEGylated gold- poly (β-amino ester), Thiolated ssDNA of RNA I gene, antisense DNA oligonucleotides	Targeting Prostate cancer cells , siRNA carrier, binds to antisense RNA of p53	Bioimaging, gene delivery RNAi-regulation of transgene expression, detection of specific genes e.g., for microbial detection	Javier et al., 2008; Lee et al., 2009; Kim et al., 2010; Rink et al., 2010; Javier et al., 2009
6	RNA	Polyvalent RNA-gold nanoconjugates	-	RNAi	Giljohann et al., 2009
7	Antibodies	scFv Antibodies against various pathogens	Smaller size, label fidelity	Immunoassays treatment and diagnosis e.g., antibodies against aflatoxins	Liu et al., 2009; Shen et al., 2008; Sharma et al., 2010

1.4.2.1 *f*AuNPs for Targeted Delivery

Functionalized gold nanoparticles (*f*AuNPs) have been employed to guide drugs and

biomolecules toward specific cell types and even organelles such as the nucleus or mitochondria. The utilization of AuNPs modified with PEG and 3-mercaptopropionic acid has been shown to enable their entry into the nuclei of HeLa cells without causing notable cytotoxicity. This characteristic establishes their suitability as a vehicle for delivering drugs specifically to the cell nucleus (Gu et al., 2009). Similarly, the utilization of liposomes encapsulating AuNPs has been investigated for their potential in targeting and entering cells effectively, all the while transporting drugs or other payloads (Chithrani et al., 2010). It has been demonstrated that AuNPs as small as 1.4 nm can be taken up into cells, 1000-fold increasing internalization. Such nanoparticles have a great deal of potential for usage as carriers for drugs, gene delivery and other biomolecules.

1.4.2.1 (a) *Gene Delivery*

As illustrated in Figure 1.8, PEGylated AuNPs are one of the most frequently employed nanoparticles for gene delivery. The effectiveness of a PEGylated AuNPs-based delivery method for its transfection efficiency was assessed utilizing plasmid DNA mediated through electroporation (Kawano et al., 2006).

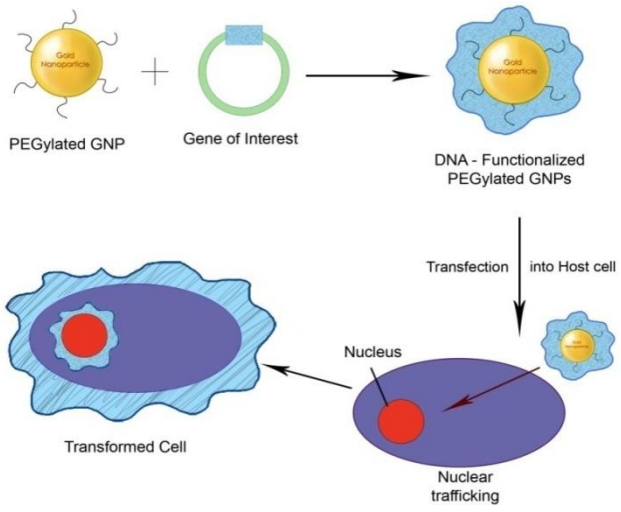

Figure 1.8 *PEGylated AuNPs for gene delivery*

After intravenous injection, gene expression was 100 times more upregulated with DNA-PEGylated AuNPs than with naked DNA. The DNA was liberated and passed across the cellular membranes, and the transgenes were stable in circulation.

In a correlated research, researchers combined positively charged colloidal AuNPs with plasmid DNA containing murine interleukin-2 (pVAXmIL-2) to greatly increase transgenic expression with minimal toxicity (Noh et al., 2007). Efficacious gene delivery vectors without cytotoxicity have also been created employing AuNPs functionalized with amino acids. Similar to this, PEGylated gold-poly (β-amino ester) nanoparticles were used to effectively deliver siRNA to the host cells, where the poly (β-amino ester) was the crucial molecule in the intracellular targeting of the DNA (Lee et al., 2009).

The use of AuNPs anchored on magnetic nanoparticles has led to the development of a gene delivery system based on an adenoviral vector (Kamei et al., 2009). This method allowed for effective gene delivery without the issue of viral tropism in the host. These conjugates of functionalized AuNPs and DNA proved to be reliable and effectively controlled substantial trans-gene expression. Didodecyldimethylammonium bromide (DDAB), a cationic lipid, coated AuNPs have recently been found to offer increased gene transport efficiency with decreased toxicity. It was seen that the expression of the luciferase gene increased 48-fold and that of the green fluorescent protein increased by more than twofold, after transfection (Li et al., 2010). Research has shown that by utilizing the layer-by-layer technique, *f*AuNPs with reversed charges can effectively transport siRNA and plasmid DNA into cancer cells (Guo et al., 2010). These charge-reversed AuNPs were utilized to convey Lamin A/C-siRNA, which targets the Lamin A/C gene accountable for a nuclear envelope protein. This approach displayed greater effectiveness in gene knockdown

compared to Lipofectamine 2000. This research reveals that such dual purpose functionalized AuNPs can be used for gene silencing along with targeted delivery (Guo et al., 2010). Successful targeted delivery of specific genes has been achieved using a diverse range of nanoparticles. In a recent work, DNA functionalized AuNPs were employed to control the expression of genes in mice bearing xenograft tumors (Kim et al., 2011). In mammalian cells and mouse models in vivo, functionalized gold nanoparticles (*f*AuNPs) modified with thiolated RNA I were effective in loading and transporting antisense DNAs to redirect gene splicing, as well as double-stranded DNAs to act as decoys for gene transcription factors. This successful utilization demonstrates their potential in gene regulation and delivery. Elevated transfection efficacy for gene delivery was attained when DNA was coupled on AuNPs functionalized with polyethyleneimine (PEI) and chitosan (Tencomnao et al., 2011). PEI functionalized AuNPs have been emplyoed as a gene delivery vector with minimal cytotoxicity in the rabbit cornea (Sharma et al., 2011). Gold nanoparticles were detectable in significant levels in the keratocytes and extracellular matrix of rabbit corneal tissues that were collected after12 h, 72 h or 7 days. In another work, the in vivo gene expression efficiency in the mouse lung was increased up to 26-fold by AuNPs functionalized with PEIs with different alkyl chain lengths and levels of substitution (Fortune et al., 2011).

