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Progress of Nanobiomaterials for Theranostic Systems

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Abstract

Since its conception, nanotechnology has gained substantial momentum and transformed nearly every field of science and technology. In the field of nanomedicine, a variety of nanobiomaterials have been developed as unique platforms to detect and deliver therapeutic agents in the treatment oflife-threatening diseases.A brief review of recent publications reveals that these therapeutic platforms can be nanoparticles, micelles, nanogels, liposomes, metallic nanoparticles and semiconductor derived quantum dots. When compared to traditional therapeutic systems, theranostic nanomedicine is an emergingfield that employs a multifunctional approach to specifically target, diagnose and deliver therapeutic agents using a single platform to treat diseases such as cancers. This chapter will focus on the recent progress of nanobiomaterialstoward theranostic nanomedicine fortackling unmet clinical problems.

Keywords: Nanomedicine, nanomaterials, drug delivery, imaging, theranostic system, fluorescent polymer, quantum dots, gold nanoparticles, iron oxide nanoparticles

15.1 Introduction

15.1.1 Nanomaterials and Nanomedicine

In 1959, physicist Richard P. Feynman laid out avision of what would one day be called nanotechnology in his talk titled "There's Plenty of Room at the Bottom" at a meeting of the American Physics Society [1]. Although as revolutionary and groundbreaking as his ideas were at the time, it was doubtful that his idea would be translated into the multidisciplinary field of applied science and technology of today. The term nanotechnology was first used at a conference in 1974 by Japanese scientist, Norio Taniguchi [2]. Since that time there have been numerous attempts to define nanotechnology, but Mihail Roco, who was the current head of the National Nanotechnology Initiative

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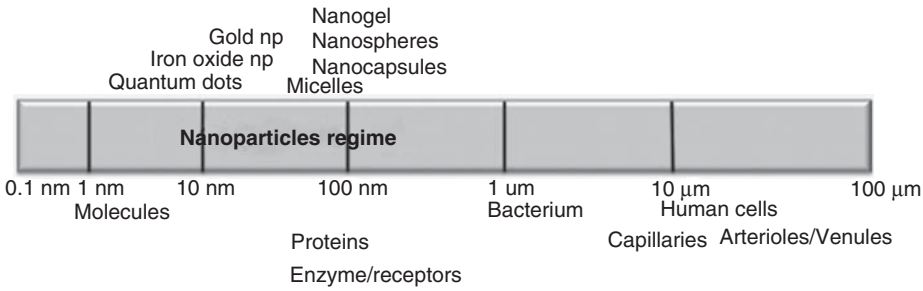

Figure 15.1 The comparison of size of nanostructures with biological components.

(NNI), proposed the most straightforward definition. "Nanotechnology is the creation of functional materials, devices, and systems through control of matter on the nanometer length scale, exploiting novel phenomena and properties (physical, chemical, biological) present only at that length scale"[1].Since then, nanotechnology has garnered significant attention in diverse fields such as cosmetics, textile, energy, electronics, biomedicine, pharmaceuticals, imaging and diagnostic industries. As estimated in 2008, the global annual investment in nanotechnology research and development, from private and public sectors collectively, exceeded $15 billion[3].

A nanometer (nm) is one billionth of a meter, which is equivalent to the diameter of a molecule of glucose. For comparison, the spacing between atoms in a molecule are in the range of one tenth of a nanometer [4]. The genetic instructions for development (a deoxyribonucleic acid double-helix) has a diameter of 2 nm [5]. The smallest cellular lifeforms (*Mycoplasma genitalium*) have a length of 200 nm [6]. Although, particles within the range of 10–1000 nm in diameter are considered nanoparticles [7], for biomedical applications it is preferred that particles are within the range of 10–200 nm, particularly in drug delivery, due to their longer circulation time and improved tissue penetrability.In the human body, most biological functions occur at the molecular level. Thus, nanostructures can be designed to interface and interact with cells and tissues at the molecular level (Figure 15.1).

Today scholars from diverse scientific communities are actively studying a broad range of materials in the nanoscale-size range and have observed new material phenomena that are absent in the microscale. Gold nanoparticles (GNPs), for example, when synthesized in various shapes, sizes and dimensions, demonstrate a wide range of optical properties that are not observed in the bulk material [8]. The advances in nanotechnology and other related fields are based upon this set of "new properties" in the nanoscale, which are not observed in the bulk material.Nanomaterials can be produced from a wide range of bulk materials such as natural polymers, synthetic polymers, semiconductors, metals and ceramics. In general, there are two approaches available to fabricate nanomaterials: top-down [9] and bottom-up [10]. The top-down approach, which is simply cutting, grinding and etching of a bulk piece of the material into the nanostructure, creates imperfections in the surface structure

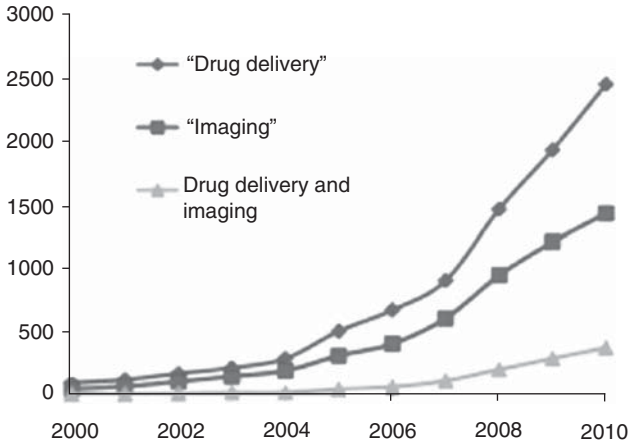

Figure 15.2 The total number of publications of nanomaterials for drug delivery, imaging, and dual purpose(Key word- Drug Delivery Nanoparticles, Imaging Nanoparticles, and Drug Delivery and Imaging Nanoparticles)(Source: Science Citation Index Expanded [SCI-EXPANDED]).

that significantly affects the overall physical properties and surface chemistry of the material's nanostructure. The bottom-up approach, which includes assembly of atomic or molecular species via weak noncovalent molecular interactions, creates nanostructures with homogeneous chemical composition and minimal defects. Most of the nanomaterials used in biomedical applications discussed in this chapter utilize the latter approach.

Nanomedicine is an emergent field which has produced new approaches to improvethe diagnosis of and treatment of medical problems. The ability of nanomaterials to incorporate biologically important molecules within a matrix, selectively deliver these factors to tissues or cells, and demonstrate coveted optical properties that are easily identifiable with current imaging technology, has garnered interest and interdisciplinary research from proximal fields. One indication of this interest is the rapid increase in related publications since 2000 (Figure15.2). Investigations and publications have doubled every year for the last decade. The clinical translation of this large body of research into commercial products has already begun. According to the April 2006 issue of *Nature Materials*, it was estimated that 130 nanotech-based drugs and delivery systems were in development worldwide [11].

15.1.2 Drug Delivery, Imaging, and Targeting

Nanoparticles are structures that are assembled either by physical or chemical crosslinking of several molecules in the range of 1–1000 nm. Small molecules such as drugs, imaging agents, and biomolecules can be adsorbed, dissolved, or dispersed throughout the matrix (as in nanosphere) or confined to an aqueous or oily core surrounded by a shell-like wall (as in nanocapsules). These entrapped molecules can be transported securely to the site of infection without

diffusing across the body's blood-brain barrier or being sequestered by various organs. In the clinical setting, the nanoparticles show therapeutic effect upon releasing the drugs from the matrix by diffusion, swelling, erosion or degradation. When nanoparticles are conjugated to targeting molecules, the delivery efficiency can increase by two orders of magnitude [12]. Researchers are currently studying incorporation of multiple drugs and/or other small molecules within a single nanoparticle[13].

As with any field, research in nanomedicine is sometimes thwarted by limitations such as the resolution limits of diagnosis and detection of biomolecules or microorganisms, localization to an infection site, and determining the fate of drug delivery vehicles. A thorough understanding of both biology and medicine at the molecular level is required to overcome the present challenges and limitations within the field [14]. Nanomaterials that possess unique optical, electronic and magnetic properties, which are amenable to detection by existing imaging modalities, are a promising solution to address these issues [15]. Nanoparticle-based imaging systems are believed to one day revolutionize how medicine is practiced. This is due to their ability to substantially improve the specificity and sensitivity of noninvasive diagnostic agents and detection of tissue specific biomolecules *in vitro* as well as *in vivo*. Further, pharmacokinetics can also be controlled through variations of size, shape and surface properties.

Cell surfaces are decorated with numerous biomolecules and are distinct for different types of tissue. In pathophysiological states some markers are overexpressed, or even in some cases, different sets of biomarkers are expressedwhen compared to healthy tissue[16]. A targeting agent that can recognize these diseased biomarkers located on the cell surface can be conjugated on the nanoparticles to selectively guide therapeutics and/or contrast agent to accumulate at the target molecules in the lesion.Targeting techniques are powerful tools to formulate individual effective dose treatment. However, this simple idea has practical difficulties in complex multi-compartmental organisms. For example, antibodies that specifically bind to target molecules can only recognize its counterpart only within a distance less than 100 nanometers, but nanoparticles decorated with these antibodies are thousands of centimeters away from the target site when in the blood stream [12]. Another difficulty often recognized in targeting techniques is that diseased sites that are located deep within an organ aresegregated from the bloodstream with multiple stages of physical and functional barriers[17]. So, special consideration has to be made while formulating a targeted therapeutic or imaging system. Nonetheless, numerous monoclonal antibodies are already in clinical application and others are under investigation in clinical trial.

15.1.3 Theranostic Nanomedicine

Substantial progress of nanomaterials for delivering drugs or providing contrast agents within the vicinity of a diseased site has already been

translated into clinical uses. However, diseases which are heterogeneous or adaptive, such as cancer, require a therapeutic protocol which is tailored to the individual patient [18]. Recently, the therapeutic benefits of nanoparticle drug delivery and imaging functions were combined to yield particles that can diagnose, deliver targeted therapy and monitor the process and response of the treatment before, during and after treatment regimen to significantly improveprognoses. The marriage of diagnosis and therapeutics within a nanosystem is defined as a theranostic nanosystem and has been extensively investigated and reviewed within the past few years [19–22].

