Keerti Jain · N. K. Jain *Editors*

Multifunctional And Targeted Theranostic Nanomedicines

Formulation, Design And Applications

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Preface

Nanotechnology-based therapeutic systems are extensively being explored for targeted delivery of drugs as well as for diagnostic purposes. At present, nanotechnology-based therapeutic approaches are being explored in pharmaceutical, biomedical, and biotechnological research and innovation to design unique nanomedicines with different ability to treat and diagnose the diseases like cancer, autoimmune disorders, AIDS, and other deadly infectious as well as non-infectious diseases. Further, various multifunctional nanomedicines, functionalized with different ligands or targeting agents or some other active moiety to make it versatile carrier with different beneficial abilities including targeted delivery, gene delivery, immunotherapy, diagnosis/imaging/sensing, theranostic applications, etc., are also being explored extensively by researchers.

Currently, nanotechnology-based products are rapidly growing and multifunctional nanomedicines are emerging as multifunctional nano-sized engineered nanomaterials useful in the simultaneous targeted delivery of various biotherapeutics as well as diagnostic and imaging agents. Although customizing, designing, optimization, formulation, pilot scale-up, and validation of nano-formulations are considered major obstacles in delivering safe and efficacious products in the market, yet ample of nano-formulations exist for the treatment of multiple diseases and disorders. Functionalization of these formulations aids in delivering these nanomaterials to the target sites in the right amount while minimizing the dose and adverse effects.

This book titled *Multifunctional and Targeted Theranostic Nanomedicines—Formulation, Design and Applications*, covers various aspects of multifunctional nanomedicines for theranostic applications such as methods of functionalization, characterization, applications, and regulatory aspects. Chapters 1 and 2 deal with introductory knowledge on nanomedicines, theranostics, functionalization, and design of functionalized theranostic nanomedicines. The safety and toxicity aspects along with regulatory perspectives of functionalized theranostic nanomaterials are also discussed in Chap. 1. Chapters 3–6 provide advanced information on vesicular, polymeric, metallic, and lipid-based nanomedicines for theranostic applications and their functionalization. Chapters 7–10 discuss the engineering, functionalization, and theranostic applications of nanoemulsions, dendrimers, carbon-based nanomaterials, and quantum dots. Chapters 11–14 deal with different

multifunctional nanomaterials including nanogels, exosomes, polymeric micelles, and nanocrystals in theranostic applications. Chapters 15 and 16 are focused on magnetic and mesoporous silica nanoparticles and their functionalization for theranostic applications. This book is a compilation of vivid chapters contributed by renowned formulators, researchers, and academicians across the world with their specialized area of interest in the field of chemistry, biology, pharmacy, diagnosis, and nanomedicine.

We firmly believe that this book, *Multifunctional and Targeted Theranostic Nanomedicines—Formulation, Design and Applications*, will be useful for the postgraduate students, doctorate, and postdoctoral research fellows, while scientists, researchers, and academicians working in nanomedicines, pharmaceutical nanotechnology, and theranostics can enrich and upgrade their knowledge. It will be equally insightful for industrial, scientific, and academic purposes and will also assist formulation scientists and academicians working in the field of pharmaceutical product development to upgrade and enhance their knowledge on the nanotechnology-driven product development. This book should primarily address the challenges in realizing the simultaneous therapeutic and diagnostic benefits of optimized pharmaceutical delivery systems, exceeding the boundaries of "magic bullet" concept.

We express our sincere thanks to all the authors for their contributions. We are also extremely grateful to our respective institutions, colleagues, students, and family members for their support during the compilation of this book. We also acknowledge our gratitude to Springer Nature team and all those concerned, for their untiring efforts in bringing this book to the publication and in the market.

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Functionalized Targeted Theranostic Nanomedicines

1

Mohammad Zaki Ahmad, Kalyani Pathak, Javed Ahmad, Mohammad Aslam, Archana Bagre, Parth Patel, and Keerti Jain

Abstract

Nanotechnology has a substantial impact on the development of both therapeutic and diagnostic agents in the health sector. Nanocarriers were widely explored for therapeutic purpose by scientific community due to its unique ability to improve the solubility, bioavailability, and cellular uptake of active pharmaceutical ingredients. Nowadays, nanomedicines became more popular for its ability to serve as carrier to get imaging of various biological systems or deliver the image-guided treatment options in treatment of various life-threatening diseases. Clinically effective formulations that combine treatment and diagnostics are widely attractive at the nexus of these two paradigms: This notion, recently termed as nanotheranostic, is significantly important for the ligand decorated nanocarriers, which accumulated at diseased area more potentially and can give customized or image-guided treatment. Numbers of theranostic nanoparticles with various

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combination of imaging agents and therapeutic agents were thoroughly investigated in past few years. These include, for example, liposomes; polymeric nanoparticles; micelles; drug conjugates and complexes; dendrimers; vesicles; micelles; core-shell particles; microbubbles; and carbon nanotubes. The current chapter gives detailed overview of various imaging techniques that are usually used in clinical setups along with recently explored theranostic nanocarriers and regulatory obstacles behind its commercialization.

Keywords

Nanomedicines · Theranostic nanoparticles · Imaging agents · Quantum dots · Diagnostic techniques

Abbreviations

AuNPs	Gold nanoparticles
GO	Graphene oxide
MRI	Magnetic imaging resonance
NIR	Near infrared
PDT	Photodynamic therapy
PEG	Polyethylene glycol
PET	Positron emission tomography
PLA	Polylactic acid
PTT	Photothermal therapy
SPIONs	Superparamagnetic iron oxide nanoparticles

1.1 Introduction

There is a tremendous demand in clinical trials for addressing differences in drug responsiveness induced by genetic diversity in large patient populations. Therefore, as a result, tailored treatment is the current strategy for resolving this issue (Moghimi et al. 2005). Hood invented the Predictive, Personalized, Preventive, and Participatory (P4) approach to medicine. Personalized medicine is predicated on collecting unique data from an individual's cells or biomolecules regarding their illness, health status, and therapeutic response (Hood 2013). Personal medicine or precision medicine can be defined as, "a customized medical care based on the detailed study of genomic, epigenetic changes and other data to treat the disease in best possible way" (National Research Council (US) Committee on A Framework for Developing a New Taxonomy of Disease 2011). The vast pharmacokinetic diversity of drug has opened the doors for personalized medicine in treatment of life-threatening diseases. Because of the unique characteristics of personalized medicine, it has gained considerable attention (Kim and Nie 2005). Numerous techniques, including genomics,

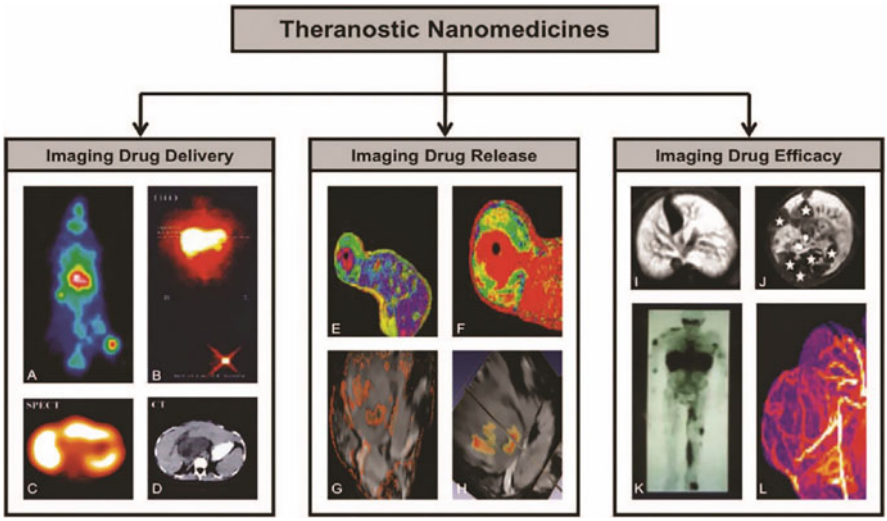

Fig. 1.1 Applications of theranostic nanomedicine formulations (Adapted with permission from Lammers et al. (2011))

proteomics, and metabolomics, can be used to decode and collect data at the molecular level for a person. Over the last few decades, the traditional Evidence-Based Medicine paradigm has transitioned steadily toward an individualized or customized medicine system. In general, the main objective behind the phase IV clinical trial was to optimize the medications for a large group of population in conventional treatment strategy. But the newer approaches focused on the individual's genetic peculiarities, which not only minimize the side effects associated with conventional medicines but also improve the therapeutic outcome. Additionally, real-time monitoring of pharmacokinetics of drug and pathological conditions will give insights for future planning of treatment strategy. This provides chance to manage the dosage of medications so that therapeutic response will get better and side effects will decrease (Lammers et al. 2012).