1.4.2.1 (b) *Drug Delivery*

Due to their simplicity in synthesis, functionalization, and biocompatibility, AuNPs are appropriate for the drugs delivery to cellular targets. When AuNPs are functionalized with certain biomolecules, they can efficiently kill bacteria or cancer cells (Figure 1.9) **(**Duncan et al., 2010).

A significant number of drug molecules can be transported by AuNPs due to their

high surface to volume ratio (Grace et al., 2007). Gold nanoparticles (AuNPs) have been employed for co-administering protein drugs owing to their ability to effectively penetrate cellular membranes, probably because of the interaction of AuNPs with lipids on cell surfaces.

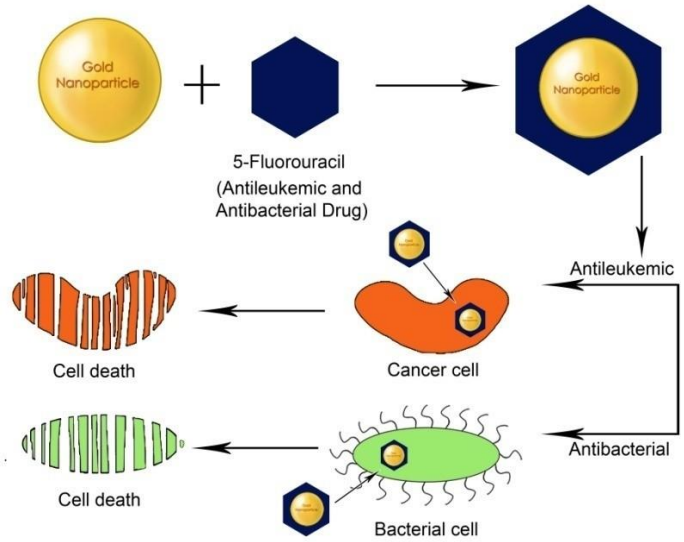

Figure 1.9 *Functionalized AuNPs (fAuNPs) for drug delivery: Precisely targeting specific cells with enhanced loading efficiency, achieving targeted drug delivery, and ensuring efficient drug release.*

Likewise, high efficacy vectors for vaccine administration have been created using AuNPs covalently bonded to low molecular weight chitosan (Zhou et al., 2008). The effectiveness of these Chito6-AuNPs has been investigated both in vitro and in vivo. These conjugates were discovered to be more effective than a naked DNA vaccination when administered intramuscularly to BALB/c mice. Compared to naked DNA, chito6-AuNPs conjugates also strongly stimulated cytotoxic T cell responses.

The antibacterial and antifungal properties of AuNPs functionalized with 5-fluorouracil (5-FU), an anti-leukemic medication, were examined against *Micrococcus luteus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia*

coli, *Aspergillus fumigatus*, and *Aspergillus niger*. Due to their easier permeability into the cells, 5-FU-AuNPs were discovered to be more efficient against Gram negative bacteria than Gram positive bacteria. They additionally demonstrated antifungal activity on *A. fumigates* and *A. niger.* Although, the mechanism behind this phenomena is still unclear. In a related investigation, AuNPs have also been utilized to identify a number of aminoglycosidic antibiotics including streptomycin, gentamycin and neomycin (Grace et al., 2007). AuNPs functionalized with these antibiotics have been tested against a variety of strains of Gram positive and Gram negative organisms, namely *Staphylococcus aureus, Micrococcus luteus, E. coli* and *Pseudomonas aeruginosa,* as evidence of their effective function in drug delivery. Gold nanoparticles (AuNPs) synthesized using cofactors as both reducing and capping agents were subsequently encapsulated with PEI (polyethyleneimine). These functionalized AuNPs were then studied for their impact on E. coli growth. The results indicated that these AuNPs hindered the synthesis of the peptidoglycan layer in E. coli, leading to an increase in cell wall permeability (Rai et al., 2010).

To halt the transmission of the H1N1 influenza virus, gold nanorods functionalized with activators of innate immune responses were employed. This involved the activation of the retinoic acid-inducible gene I pathogen recognition pathway through the application of short hairpin RNA (ssRNA). This activation enhanced the expression of interferon-gamma (IFN-) and other IFN-stimulated proteins, subsequently curtailing the replication of H1N1 influenza viruses (Chakravarthy et al., 2010).