Traditional pharmacological agents (mostly small drug molecules) distribute not only into the diseased site but also into almost all organs. Recent advancement of nanomedicine includes the use of drug carrying nanovehicles with the hope of saturating the sight of interest with significant amount of drugs with minimal systemic distribution. These nanovehicles can further be decorated with targeting molecules to enhance targeting efficiency. However, diseases like cancer, which is described as uncontrolled growth of cells,is not only malignant, but also is highly adaptive and heterogenic. For instance, most chemotherapeutic drugs work by impairing cell division in fast-dividing cells. However, in succeeding generations of tumor cells (typically at the center of the solid tumors), cell division is typically lost, making them less sensitive to most chemotherapeutic agents. Further, in addition to cancerous cells, other fast-dividing cells, such as bone marrow cells, intestinal epithelium, and hair follicles, show rapid cell division in normal physiological conditions making themalso highly affected by chemotherapy. So, it is extremely important to provide real-time validation and monitoring of the therapy in addition to targeting therapeutics to the tumor site. The numerous advantages of designing concurrent imaging systems in combination with therapeutic interventions are listed below.

1. Acquire complementary information such as systemic distribution, toxicity and pharmacodynamic of the pharmacological agents.
2. Track drug delivery vehicles systemically and understand the targeting capability.
3. Validate the treatment strategy.
4. Real-time monitoring of disease response to the therapy.
5. Provide early feedback of the therapeutic efficacy.
6. Track the expression pattern of surface markers in response to therapeutics.
7. Help to understand the adaptive nature of disease and alternate the treatment strategy.
8. Understand mechanismunderlying regression of the disease and therapeutic effect.
9. Indication of the complete eradication of the disease.

15.2 Design Concerns for Theranostic Nanosystems

The efficiency of therapeutic or diagnostic particles is governed by parameters such as biodistribution (BioD), pharmacokinetics (PK), and pharmacodynamics (PD) [23]. For example, the extended blood circulation time is the primary determinant of attainable particle-plasma concentration. Elevated plasma levels result in an enhanced permeation and retention (EPR) effectfor ligand-mediated targeting particles as the elevated localized drug concentrations shift binding equilibrium towards a bound configuration. On the other hand, for imaging agent-containing particles, it is important to excrete the excess nanoparticles from circulation after significant accumulation at the target to reduce signal-to-noise ratio prior to obtaininga diagnostic image. To design a functional theranostic system, careful optimization of the chemical synthesis methods and fabrication techniques to address many important parametersarediscuss below.

15.2.1 Sizeand Stability

Nanoparticles with dimensions in the range of 10–1000 nm can cross the blood-tissue barriers, which is a significant barrier for other drug delivery and imaging approaches. Particles smaller than 150 nm diameters are also able to avoid macrophage engulfment [12]. The blood-brain barrier can be infiltrated by nanoparticles smaller than 100 nm [24]. Nanoparticles smaller than 150 nm can cross cell membranes in the caveolae, and particles 20–60 nm in diameter can specifically cross tissue layers by transcytosis [12]. Nanoparticles larger than 15 nm are not excreted renally, whereas nanoparticles smaller than 5.5 nm are rapidly cleared by urinary excretion[25]. Thus, the size of the nanoparticles should be carefully selected to avoid unwanted barriers, sequestration or excretion and to selectively target the intended site. Later in this chapter we will discuss various synthesis techniques for each different types of materials to fabricate nanoparticles with different sizes.

Another prime concern about nanoparticles utility is the stability of nanoparticles over the period of time (kinetic stability), and stability against dilution and other environmental changes (pH, charge, and temperature) [26]. First, nanoparticles havehigh tendency of adhesion and aggregation as compared to the submicron sized particles. Second, in clinical settings, nanoparticles encounter mechanical stress due to bloodstream turbulences. Nanoparticles with low thixotropic characteristics may be disrupted during their navigation. Third, nanoparticles will be attacked by many enzymes (amidohydrolases, esterases, and other proteolytic enzymes), which cause a premature breakdown of the nanoparticles prior to intended application.Thus, it is quite important to develop techniques to provide stability to the nanoparticles prior to formulatingthem as functional materialsand products. One such technique can be surface modification of particles with polymers to generate an effective repulsive force between nanoparticles[27]. Another technique can be the use of stabilizer such as PEG, PVA, and polysaccharides such as dextran[28].

15.2.2 Surface Area and Chemistry

The large surface-area-to-volume ratio intrinsic to nanoparticles allows for a high density of functional moieties at the surface. The large surface area facilitates the development of "smart" nanoparticles within the system by conjugating multiple, complimentary targeting molecules to the surface. Throughout the journey, from injection to target cells, a nanoparticle will encounter multiple cell types and physiological conditions prior to reaching the destination. The presence of a high density of multiple binding epitopes specific to a particular cell type increase the odds of binding to the target cell type while concurrently minimizing off-target binding. The surface-area-to-volume ratio also modulates the elution rates of drugs. As the size of the particles decrease, the surface-area-to-volume ratiosignificantly increases, which results in a faster diffusion of encapsulated drugs from the nanoparticle matrix. Finally, the surface chemistry of the particles can produce a wide range of optical properties [29] (as in the case of quantum dots [QDs] and GNPs), which is critical for bioimaging applications. By simply changing the size of the nanoparticle, a volumetrically proportional change in the surface area is created to modify these important qualities. Numerous surface modification techniques are reported to create a wide range of surface chemistry. In general, surface modification can be done as post synthesis or controlled *in situ* modification.The post-synthesis technique has a benefit of easy processability, whereas the controlled *insitu*modification technique has a benefit of creating nonaggregated nanoparticles[27].

15.2.3 Drug Loading and Release

A successful drug carrier has highdrug carrying capacity, feasibility of incorporationof both hydrophilic and hydrophobic drugs, and enablesthe controlled (sustained) drug release from the matrix. Basically, drugs can be incorporated within nanoparticles by one of three methods: one by conjugating drugs to the material of the nanoparticles prior to nanoparticle formulation, second by encapsulating during the course of production, and third adsorbing the particles after the nanoparticles formation[30]. In these strategies, drug molecules may be encapsulated, dispersed, absorbed, or chemically bound within the polymeric matrix. In any nanoparticle system, the chemical structure of drug molecules,the nanomaterials,and the conditions of drug loading determine the efficiency of drug incorporation[31]. It is critically important to understand the loading/binding rate to create the best possible formulation. In general, linear sorption isotherms can be employed to determine loading rate in case of drug encapsulation and Langmuiror S-type Isotherm for surface adsorption[32].

Drug-loaded particles when delivered to targeted site by targeting strategies have the ultimate goal of demonstrating sustained release. Drug release can be achieved by desorption of surface bound drugs, diffusion through the nanoparticles matrix, degradation of the matrix, and combination of either of these. So it is critically important to understand the release mechanism and

factors affecting the drug release from the particular nanoparticle system. For example, smaller particles have higher burst release than larger particles[32], and nanoparticles composed of hydrophilic polymers show release rates largely controlled by diffusion[33]. If the drugs are conjugated to the nanoparticles system, then the drug release predominantly occurs due to the degradation of the matrix. When drugs are entrapped within low molecular weight polymers, the initial burst release is increased[34]. So, careful selection of both materials to formulate nanoparticles for specific drug types and drug loading strategiesmay be critical to design an effective therapeutic drug delivery system.

15.2.4 Imaging

Contrast agents that are tagged within the nanoparticle system should provide adequate signal that can be detected by currently existing imaging modalities such as fluorescent microscopy, magnetic resonance imaging (MRI), positron emission tomography, computer tomography, X-ray, radiography, and ultrasound. For example, as in the case of fluorescent technology,contrast agent demonstrate excitation/emission as a result of incident photons of a specific wavelength. As electrons decay from this excited state, they emit light with higher wavelength (fluorescent light) [22]. Fluorescent light that is emitted can be captured to quantify the illumination of the sample. The wavelength of the emitted light ranges from visible to the near-infrared (NIR) range, thus providing good spatial resolution. However, autofluorescence and light absorption from biological molecules such as proteins (257–280 nm), heme groups (max absorbance at 560 nm), and water (above 900 nm) [35] can produce significant background noise. These sources of noise must be considered when deciding which wavelength is selected for excitation and emission. Therefore NIR fluorescence imaging within the wavelength range of 700–1000 nm are central toresearch in order to avoid autofluorescence. Another known problem with flourecent dyes such as fluorescein and sulforhodamine is that the fluorescence is considerably quenched after conjugation with biological molecules, particularly to proteins[36]. This quenching effect can be troublesome in the case of traditionalimaging use but can be very beneficial while developing a "smart" theranostic system. Similarly in the case of MRI, contrast agent such as gadolinium diethylenetriaminopentaacetic acid (Gd-DTPA) is widely accepted in clinical use due to strong T1 shortening effect but suffers with low contrast effects and a very short retention time*in vivo[37]. So as an alternative,* magnetic iron oxide (IO) nanoparticle, whose magnetic properties can be manipulated by controlling the sizes of core and coating surface hasemerged as an exciting target-specific MRI T2 contrast agent[38].

15.2.5 Targeting

Designing a targeting theranostic system has multiple advantages, such as maximum therapeutic efficiencies, minimized side effects by reducing toxicity

and immunogenicity, avoiding non-target specific treatment, and being rapidly cleared from the system upon target failure. However, except for some superficial lesion and haematological malignancies, as in the case with most other tumor tissue, nanoparticles have to navigate through a multistage path prior to reaching the final target molecules[12]. In order to invade all the barriers and reach the destination, natural nanoparticles (the wild-type human immunodeficiency virus HIV-1 is a natural nanoparticle) of diameter 126 nm bears 8–10 trimers targeting spikes. These spikes are 8 nm long and 16 nm apart oriented correctly around the 5000 nm^2 surface area. To engineer an ideal targeted particle, it is critically important to understand the surface chemistry of the nanoparticle and its conjugation with targeting chemistry[39].