In the realm of medical science, nanotechnology has developed a distinct position. Due to their unique physical and chemical attributes, nanomaterials imparted the desired characteristics like large surface area for improving the solubility of lipophilic drugs; ease of functionalization will provide better cell uptake and side specific delivery of drug, high loading capacity, etc. This enables them to be applied in a broad range of technological disciplines. Theranostics is a term that refers to a method that combines diagnostic and therapeutic aspects. The concept of tailored nanomedicine lies at the heart of nanotheranostic. In 2002, Funkhouser coined the term "Theranostics," which includes therapeutic as well as imaging moieties in a single carrier to track the unwanted disposition of drug or its carrier and side effects associated with it (Moghimi et al. 2005). After it is injected into the body, the pharmacokinetics and pharmacodynamics can be tracked using theranostic materials. There was an initial focus on cancer treatment, but it has now been

broadened to other life-threatening diseases as well, which include autoimmune disorders such as type 1 diabetes, cardiovascular diseases, inflammatory diseases, and many more (Gollavelli and Ling 2014). Figure 1.1 showed the various applications of theranostic nanocarrier system. It is possible to perform both treatment and diagnosis simultaneously using tailored multifunctional theranostic nanomaterials, such as magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET) scans, or fluorescence imaging. Smart and new biomaterials will steadily improve the theranostic efficacy of nanoparticles (Choi et al. 2011).

Therapy is the process of resolving a problem following its discovery. The methods used will differ according to the patient's condition. Effective treatments strategy for cancer includes radiation therapy, immunotherapy, chemotherapy, targeted therapy, stem cell therapy, and surgery. Conventional therapy approaches have numerous disadvantages like toxicity to normal cells, lengthy processing times, high dosage requirements including nonspecific targeting, etc. (Gollavelli and Ling 2014). The major side effects associated with chemotherapy include neurotoxicity, immune system suppression, hair loss, fatigue, muscle pain, headache, etc. This showed the requirement of development of more precise and effective dosage form, which significantly reduces the dose of anticancer agents by lowering the unwanted disposition of drug and increasing the cellular uptake at tumor site (Li et al. 2014). Nanotherapeutics have potential to precisely target the infection location; they reduce undesirable side effects, increase effectiveness, and improve patient compliance and prognosis. This chapter discussed in detail the importance of theranostic nanocarrier and various imaging techniques along with various therapeutic and imaging agents, along with novel nanocarrier explored to get theranostic application in treatment of various life-threatening diseases.

1.2 Design of Nanotheranostic

Nanotheranostic is a term that referred as combination of nanotechnology, diagnostics agent, and therapeutic moiety. Several scientific fields have put significant efforts in developing theranostic nanomedicines during the past several years.

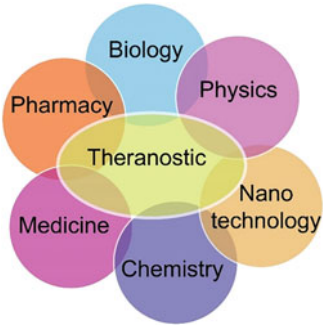

Fig. 1.2 Schematic representation of the highly interdisciplinary field of nanotheranostics

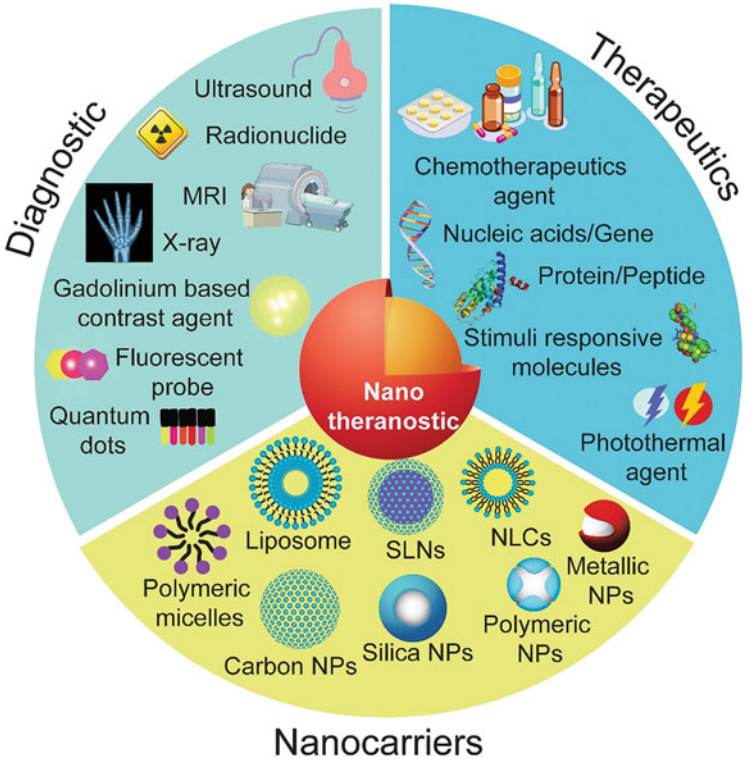

Fig. 1.3 Schematic representation of nanotheranostic system

New and exciting applications for nanotheranostic-based nanomedicines are the results of this collaboration (Akhter et al. 2013; Bukhari et al. 2021; Ahmed et al. 2022). As shown in Fig. 1.2 theranostic is an interdisciplinary approach, which required sincere involvement of a pharmacist, pharmacologists, a medicinal chemist, and other technical personnel.

There are three primary components to nanotheranostic agents: a therapeutic drug, an imaging agent, and a carrier that envelopes both. This theranostic system is targeted explicitly by connecting the ligands to carrier molecule. Figure 1.3 depicts a straightforward schematic illustration of a nanotheranostic system.

1.3 Therapy in Nanotheranostic

1.3.1 Drug Therapy

There are a lot of things that make it hard for drugs to get to the sites they need to go, like anatomical barriers, cellular membranes, blood-brain barriers, nuclear membranes, physiological barriers, and chemical and physical barriers (Lammers

et al. 2012; Bai et al. 2015). Nanotechnology plays an integral part in the rapidly expanding field of personalized medicine by clubbing therapeutic and diagnostic functions in a single system (Kim et al. 2013; Akhter et al. 2011). As nanocarrier-based delivery systems have the ability to deliver the drug directly to the desired site, thus reducing the dose and frequency of medication (Bai et al. 2015; Ahmad et al. 2013, 2015). Additionally, cytotoxicity associated with the plain drug also decreases significantly upon its entrapment inside ligand decorated nanocarrier due to selective delivery to diseases site sparing normal cells. Cisplatin, 5-fluorouracil, carboplatin, bleomycin, dactinomycin, paclitaxel, topotecan, vinblastine, doxorubicin, etoposide, mercaptopurine, and other chemotherapeutics are commonly employed as therapeutic drugs in nanotheranostic system for treatment of cancer (Ali et al. 2011).

1.3.2 Gene Delivery

Gene delivery includes transfer of specific gene to the infected cells of human body to treat the specific disease. One such approach is RNA interference (RNAi), which includes usage of siRNA (small-interfering RNA) or miRNA (micro-RNA) to reduce the expression of physiologically overexpressed proteins. Circulating miRNA is an important biomarker of cancer (Wan et al. 2012). Due to siRNA's low stability and inadequate distribution to target cells, its therapeutic potential is restricted. Inhibition of overexpressed miRNA and its reconditioning were the two major objectives behind the RNAi treatment (Muthiah et al. 2013). To overcome the challenges affiliated with the in vivo delivery of genes, Kenny et al. developed theranostic siRNA attached PEGylated nanoparticles. These magnetic resonance sensitive nanoparticles were used in combination with fluorescent markers to get the image-guided delivery of siRNA in tumor. In vivo administration of this formulation in tumor-bearing mice resulted in a significant decrease in tumor growth (Kenny et al. 2011). Overall, gene delivery is a novel approach to treat a range of diseases, but its high cost and lack of stability limit its application in clinical application.

1.3.3 Photodynamic Therapy

Historically from very ancient time phototherapy was used for treatment of various skin and other diseases; for instance, vitiligo was treated by applying plant extract of *Ammi majus L.*, followed by exposing them to sunlight (Hönigsmann 2012). Nowadays, development of focusable carbon arc torch to cure the lupus vulgaris by Danish physician Niels Finsen made the phototherapy popular, again (Gøtzsche 2011). Photodynamic therapy (PDT) can be used in the treatment of a wide range of malignant as well as non-malignant disorders. Photosensitizers are used in PDT to absorb light energy and transfer it to surrounding cells, resulting in the formation of reactive oxygen species inside the cells, which can eventually lead to cell death (Bai et al. 2015). In PDT, photosensitizing agents absorb lights at a specific wavelength