1.4.2.2 As Cancer Diagnostic and Therapeutic Agents

In recent years, the use of nanoparticles in cancer treatment has grown in favour (Scheinberg et al., 2010). AuNPs (2 nm) coupled with cyclodextrin and admantane

have been reported to exhibit photothermal effects on cancer cells (Wang et al., 2010). Conventionally, gold nanoparticles (AuNPs) have been integrated with magnetic nanoparticles to selectively target specific cell types, enhancing the efficacy of cancer cell imaging. Utilizing a process of aggregation and entrapment termed the "permeation and retention effect," facilitated by angiogenic arteries and limited lymphatic flow, nanoparticles can effectively zero in on tumor cells. As a result, the nanoparticles can concentrate more readily inside malignant cells than in healthy cells. At a concentration of 5 g/mL, iron nanoshells coated with gold were found to impede the growth of colorectal and oral cancer cells without being hazardous to healthy cells before oxidation (Wu et al., 2011). However, it was shown that the cytotoxicity was based on the age of the nanoparticles. The release of these particles within human cell lines was observed to occur gradually over a span of 48 hours. This release was easily detectable through various biomedical techniques, mainly due to the presence of iron.

Recently, AuNPs functionalized with fluorescently tagged heparin have been employed to specifically identify and kill metastatic cancer cells through apoptosis (Lee et al., 2010). The approach employed in the study is based on the heightened presence of heparin-degrading enzymes within metastatic cancer cells. Heparin's fluorescence diminishes when it is bound to AuNPs, but this fluorescence is reinstated after digestion by heparinase and heparanase. This mechanism enables the detection of cancer cells. The research also illustrated that heparin binds to the excessively expressed RGD peptide in cancer cells, leading to apoptosis. Consequently, these functionalized AuNPs hold potential for both diagnosing and treating cancer.

According to reports, AuNPs exhibit excellent colloidal stability and biocompatibility, rendering them a superior surface for modifications such as PEGylation and Thiol-PEGylation etc. A review of SH-PEGylated AuNPs has been conducted as a contrast

agent for tumor vascular agents. AuNPs can be used to diagnose several malignant cell types that are difficult to detect at an early stage, such as oral squamous epithelial cells (Kah et al., 2007).

Gold nanoparticles (AuNPs) possess the capability to exhibit distinct surface plasmon resonances when positioned in close proximity to one another. As a result, AuNPs have been effectively used to discriminate between normal and malignant cells when linked to anti-epidermal growth factor receptor antibodies functioning as biomarker agents. Given their straightforward synthesis, chemical stability, biocompatibility, and unique optical attributes, gold nanoshells with iron oxide cores, referred to as Gold-iron oxide nanoshells, have been developed as a practical instrument for both targeting and treating tumor cells. (Melancon et al., 2009). Gold provides optical characteristics while the iron oxide core provides magnetic properties in gold-iron oxide nanoshells for their application in diagnosis and therapeutic use i.e.; in MRI of tumor tissues and in hyperthermic treatment, respectively. Though these nanoparticles must overcome biological obstacles, careful consideration of their size, shape, and functionalization process can improve their guided entry into particular tumor targets. The retention duration in tumor tissue can be extended by choosing smaller nanoparticles (passive targeting). To enhance the duration of stay of these nanoparticles and facilitate their applicability in both diagnosis and therapy, it is feasible to functionalize these nanoshells by attaching them to the gold surface through cell surface receptors (such as epidermal growth factor receptors, EGFRs), peptides, and antibodies specifically targeting tumor cells.

Targeting and imaging of the tumor cells have been accomplished using AuNPs functionalized with polyamidoamine (PAMAM), dendrimer-folic acid, and/or flourescein isothiocyanate (FITC) conjugates (Shi et al., 2009). These dendrimers'

surface characteristics and the acetylation of their terminal amines make it possible to produce *f*AuNPs with a variety of ligands, creating multifunctional nanoparticles. The folic acid attached to these nanoparticles aids in their ability to target the tumor cells by interacting with the folic acid receptors on the cell membrane *in vitro*. In another related work, AuNPs having a glutathione cap with COOH groups and folic acid along with a FITC tag were employed to target cancerous cells (Zhang et al., 2010). Due to the presence of the folic acid receptors, these *f*AuNPs specifically interacted exclusively with HeLa cells, excluding non-cancerous cells and offering a quick and accurate technique of cancer cell detection. Folic acid has also been utilized to target cancer cells when it is coupled to PEGylated AuNPs (Bhattacharya et al., 2010). For targeted drug delivery, PEG backbones such as PEG diamine, PEG tetramine, PEG dithiol, etc. were employed. To specifically target and deliver AuNPs to breast cancer cells with up to 2.7-fold increased therapeutic potency *in vitro*, a thiol-PEGylated tamoxifen derivative was synthesized (Dreaden et al., 2009). Thioctic acid PEG-folate AuNP conjugates were employed to target the ovarian cancer cells at the subcellular level. Depending on the drugs or compounds carried on them, these *f*AuNPs could enter different subcellular compartments. Doxorubicin and cisplatin loaded AuNPs targeted the nucleus of the cancer cells whereas their mitochondria were targeted by gamitrinibs loaded AuNPs (Rajendran et al., 2010). Therapeutic agents functionalized AuNPs can be activated by exchange with complementary molecules, as a result cytotoxicity is reduced, subcellular sites are targeted, and eventually the drug is released for the desired therapeutic effect (Kim et al., 2010). Similar to this, hollow gold nanospheres have been created with the dual ability to release doxorubicin when exposed to near infrared light and photothermally destroy cancer cells (You et al., 2010). Similarly, cylindroid AuNPs have been used to deliver drugs to tumor cells

when they are combined with fluorescein or doxorubicin (Kim et al., 2010). Porphyran capped gold nanoparticles were employed to deliver the anticancer medicine (doxorubicin) in the human glioma cell line LN-229. When this drug was conjugated to AuNPs as opposed to the drug alone, its cytotoxicity was increased (Venkatpurwar et al., 2011).