Biomolecules such as antibodies, aptamers, ligands, enzymes and nucleotides are capable of binding with cell surface molecules with high stereospecific affinity. Nanoparticles with appropriate chemistry such as amine, carboxyl, thiol, carbonyl and many more can be easily conjugated with these biomolecules to create targeting ability of the nanoparticles. On the other hand, unwanted uptake of nanoparticles into elements of recticulo-endothelial system and attachment to non-target site should be avoided. Surface modification of nanoparticles with appropriate electric charges and hydrophilic group, such as PEG molecules, may confer stealth properties within the nanoparticles[40]. Another important concern while designing nanoparticles is to minimize immunogenicity. The most widely accepted technique in this regard is to construct the nanoparticle system with humanized glycosylated components[41].

15.3 Designing a Smart and Functional Theranostic System

Despite muchprogress, drug delivery systems still face numerous challenges, particularly nonspecific targeting and drug release. From administration to pharmacological activity, nanocarriers navigate through very different biologicalenvironments. To address this issue researchers are actively investigating various environmental responsive therapeutic nanocarriers. In the following section, we review studies in which environmentally responsive nanoparticles that change size, shape, degradation, surface chemistry and targeting molecules are discussed.

15.3.1 Tailoring Size and Shape of the Particles

Particle size is the prime determinant of a particle's physical properties. A small change in the diameter of the particles from 15 nm to 25 nm translates into an increase in the surface area equivalent to more than three hundred human bodies in length [12]. Thus, changing the size of a particle during the course of therapy by various stimuli such as pH, temperature, light and hydrophobic-hydrophilic switching of the surface can create a "smart" theranostic system [42]. In most cases, these parameters alter the particle swelling, resulting in a

change of the particles size. Particles that are composed of PEG and polycarbonate block copolymers (polymersomes) show significant swelling behavior (expanding from 120 nm to over 1000 nm within 24 hours) when suspended in a buffer of pH 4.0 [43]. Changes in particle size, in response to varied pH of the surrounding environment, also improves cytosolic delivery of membrane-impermeable proteins by disrupting the endosomal membrane, as in the case of pH-sensitive PDEAEMA-core/PAEMA-shell nanoparticles [44]. Similarly, particles composed of poly(NIPAAm) based [45], Pluronic [46] and pluronic/poly(ethylenimine)[47] also show changes in size in response to temperature changes. In the case of pluronic/poly(ethylenimine), fast and reversible swelling induced by a short cold shock treatment (20°C) in the endosome increase the size of particles from 100 nm to 300 nm resulting in rupture of the endosomal membrane [47]. Another example of swelling induced size change occurs in virus mimicking particles composed of a pH-sensitive hydrophobic core (poly (His-co-Phe)) and two layers of hydrophilic shells (PEG and BSA). The particle hydrophobic core at pH 7.4 becomes hydrophilic at pH 6.4, and exhibits a reversible size change from 55 nm to 365 nm due to instantaneous swelling [48]. Another interesting size change behavior has been observed in poly(4-cinnamicacid)-co-poly(3,4-cinnamic acid) (PCA) particles in response to radiation wavelength. These particles decrease in size when exposed to UV irradiation above 280 nm and reversibly increase when exposed to 254 nm wavelengths [49, 50].

Similar to size change, particles that can undergo shape deformation in response to environmental stimuli have also been reported. In an *in vitro* study led by Yoo and Mitragotro, macrophages were unable to phagocytose particles when in the elliptical disk-like form. However, when particles were switched from elliptical disk-like to sphere shape macrophages, the ability to phagocytose them was regained. These particles, composed of PLGA, demonstrate switching behavior in response to high temperature, low pH or chemicals [51]. Another shape change in the nanoparticles system was observed in amphiphilic DNA-brush copolymers. In this system, amphiphilic polymer chain assemble into 25 nm spherical micelles that transform into cylindrical shape in the presence of DNAzyme and return to their original spherical shape when a specific DNA sequence is added to the micelle [52].

15.3.2 Degradation and Drug Release Kinetics

The ultimate goal of therapeutic nanoparticles is to carry maximal drug payloads to the targeted disease site and demonstrate a controlled, "smart" drug release profile. Controlled release can be achieved by degradation/dissolving of the nanocarriers or through diffusion. Tailoring these two parameters through environmental stimuli can optimize smart drug release kinetics. For example, poly (ß-amino ester) exhibits different solubility at different pH level (completely soluble below pH value of 6.5 and insoluble at pH 7.4) [53, 54]. This property can be utilized to design a smart delivery system with a rapid payload release when it encounters an acidic microenvironment. The

drug otherwise remains safely encapsulated within the mechanically stable, insoluble nanoparticles. This system was further illustrated in poly(B-amino ester) based mPEG-PbAE block copolymer where stable micelles (at pH 7.4) were demicellized selectively at tumoral acidic pH. In two separate studies, this micelle system was used to deliver anticancer drug (Doxorubicin) to the tumor site [55] and MRI contrast agents (Fe_3O_4) to cerebral ischemic area [56]. As mention earlier, other factor such as pH, temperature, hydrophobic-hydrophilic switching, and light induced swelling also effect on the over drug release kinetics.

15.3.3 Surface Properties and Placement of Targeting Molecules

While travelling to targeted tissues, nanoparticles interact with numerous living components and tissue barriers. The surface chemistry determines the fate of each particle. For example, hydrophilic surfaces (PEG and dextran coated particles) show poor protein adsorption and delayed circulation clearance [57]. Particles with positively charged surfaces exhibit improved cellular attachment and internalization. Hydrophilic surface of the particles can prolong circulation but also may inhibit particles-cell interaction. Surface modification of particles with moieties such as antibodies, aptamers, proteins, and peptides sequences results in selective localization to a target tissue[58–61].

Surface chemistry can also be altered to create smart delivery systems. Smart nanoparticles with PEGylated surfaces can be designed in such a way that de-PEGylation occurs only within the vicinity of diseased tissues. Sun and coworkers formulated a drug carrying micelle system by conjugating PCL chains to PEG molecules through a disulfide bond. These disulfide bonds can be reduced to achieve rapid drug release at target cells [62]. In two separate studies, matrix metalloproteinase-2 (MMP-2) (an enzyme released by tumor cells) liable PEG was coated in hepatic tumor-targeted liposomes [63] and cell penetrating peptide (CPP) conjugated QDs[64] to enhance the cellular uptake in presence of MMP-2 molecules. In addition to this, surface charge switching particles are also studied to avoid off-site nonspecific binding of particles. For example, polymeric micelles composed of a hydrophobic core, poly-L-lactide, and two hydrophilic shells, PEG and poly-L-lysine (PLys)-dimethlymaleic anhydride (DMA) show surface charge switching according to the change in pH of the environment. In this system, negatively charged, DMA molecules at the outmost surface molecule can be cleaved (at pH 6.5–6.0), exposing the underlying positively charged, PLys molecules to enhance cellular uptake [65].

Alternatively, targeting molecules can be hidden by PEGylation to avoid off-site recognition. Lee and coworkers have developed a polymeric micelle system to deliver doxorubicin to tumor cells based upon masking and unmasking of functional ligands. These micelles are composed of two block copolymers, polyHis-b-PEG and pLLA-b-PEG-b-PolyHis-biotin. Above pH 7.0 (physiological pH), the hydrophobic polyHis segment sequestered biotin and exposed hydrophilic PEG as the outer shell. However, below pH 7.0 (tumoral pH), ionized polyHis was liberated from the hydrophobic core, extending the biotin

beyond the PEG shell to interact with tumor cells. Furthermore, below pH 6.5 (endosomal pHs), the micelles dissociate and disrupt the endosomal membrane to release doxorubicin into the cytosol [66].

15.4 Materials for Theranostic System

15.4.1 Polymeric Systems

Research on biodegradable nanoparticles (NPs) has intensified over the last 10 years [14, 67]. These NPs are formulated with biodegradable and biocompatible polymers that can be further surface modified or functionalized with targeting molecules, drugs and imaging agents [68]. Factors such as particle surface charge, functionalities and hydrophilicity/hydrophobicity ratios are easily manipulated in polymeric nanoparticles, which are critical determinants of drug release pharmacokinetics [69]. Polymeric nanoparticles are on the front line of development for diverse therapeutic applications, ranging from cancer therapeutics to antimicrobial actions, vaccine delivery to gene delivery or site-specific targeting. The following section will be subdivided according to polymer hydrophobicity or hydrophilicity in order to understand their role in drug delivery.

15.4.1.1 Hydrophobic Polymers

Polymers that are miscible in organic solvent, but not aqueous solvents, are called hydrophobic polymers. Polymers such as poly(lactic acid) (PLA) [70–74], poly(lactide-co-glycolide) (PLGA) [75–78], poly(e-caprolactone) (PCL) [79–83], and poly(alkyl cyanoacrylate) (PACA) [84–88] are the most extensively characterized hydrophobic polymers that can be used to fabricate nanoparticles. The surface hydrophobicity of nanoparticles is an important factor in drug delivery applications. Nanoparticles made up of hydrophobic polymers have limited water absorbing capacities (5–10%) [33] prolong drug release by preventing rapid diffusion, and have demonstrated higher cellular uptake [69]. However, these polymers also elicit unintended protein absorption [89] and rapid clearance by macrophages [90]. Due to their ability to dissolve in organic solvent but not in aqueous medium, these polymers are easily fabricated into nanoparticles with desired size, morphology, polydispersity, stability, and drug-loading ability by dispersion of preformed polymers.

Solvent evaporation [91–93], salting-out [72, 94], nanoprecipation [95], and dialysis [96-98] are commonly used techniques that utilize dispersion of preformed polymers. The general principle shared by these techniques is that the polymer is dissolved in organic solvent and subsequently subjected to a nonsolvent system (typically water) with a surfactant (optional). Once the solvent leaves the system (by displacement or evaporation), the polymer is precipitated into a nanostructure (nanospheres or nanocapsules). The organic solvents used in these techniques are typically toxic and recent research has highlighted the need for organic, solvent-free methods. This, in turn, led to the

use of supercritical fluid technology for the production of nanoparticles [99, 100]. Techniques such as rapid expansion of supercritical solution (RESS) [101] and rapid expansion of supercritical solution into liquid solvent (RESOLV) [102] are based upon the ability of supercritical solvent to expand and decrease the solvent density dramatically from liquid-like to gas-like density. When the nanoparticle precursor (polymer) is dissolved in these solvents, the polymer precipitates into a nanostructure upon expansion. Nanoparticles precipitate into ambient air via the RESS technique while RESOLV fabricated nanoparticles precipitate into a liquid medium [103].