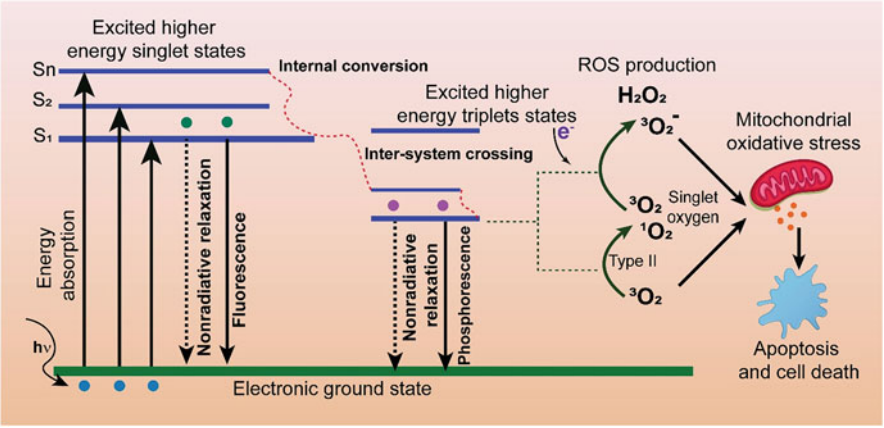

Fig. 1.4 Schematic representation of photosensitizer associated ROS generation and its role in causing apoptosis of cancer cells

and convert molecular oxygen present inside the cytoplasm of cells to singlet oxygen, which cause apoptosis of tumor cells (Paszko et al. 2011). Examples of photosensitizing agents used in PDT include napthalocyanines, photofrin, phorphine, phthalocyanine derivatives, and chlorins (Gollavelli and Ling 2014; Li et al. 2014; Allison et al. 2004). Figure 1.4 showed the photochemical reaction as per Jablonski's rule. In the case of a photosensitizer material, an excited electron crosses over the other molecules instead of coming to its ground or low energy state, which may produce hydrogen peroxide or superoxide as shown in Fig. 1.5. This hydrogen peroxide or superoxide was able to kill the tumor cells by damaging nucleus or cell organelles like mitochondria, resulting in cell death (Calzavara-Pinton et al. 2007). However, these types of photosensitizing agents tended to stay in the human body for longer period of time, which made the patients more susceptible to sunlight. To avoid any unwanted phototoxicity, patients were advised to stay in a dark environment (Zhao and He 2014). The ligand decorated theranostic nanocarriers can make the PDT safe as well as effectively improving the cellular uptake in diseased cells.

1.3.4 Photothermal Therapy

Photothermal therapy (PTT) is also called heat ablation technique, which uses light radiation to generate heat within the tissue (Gellci and Mehrmohammadi 2014). Heat is a highly effective weapon for destroying tumor cells. However, inducing a moderate temperature of 41–50 °C in the body for a brief period (5–10 min) without damaging normal tissue is a challenging task. PTT uses the near infrared (NIR) light waves to get the better tissue penetration and a photosensitive agent with good light absorbing capacity. PTT can selectively kill the pathologic cell upon excitation of photosensitizing agent by NIR light by converting the resonance energy of

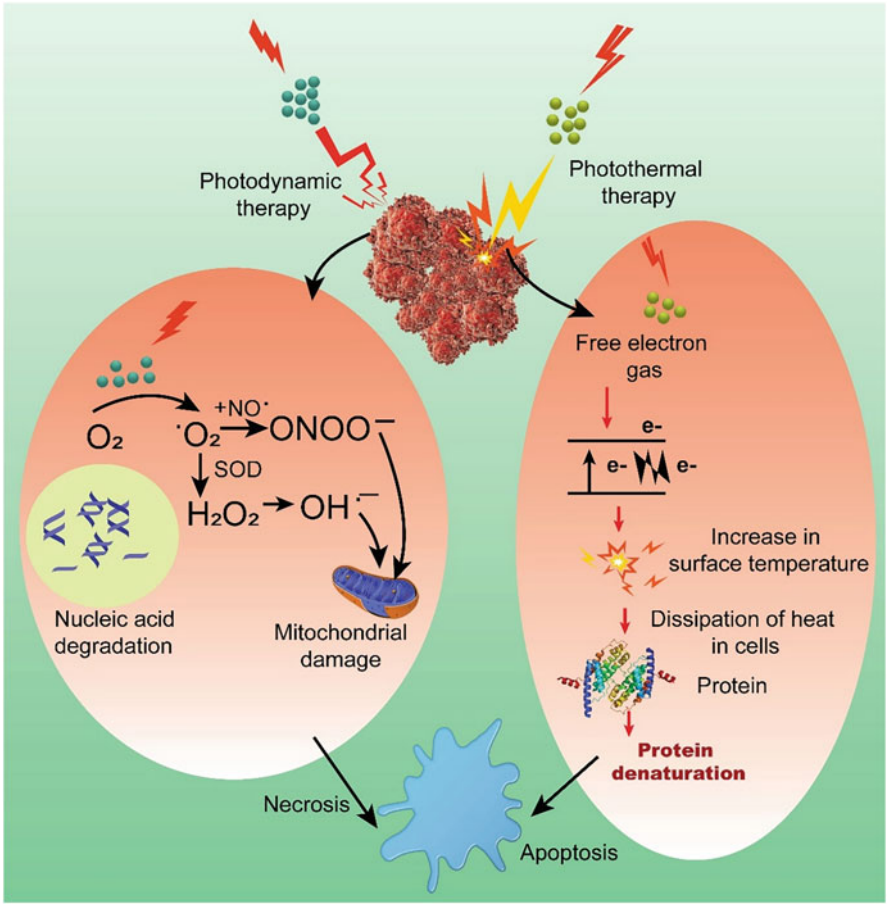

Fig. 1.5 Schematic representation of PTT and PDT using nanotheranostic

electromagnetic rays into heat production as shown in Fig. 1.5. This heat causes the irreversible changes in tumor cells by cellular mutilation (Gellci and Mehrmohammadi 2014). However, use of PTT in clinical practice is very limited due to its no specificity in identification between malignant and non-malignant cells. Clear demarcation between diseased and non-diseased cell is important to protect the normal body cells. To overcome this problem, ligand decorated nanotheranostic agents were explored in recent years. Due to its ability to significantly improve the cellular uptake at diseased tissue, side effect associated with unwanted disposition of photosensitizing agents was reduced significantly, and its therapeutic potential will improve (Huang et al. 2021; Liu et al. 2020; Dheyab et al. 2021). Such types of nanomaterials along with suitable targeting ligands were preferable for the clinical application of PTT.

Many interesting theranostic nanomaterials are made from noble metals such as gold. Gold nanoparticles (AuNPs) were widely explored for the PTT due to its low toxicity and easy renal clearance (Akhter et al. 2012). Gold has been the most extensively studied PTT agent, which showed promising results in treatment of various cancers in many research articles.

1.4 Nanotheranostic for Imaging

Theranostic agents must contain some imaging agents to provide in vivo diagnosis. Many approaches are being investigated to meet these goals using various materials. Nanotheranostic can be imaged using a variety of techniques such as optical imaging, nuclear imaging, CT, MRI, PET, and ultrasound (Fig. 1.6) (Debbage and Jaschke 2008; Janib et al. 2010). Each technique has its own advantages and disadvantages, which are discussed in the following subsections.

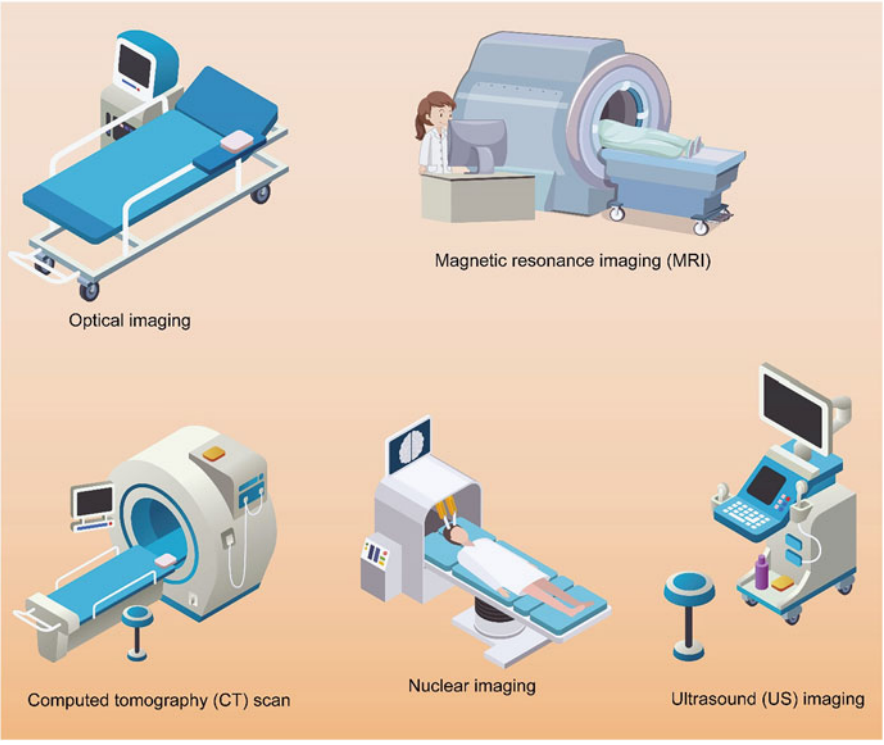

Fig. 1.6 Typical molecular imaging instruments

1.4.1 Optical Imaging

Optical imaging is the cost-effective and most widely used imaging technique in preclinical studies. It has proven helpful for non-invasive, sensitive, real-time molecular recognition and imaging investigations. Photons generated by bioluminescent or fluorescent probes are used in optical imaging (Janib et al. 2010; Sarbadhikary et al. 2021; Jain and Zhong 2022). It offers advantages over other imaging modalities in that it is relatively inexpensive to detect low-energy photons; also, the visible to near-infrared (NIR) light spectrum gives excellent spatial resolution without the use of ionizing radiation (Janib et al. 2010). To get the better resolution optical imaging can be integrated with other imaging agents such as PET or MRI. Bioluminescence and fluorescence imaging are the two most commonly explored technique in tumor diagnosis and image-guided surgery, in vivo (Lim et al. 2020). Metal nanoparticles can also be used in nanotheranostic to provide anticancer therapy with fluorescence imaging. For example, silver nanoparticles with strong metal enhanced fluorescence are helpful for cell imaging (Li et al. 2015a). Unfortunately, this modality has limited application in biological system due to poor tissue penetration. Additionally, fluorescence imaging is sensitive to noise due to heme groups (λ_{max} 560 nm), protein (257–280 nm), and even water (around 900 nm) (Debbage and Jaschke 2008). However NIR probe due to its identical structural properties could improve the efficiency due to good tissue penetration capacity.