Drug delivery into cells without internalization has been accomplished using biocompatible AuNPs with two functional domains (Kim et al., 2009). These domains consist of a hydrophobic alkanethiol interior enclosed by a hydrophilic shell composed of a tetraethylene glycol (TEG) unit, terminated with a zwitterionic head group. These modified particles effectively minimize nonspecific binding with bio-macromolecules. The study revealed that hydrophobic dyes or drugs can be efficiently encapsulated within the hydrophobic pockets of AuNPs and subsequently released into cells through membrane-mediated diffusion, following the uptake of the carrier nanoparticles. The compact size of these nanocarriers, combined with their biocompatible surface properties, contributes to extended circulation duration in the bloodstream and a preference for accumulating in tumor tissues due to the enhanced permeability and retention effect. Furthermore, these systems are particularly suitable for targeted approaches due to the non-interacting nature of their monolayer. B-Chronic Lymphocytic Leukemia (BCLL) is characterized by heightened resistance to apoptosis. In comparison to utilizing these antibodies in isolation, it has been reported that AuNPs conjugated with anti-VEGF (Vascular Endothelial Growth Factor) can enhance the induction of apoptosis in CLL cells (Mukherjee et al., 2007).

Research have been carried out on nanoparticles functionalized with peptides, fluorophores, cell adhesion molecules, aptamers or other biomolecules to target particular tissues and they show potential as tools for tumor imaging, drug

administration, and apoptosis detection (Sun et al., 2008; Kim et al., 2010; Choi et al., 2010) (Figure 1.10).

It has been demonstrated that human breast cancer cells can successfully absorb AuNPs functionalized with coumarin and PEG without experiencing any toxicity. This dual functionalization of AuNPs with biomolecules and fluorescent dyes can be exploited to specifically target cells for drug delivery and bioimaging applications.

Similar to this, AuNPs functionalized with the synthetic somatostatin analogue octreotide peptide have been investigated as prospective bioimaging agents for a variety of neuro-endocrine carcinomas (Surujpaul et al., 2008).

Somatostatin receptors are over expressed in these carcinomas, and the creation of bioimaging agents based on these receptors can aid in the early detection of such cancers. Due to their improved protein receptor recognition and higher fluorescence qualities, AuNPs functionalized with the octreotide were found to interact more with tumor cells than AuNPs alone.

Radioactive AuNPs functionalized with gum arabic glycoprotein (GA-^{198}AuNP) were examined for their biocompatibility and potential as cancer therapeutics in severely compromised immuno-deficient (SCID) mice (Chanda et al., 2010). Targeting specific tumor cells allowed nanoparticles to penetrate through tumor pores and capillaries with little to no radioactive leakage.

1.4.2.3 As Biosensors

AuNPs have been investigated and used in the creation of a variety of biosensors to identify particular biomolecules important in the genesis of disease. Clinically significant choline determination in various human samples is often assessed by measuring the activity of the choline esterase enzyme. An alternative, significantly

sensitive, quick, and effective method of detection was made possible by a biosensor created by combining choline oxidase (ChOx), multi-wall carbon nanotubes (MWCNTs), gold nanoparticles (AuNPs), and poly-diallyl dimethyl ammonium chloride (PDDA) for the specific detection of choline (Qin et al., 2010). Similar to that, AuNPs helped in uric acid (UA) detection (Figure 1.11).