15.4.1.2 Hydrophilic Polymers

Polymers that have high affinity for aqueous solvent systems are called hydrophilic polymers. Polymers based upon poly(ethylene glycol) (PEG) [104-109], poly(vinyl alcohol) (PVA)[110–112], poly(ethyleneimine) [113], poly(vinyl pyrrolidone) [114–117], and poly-N-isopropylacrylamide [118–122] are the most common synthetic hydrophilic polymers. Polymers based on chitosan [123–127] and alginate [128–130] are examples of natural hydrophilic polymers that are frequently investigated in nanoparticle fabrication studies (recently referred to as nanogels) for drug delivery system. NPs made up of hydrophilic polymers do not require toxic organic solvents and surfactants, and demonstrate extended life spans in circulation [131, 132]. However, their hygroscopic nature may result in rapid dissolution of the drugs from the system through diffusion [33]. Despite an affinity for water hydrophilic polymer chains they do not rapidly dissolve in an aqueous environment. Dissolution is prevented by the critical crosslinks present in these nanostructure. Nanoparticle crosslinking is created by either chemical bonds between the polymer chains or physical entanglements of the polymer chains.

Several strategies are used to crosslink polymer chains by covalent bonding such as radical (induced either by photosensitive or chemical initiators) initiated polymerization, high-energy irradiation, enzymes, complementary group chemical reaction (aldehydes, Michael addition, and click reactions) [33]. The general principle behind covalent crosslinking assumes that the polymer chains are dispersed in aqueous solvent at low concentration, under continuous stirring. When a crosslinking initiator is introduced these polymeric chains crosslink, randomly, into nanoparticles. These crosslinking strategies, which rely on specific functional group reactions, preserve other functionalities present in the polymer networks. Thus, crosslinked nanoparticles retain the "smart" functionality for drug delivery or conjugation to other biomolecules of interest [133]. Chemical-based crosslinking carries with it toxicity issues associated with the crosslinking initiators. In contrast to this, physical gelation strategies eliminate the need for external crosslinking agents. During gelation, polymeric chains uniformly disperse in the aqueous system, and physically entangle in response to an environmental change (pH, temperature, and ionic strength within the polymeric chain) [133–135] of the aqueous system to create nanostructures. Since the nanostructures are assembled from

water-soluble polymeric chains in response to an environmental change, this polymeric assembly is highly reversible [33].

15.4.1.3 Amphiphilic Polymers

Polymers composed of both hydrophobic and hydrophilic segments in the same polymeric chain are known as amphiphilic polymers. Polymers such as poly(ethylene glycol)-poly(amino acid) (PEG-PAA) [136, 137], poly(ethylene oxide)-b-poly (propylene oxide) [138–140], and poly(ethylene oxide)-b-poly (ester)s [141–144] are examples of synthetic amphiphilic polymers. Liposomes represent natural amphiphilic lipid core polymers [145]. Micelles are formed when the hydrophobic core of amphiphilicchains assemble together to minimize hydration, thus creating a nonpolar core, which can act as a reservoir for hydrophobic drugs. The hydrophilic segment of the block polymer preserves the thermodynamic stability of the system in aqueous solvents [146, 147]. Micelle preparation from amphiphilic polymers is a simple process. When hydrated in an aqueous solvent amphiphilic polymeric chainsself-assemble, due to the desolvation, collapse, and the intermolecular association of the hydrophobic portions and electrostatic interaction between charged block copolymers of monomers [148]. The following features of micellar delivery systems distinguish them from other drug delivery systems: 1) they are of small size (< 100 nm) with a narrow size distribution [149]; 2) they can be prepared in large quantities easily and reproducibly [150]; 3) they can physically entrap hydrophobic therapeutics at concentrations above their intrinsic water solubility [150]; and 4) the hydrophilic corona, usually composed of PEG molecules, increases the thermodynamic stability and also increases circulation time [151].

15.4.1.4 Review on Application of Polymeric Nanomaterials in Drug Delivery

Polymers have been utilized as a therapeutic agent since 1940. Products such as Povidone (PVP-iodine), Stimaler and Adagen (polymer-protein conjugates) are early works that have found approval for human clinical use [152]. However, polymeric nanomaterials as drug carriers have only garnered significant interest over the last two decades. These nanoparticles can act as a drug reservoir and can also be functionalized with targeting molecules for controlled and targeted drug delivery, thus making them a promising approach within the medical field. Molecules of therapeutic agents are either dispersed within the polymeric matrix or conjugated to the polymeric backbone of the nanoparticles. In the former case, the encapsulated drugs can diffuse out of polymer matrix by fluid uptake or in response to environmental changes. In the latter case, degradation of the polymer matrix or cleavage of polymer-drug conjugating bond determines drug release kinetics. In recent years, various techniques have been developed to adjust the release rate and to avoid premature drug release prior to reaching the targeted tissue. As a result, numerous polymeric

particles are currently in the early stages of clinical evaluation to characterize their therapeutic relevance. Polymeric nanoparticles can be formulated into nanospheres, nanocapsules, dendrimers, micelles, nanogels, vesicles or liposomes according to the nature of the polymeric chain (hydrophilicity and hydrophobicity). In all cases, however, these particles have proven to be effective drug carriers by increasing the efficacy of the drug and safely delivering highly toxic, poorly soluble, or relatively unstable drugs [153]. Polymeric nanoparticles are the leading nanocarriers for wide range of drug molecules as tabulated in Table15.1.

Two clinical advances are particularly noteworthy given their low therapeutic toxicity and ability to extend the product life cycle. Liposomal drugs and polymer-drug conjugates account for greater than 80% of the nanoscale-based therapeutics market [154]. The first liposome-based drug delivery system approved for human use was Doxil (doxorubicin liposomes) for the treatment of acquired immunodeficiency syndrome (AIDS)-associated Kaposi's sarcoma. Since that time the FDA has cleared other liposome-based drug delivery systems. In addition to this, several other modified liposome-based drug delivery systems are also under investigation, such as surface functionalized targeted liposome, polymer combined liposome, photosensitive liposomes for stimuli responsive drug release and cationic liposomes for nucleic acid-based therapeutics delivery [153]. Similarly, Abraxine (paclitaxel bound albumin) is an example of an FDA approved drug conjugate for the treatment of breast cancer [155]. Abraxane is a nanosystem (130 nm) that significantly increases the therapeutic response rate at the tumor site, retards disease progression and ultimately increases the survival rate of breast cancer patients [156]. Paclitaxel albumin-bound systems have been clinically investigatedfor targeting metastatic breast cancer by conjugation with transtuzmab (antibody) [157, 158]. More recently Nanoxel, a nanoparticle-based delivery system composed of synthetic biodegradable amphiphilic block copolymer mPEG and PDLLA, has been approved for human use. Nanoxel increases the efficacy and minimizes the cytotoxicity of paclitaxel in breast cancer and ovarian cancer treatment [14].

15.4.2 Diagnostic and Imaging Materials

Nanotechnology has also become increasingly prevalent in the development of many innovative biological applications, such as biosensors, labeling of cells or cellular component, detecting biomarkers and diagnosing diseased site. Materials such as semiconductor nanocrystals (QDs), metallic nanoparticles (gold), polymers (Biodegradable photoluminicient polymer (BPLP), Poly(amido-ester)(PAMAP)), and small molecules (fluorescent dyes) have demonstrated optical properties that can be detected by fluorescent technology. Alternatively, iron oxide is a powerful imaging agent in magnetic resonance imaging (MRI) technology. This technology relies upon the precession of water hydrogen nuclei by pulses of radiofrequency under a magnetic field. In this process the difference in energy will be released as a photon that can be detected as an electromagnetic signal [22]. Although this technology

demonstrates excellent spatial resolution, it suffers from low sensitivity. Other imaging modalities such as positron emission tomography, computer tomography, X-ray, radiography, and ultra-sound are used in a clinical setting but lack specificity, sensitivity, or may suffer from radioactivity-associated health risks [202]. In this chapter we have limited our discussion on fluorescence- and magnetic resonance-based contrast agents.

15.4.2.1 QDs

Quantum dots(QDs) are nanocrystals composed of semiconductor matter that exhibit optical and electrical properties according to the size and shape of the individual crystal [29]. Binary alloys such as cadmium selenide, cadmium sulfide, indium arsenide, and indium phosphide are semiconductor material that can be grown into nanocrystals ranging from 2–10 nanometers in diameter (about the width of 50 atoms), collectively known as QDs[29]. In semiconductors, electrons can have energies only within certain bands. When an electron is excited and moves from the valence band into the conduction band, due to photon impingement of energy higher than the band-gap energy, it generates a short-lived electron–hole pair (exciton). When the crystal returns to its resting state, the electrons and holes recombine quickly and a fluorescent emission of photons are detected with a lower energy than that of the excitation energy [203]. As the size of the crystal grows bigger, the band gap gets smaller; the energy between the highest valance band and the lowest conduction band significantly decreases. As a consequence a lower energy level is required for excitation and lower energy fluorescent light (such as red light) is emitted.

15.4.2.1.1 Synthesis of QDs

Techniques such asphotolithography, chemical synthesis and self-assembly are some of the common routes used to synthesize QDs. Numerous improvements in the synthesis process have resulted in low size dispersity and higher florescence efficiencies since the initial report by Efros and Ekimov in 1982 [204, 205]. For example, preparation of QDs in aqueous solution mixed with stabilizing agents (e.g., thioglycerol or polyphosphate) was a common practice prior to 1993. The resulting QDs had large size variations and poor florescence efficiencies [29]. In 1993, the size variability was significantly reduced by the use of a high-temperature organometallic procedure, though products still suffered from low quantum yields [206]. Later techniques, such as layer deposition of surface-capping agents (ZnS or CdS), significantly improved the quantum yield of QD particles [207]. In addition to this, the quality of particle was improved by varying the precursor materials (such as CdO) [208]. As discussed earlier, variations in the size of QDs has a significant impact on tuning the emission wavelength from the blue to the near infrared. It is also critical to understand the techniques that control the size of the particles [209].