1.4.2 Magnetic Resonance Imaging

The precession movement given by hydrogen nuclei of water within the applied magnetic field is the basis for the MRI signal (Janib et al. 2010; Richard et al. 2008). This modality produced a high-quality cross-sectional image of the body in any plane using radiofrequency pulses and a controlled magnetic field (Anani et al. 2021; Zhou et al. 2021). All hydrogen atoms align and become excited along the applied external magnetic field after the application of radiofrequency. The wire coils in the MRI unit will capture the energy released by the excited atoms, and with the aid of a computer system, the MRI mapping will be performed. The relaxation process, which takes place when the nuclei return to their initial aligned condition, can be used to produce an image (Li et al. 2015a). MRI contrast agents helped in reducing the relaxation parameters to increase the tissue differentiation. MRI contrast agents can be divided as paramagnetic agents, such as manganese or gadolinium, and superparamagnetic agents like iron core or manganese core polymeric matrix, which were considered as better MRI contrast agents (Avasthi et al. 2020; Bonnet and Tóth 2021; Xiao et al. 2016).

Due to its great spatial resolution and sensitivity, MRI is frequently used to diagnose solid and brain tumors, which is regarded as the most efficient and non-invasive imaging technology. It has superior spatial resolution when compared to other imaging techniques. However, it has low sensitivity. To compensate, relatively large contrast agent concentrations are needed to provide a discernible

signal. Concerns about accumulation and toxicity have arisen due to the use of high dosages of these contrast agents, which has become a substantial issue for Gd (III) complexes. While Gd (III) provides more excellent contrast for tumor and vascular imaging, sluggish excretion and toxicity from long-term accumulation may limit its clinical application.

1.4.3 Computed Tomography

A CT scan employs computer processing to build cross-sectional images from a series of X-ray images obtained from various angles around the body. The capacity of CT to differentiate tissues is dependent on the degrees of X-ray attenuation, and the attenuation coefficient depended on the electron density and atomic number of the tissues. Absorption differences between identification of air, fat, and bone depended on the absorption differences, and produced high contrast images of anatomical components (Janib et al. 2010; Weissleder 2002). The CT contrast agents that are currently available have a low molecular weight and exhibit quick extravasation as well as clearance. However, macromolecular and nanoparticulate agents may be more suited for vascular CT imaging because of their long-lasting presence in the blood (Janib et al. 2010). Most CT contrast research is focused on solid nanoparticles or liposomes containing iodinated molecules since these ingredients are required in high concentrations (Cormode et al. 2009).

1.4.4 Ultrasound Imaging

In ultrasound imaging, a transducer (probe) is used to convert the electrical energy into mechanical energy based on the piezoelectric effect. A thin layer of gel was applied over the skin to transmit the ultrasound waves generated by transducer (Janib et al. 2010). This ultrasound imaging approach is well established, non-invasive, adaptable, and commonly utilized in human clinical imaging modalities as a diagnostic tool (Janib et al. 2010; Zhou et al. 2020). Because of the advantages of real time, portable, non-ionizing, and deep tissue-penetrating abilities, Ultrasound has already become a widely demanded approach for tumor diagnosis due to advantages like portable machinery, real time imaging and deep tissue imaging abilities (Zhou et al. 2020; Frinking et al. 2000).

Furthermore, the advent of US contrast agents has accelerated its use in diagnosis and treatment of various diseases (Campbell 2006). Microbubble-based ultrasound imaging offers good contrast effect, which is the need for in vivo imaging. Additionally, it can function as an efficient delivery mediator of genes and medications for treating tumors by affecting cellular membranes and vascular endothelial integrity in the case of an excessive collapse of microbubbles at high-pressure amplitude (Papachristodoulou et al. 2019; Xu et al. 2017). Furthermore, these gas-filled microbubbles have poor stability and a short half-life due to the materials used and the process used to produce them (Zhou et al. 2020). Nanotheranostic agents have

some eye touching features like ultrasmall size and ability to bind specific receptors to target cells or tumor microenvironments with prolonged circulation time and high stability (Ahmad et al. 2013, 2020, 2021; Akhter et al. 2012). Tremendous possibilities available behind the development of novel theranostic agents to treat life-threatening diseases attract lots of scientists to research in this particular area (Bajwa et al. 2015; Jain et al. 2015a). Such nanotheranostics carriers are discussed in the following sections.

1.5 Different Types of Nanotheranostics

The "theranostics" word is referred to systems that can be both applied as therapeutics and imaging agents. Ideally, a nanotheranostic should be designed in such a way that it could circulate for a longer duration in the biological system, depict desired release as per the requirement, have target specificity and ability to deliver the drug and theranostic agent in desired concentration at target site, and have imaging ability and a larger target to background ratio.

Based on nanoplatform used in design of nanotheranostics, nanotheranostics could be classified into two categories. One is inorganic theranostic, where inorganic materials are used as theranostics like superparamagnetic iron oxide, gold, carbon

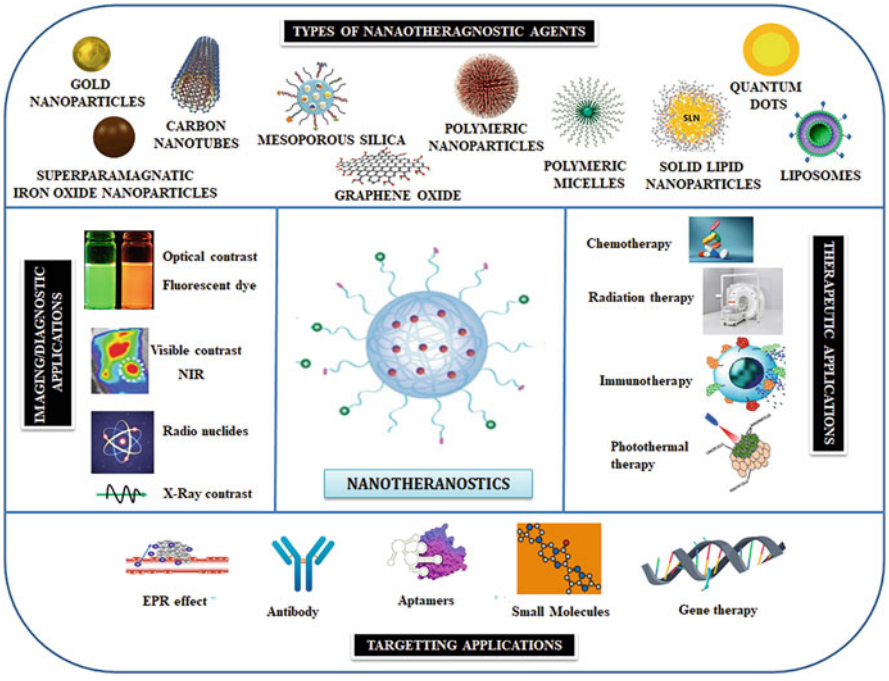

Fig. 1.7 Types and applications of nanotheranostics

nanomaterials, graphene oxide, quantum dots, etc. Another category is organic nanotheranostics in which organic materials like polymeric nanoparticles, polymeric micelles, liposome, lipids, etc. are used. We have shown different nanotheranostic systems graphically in Fig. 1.7 along with their three main biomedical applications, i.e., therapeutic delivery, diagnostic/imaging applications, and targeted delivery of bioactive if we modify or engineer the nanotheranostics with targeting ligands/moieties also depicted in Fig. 1.7. Additionally, the summary of recently researched nanotheranostic agents is showcased in Table 1.1.

Inorganic nanocarriers have offered wide range of benefits due to their properties like diverse surface chemistry, controllable structures, large surface area, and tunable optical characteristics in delivery of therapeutic and diagnostic agents. Furthermore, research work published in last decade proved the efficiency of inorganic nanoparticles as theranostic agents (Feliu et al. 2016).