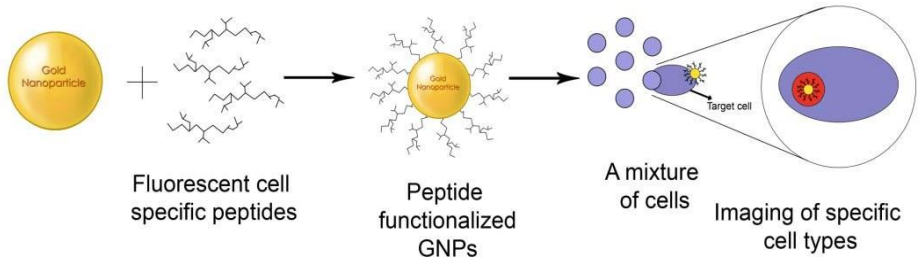

Figure 1.10 *AuNPs functionalized with cell specific peptides for bioimaging.*

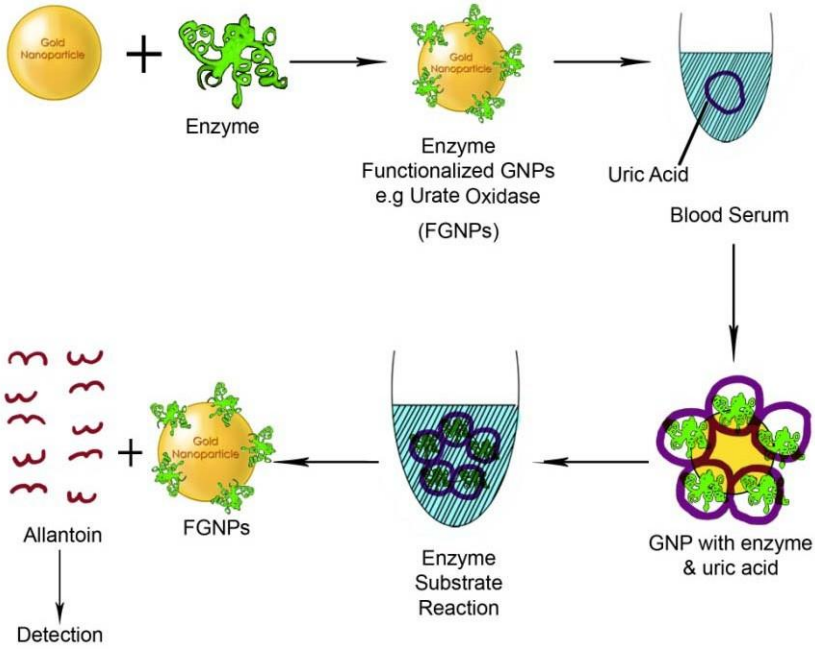

Figure 1.11 *fGNP based biosensor for the detection of serum proteins.*

Abnormal levels of UA, an essential byproduct of purine metabolism, have been linked to a number of metabolic illnesses, including gout, hyperuricemia, pneumonia, kidney damage, cardiovascular ailments, and Lesch-Nyhan syndrome. For the measurement of UA content in human fluids, a number of techniques are available, including colorimetric, enzymatic, and electrochemical approaches.

Moreover, UA can be detected in the blood serum and urine by an amperometric technique using AuNPs, with detection limit as low as 50 nM (Kannan et al., 2009). Accordingly, cholesterol oxidase was immobilized on the basis of amperometric shifts using a gold-platinum alloy nanoparticles-based nanosensor with high selectivity, quick response time, sensitivity, and good reproducibility (Safavi et al., 2010).

The detection method was based on the activity of hydrogen peroxide. Another work used gelatin-coated AuNPs with 6-mercaptohexan-1-ol (MCH) for proteinase activity testing, where gelatin serves as a proteinase substrate, to build a straightforward but important colorimetric biosensor (Chuang et al., 2010).

Due to the presence of MCH, proteinase digestion separates the gelatin and draws the nanoparticles together, which causes the AuNPs to aggregate and subsequently alter their surface plasmon resonance. The final outcome of proteinase activity is a change in the SPR and alters the colour of the solution that can be clearly identified through the change in absorbance ratio. Such a technique has a great deal of potential for detecting proteinase activity in various biological materials. A colorimetric "universal" biosensor that can detect DNA, proteins, small molecules, ions, and other substances was developed utilizing ssDNA, AuNPs, and a water-based polyelectrolyte (Xia et al., 2010).

Other biosensor model was created based on changes in surface plasmon resonance, and the effectiveness of the model was examined using streptavidin (Nusz et al.,

2009).

Here, the amount of molecules of a particular analyte attached to individual nanoparticles was correlated with the absorption maxima of the scattered light by those nanoparticles. Depending on the shape of the nanoparticles and other system characteristics, the biosensor model was able to estimate the molecular detection limits (minimum number of detectable molecules) and dynamic range (highest number of analyte molecules attached to a nanoparticles).

1.4.2.4 Detection

Additionally, AuNPs are utilized to detect a wide range of biological substances, including as proteins, enzymes, DNA, antigens, and antibodies, *etc*.

1.4.2.5 (a) Detection of Biological Molecules

Based on the distinctive surface plasmons of AuNPs, proteins have been detected using these particles (Bizzarri et al., 2007).

In this context, the functionalization of AuNPs has been achieved through bifunctional molecules. These molecules are attached to the AuNPs via their thiol group on one end and connected to the electron-rich aromatic side chains of proteins through a diazonium moiety on the other end. The model was validated using thrombin as the target protein.

The coupling of AuNPs, which serve as the Raman marker, tends to amplify the vibrations of the diazo bond formed between the bifunctional molecule and the target protein. This enhancement is observed in Surface-enhanced Raman Spectroscopy (SERS). The presence of thrombin is detectable when the functionalized AuNPs interact with anti-thrombin, which acts as a sensitive recognition element immobilized on a substrate. This interaction demonstrates the capability of the approach to identify

and detect thrombin effectively. Electro-deposition on screen-printed AuNPs has been used to create a chip-based array using selectively immobilized oligonucleotide modified AuNPs (Moreno et al., 2009). The technique also eliminates non-specific contacts while enabling multimodular detection based on the usage of several oligonucleotides. Similar to that, DNA functionalized AuNPs were used to create a straightforward optical detecting device (Wu et al., 2006). The technique uses fluorophores coupled to the detection sequences to quench their fluorescence using AuNPs. The procedure is simple because it avoids the stem loop shape that characterizes conventional molecular beacons and produces less background noise as a result of the electrostatic attraction of fluorescent dye to the AuNPs and the latter's repulsion to DNA. Additionally, it offers potential automation, real-time monitoring, and a reduced danger of contamination because there are no washing procedures. The degree to which the detection sequence binds to the target DNA sequence is determined by the decrease in fluorescence. For the purpose of detecting desired DNA sequences, AuNPs-based nanobeacons or nanobeacons functionalized with DNA sequences were developed recently (Song et al., 2009; Han et al., 2011).

Similar to this, AuNPs have been utilized to detect other biological compounds like antioxidants which have been investigated for their roles in diseases like cancer, atherosclerosis, etc., in inhibiting free radicals. It is generally known that vitamin E (tocopherol) has antioxidant properties. Using self-assembly of the thiol ligand, AuNPs functionalized with Trolox, an analogue of vitamin E, have been created and tested for their capacity to scavenge free radicals. The antioxidant activities of the AuNPs functionalized with Trolox was determined to be greater in comparison to Trolox alone, demonstrating a potential of these antioxidant-functionalized AuNPs in the treatment of several disorders (Nie et al., 2007).