Though QDs are attractive vehicles because of their superior fluorescent properties, such as tunable emission, wavelength, quantum yield, molar absorption coefficients and low photo bleaching, they are frequently criticized for toxicity

[210]. Toxicity issues commonly associated with QDs include desorption of free Cd (QD core degradation) [211], free radical formation, and interaction of QDs with intracellular components [212]. The extent of cytotoxicity depends upon factors such as the choice of precursor Cd materials, capping materials, surface chemistry, processing parameters, size, color, and dose of QDs [213–215]. Despite persistent effort the toxicity associated with QDs remains; a technique which yields highly monodisperse, luminescent and nontoxic QDs remains to be discovered [210].

15.4.2.1.2 Review of QDs for Diagnosis and Imaging

Since its first discovery almost 30 years ago, QDs have been employed in diverse biomedical applications such as *in vitro* cell labeling, *in situ* tissue diagnostics, targeted *in vivo* imaging, monitoringintracellular drug/gene trafficking, and more recently as a theranostic tool [209, 216–218]. It was not until 1998 that Bruchez and coworkers reported for the first time the use of QDs in immunofluorescence to detect actin filaments in mousefibroblasts [219]. Subsequently, Wu and coworkers reported the use of QDs in static immunostaining of specific cellular targets. In this work, it was demonstrated that the QDs conjugated to immunoglobulin G (IgG) and streptavidin can label breast cancer marker (Her2) on the surface of fixed and live cancer cells, stain actin and microtubule fibers in the cytoplasm, and detect nuclear antigens inside the nucleus. They concluded that QD-based probes could offer substantial advantages over organic dyes in cellular imaging [220]. During the same year,Dahan and coworkers reported the use of QDs to track individual glycine receptors (GlyRs) and characterized multiple diffusion domains in relation to the synaptic, perisynaptic, or extrasynaptic GlyR localization [221]. It was concluded that this real-time visualization of single-molecule movement in single living cells was possible only with QDs which, unlike organic dyes, are not susceptible to photobleaching and can be effectively applied in time-lapse studies [218]. Since that time,QDs have been frequently reported in *in vitro* cell labeling, receptor diffusion dynamics, ligand–receptor interactions, biomolecular transport and enzyme activity studies.

There has been considerable interest in the use of QDs for mapping lymph node and vascular structure, tracking cells *in vivo* and tumor imaging. This niche within the field continues to expand at a greater rate than *in vitro* diagnosis. QDs demonstrate strong fluorescence, good photochemical stability and, most importantly, a narrow and tunable emission spectrum (from near-ultraviolet to near-infrared region) that reduces background noise from tissue autofluorescence [222]. The first study, reported by Akerman, to use peptide conjugated to QDs targeted tumor vasculature for imaging. Although *in vitro* histological results revealed that detectable amount of QDs localized to tumor vessels, the level of QDs were below the range of detection *in vivo*[223]. Since that time many successful approaches have been reported with improved QDs systems. QDs encapsulated in phospholipid micelles were injected into frog oocyte cells for real-time tracking of embryonic development [224].

QD encapsulated triblock polymer conjugated with tumor-targeting ligands have successfully imaged human prostate cancer grown in nude mice [225]. QDs conjugated to arginine-glycine-aspartic acid (RGD) peptide were used to image the tumor vasculature in living mice [226]. Similarly, numerous lymph node mapping studies were successful with the assistance of QDs [227–229] and were observable for longer than 4 months [230]. Recently, a QD-based *in vivo* imaging system was reported in coordination with bioluminescent protein. In this system, external illuminating sources were completely abandoned and QDs were illuminated by resonance energy transfer (BRET) system. This system aims to eliminate autofluorescent background noise and avoid superficial location of excitation light, which is a major disadvantage of external light-source-dependent systems [231].

QDs have also been evaluated for pharmacokinetic and pharmacodynamic modulation of drug and intracellular drug/gene trafficking mechanisms. Manabe and coworkers developed a QD-captrophil (antihypertensive drug) conjugated system to characterize the half-life and plasma concentration of captrophil in blood. However, nonspecific uptake by macrophages and endothelia cells of QD-captrophil significantly reduced the efficacy of drug performance [232]. Significant efforts have also been made to understand intracellular drug/gene trafficking by utilizing QD-mediated Forster Resonance Energy Transfer (FRET). Ho *et al.* investigated the structural composition and dynamic behavior of polymeric-DNA nanocomplexes intracellularly based on a QD-FRET system. In this system, plasmid DNA (pDNA) was conjugated with 605QD (donor) and a cationic polymer (chitosan) conjugated with Cy5 dye (acceptor). When the pDNA-chitosan nanocomplexes formed, fluorescence from both 605QD and Cy5 were simultaneously obtained, but when disrupted, only fluorescence was observed from the donor indicating the release of drugs (pDNA) from the system [233]. In addition to this, Chen and coworkers developed a two-step QD-FRET system by pairing QD and nuclear dye on the pDNA to study both polyplex dissociation and DNA degradation in a simultaneous and noninvasive manner [234]. These approaches are invaluable for studying mechanisms involved in polyplex unpacking and trafficking within live cells.

15.4.2.2 Gold Nanoparticles (GNPs)

Like QDs, gold nanoparticles (GNPs) also exhibit optical properties that are not observed in the bulk metal or in molecular compounds. These optical properties are dependent upon factors such as the shape and size, interparticle distance, and nature of the protecting organic shell [8]. Nanoparticles composed of metals such as gold, silver, and copper have the ability to produce an intense absorption (530 nm in the 5–20 nm diameter range of GNPs) due to the characteristic collective oscillation of electrons on the surface (plasmon resonance), when subjected to an electromagnetic field [235]. Absorption wavelength of these gold particles can be tuned (redshift) according to the size and shape of the particles. For example, spherical particles with 48.3 and 99.4 nm in diameter exhibit the maximum absorption of Au at 533 nm and 575 nm, respectively

[236]. In addition to this, if the shape of the particles is changed into rod-like particles, absorption can be pushed to the NIR region (650–900 nm) [237]. Another interesting property of GNPs is the ability to convert light energy into heat energy (energy transducers) upon laser irradiation, which makes them desirable candidate materials in photothermal therapy [235].

Apart from these, GNPs are gaining interest in surface-enhanced Raman scattering (SERS) technology. In general, every molecule when subjected to laser light shows signals within the 400–1800 cm^{-1} range that is unique to each molecule, due to their characteristic molecular vibrations [238]. This technique shows clear differences in the raman spectra of the cell nucleus and the cell cytoplasm [239], however, identification of other low concentration biomolecules or drug molecules within the cell suffer from extremely weak signal for detection. In order to solve this problem, GNPs can be attached to a probe molecule to increase the local optical fields of these structures by 10- to 12-fold in magnitude (SERS) [240, 241]. This property of gold particles can help identify the fate of a therapeutic agent within the infected cells or tissues.

15.4.2.2.1 Synthesis of Colloidal Gold Particles

Discovery of "soluble" gold has been dated as early as the 5th century B.C. [242]. However, the first formal report ofcolloidal gold formulation (deep red solutions) by reduction of an aqueous solution of chloroaurate ($AuCl4^-$) was purposed by Michael Faraday in 1857 [243]. Consequently, various methods for the preparation of gold colloids have been reported and reviewed [242, 244–246]. For example, the Turkevich method [247], Brust-Schiffrin method [248], Perrault Method [249] and Martin Method [250] are a few of the pioneering works which led to increasingly stable, monodispersed and nanosized gold particles. Although each method has furthered the goal "high quality" gold particles, the principle of synthesis still follows Faraday's (reduction of $AuCl_4^-$) concept, though with novel reducing agents and stabilizing agents.

15.4.2.2.2 Review of Collodial Gold Particles for Diagnosis and Imaging

Colloidal gold particles are another promising nanomaterial used in various aspects of biomedical application such as *in vitro* assays, *ex vivo* and *in vivo* imaging, cancer therapy (photothermal), radio/photo sensitizers and drug delivery. In early attempts by Mirkin and coworkers, it was reported that gold particles (13 nm diameter) coated with 5 -(alkanethiol)-capped oligonucleotide and coupled with a complementary target oligonucleotide sequence absorbed at longer wavelengths when aggregated. This technique was translated into a colorimetric DNA-hybridization assay that can distinguish sequences containing one base-end mismatch, a deletion or an insertion from a fully complementary target [251]. GNPs are now routinely used in many other *in vitro* assays such as immunoassays, protein assay, time-of-flight secondary ion mass spectrometry, capillary electrophoresis and detection of cancer cells. Gold particles have found success in the handheld immunodiagnostics and histopathology fields because they do not alter the biological activity of bound proteins.

Over the last three decades endoscope-compatible microscopies, such as optical coherence tomography and reflectance confocal microscopy, have been

used to obtain three-dimensional pictures of tissue microanatomy with excellent spatial resolution (1–10 μm). However, to identify molecular biomarkers associated with diseases such as cancer, specific molecular contrast agents are required. Contrast agents based on GNPs have been used in techniques such as reflectance microscopy, optical coherence tomography, photoacoustic tomography and SERS to acquire *ex vivo* and *in vivo* imaging of disease state. Sokolov and coworkers realized this limitation and developed a GNPs-based probe molecule that can image precancerous stage via reflectance laser-scanning confocal microscope [252]. Similarly, numerous studies werereported in tissue phantom to study GNPs as a contrast agent in OCT until Kirillin and coworkers demonstrated real-time *in vivo* skin images comparable to histology (gold standard) by Monte Carlo calculations [253].
In another study, Zharov and coworkers developed a photoacoustic flow cytometry model for real-time detection of circulating cancer cells labeled with gold nanorods in the vasculature of mouse ears. This technique is expected to expand to study pharmokinetics and the immune response to apoptotic and necrotic cells because the estimated threshold sensitivity was one cancer cell in the background of 10^7 normal blood cells [254]. Another promising technique for *in vivo* imaging is based on Raman spectroscopy and SERS nanoparticles. In clinical settings, optical imaging using Raman spectroscopy is only relevant for superficial tissues such as breast or tissues and those accessible by endoscopy such as the esophagus and colon. However, Keren and coworkers demonstrated a deep tissue whole-body Raman imaging by using SERS nanoparticles composed of a gold core, a Raman-active molecular layer, and a silica coating [255].