1.5.1 Superparamagnetic Iron Oxide Nanoparticles

SPIONs are the particles of submicron size, consisting of iron oxide core, which imparts them magnetic properties under influence of external magnetic field. The colloidal stability of SPIONs is improved by either surface functionalization or surface coating with suitable polymeric or non-polymeric capping agents such as carboxymethyl cellulose, chondroitin sulfate, starch, chitosan, etc. (Sun et al. 2019; Mallick et al. 2015, 2016). SPIONs can be used as efficient multimodal nanotheranostics carrier due to its small size, good biocompatibility, ability of surface functionalization, sensitivity, and their capacity to be used as multimodal contrast agent (Cai et al. 2020). The FDA authorized "Ferumoxides," the first SPION-derived MRI contrast agent, in 1996. Several iron oxide nanoparticles, notably "Feridex" and "Feraheme," have so far obtained confirmation for use in clinical settings (Wahsner et al. 2019). Iron oxide nanoparticles capped with specific polymers have shown ability to serve as efficient contrast agents in ultrasound/MRI (Sun et al. 2019).

SPIONs are used as multimodal MRI contrast agent for imaging of brain cells, liver cells, lung cells, heart cells, etc. (Yoon et al. 2017; Reczyńska et al. 2020; Dao et al. 2017). Conjugation of SPIONs with aptamers has been explored for colorectal and prostate cancer therapy and imaging. High-intensity MRI signals were observed from tumor area upon intravenous administration of aptamer conjugated SPIONs. Scientists have also observed that SPIONs were metabolized into non-toxic iron ions in in vivo experiments (Jalalian et al. 2013; Wang et al. 2008). Cai et al. developed ultrasmall SPIONs coupled with phenothiazine-based NIR fluorescent dye and evaluated for Alzheimer's disease. The SPION coupled with novel theranostic agents showed strong binding with Aβ species as well as an enhancement of fluorescence in the NIR window. They were highly stable in bovine serum with low cytotoxic effect toward human neuroblastoma cells. The in vivo NIR fluorescence and MRI images showed a big difference between double transgenic mice and wild-type control mice. Histological staining in slices of brain confirmed the specific

Table 1.1 Summary of few nanotheranostic agents explored for biomedical applications

S. no.	Types	Functionalization	Targeted disease	Imaging technique	References
1.	Superparamagnetic iron oxide nanoparticles (SPIONs)	SPIONs-aptamer bioconjugates	Cancer	MRI	Jalalian et al. (2013)
2.	SPIONs	Chitosan-coated SPION	Cancer	Ultrasound/ MRI	Sun et al. (2019)
3.	SPIONs	Chondroitin sulfate-capped SPIONs	Cancer	MRI	Mallick et al. (2016)
4.	Ultrasmall SPIONs	Phenothiazine-based near-infrared (NIR) fluorescent dye	Alzheimer's disease	NIR	Cai et al. (2020)
5.	Ultrasmall SPIONs	Epirubicin (EPI)-loaded ultrasmall SPIONs with poly (aspartic acid) graft copolymer	Cancer	MRI	Yoon et al. (2017)
6.	Gold antennas	Cetuximab loaded in gold nanoantenna	Tumor	Raman spectroscopy	López-Lorente (2021)
7.	Hollow gold nanocages	Ac-Glu-Glu-Cys-NH_2 tripeptide-linked AuNPs loaded with cisplatin Pt (II)	Cancer	NIR	Xiong et al. (2018)
8.	Carbon nanomaterials	Functionalized fullerene with cytokine interleukin-13 encapsulated	Cancer	MRI	Li et al. (2015b)
9.	Nanodiamonds	Human serum albumin-based biopolymer (polyethylene glycol) coating (dcHSA-PEG)	Brain targeting	Fluorescent dye	Moscariello et al. (2019)
10.	Polymeric nanoparticles	Poly (vanillin oxalate) nanoparticles	Hepatic ischemia/ reperfusion (I/R) injury	H_2O_2	Kang et al. (2016)
11.	Polymeric nanotheranostics	Autofluorescence polymer polyethylene imine-polylactide (PEI-PLA)	Angiogenesis and tumor cell growth	Polymer with NIR dye	Shao et al. (2019)
12.	Liposome	PEG-coated and folate-PEG-coated	Cancer	^{159}Gd	Siafaka et al. (2021)

binding of nanoparticles to Aβ plaque. The SPION coupled with novel theranostic agents blocked the seeding-mediated aggregation with an IC_{50} of 11.7–32.1 ng/mL, which is much better than observed in phenothiazine-based small molecules (Cai et al. 2020). Also, the research published in last few years confirmed that conjugation of SPIONs with antibodies, ligands, polymers, folic acid, PEG, or peptide gave promising results of radiosensitivity in ovarian cancer, cervical cancer, and breast cancer (Fakhimikabir et al. 2018; Zhang et al. 2016; Song et al. 2018; Pan et al. 2018).

Excellent blood–brain barrier permeability of SPIONs without disturbing other brain cells under low radio radiofrequency field is an important feature in brain imaging. Phenothiazine-based and PEGylated ultrasmall SPIONs worked as novel theranostics agents for amyloid plaques in Alzheimer's disease (Dao et al. 2017; Yallapu et al. 2010). Next generation SPIONs act as a Trojan horse (administered intravenously) for the delivery of therapeutic drugs to cancers. Recently, ultrasmall SPIONs working as a multimodal contrast agent have been recognized as a promising material for development of nanotheranostic carriers due to its good biocompatibility and high sensitivity (Chen et al. 2022). SPIONs have tremendous potential to study the in vivo behavior of nanocarriers, as they are inherently holding the MRI contrast agents.

1.5.2 Gold

AuNPs have been widely explored for applications as imaging agents, drug delivery carrier, for targeted delivery, theranostics, etc., because they are capable of conjugating and delivering drugs and bioactive molecules (ligands) to targeted cells. AuNPs were an attractive alternative amidst various inorganic systems exploited by research community due to their high surface-area-to-volume ratio, unique and tunable optical properties, as well as easy surface functionalization along with high loading capacity of biomolecules. When AuNPs are in contact with a biological medium, they may rapidly be coated with nonspecific serum proteins. This process has been known as the *corona effect*. To diminish corona effect AuNPs were frequently coated with PEG (Albertini et al. 2019; Groysbeck et al. 2019) or multilayer coating of albumins (Achilli et al. 2022).

In addition to this, AuNPs can be explored for the PDT, PTT, and photoacoustic treatments. Therefore, we can use AuNPs in various fields, e.g., bioimaging (Demiral et al. 2021; Nicholls et al. 2016), targeted delivery of therapeutics (García et al. 2022; Li et al. 2018), and plasmonic PTT (Ali et al. 2022; Taylor et al. 2022). Demiral et al. formulated PEGylated AuNPs by attaching cell penetration enhancer D-α-Tocopherol succinate to detect and treat drug-resistant micro tumors through PTT by using verteporfin as photosensitizer. The theranostic system was not only used for drug delivery and imaging in vitro/vivo, but it can also be used for other fluorescence-based biological and medical purposes. Covalent attachment of ligand and imaging agent to the system makes the theranostic agent work better against tumors, and observed to be the most promising candidate, causing 4 times as many

cells to die. The cell studies showed that the theranostic agent improves the apoptosis process in 61% of the cells (Demiral et al. 2021).

Gold nanocarriers involved in diagnosis, therapeutic, and theranostic application showed different morphology such as spherical, nanorods, nano shells, hollow nanocages, nanoantenna, nanoplates, nano prisms, etc. (López-Lorente 2021; Xiong et al. 2018; Vines et al. 2019; Gharatape and Salehi 2017). Gold-based nanotherapeutics can be remodeled for tumor microenvironment for targeting by changing unfavorable therapeutic conditions into therapeutically accessible by imparting them different external (temperature, laser, or ultrasound) and internal (pH, enzymes, and glutathione) stimuli responsive drug release mechanisms (Mohapatra et al. 2021; Rajendrakumar et al. 2018). AuNPs have inherent property to provide catenate sites for coating or conjugation of various active pharmaceutical ingredients, ligands, proteins, and imaging agents, which provide immense potential to use AuNPs as nanocarrier in biological system.

1.5.3 Carbon Nanomaterials

Carbon allotropes such as nanodiamonds, carbon nanoparticles, fullerenes, carbon nanotubes, graphene, etc., have tremendous potential to serve as delivery carrier due to their unique physiochemical properties, chemical nature, handy fabrication, facile surface modification, thermal stability, optical properties, great mechanical strength, and electrical conductivity (Kaur et al. 2016). Researchers already explored carbon-based nanomaterials as theranostics nanocarrier in image-guided treatment of cardiovascular diseases and cancer (Alagarsamy et al. 2021; Gao et al. 2019).

Nanodiamonds have an octahedral architecture (size 5–50 nm), known for its unique properties like low toxicity, stable fluorescence, easy functionalization, and intrinsic biocompatibility (Qin et al. 2021). Similarly, carbon nanotubes have a unique architectural form of fullerene (cylindrical fullerene) broadly classified into single-walled carbon nanotubes and multi-walled carbon nanotubes. They are highly ordered, pseudo-one-dimensional carbon allotropes that can easily penetrate various cells to deliver the drugs or bioactive molecules (Augustine et al. 2017).