Additionally, *f*AuNPs have been used to detect aflatoxins, which are mycotoxins linked to a variety of pathophysiological issues in humans. Aflatoxin AFB1 is correlated with cancer. By electro-depositing the antibodies against AFB1 on cysteamine-functionalized AuNPs, AuNPs functionalized with antibodies against AFB1 have been created (Sharma et al., 2010). These *f*AuNPs were discovered to be highly effective and quick to respond when detecting AFB1.

1.4.2.4 (b) Detection of Microorganisms

Detection of microorganisms can be accomplished by numerous biochemicals, molecular and microbiological methods. Recent developments in the area of nanotechnology have made it feasible to identify microorganisms by using nanoparticles functionalized with oligonucleotides corresponding to the gene tags of the microorganisms. In one such study, AuNPs were functionalized using oligonucleotides complementary to the specific sequences of the heat shock protein 70 (HSP 70) of Cryptosporidium parvum, which could be used to detect the Cryptosporidium oocytes in a colorimetric assay, providing a quick and reliable molecular detection method (Javier et al., 2009).

AuNPs were used to trace *Listeria monocytogenes* and *Salmonella enteritidis*, where AuNPs accumulated within the flagella and in the biofilm network (Sawosz et al., 2010). Similar to this, AuNP-Poly(para-phenyleneethynylene) effectively distinguished between Gram positive and Gram negative bacteria based on the differences in responses by each bacteria (Phillips et al., 2008). In a different work, AuNPs functionalized with hairpin DNA was employed to visualized Respiratory syncytial virus infected live HEp-2 cells (Jayagopal et al., 2010). Further immunoassay based on multi-functionalized AuNPs was created with antibodies against protein A, a cell wall protein of the bacterium *Staphylococcus aureus*, to

identify it in food samples (Lin et al., 2008). This involved the modification of a gold electrode through the sequential adsorption of 1, 6-hexanedithiol, AuNPs, and IgG, and the correlation between changes in the electron transfer resistance and the deposition of functionalized AuNPs. The increases in the amplified impedance exhibit strong correlation with the protein A detection limits.

A chemiluminescence assay based on gold nanoparticles was created to detect *Staphylococcus* enterotoxin B (SEB) (Yang et al., 2009). AuNPs were bioconjugated with antibody against SEB via physical adsorption; following this the complex was then adsorbed on a polycarbonate surface. Then, using a sandwich-type ELISA and a secondary antibody-generated chemiluminescence signal, the SEB was detected. The detection limit of the approach was determined to be 0.01 ng/mL, which make this approach easy, simple, and extremely sensitive.

It is now vital to develop appropriate methods for the detection of antibiotic resistant organisms due to the recent expansion of antibiotic resistance among numerous microbial pathogens. Using AuNPs functionalized with β-lactam antibiotics, a robust colorimetric assay was created (Liu et al., 2007). Depending on the connected linker groups, β-lactamase can cause the AuNPs to either aggregate or disaggregate, causing a noticeable colour change. For instance, when utilized as a linker between AuNPs and the antibiotic, the thiol group is cleaved, causing the AuNPs to disaggregate and change colour.

1.4.2.6 Enzyme Immobilization

AuNPs have been employed as immobilization matrix for enzymes. AuNPs having a carboxyl terminated thiol group were functionalized by the binding of the enzyme glucose oxidase (Li et al., 2007). Thermal stability tests revealed that the immobilized enzyme was more thermally stable then the free enzyme. Such immobilized systems

have several applications in biotechnological processes in food and environmentfields. Hollow gold nanoshells enclosing horse radish peroxides have been developed for the purpose of determining small molecules that can enter the nanoshells (Kumar et al., 2005). Because of this technique, the enzyme can be used for a variety of biotechnological applications and retain its activity in nanoshells.

Bi-enzyme functionalized magnetic nanoparticles were developed using three layer nanoparticles with a magnetic Fe_3O_4 core, a prussian blue interlayer and a gold nanoshell connected to the enzymes glucose oxidase and hydrogen peroxide (Zhuo et al., 2009). The carcino-embryonic antigen (CEA) and -fetoprotein (AFP) were used as model systems for testing this biosensor since they produce amplified signals in terms of electrochemical activity and enzyme catalysis. An external magnetic field can be used to renew these magnetic nanoparticles. Such a biosensor offers a wide range of different detection techniques with great sensitivity and reproducibility.

1.5 Challenges in synthesis of *f*AuNPs and their biomedical application

Synthesizing functionalized gold nanoparticles (*f*AuNPs) and subsequently applying them in the biomedical realm presents several intriguing yet complex challenges. These challenges encompass both the synthesis process and the subsequent utilization of *f*AuNPs in various biomedical applications. Here, we outline some key challenges in both areas:

Challenges in Synthesis:

- **Controlled Synthesis:** Achieving precise control over the size, shape, and surface properties of *f*AuNPs is often intricate. These factors are critical as they influence the nanoparticles' interactions with biological systems, stability, and performance.

- **Functionalization Strategy:** Developing an efficient functionalization approach that attaches biomolecules, drugs, or targeting agents to the nanoparticles surface while preserving their functionality and stability can be demanding.
- **Biocompatibility:** Ensuring that the synthesis process and any added functional groups result in *f*AuNPs that are biocompatible and non-toxic to cells and tissues.
- **Scalability:** Scaling up the synthesis process while maintaining the same level of control over particle properties is a significant challenge for translational purposes.