15.4.2.3 Iron Oxide Nanoparticles

Similar to QDs and GNPs, iron oxide nanoparticles (IONPs) are otherinteresting metallic nanoparticles that have demonstrated significant importance in biomedical applications. In the past few decades, many IONPs (bare nanoparticles or polymer, liposome or micelles coupled) have been reported, some of which are in clinical trials today. A fewof these candidates have also entered the market as pharmaceutical products [256]. It is the superparamagnetic property of IONPs (particles less than 20 nm), which are not observed in bulk material, that makes them an attractive contrast probe for magnetic resonance imaging (MRI) [235]. Individual atomic magnetic moments present in these particles are typically oriented randomly, resulting in a net zero magnetic moment in the particles absent of external magnetic fields. In the case of nanoparticles, a small change in thermal energy can cause the magnetic moment to fluctuate, resulting in a superparamagnetic state. The high magnetic moments of each particle can further reduce the T2 relaxation time leading to signal attenuation on a T2 or T2*-weighted mapping system. Materials such as magnetite or maghemite are the most commonly used metals for the formulation of IONPs due to their superior magnetic properties, biocompatibility, and low cost compared to other magnetic materials (oxides and pure metals).

15.4.2.3.1Synthesis of Iron Oxide Nanoparticles
Since 1970, extensive research on the synthesis and characterizations of magnetic particles has been reported. Many of these techniques rely on the ability of ferrous, ferric ions, or salt to precipitate into nanocrystals in potassium nitrate and potassium hydroxide (in Sugimoto's method), oxygen-free-base (in Massart's method), or water in oil phase (microemulsion method). Recently, advanced techniques have reported sonochemical routes, electrochemical deposition under oxidized conditions (EDOC), hydrothermal method and synthesis by magnetotactic bacteria. Apart from the synthesis, another important process is surface coating. Polymer surface coating can occur either during the process of synthesis or post-synthesis. Polymer coatings modulate the solubility, stability, biocompatibility, and circulation time of ferrous particles *in vivo*.

15.4.2.3.2 Review of Iron Oxide Nanoparticles for Diagnostic and Imaging
Over the past two decades numerous IONPs systems have been translated into medical practice and many more are under investigation in clinical trials. These particles are typically employed as contrast agents in MRI for imaging bowel, liver, spleen, lymph node, bone marrow, MR angiography, tissue perfusion, and many other tissues. IONPs, when administered intravenously, show rapid accumulation in the reticuloendothelial system (RES) and cells of the liver and spleen. Owing to this property, IONP-based contrast agents such as Feridex IV® and Endorem® have already been approved for hepatic imaging [257]. These contrast agents are capable of distinguishing lesions as small as 2–3 mm of liver tumor and metastases [258]. *Seneterre and coworkers*demonstrated through their study that ferumoxides-enhanced T2-weighted MR imaging wascomparable to that of CT during arterial photography in identifying liver metastases [259]. Another contrast agent, Combidex®, is in late stage clinical trials for use in the detection of lymph node metastase [260] and has effectively identified lymph node metastases in prostate cancer with a diameter of 5–10 mm under MRI. Similarly, Lumiren® and Gastromark® are examples of other IOPN available for medical doctors as bowel contrasts [261]. More recently, I0PNs have also been proposed as contrast agents (MRI) to diagnose cardiovascular diseases such as myocardial injury, atherosclerosis and other vascular disease. Kelly and coworkers have identified the adhesion molecule (VCAM-1) of endothelial and macrophage cells as a target of IONPs, to study its role in atherosclerosis and more than 30 families of new peptides which bind to atherosclerotic lesions [262]. In two separate studies, it was concluded that MR images obtained by the use of IOPNs could be valuable tools to evaluate the risk of acute ischemic events [263, 264].

15.4.2.4 Fluorescent Dyes

Fluorescent dyes are small molecules that cannot be fabricated into nanoparticles, but can be incorporated into a nanoparticle system either by encapsulation or conjugation. In general, fluorescent dyes can be categorized into two groups according to the underlying process of emission. First, resonant dyes exhibit

emission that originates from the process of an optical transition delocalized over the whole chromophore. Fluoresceins, rhodamines, most 4,4´-difluoro-4-bora-3a,4a-diaza-s-indacenes (BODIPY dyes) and most cyanines are some examples of resonant dyes. Alternatively, in intramolecular charge transfer (CT) dyes the emission originates from an intramolecular charge transfer transitions. Coumarin and coumarin-based dyes are examples of CT dyes. Resonant dyes show poorly separated but slightly structured absorption and emission bands, and higher molar absorption and fluorescent quantum yield, whereas CT dyes show well-separated, broader and structureless absorption and emission bands [210].

15.4.2.4.1 Synthesis of Fluorescent Dyes

Synthesis of *De Novo* fluorescent dyes with the required properties can be complicated by an incomplete understanding of how the molecular structure determines the fluorescent properties, despite similarity to known molecules. Fortunately,existing molecules can be derivitized to yield molecules with desired fluorescent properties [265]. The monomethine and trimethine cyanines (Cy3), with absorption bands in the visible region, can be shifted by about 100 nm just by extending the chromophore by one vinylene moiety (CH = CH) [266]. As a result, pentamethine (Cy5) derivatives and heptamethine cyanines (Cy7) have absorption at a near-infrared region (>700 nm) and beyond 1000 nm, respectively. One of the advantages of organic fluorescent dyes is ease of functionalization with other nanomaterials [267]. The commercial availability of functionalized dyes in conjunction with established labeling, purification, characterization protocols, and information on the site-specificity of the labeling procedure has facilitated the conjugation of fluorescent molecules to nanoparticles of interest.

15.4.2.4.2 Review of Fluorescent Dyes for Detection and Imaging

In most *in vivo* applications it is desirable to use near infrared (NIR) fluorescent dye to avoid background interference from tissues, to improve tissue penetration depth, imaging sensitivity and noninvasiveness. Fluorescent dyes can be conjugated with targeting ligands, encapsulated within or conjugated with nanoparticles, and used in coordination with other contrast agents to achieve quenching/non-quenching effects. For example, Cy5, Cy5.5 and Cy7 have been conjugated with various chemical ligands, peptides, proteins and antibodies to target different types of cancers. For example, Garanger and coworkers reported a Cy5.5-based formulation that was conjugated with cRGD cyclopeptide and drug molecules. This system was employed in a mouse model and demonstrated significant reduction of tumor even at low doses [268]. Derycke and coworkers encapsulated aluminum phthalocyanine tetrasulfonate (AlPcS4), a photosensitized agent that shows absorption at 672, to visualize bladder cancers and to provide photodynamic therapy [269]. Conditionally activated fluorescent dyes lack fluorescence without the presence of an enzymes associated protease, predominantly expressed in inflammatory condition and in tumor cells, which stimulates excitation. These types of dyes are particularly suited

for disease severity characterization because the fluorescence intensity can be directly related to the quantity of enzyme present within the inflamed zone [267]. Similarly, many other fluorescent dyes are reportedly sensitive towards small fluctuation in the pH value of the environment [270]. Recently, Lee and coworkers illustrated the ability of pH-sensitive cyanine dye conjugated to a cyclic arginine-glycine-aspartic acid (cRGD) to detect primary and metastatic breast tumors with high sensitivity and specificity [271].

15.4.2.5 Fluorescent Polymers

A large number of light emitting polymers have been discovered over the last two decades [272, 273]. However, none of them have been translated in biomedical applications. These polymers typically contain complex conjugated systems of multiple benzene rings with nondegradable bonds [274] and unknown toxicity to living cells. Researchers have only recently realized the potential of fluorescent polymers for drug delivery purposes. Polymers such as poly(amino ester)s (PAMAM)[275] and biodegradable photoluminescent polymer (BPLP) [276] are some of the more interesting biodegradable polymers that show inherent fluorescent properties. As discussed earlier, polymeric materials can be fabricated into numerous types of nanostructures and are superior in formulating drug delivery vehicles. Thus, the use of fluorescent polymers to fabricate these nanostructures can achieve additional visualization capability within the same nanostructures. Traditionally, drug delivery vehicles possessing therapeutic and diagnostic functions (theranostic system) are fabricated by conjugation or encapsulation of a drug molecule and fluorescent molecules [235]. However, this methodology suffers for numerous limitations, such as tedious multiple fabrication steps, nonhomogeneous distribution of drug and fluorescent molecules to each particle, low loading efficiency for each molecule, low dye-to-particle ratio and available surface functionalities for further conjugation with targeting units.

Polymers such as BPLPs[276], which have demonstrated high quantum yields,intrinsic bright and tunable fluorescence (up to near infrared)without adding any organic dyes and QDs, photobleaching resistance, cytocompatibility and pendent functionalities can be synthesized as hydrophobic, hydrophilic and amphiphilic polymers by simple and cost-effective one-pot-polycondensation reactions. BPLP polymers can be synthesized by reacting citric acid with any a-amino acid in presence of any aliphatic diol to create a fluorescent, low-molecular-chain polymer. These fluorescent chains can be further crosslinked into thin film or fabricated into nanoparticles. The primary advantage of the BPLP system is that it is strictly composed of metabolites and aliphatic diols and can be biodegraded back to the respective monomers (citric acid and amino acids). The major concern for existing contrast agents is the creation of toxic degradation compounds, which are incompatible with human use. The BPLP system may ameliorate these toxicity concerns. In our laboratory, we are investigating the utility of versatile BPLPs to formulate next generation theranostic nanoparticles.