Fullerenes have lots of free active groups on its surface, which provide them immense potential for functionalization (Shi et al. 2014). Li et al. formulated surface functionalized fullerene with cytokine interleukin-13, and encapsulated Gadolinium, to increase the intracellular uptake of anticancer agent. When this fullerene was attached to an interleukin-13 peptide, this hydrophilic nanoparticle showed a better uptake in human brain cell lines (U-251 GBM). These results support the idea that the positively charged (amino)-I nanoparticle has a stronger charge attraction for human brain cellular endocytosis on the metallofullerene cage surface (Li et al. 2015b).

A wide range of carbon-based materials provide good fluorescence and less toxicity compared to the organic dyes, although their cytotoxicity-related concerns and poor knowledge regarding pharmacokinetic behavior limit their commercial application.

1.5.4 Graphene Oxide

Graphene oxide (GO) is a form of graphene that has been oxidized, widely explored in biotechnology and medicine field to treat cancer, drug delivery, and easy penetration through cells. GO also has many physical and chemical properties, such as a nanoscale size, a large surface area, and an electrical charge (Esmaeili et al. 2020). GO is more hydrophilic compared to graphene, hence showed more solubility and colloidal stability in aqueous media. GO-based nanocarriers can also be explored as antimicrobial agents due to their dose-dependent cytotoxicity and bactericidal activity. Although GO was toxic to living cells and organs, which makes it hard to use in the biomedical field, surface functionalization of GO decreases the toxicity significantly. Two graphene-based materials, GO and reduced GO nanosheets, which significantly inhibited the *E. coli* bacterial growth were reported by Kumar and co-workers (Kumar et al. 2019).

GO nanoparticles were explored for the non-invasive bio-imaging and targeted therapy (Syama and Mohanan 2019) due to their unique advantages such as low cytotoxicity, tunable optical properties, high photostability, brightly emissive for high-contrast imaging, and facile surface functionalization for specific targeting (Dong et al. 2018). GO as fluorescence probe is highly suitable for fluorescent probe, due to its advantages like biological compatibility, resistance to photobleaching, and efficient light emission (Esmaeili et al. 2020). More advancement in GO nanoparticles was performed to improve its hydrophilicity and subsequently the in vivo circulation time by attaching the low molecular weight PEG. Results of the in vivo study showed improved blood circulation time and lowered cytotoxicity (Ghosh and Chatterjee 2020). To make the most of the benefits of nanotechnology and to reduce the risks to human health, it is important to figure out the molecular targets involved in toxicity and to weigh the pros and cons of GO.

Nanomedicine can be formulated using varieties of organic materials, which majorly include carbohydrates, proteins, lipids, and synthetic or semi-synthetic polymers. The commonly used nanoparticles are discussed in the following subsections.

1.5.5 Polymeric Nanoparticles

Polymeric nanoparticles are widely explored due to their easy availability, cost effectiveness, sustained type drug release pattern, and inertness. Commonly used polymers for formulating theranostics nanoparticles are chitosan, gelatin, albumin, sodium alginate, poly (lactic-co-glycolic acid), PLA, poly-glutamic acid, etc., used very promisingly for delivering the drug to cardiovascular and central nervous systems (Kang et al. 2016). Shao and co-workers formulated the triple-collaborative nanotheranostics nanocarrier for combining the therapeutic benefits of anti-angiogenesis, RNA interference, and PTT using PLA as polymer. In vitro cell line study showed self-fluorescence activity and significant increase in cellular uptake (Shao et al. 2019). Chio et al. made chlorin e6, a second-generation photosensitizer

and camptothecin-loaded polymeric nanoparticles that were decorated with hyaluronic acid-grafted monomethoxy PEG for imaging and treating triple negative breast cancer. In vitro cell line studies with MDA-MB-231 cells showed that hyaluronic acid-grafted monomethoxy PEG-based nanoparticles were taken up much more rapidly than nanoparticles without a coating. The therapeutic effectiveness was measured by putting free and camptothecin-loaded nanoparticles into MDA-MB-231 cells for 6 h and then emitting a 670 nm laser on them. The results showed that nanoparticles killed 28% of the cells while same concentration of camptothecin has killed 12% of cells (Choi et al. 2015). Polymeric nanoparticles have been explored widely for theranostic application, and due to the inertness of polymers, it provided stable nanoparticles for treatment of various diseases.

1.5.6 Polymeric Micelles

Polymeric micelles are self-assembled aggregated colloidal nano-constructs of amphiphilic polymers with a core-shell structure. These have been used as versatile carriers for delivery of diagnostic agents and drugs. They have gained immense popularity due to their unique features such as smaller size, good solubilization properties, easy surface functionalization, biocompatibility, longevity, enhanced permeation and retention effect, good encapsulation efficiency, high stability (in vitro and in vivo), and the ability to accumulate into tumor through compromised vasculature. The core of the micelles are formed by various amphiphilic copolymers including di-block, triblock, as well as graft copolymers of PEG, poly-L-aspartic acid, poly(2-hydroxyethyl-L-aspartamide), etc. (Kang et al. 2016). Gregoriou and his team used a single emulsification-based approach to make resveratrol-loaded micelles from pluronic F127 block copolymer and D—tocopheryl PEG succinate. This was done to improve cellular uptake and get more desirable pharmacokinetics for breast cancer treatment. Cellular uptake studies were performed on MCF-7 and MDA-MB-231 cells; 4 h cellular uptake data showed significantly higher cellular uptake by MDA-MB-231 cells compared to MCF-7 cells (Gregoriou et al. 2021). Micelles were widely explored for their simplicity and easy modification capacity. Results published regarding the theranostics micelles in scientific literature have potential to give therapeutic and image-guided treatment to patients.

1.5.7 Liposome

Liposomes are one of the widely accepted, biocompatible, biodegradable lipidic nanocarriers and first approved nanocarrier by FDA. Easy preparation, feasibility to scale up, biocompatibility, ability to load hydrophilic, as well as hydrophobic drugs and easy surface functionalization (PEG or vitamins) established it as a superior nanocarrier. Recently, liposome was explored for various applications of in vivo imaging through optical, MRI, PET, CT, PDT and PTT, etc., by scientific community (Lee and Im 2019).

Skupin-Mrugalska et al. formulated liposomes by using lipid derivatives of gadolinium (III) diethylenetriaminepentaacetic acid salt, which can also serve as MRI contrasting agent and a photosensitizer agent zinc phthalocyanine. This hybrid nanocarrier was capable to kill cancer cells by PTT as well as diagnosis through MRI. Confocal microscopy images showed the internalization of nanohybrid inside the fibroblast cells (Skupin-Mrugalska et al. 2018). Lozano et al. made a nanotheranostic system for TNBC using doxorubicin and indocyanine green loaded monoclonal antibody decorated PEGylated liposomes that target the mucin1 receptor. In vivo monitoring of this antibody-coated nanotheranostic formulation showed that the liposomes quickly gathered in a tumor model, unlike the non-targeted formulation (Lozano et al. 2015).

There are numbers of liposomal products currently in the market as well as in clinical trials, which impart them immense value to develop as novel commercial product. But clinical application of theranostic liposomes or other nanocarriers requires detailed clinical studies regarding their behavior in biological system, metabolism profile, toxicological studies, and many more. Although few theranostic-based nanocarriers are currently under clinical trials, outcomes of such clinical studies will guide researchers in the future.

1.6 Regulatory Aspects

Nanomedicine has been a massively explored research area worldwide, due to its ability to improve therapeutic outcome of active pharmaceutical ingredients by transforming polymers, lipids, carbohydrates, etc., into nanocarriers, ligand decorated nanocarriers, inorganic nanoparticles, dendrimers, and quantum dots (Jain et al. 2015b; Ojha et al. 2021). However, most of the regulatory authorities do not have clear regulatory guideline for the marketing approval of nanomedicine, which restricts the easy translation of nanomedicine and creates a lack of clarity for research institutes and manufacturer in development of novel nanocarriers (Sainz et al. 2015).

There are lots of challenges in preparation of clear regulatory guidelines for nanomedicines. For instance, (1) at physiological level nanomedicine demonstrated contrasting pharmacokinetic behavior compared to small drug molecules, (2) unavailability of clear evidence regarding its penetration through blood-brain barrier due to its small size, which may compromise the normal brain function; (3) another problem was associated with nanomaterial is explanation of the accumulation of nanoparticles in high blood perfusion containing organs upon systemic administration, and (4) lack of standard nanotoxicology tests cumulatively limits the translation of nanomedicine from bench to bed (Hejmady et al. 2020). Furthermore, insufficient and ununified regulatory guidelines (like different interpretations of definition and classification of nanomedicine in different countries) made regulatory approval of nanomedicine more stringent and time-consuming. One of the major concerns was scale up of laboratory batches at commercial level, and stability throughout its shelf life also created muddle in regulatory approval. Genotoxicity

and environment-related concerns have further aggregated, which required separate guideline and analytical tests for regulatory clearance (Foulkes et al. 2020).