Challenges in Biomedical Application:

- **Cellular Uptake and Internalization:** Understanding how *f*AuNPs are taken up by cells and their subsequent fate within the cellular environment is crucial for safe and effective applications.
- **Targeting Specificity:** Designing *f*AuNPs that can effectively target specific cells or tissues, and studying the impact of targeting on their therapeutic or diagnostic efficacy.
- **Biological Barriers:** Overcoming biological barriers such as the reticuloendothelial system (RES) and mucosal barriers that can influence the distribution and accumulation of *f*AuNPs in vivo.
- **Biodistribution and Clearance:** Investigating the distribution of *f*AuNPs in different organs and tissues, as well as their clearance pathways, is essential for assessing their overall safety profile.
- **Therapeutic Efficacy:** Evaluating the effectiveness of *f*AuNPs as drug carriers, considering factors like drug loading, controlled release, and therapeutic outcomes.

- **Diagnostic Accuracy:** Assessing the accuracy of *f*AuNPs in diagnostic applications, such as imaging or sensing, and determining their sensitivity and specificity.
- **Long-Term Stability:** Ensuring that *f*AuNPs maintain stability over extended periods, especially in physiological environments, is vital for their reliable application.
- **Clinical Translation:** Bridging the gap between laboratory research and clinical practice involves addressing regulatory requirements, safety concerns, and demonstrating reproducibility and reliability of *f*AuNPs.
- **Multifunctionality:** Exploiting the potential of *f*AuNPs for multifunctional applications (e.g., combined therapy and imaging) while maintaining their individual functionalities and minimizing unintended interactions.
- **Ethical and Societal Considerations:** Reflecting on the ethical implications of using nanoparticles in medicine, including considerations around patient consent, data privacy, and equitable access to emerging technologies.

1.6 Origin of the present work

The diminutive size of nanoparticles makes them suitable for biolabeling purposes. These nanoscale particles can interact both on the surface of biomolecules and within cells, generating valuable signals and specific targets for diagnostic and therapeutic processes. As a result, a wide array of nanoparticles, offering numerous possibilities for modification with other bio-based materials, has been extensively investigated for further biomedical testing. These novel nanoparticles hold potential for applications such as thermal ablation, imaging assays, drug delivery, radiotherapy enhancement, and gene delivery (Bushra et al., 2014). Notably, various types of metallic nanoparticles and their derivatives (such as silver, gold, copper, nickel, platinum, titanium, and zinc

nanoparticles) have garnered significant attention due to their potent antimicrobial properties. Additionally, the antimicrobial activities of Nanomaterial have been attributed to their physicochemical properties and morphology (Mohammadi et al., 2011; Fellahi et al., 2013; Besinis et al., 2014). The bactericidal effect of nano-sized particles has been established, with the positively charged metallic nanoparticles exhibiting binding affinity toward negatively charged bacterial surfaces, thereby augmenting their bactericidal effectiveness. Nanoparticles shape also exerts a substantial impact on antimicrobial activity (Bera et al., 2014).

The synthesis of nanoparticles can be achieved through diverse synthetic routes, finding utility in various nanomedical and biological applications. However, the commercial-scale production of these nanoparticles remains imperative to reduce costs. Utilizing sustainable, cost-effective, eco-friendly, and non-toxic resources for nanoparticles preparation is crucial. Producing monodispersed nanoparticles is vital for future research, even though the exact mechanism of nanoparticles synthesis is not fully elucidated. Thus, forthcoming research should focus on controlling nanoparticles shape and size. Furthermore, the broader utilization of nanoparticles in therapeutic applications while mitigating toxicity presents a major challenge. Innovative strategies involving noble metal nanoparticles are being developed to overcome these challenges. Careful consideration of anthropological health impacts is necessary prior to widespread deployment. Nanoscience holds great promise in reshaping biomedicine and warrants comprehensive exploration in the future.

Noble metal nanoparticles possess unique atomic and supramolecular properties, rendering them valuable agents in diagnosis and therapeutic applications. The demand for various Nanomaterial and composites is high across healthcare, medical, and biological sectors. Consequently, ensuring safety precautions is paramount to protect

human health, with rigorous safety profile studies essential for metallic nanoparticles used in healthcare. Future research should delve into the application of specific metal nanoparticles for distinct purposes.

The scaling up of noble metal nanoparticles synthesis from laboratory to commercial medical sizes is a future necessity. These metallic nanoparticles have progressed and are at an advanced testing stage across multiple applications, particularly in cancer treatment. Their potential cytotoxicity or biocompatibility remains a pivotal question, urging investigations into nanoparticles-cell interactions and modification for enhanced biocompatibility with cancerous cells to minimize harm to normal cells. Modern research should pivot towards exploring the commercial-level potential of nanoparticles, as most current investigations are confined to laboratory settings. Such exploration at the commercial scale could potentially revolutionize human life.

The natural secondary metabolite, derived from plant, exhibits anti-inflammatory, antioxidant, and antibacterial properties, as indicated by various studies (Lee et al., 2013; Chattopadhyay et al., 2004; Senft et al., 2010). This plant's rhizome has diverse medical applications, including the treatment of burns, inflammations, smallpox, as well as oral and gastric ulcers (Al Kashmiri et al., 2010; Khanna et al., 1999; Nadkami et al., 1976). Despite its potential in medicine, these biomacromolecules faces challenges due to its limited solubility in water and poor bioavailability, raising concerns about safety. Different strategies have been explored to enhance its bioavailability, such as loading it into micelles, encapsulating it in liposomes, and even functionalizing it by attaching hydrophilic molecules (Agashe et al., 2011; Mohanty et al., 2010; Mohanty et al., 2012; Yadav et al., 2020). However, some of these methods can hinder the therapeutic activity of the molecule, conflicting with the main goal of conjugation. An alternative technique involves bonding these biomacromolecules to noble metal nanoparticles, like gold

nanoparticles, which can improve its activity, stability, and metabolic pathways, particularly in aqueous conditions (Sindhu et al., 2011). Many studies support the use of biomacromolecules-functionalized or -coated gold nanoparticles, often involving the direct reduction of gold ions (HAuCl4). However, this method has limitations, including time-consuming procedures, excessive reagent usage, and the need for a lengthy purification process. Therefore, we proposed a straightforward approach based on electrostatic interactions to conjugate biomacromolecules onto the surface of AuNPs without disrupting its therapeutic properties. In this method, we utilized AuNPs that had been previously synthesized in our laboratory.