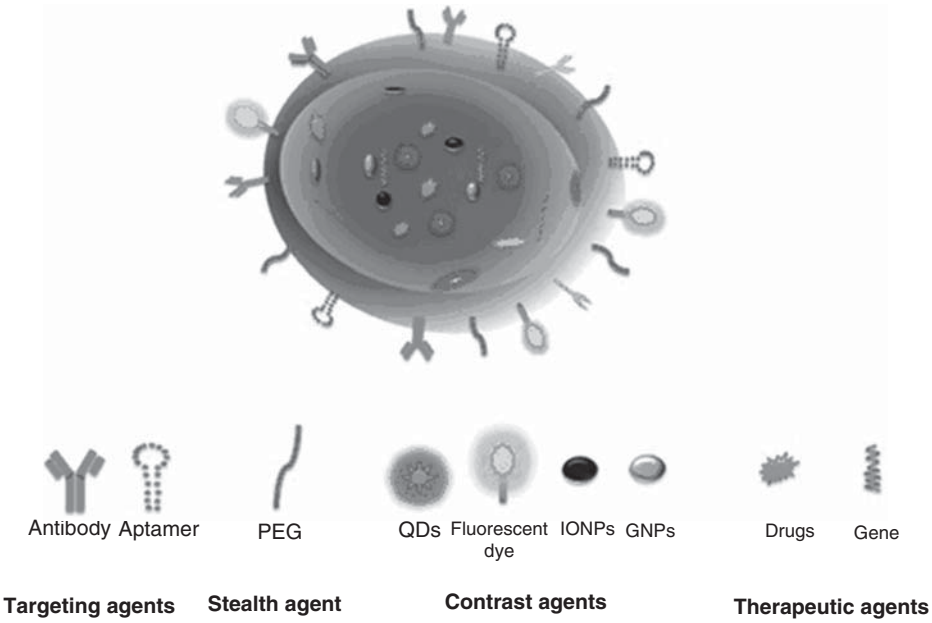

Figure 15.3. Theranostic nanoparticle. The theranostic nanoparticle has the capability to simultaneously carry therapeutic agents (drugs, gene or other pharmaceutical agents), targeting agents (antibodies, aptamer or other recognition agents), contrast agents (QDs, flourecent dyes, IONPs, GNPs or other imaging agents) and stealth agents (PEG or other hydrophilic polymers).

15.5 Theranostic Systems and Applications

As the potential for nanoparticles in biomedical applications, such as drug delivery and imaging, continues to expand, researchers are intensely investigating the development of an imaging system with therapeutic intervention via a targeting delivering system (Figure 15.3). One theranostic design approach is to integrate existing nanomaterials or imaging agentsfor simultaneous drug delivery and imaging[235]. A recent approach employs intrinsicallyfluorescent polymers to create visually identifiable drug carrying vehicles.

15.5.1 Polymeric Nanoparticle-based Theranostic System

Polymers are the first line materials for multifunctional nanoparticle and theranostic systems. The versatile functions of polymers allow for coating, encapsulation and conjugation to any contrast agent and therapeutic agent, in combination with targeting moieties within the same system. Researchers are also developing fluorescent polymers as theranostic system. For example, Choi and coworkers reported that folate-targeted PAMAM dendrimers can release DOX molecules in the presence of light [277]. In another study, Feng and coworkers characterized a self-assembly fluorescent cationic polyfluorene polymer with DOX conjugated anionic poly(L-glutamic acid) polymer. In a

nanoassembly system (50 nm), the fluorescence is in a quenched state due to an electron transfer mechanism. Upon hydrolyses of the anionic polymer the system disintegrates, releasing DOX molecules and exciting PFO to monitor the DOX release [278].

In our laboratory, we are currently designing a theranostic system based upon biodegradable photoluminescent polymers (BPLPs). The BPLP system can also be incorporated into any other polymeric system to provide an innate fluorescence property and can be completely degraded into its starting monomers (citric acid and amino acid). This ability of BPLP to degrade into cytocompatible monomers may solve the issue of toxicity accompanied with most of the imaging agents.In a recent study, we formulated an amphiphilic copolymer (ABPLP) based on BPLP (hydrophobic block) and PEG (hydrophilic block). ABPLPs demonstrated unique intrinsic fluorescence that can be visible *in vivo* and able to self-aggregate into nanostructures (average diameter of 50–120 nm) encapsulating anticancer drug (paclitaxel). Upon incubation of micelles with cells, they were rapidly internalized with minimal toxicity (below 5%), but induced cell death (80%) upon paclitaxel delivery over a 5 day period. This micelle system can potentially serve as an *invivo* safe theranostic nanodevice for cancer management. In a separate study, we have also synthesized hydrophilic BPLP containing radical liable bonds. These BPLPs can be photo-crosslinked into fluorescent nanogels (80–200 nm) and further loaded with anticancer drug (5-fluorouracil). These nanogels alsopossessrich surface functional groups (carboxylic groups) that can be further functionalized with targeting molecules. *In vitro* studies suggested that these nanogels demonstrated effective delivery of drugs to cancer cells inducing significant cytotoxicity (92%) over 48 h.

Apart from these intrinsicallyluminescent polymeric systems, any other polymeric system can be conjugated to, or encapsulated with,existing fluorescent dyes to acquire imaging ability within a polymeric nanoparticle system. In fact, dyes such as FITC, Cy7, Cy5, and ICG are actively used in monitoring drug release from nanoparticles and detecting various tumor locations [267]. In addition, polymeric systems are also used for coating or surface modifying other contrast agents such as QDs and IONPs to construct theranostic systems that will be discussed in a later section.

15.5.2 QD-based Theranostic System

Another application of QDs is the integration of therapeutic interventions, also known as theranostic QDs [279]. The innate toxicity of QDs has inspired researchers to coat the particles with polymeric materials, thereby reducing toxicity, prolonging the circulation time, and diversifying the surface chemistry for further modifications. In addition to this, the coated polymeric shell can also be loaded with drug molecules to induce therapeutic effects within these contrast agents as tabulated in Table 15.2. QD drug delivery systems were first reported by Lai and coworkers, where they used CdS particles to cap pores of mesoporous silica particles after encapsulating drug molecules

Table 15.1 Examples of formulation methods used for fabricating polymeric nanoparticles for drug delivery.

Polymer	Formulation Method	Drug Type	Reference
PLGA	Solvent evaporation Nano precipitation Emulsion diffusion Solvent displacement	Paclitaxel, Doxorubicin, 5-fluorouracil, 9-nitro-camptothecin, Cisplatin, triptorelin, dexametha-sone, xanthone,Estradiol, Docetaxel, 2-Aminochromone, Thymopentin, Etanidazole, Insulin, Haloperidol, Tetanus drug	[159–167]
PLA	Solvent evaporation Emulsion diffusion Double emulsion Salting out Spray dying	Dexamethasone, Hemoglobin, Ellagic acid, Haloperidol, Savoxepine, Tyrphostins, Progesterone, Vinblastin, Insulin, Oridonin, BSA, Neurotoxin-1, Protein-C	[34, 168–175]
PCL	Solvent evaporation Nano precipitation Emulsion diffusion Solvent displacement Micelles	Tamoxifen, Taxol, Insulin, Clonezepam Amphotericin B, Saquinavir, Docetaxel, Vinblastine	[31, 176–181]
PACA	Anionic polymerization	Ampicillin, Indomethacin, Ftorafu, Doxorubicin, 5-Flourouracil	[182–186]
Chitosan	Ionic gelation, Desolvation, Emulsion-droplet coalescence, Reverse micellar, Self-assembly	Glycyrrhizin, Insulin, Cyclosporin, BSA	[187–190]
Alginate	Solvent evaporation, Emulsion diffusion, Double emulsion, Salting out, Spray dying	5-fluorouracil, doxorubicin, antisense oligonucleotide, Insulin, isoniazid, rifampi-cin, pyrazinamide, etham-butol, econazole,	[128, 191–195]

PNIPA-based	Chemical crosslinking, Free radical initiating, Seeding and feeding precipitation polymerization, Atom transfer radical polymerization	Dextran,clonazepam, naltrexone	[178, 181, 196–199]
PVA-based	Chemical crosslinking agents, electron beam, γ-irradiation, Water-in-oil emulsion/cyclic freezing–thawing	human serumalbumin, tetanous toxoid and cytochrom C, ibuprofen, nandolo, propranolol, and tacrine	[30, 200, 201]

and neurotransmitters. In response to stimuli, these caps open and release the drugs. A decrease in the fluorescence intensity of CdS particles within the silica particles confirms the release of the drug's molecules [280]. Nurunnabi and coworkers formulated a QD-based Herceptin conjugated micelle system for cancer therapy and imaging. This theranostic system achieved significant reduction of tumor size and good visualization of the tumor site, thereby allowing both treatment and monitoring of the tumor [281].

In another study, Wu and coworkers immobilized QDs in polysaccharide-based nanogels, which could perform optical pH sensing, tumor cell imaging, and anticancer drug (temozolomide) delivery [282]. Park and coworkers reported a PEGylated phospholipid micelle system thatco-encapsulate QDs and IONPs, along with DOX, and further conjugated a tumor homing peptide for simultaneous magnetofluorescent imaging and drug delivery [283]. Recently, Xu and coworkers used Mn-doped ZnS QDs (core) and glycopolypeptides (shell) to deliver an anti-inflammatory drug (ibuprofen). This study, however, did not utilize the optical properties of QDs [284].Bagalkot and coworkers reported an interesting variant of the theranostic system composed of QDs conjugated to an aptamer (Apt), loaded with DOX molecules as intercalating agents. In this nanosystem, the fluorescent properties of both Qds and DOX, intercalated within the aptamer sequence, were both in a quenched state. However, commensurate with the gradual release of DOX molecules at the targeted tumor cells, QDs recovered fluorescence, signaling the delivery of the anticancer drug [285]. A similar photoluminescent quenching effect was also reported by Yuan and coworkers when theyconjugated mitoxantrone (MTX) onto QD surfaces. The photoluminescent property of QDs can be restored when this nanosystem is exposed to a species with higher affinity (DNA) for MTX [286].