Besides all these regulatory-related problems, more than 50 nanomedicines were already deployed in the market till date, and this number raises steadily (Sarwal et al. 2019). Most of the regulatory authorities do not consider nanomedicine harmful but choose to evaluate and provide approval on case-to-case bases. In recent time many regulatory authorities have created committee or declared some sort of guidelines to make the regulatory approval process easy. For example, the US Food and Drug Administration has formed a task force and a "Nanotechnology Interest Group." The task force noticed that the current regulatory guidelines were broad enough to ensure the safe production of nanomedicines, which can go to the preclinical and clinical trials. But no specific points were discussed about the clinical evaluations (Nanotechnology Task Force 2022). Similarly, the European Union and the healthcare product authority of the United Kingdom have established a European nanomedicine characterization laboratory for providing constantly refined intellectual results in preclinical studies of nanomedicine to manufacturers and regulatory authorities. Furthermore, few of the health-related government agencies of the United States and European Commission have introduced a project titled "REFINE" to set the criteria for regulatory approval of nanomedicine with their objective "Development and validation of new analytical or experimental methods." To set the definite criteria for manufacturing of nanomedicine, the Department of Science and Technology, Government of India, has also prepared a draft. This contained the three-tier governance framework to regulate the nanomedicine (Foulkes et al. 2020).

It was now clear toward the regulatory authorities present worldwide that a proper set of guidelines is a must for smooth approval of nanomedicine and for guiding the manufacturers as well as health workers for development of new nanomedicines. Academicians, clinicians, manufacturers, and regulatory personnel should combinedly form a defined set of criteria, which will make approval process for nanomedicine easy.

1.7 Conclusion

The current chapter discussed in detail the various ligand decorated nanotheranostic drug delivery carrier. Various imaging techniques like fluorescence, PET, CT, MRI, etc., used for in vivo imaging were also explored. The novel techniques like PTT and PDT have opened the new door for the treatment of various life-threatening diseases like cancer. Also, the numerous research articles published by scientific community proved the efficiency of ligand decorated theranostic nanocarrier for the treatment of cancer, infectious diseases, and neurodegenerative diseases. Some of the ligand decorated nanocarriers have also entered the initial stage of clinical trials, which proved the importance and feasibility of this type of nanocarriers in clinical applications.

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References

Achilli E, Flores CY, Temprana CF, del Alonso S, Radrizzani M, Grasselli M (2022) Enhanced gold nanoparticle-tumor cell recognition by albumin multilayer coating. OpenNano 6:100033

Ahmad MZ, Akhter S, Rahman Z, Akhter S, Anwar M, Mallik N et al (2013) Nanometric gold in cancer nanotechnology: current status and future prospect. J Pharm Pharmacol 65(5):634–651

Ahmad MZ, Alkahtani SA, Akhter S, Ahmad FJ, Ahmad J, Akhtar MS et al (2015) Progress in nanotechnology-based drug carrier in designing of curcumin nanomedicines for cancer therapy: current state-of-the-art. J Drug Target 24(4):273–293

Ahmad MZ, Ahmad J, Haque A, Alasmary MY, Abdel-Wahab BA, Akhter S (2020) Emerging advances in synthetic cancer nano-vaccines: opportunities and challenges. Expert Rev Vaccines 19(11):1053–1071

Ahmad MZ, Ahmad J, Alasmary MY, Abdel-Wahab BA, Warsi MH, Haque A et al (2021) Emerging advances in cationic liposomal cancer nanovaccines: opportunities and challenges. Immunotherapy 13(6):491–507

Ahmed F, Khan MA, Haider N, Ahmad MZ, Ahmad J (2022) Recent advances in theranostic applications of nanomaterials in cancer. Curr Pharm Des 28(2):133–150

Akhter S, Zaki Ahmad M, Singh A, Ahmad I, Rahman M, Anwar M et al (2011) Cancer targeted metallic nanoparticle: targeting overview, recent advancement and toxicity concern. Curr Pharm Des 17(18):1834–1850

Akhter S, Ahmad MZ, Ahmad FJ, Storm G, Kok RJ (2012) Gold nanoparticles in theranostic oncology: current state-of-the-art. Expert Opin Drug Deliv 9(10):1225–1243

Akhter S, Ahmad I, Ahmad MZ, Ramazani F, Singh A, Rahman Z, et al (2013) Nanomedicines as cancer therapeutics: current status. Curr Cancer Drug Targets 13(4):362–378

Alagarsamy KN, Mathan S, Yan W, Rafieerad A, Sekaran S, Manego H et al (2021) Carbon nanomaterials for cardiovascular theranostics: promises and challenges. Bioact Mater 6(8): 2261–2280

Albertini B, Mathieu V, Iraci N, van Woensel M, Schoubben A, Donnadio A et al (2019) Tumor targeting by peptide-decorated gold nanoparticles. Mol Pharm 16(6):2430–2444

Ali I, Rahis-Uddin SK, Rather M, Wani W, Haque A (2011) Advances in nano drugs for cancer chemotherapy. Curr Cancer Drug Targets 11(2):135–146

Ali MRK, Warner PE, Yu AM, Tong M, Han T, Tang Y (2022) Preventing metastasis using gold nanorod-assisted plasmonic photothermal therapy in xenograft mice. Bioconjug Chem 33:2320

Allison RR, Downie GH, Cuenca R, Hu XH, Childs CJH, Sibata CH (2004) Photosensitizers in clinical PDT. Photodiagn Photodyn Ther 1(1):27–42

Anani T, Rahmati S, Sultana N, David AE (2021) MRI-traceable theranostic nanoparticles for targeted cancer treatment. Theranostics 11(2):579–601

Augustine S, Singh J, Srivastava M, Sharma M, Das A, Malhotra BD (2017) Recent advances in carbon based nanosystems for cancer theranostics. Biomater Sci 5(5):901–952

Avasthi A, Caro C, Pozo-Torres E, Leal MP, García-Martín ML (2020) Magnetic nanoparticles as MRI contrast agents. Top Curr Chem 378(3)

Bai RG, Muthoosamy K, Manickam S (2015) Nanomedicine in theranostics. Nanotechnology applications for tissue engineering, pp 195–213

Bajwa N, Kumar Mehra N, Jain K, Kumar JN (2015) Targeted anticancer drug delivery through anthracycline antibiotic bearing functionalized quantum dots. Artif Cells Nanomed Biotechnol 44(7):1774–1782

Bonnet CS, Tóth É (2021) Metal-based environment-sensitive MRI contrast agents. Curr Opin Chem Biol 61:154–169

Bukhari SI, Imam SS, Ahmad MZ, Vuddanda PR, Alshehri S, Mahdi WA et al (2021) Recent progress in lipid nanoparticles for cancer theranostics: opportunity and challenges. Pharmaceutics 13(6):840

Cai J, Dao P, Chen H, Yan L, Li YL, Zhang W et al (2020) Ultrasmall superparamagnetic iron oxide nanoparticles-bound NIR dyes: novel theranostic agents for Alzheimer's disease. Dyes Pigments 173:107968

Calzavara-Pinton PG, Venturini M, Sala R (2007) Photodynamic therapy: update 2006 part 2: clinical results. J Eur Acad Dermatol Venereol 21(4):439–451

Campbell RB (2006) Tumor physiology and delivery of nanopharmaceuticals. Anti Cancer Agents Med Chem 6(6):503–512

Chen C, Ge J, Gao Y, Chen L, Cui J, Zeng J et al (2022) Ultrasmall superparamagnetic iron oxide nanoparticles: a next generation contrast agent for magnetic resonance imaging. Wiley Interdiscip Rev Nanomed Nanobiotechnol 14(1):e1740

Choi J et al (2011) Gold nanostructures as photothermal therapy agent for cancer. Anti-Cancer Agents in Med Chem 11(10):953–964

Choi J, Kim H, Choi Y (2015) Theranostic nanoparticles for enzyme-activatable fluorescence imaging and photodynamic/chemo dual therapy of triple-negative breast cancer. Quant Imaging Med Surg 5(5):656–65664

Cormode DP, Skajaa T, Fayad ZA, Mulder WJM (2009) Nanotechnology in medical imaging: probe design and applications. Arterioscler Thromb Vasc Biol 29(7):992–1000

Dao P, Ye F, Liu Y, Du ZY, Zhang K, Dong CZ et al (2017) Development of phenothiazine-based theranostic compounds that act both as inhibitors of β-amyloid aggregation and as imaging probes for amyloid plaques in Alzheimer's disease. ACS Chem Neurosci 8(4):798–806

Debbage P, Jaschke W (2008) Molecular imaging with nanoparticles: giant roles for dwarf actors. Histochem Cell Biol 130(5):845–875

Demiral A, Verimli N, Goralı Sİ, Yılmaz H, Çulha M, Erdem SS (2021) A rational design of multi-functional nanoplatform: fluorescent-based "off-on" theranostic gold nanoparticles modified with D-α-tocopherol succinate. J Photochem Photobiol B 222:112261

Dheyab MA, Aziz AA, Khaniabadi PM, Jameel MS (2021) Potential of a sonochemical approach to generate MRI-PPT theranostic agents for breast cancer. Photodiagn Photodyn Ther 33:102177