The use of organic amines linked to alkoxysilane has been demonstrated for controlled synthesis of noble metal nanoparticles (Pandey et al., 2012; Pandey et al., 2014a; Pandey et al., 2014b; Pandey et al., 2014c; Pandey et al., 2015; Pandey et al., 2015b). It has been shown that 3-aminopropyltrimethoxysilane (3-APTMS)-capped gold ions are precisely converted into nanoparticles in the presence of wide range of reducing agents (e.g., 3-glycidoxypropyltrimethoxysilane (3-GPTMS), cyclohexanone, tetrahydrofuran hydroperoxide, and formaldehyde). Our initial study demonstrated the synthesis of functional AuNPs, which involved the active participation of 3-APTMS and 3-GPTMS. The synthesis of gold nanoparticles (AuNPs) that are surface functionalized with a cationic polymer such as

polyethylenimine (PEI) has potential advantages for imaging, targeting, and drug delivery (Wiseman et al., 2003; Xia et al., 2009). PEI was chosen as the polymer coating since the optical properties of PEI-functionalized AuNPs can be modified. We have recently demonstrated the role of a cationic polymer, PEI, which not only allows the rapid reduction of gold cations into gold nanoparticles in the presence of formaldehyde but also allows the formation of cationic coating for drug loading. The use of AuNPs for

medical applications has been previously demonstrated. It should be noted that AuNPs in a homogeneous suspension have limited capabilities for targeted drug loading. A robust framework in which two or more components can be incorporated to provide multifunctional capabilities is preferable. PEI-capped gold ions are converted into AuNPs within 2 minutes in the presence of suitable organic reducing agents such as formaldehyde/acetaldehyde/ cyclohexanone/tetrahydrofuran hydroperoxide/acetone/ t-butyl methyl ketone (Pandey et al., 2016). The choice of the organic reducing agent precisely controls the dispersibility of AuNPs for in vitro or in vivo applications.

1.7 Objectives of the present work

- To synthesize amine functionalized AuNPs i.e.; PEI functionalized and APTMS functionalized AuNPs.
- Surface modification of as synthesized NPs with biomolecules i.e.; Curcumin and CEO
- To study the antibacterial and antibiofilm activity of synthesized functional Nanomaterial
- To study the cytotoxicity and biocompatibility of synthesized functional Nanomaterial
- To study the antioxidant properties of synthesized functional Nanomaterial.

1.8 Work plan for the present work

❖ **Introduction:** Introduce the research objectives, highlighting the synthesis and characterization of amine-functionalized gold nanoparticles (AuNPs) with a focus on PEI and APTMS functionalization.

❖ **Synthesis of Amine-Functionalized AuNPs:**

- Detail the methodology for synthesizing PEI and APTMS functionalized AuNPs.
- Explain the meticulous manipulation of the synthesis process to achieve targeted surface properties.

❖ **Surface Modification with Biomolecules:**

- Elaborate on the process of modifying the as-synthesized AuNPs with biomolecules (curcumin and CEO).
- Discuss the rationale behind selecting these biomolecules and their anticipated functional impact on the nanoparticles.

❖ **Antibacterial and Antibiofilm Activity Analysis:**

- Describe the experimental setup for assessing antibacterial and antibiofilm activities.
- Outline the procedure for subjecting synthesized Nanomaterial to diverse bacterial strains and biofilm formations.
- Present the criteria and metrics used to evaluate the effectiveness of the Nanomaterial in combating bacterial growth and biofilm adhesion.

❖ **Cytotoxicity and Biocompatibility Assessment:**

- Explain the approach to evaluating cytotoxicity and biocompatibility of the functional Nanomaterial.
- Detail the selection of cell lines, culturing conditions, and assays to measure cell viability, proliferation, and overall cellular health.

❖ **Exploration of Antioxidant Properties:**

- Discuss the experimental design for investigating the antioxidant properties of the synthesized Nanomaterial.

- Highlight the methods to assess the materials' capacity to scavenge reactive oxygen species and mitigate oxidative stress.

❖ **Data Analysis and Interpretation:**

- Present the results obtained from each experimental phase in a structured manner.
- Interpret the findings in the context of the research objectives, discussing implications and correlations.

❖ **Discussion and Integration:**

- Synthesize the outcomes of antibacterial, cytotoxicity, biocompatibility, and antioxidant studies.
- Discuss how the surface modification and functionalization impact the overall properties and potential applications of the Nanomaterial.

❖ **Conclusion and Future Directions:**

- Summarize the key findings, reaffirming the significance of the research.
- Suggest potential future avenues for expanding research on amine-functionalized AuNPs and their applications

This thesis work plan outlines a systematic approach to address the research objectives related to amine-functionalized gold nanoparticles. Through a structured synthesis process, surface modification, and comprehensive characterization, the research aims to elucidate the antibacterial, cytotoxicity, biocompatibility, and antioxidant properties of these nanomaterials. The conclusions drawn from these analyses will contribute to a deeper understanding of their multifaceted potential across various domains.