Table 15.2 Examples of QD-based theranostic system.

Drug loading dock	Imaging Agents	Theraputic	Targeting Agent	Targeted Disease	Ref
Chitosan	QDs	siRNA	HER2 antibody	breast cancer cells	[287]
Poly(ethylene glycol) -phospholipid	QDs/iron oxide nanoparticles	Doxorubicin	Tumor-homing peptides (F3)	MDA-MB-435 human cancer cells	[283]
Poly(ethylene glycol)	quantum dot	siRNA	Tumor-homing peptides (F3)	HeLa cells	[288]
Amphipol	QDs	siRNA	Her-2/neu antobody	SK-BR-3 cells	[289]
Aextran-conjugated poly(l-alanine)	QDs	Ibuprofen	Passive	HEK293 cell lines	[284]
A10 RNA aptamer	QDs/ doxorubicin	Doxorubicin	A10 RNA aptamer	Prostate cancer cells	[285]
Methoxy poly(ethylene glycol) pentacosydonic acid	QDs	Herceptin	Herceptin	MDA-MB-231 tumors	[281]
Hydroxy-propylcellulose-poly(acrylic acid)	QDs	Temozolomide	Passive	Melanoma B16F10 cells	[282]

15.5.3 Colloidal Gold-particle-based Theranostic System

Due to their intrinsic diagnostic ability, gold nanoparticles can also be incorporated into theranostic systems. In recent years, researchers have reported the therapeutic ability of GNPs such as anticancer drug delivery, gene delivery and photothermal therapy (Table 15.3). Therapeutic GNPs are an attractive approach due to their unique characteristics such as strong surface Plasmon absorption, stability, biosafety, and ease of modification [235]. In fact, a PEG-modified gold-nanoparticle-based drug delivery system, developed under the trade name of Aurimune (CYT-6091) to deliver recombinant human tumor necrosis factor alpha (TNF) (tumor-killing agent), is already showing

promising results in Phase I clinical trials. Paciotti and coworkers first reported the formulation of this system and demonstrated rapid accumulation of these particles in colon carcinoma tumors without significant accumulation in the liver, spleen, and other healthy organs of the animals [290]. Further, this conjugate system shows maximal antitumor responses with lower drug doses, proving its efficacy over native TNF vector. Subsequently, Phase Idata demonstrate that delivery of TNF in humans could achieve concentrations far beyond what was attainable in previousstudies along with manageable side effects. The particles accumulated in and around tumor sites, avoided uptake by healthy tissues and immune system detection[291].

Other studies have investigated anticancer drug delivery to the tumor sites. Modified PTX conjugated with GNPs[292], Methotrexate conjugated GNPs[293], and DOX conjugated GNPs[294] are some of the chemotherapeutics developed in recent years to target various cancers. Park and coworkers reported another interesting use of GNPs in tumor therapy. Park demonstrated the efficacy of a paired-nanoparticles system that can work together to detect a diseased site and more effectively deliver chemotherapeutics to the site than individual nanoparticle treatments. In this system, the first nanoparticles composed of Cetyltrimethylammonium bromide (CTAB)-coated gold nanorods coated with a mixed monolayer of poly(ethylene glycol) (PEG) and SERS-active reporter molecules were directed to the tumor site. Once these particles entered the tumor zone they were subjected to photothermal heating, selectively at the tumor site, by excitation with an 810 nm laser. Next, a thermally sensitive liposome and micelles carrying DOX molecules were delivered to the tumor site where selective drug accumulation was observed without systemic toxicity [295].

Numerous other photothermal therapies using GNPs take advantage of the unique surface plasmon resonance (SPR). It was found that: 1) Elevated temperature at the tumor region is capable of inducing irreversible tissue damage that cannot be achieved by GNR-free NIR light exposures [296]; 2) the absorbance wavelength (in the visible range) of small gold nanospheres is not optimal for *in vivo* applications, so particles that attain SPR peaks in the NIR region either by clustering small diameter particles [297], large (100–300 nm) nanoparticles or different shapes of particles (nanorod, nanocage, and nanoshell) [235] should be used; 3) as manyas 5000 gold nanoshells per prostate cancer cell are needed to achieve cell death [298], and; 4) monocytes can be recruited into hypoxic regions within tumors induced by gold nanoshells [299]. Although photothermal technique sounds promising in cancer therapy, future investigation and optimization are required.

15.5.4 Iron-oxide-based Theranostic Systems

IONPSs coated with other polymeric materials are easily coupled with drug molecules to provide therapeutic intervention while retaining their diagnostic capability. Many types of chemotherapeutic agents, proteins, peptides, DNA and siRNA can be stored in the IONPS particles to achieve synergic effects

Table 15.3. Examples of GNP-based theranostic system.

Drug docking zone	Imaging agent	Therapeutic	Targeting molecules	Cellular target	Reference
Poly(ethylene glycol)	GNPs	PTX	Tumor necrosis factor	Tumor	[300]
Poly ethylenimine)	GNPs	Plasmid DNA	Passive	monkey kidney (COS-7) cells	[301]
Methoxy Poly(ethylene glycol)	Fluorescein/ GNPs	Photo-thermal	Tumor homing ligand (NDP-MSH)	MC1R-positive B16/F10 tumors	[302]
Gold particles	GNPs	Photo-thermal	Anti-epidermal growth factor receptor antibody	A431 tumor	[303]
Poly(l-aspartate)/ poly(ethylene glycol)	GNPs/ doxorubicin	Doxo-rubicin	Folic acid	4T1 mouse mammary carcinoma cells	[294]
Cationic lipid bilayer	GNPs	Plasmid DNA	Passive	human embryonic kidney cells (HEK 293)	[304]
Thiol-derivatized PEG	GNPs	Tumor necrosis factor	Passive	MC-38 colon carcinoma tumors	[305]
Gold particles	GNPs	Metho-trexate (MTX)	Passive	Lewis lung carcinoma (LL2)	[306]

(Table 15.4). Anticancer drugs such as doxorubicin, paclitaxel, methotrexate, cisplatin, and gemcitabine are encapsulated or linked to IONPSs for potential diagnosis and treatment of various tumors. Kohler and coworkers formulated biostable methotrexate-immobilized iron oxide nanoparticle drug carrier coated with PEG for the real-time monitoring of drug delivery through MRI. The controlled release of MTX from the conjugated IONPS system in response to the pH changes and in the presence of lysozymes was also evaluated [307, 308]. In another study, Jian and coworkers evaluated efficiency of doxorubicin and paclitaxel (alone or in combination) loaded IONPS coated with oleic acid and pluronic. They also evaluated the MRI signal intensity of this system in the carotid arteries of mice, and reported superior sensitivity in terms of T_1 and T_2 relaxivity [309]. Piao and coworkers developed porous IONPS nanostructures and demonstrated successful loading of DOX molecules [310]. Chen and coworkers modified this technique to create porous IONPs loaded with cisplatin and coupled with Herceptin to confer targeting specificity. As a result, this IONPS system demonstrated selective affinity towards ErbB2/Neu-positive breast cancer cells and provided sustained cytotoxicity [311].

Another interesting therapy that can be aided with IONPS is hyperthermia. Yanase and coworkers demonstrated that the Fab fragment from an anti-human MN antigen-specific antibody, anchored phospholipid-coated IONPs administrated systemically, and significantly improved tumor uptake. When exposed to an AMF, the temperature of the tumor was elevated above 43°C within 15 min, resulting in tumor regression but no effect on the control group (no use of IONPs) [312]. Recently, this hyperthermia effect was further enhanced by Primo and coworkers with Zn-Pc (a photodynamic therapeutic [PDT] agent) loaded IONPSs. A synergistic toxic effect was observed in J774-A1 macrophage cells from both PDT and magnetohyperthermia[313].

15.6 Future Outlook

In the late 19th century, Nobel laureate, Paul Ehrlich, coined the phrase "magic bullet," to describe a drug that selectively attacked diseased cells without harming healthy cells. Over the past several decades, scientists have investigated and developed numerous drug types and delivery systems, but have failed to achieve a magic bullet. However, within the past two decades, scientists have revolutionized therapy systems by taking a "plenty of room at the bottom" approach. One example of this breakthrough is the use of nanomaterials that can selectively deliver therapeutic and diagnostic agents specifically to the diseased site. As this technology has matured, the need for simultaneous, noninvasive quantification of the diseased site and an individualized pharmacotherapy system has become apparent. This, in turn, has driven the development of theranostic systems.

Theranostic nanoparticle system development is still in its infancy; consequently, there remains much to investigate. From the perspective of the biomaterial scientist, it is important to characterize the safety of the biomaterial

Table 15.4. Examples of IONPs-based theranostic system.

Drug docking zone	Imaging agent	Therapeutic	Targeting molecules	Targeted tissue	Reference
IONPs	IONPs	Doxorubicin	Passive	Lewis lung carcinoma	[314]
IONPs	IONPs	Cisplatin/ Herceptin	Herceptin	ErbB2/ Neu-positive breast cancer cells	[315]
IONPs	IONPs	Epirubicin	Magnetic field	Various sarcomas	[316]
Poly (ethylenimine)	IONPs	LacZ gene	Magnetic field	Ileum lamina propria	[317]
IONPs	IONPs	Antisense oligodesoxynucleotides	Magnetic field	Cremaster vessels	[318]
Liposomes	IONPs	Silencing RNA	Magnetic field	Gastric tumours	[319]
Cationic Liposomes	IONPs	Hyperthermia	Direct injection	Solid glioma tissue	[312]

before designing a theranostic nanosystem to carry therapeutics. Future studies must focus on characterizing biomaterials with novel chemistry to impart smart functions such as stimuli sensitivity and tissue/cell-specific targeting to theranostic nanoparticle systems. Theranostic nanomedicine is at the intersections of cancer biology, diagnostic imaging, and nanobiomaterials. Integrated with the evolving understanding of cancer biology, the explosive development on nanobiomaterials and diagnostic imaging/instrumentation will be predominant in theranostic nanomedicine for the treatment of cancers and other fatal diseases.

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