Dong J, Wang K, Sun L, Sun B, Yang M, Chen H et al (2018) Application of graphene quantum dots for simultaneous fluorescence imaging and tumor-targeted drug delivery. Sens Actuators B Chem 256:616–623

Esmaeili Y, Zarrabi A, Mirahmadi-Zare SZ, Bidram E (2020) Hierarchical multifunctional graphene oxide cancer nanotheranostics agent for synchronous switchable fluorescence imaging and chemical therapy. Microchim Acta 187(10):553

Fakhimikabir H, Tavakoli MB, Zarrabi A, Amouheidari A, Rahgozar S (2018) The role of folic acid-conjugated polyglycerol coated iron oxide nanoparticles on radiosensitivity with clinical electron beam (6 MeV) on human cervical carcinoma cell line: in vitro study. J Photochem Photobiol B 182:71–76

Feliu N, Docter D, Heine M, del Pino P, Ashraf S, Kolosnjaj-Tabi J et al (2016) In vivo degeneration and the fate of inorganic nanoparticles. Chem Soc Rev 45(9):2440–2457

Foulkes R, Man E, Thind J, Yeung S, Joy A, Hoskins C (2020) The regulation of nanomaterials and nanomedicines for clinical application: current and future perspectives, vol 8. Biomater Sci R Soc Chem, pp 4653–4664

Frinking PJA, Bouakaz A, Kirkhorn J, ten Cate FJ, de Jong N (2000) Ultrasound contrast imaging: current and new potential methods. Ultrasound Med Biol 26(6):965–975

Gao G, Guo Q, Zhi J (2019) Nanodiamond-based theranostic platform for drug delivery and bioimaging. Small 15(48):1902238

García MC, Calderón-Montaño JM, Rueda M, Longhi M, Rabasco AM, López-Lázaro M et al (2022) pH-temperature dual-sensitive nucleolipid-containing stealth liposomes anchored with PEGylated AuNPs for triggering delivery of doxorubicin. Int J Pharm 619:121691
Gellci K, Mehrmohammadi M (2014) Photothermal therapy. Encyclopedia of Cancer 1–5
Gharatape A, Salehi R (2017) Recent progress in theranostic applications of hybrid gold nanoparticles. Eur J Med Chem 138:221–233
Ghosh S, Chatterjee K (2020) Poly(ethylene glycol) functionalized graphene oxide in tissue engineering: a review on recent advances. Int J Nanomedicine 15:5991
Gollavelli G, Ling YC (2014) Magnetic and fluorescent graphene for dual modal imaging and single light induced photothermal and photodynamic therapy of cancer cells. Biomaterials 35(15):4499–4507
Gøtzsche PC (2011) Niels Finsen's treatment for lupus vulgaris. J R Soc Med 104(1):41–42
Gregoriou Y, Gregoriou G, Yilmaz V, Kapnisis K, Prokopi M, Anayiotos A, Strati K, Dietis N, Constantinou AI, Andreou C (2021) Resveratrol loaded polymeric micelles for theranostic targeting of breast cancer cells. Nano 5(1):113
Groysbeck N, Stoessel A, Donzeau M, da Silva EC, Lehmann M, Strub JM et al (2019) Synthesis and biological evaluation of 2.4 nm thiolate-protected gold nanoparticles conjugated to Cetuximab for targeting glioblastoma cancer cells via the EGFR. Nanotechnology 30(18): 184005
Hejmady S, Singhvi G, Saha RN, Dubey SK (2020) Regulatory aspects in process development and scale-up of nanopharmaceuticals. Ther Deliv 11:341–343
Hönigsmann H (2012) History of phototherapy in dermatology. Photochem Photobiol Sci 12(1): 16–21
Hood L (2013) Systems biology and P4 medicine: past, present, and future. Rambam Maimonides Med J 4(2):0012
Huang X, Lu Y, Guo M, Du S, Han N (2021) Recent strategies for nano-based PTT combined with immunotherapy: from a biomaterial point of view. Theranostics 11(15):7546
Jain K, Zhong J (2022) Theranostic applications of nanomaterials. Curr Pharm Des 28(2):77–77
Jain K, Mehra N, Jain N (2015a) Nanotechnology in drug delivery: safety and toxicity issues. Curr Pharm Des 21(29):4252–4261
Jain K, Verma AK, Mishra PR, Jain NK (2015b) Surface-engineered dendrimeric nanoconjugates for macrophage-targeted delivery of amphotericin B: formulation development and in vitro and in vivo evaluation. Antimicrob Agents Chemother 59(5):2479–2487
Jalalian SH, Taghdisi SM, Hamedani NS, Kalat SAM, Lavaee P, ZandKarimi M et al (2013) Epirubicin loaded super paramagnetic iron oxide nanoparticle-aptamer bioconjugate for combined colon cancer therapy and imaging in vivo. Eur J Pharm Sci 50(2):191–197
Janib SM, Moses AS, MacKay JA (2010) Imaging and drug delivery using theranostic nanoparticles. Adv Drug Deliv Rev 62(11):1052–1063
Kang C, Cho W, Park M, Kim J, Park S, Shin D et al (2016) H2O2-triggered bubble generating antioxidant polymeric nanoparticles as ischemia/reperfusion targeted nanotheranostics. Biomaterials (85):195–203
Kaur S, Mehra NK, Jain K, Jain NK (2016) Development and evaluation of targeting ligand-anchored CNTs as prospective targeted drug delivery system. Artif Cells Nanomed Biotechnol 45(2):242–250. https://doi.org/10.3109/2169140120161146728
Kenny GD, Kamaly N, Kalber TL, Brody LP, Sahuri M, Shamsaei E et al (2011) Novel multifunctional nanoparticle mediates siRNA tumour delivery, visualisation and therapeutic tumour reduction in vivo. J Control Release 149(2):111–116
Kim GJ, Nie S (2005) Targeted cancer nanotherapy. Mater Today 8(8):28–33
Kim TH, Lee S, Chen X (2013) Nanotheranostics for personalized medicine. Expert Rev Mol Diagn 13(3):257–269
Kumar P, Huo P, Zhang R, Liu B (2019) Antibacterial properties of graphene-based nanomaterials. Nanomaterials 9:737

Lammers T, Aime S, Hennink WE, Storm G, Kiessling F (2011) Theranostic nanomedicine. Account Chem Res 44(10):1029–1038
Lammers T, Kiessling F, Hennink WE, Storm G (2012) Drug targeting to tumors: principles, pitfalls and (pre-) clinical progress. J Control Release 161(2):175–187
Lee W, Im HJ (2019) Theranostics based on liposome: looking back and forward. Nucl Med Mol Imaging 53(4):242–246
Li B, Wang Q, Zou R, Liu X, Xu K, Li W et al (2014) Cu7.2S4 nanocrystals: a novel photothermal agent with a 56.7% photothermal conversion efficiency for photothermal therapy of cancer cells. Nanoscale 6(6):3274–3282
Li H, Hu H, Zhao Y, Chen X, Li W, Qiang W et al (2015a) Multifunctional aptamer-silver conjugates as theragnostic agents for specific cancer cell therapy and fluorescence-enhanced cell imaging. Anal Chem 87(7):3736–3745
Li T, Murphy S, Kiselev B, Bakshi KS, Zhang J, Eltahir A et al (2015b) A new interleukin-13 amino-coated gadolinium metallofullerene nanoparticle for targeted MRI detection of glioblastoma tumor cells. J Am Chem Soc 137(24):7881–7888
Li Y, He D, Tu J, Wang R, Zu C, Chen Y et al (2018) The comparative effect of wrapping solid gold nanoparticles and hollow gold nanoparticles with doxorubicin-loaded thermosensitive liposomes for cancer thermo-chemotherapy. Nanoscale 10(18):8628–8641
Lim W, Kim B, Jo G, Yang DH, Park MH, Hyun H (2020) Bioluminescence and near-infrared fluorescence imaging for detection of metastatic bone tumors. Lasers Med Sci 35(1):115–120
Liu X, Zhang M, Yan D, Deng G, Wang Q, Li C et al (2020) A smart theranostic agent based on Fe-HPPy@Au/DOX for CT imaging and PTT/chemotherapy/CDT combined anticancer therapy. Biomater Sci 8(15):4067–4072
López-Lorente ÁI (2021) Recent developments on gold nanostructures for surface enhanced Raman spectroscopy: particle shape, substrates and analytical applications. A review. Anal Chim Acta 1168:338474
Lozano N, Al-Ahmady ZS, Beziere NS, Ntziachristos V, Kostarelos K (2015) Monoclonal antibody-targeted PEGylated liposome-ICG encapsulating doxorubicin as a potential theranostic agent. Int J Pharm 482(1–2):2–10
Mallick N, Asfer M, Anwar M, Kumar A, Samim M, Talegaonkar S et al (2015) Rhodamine-loaded, cross-linked, carboxymethyl cellulose sodium-coated super-paramagnetic iron oxide nanoparticles: development and in vitro localization study for magnetic drug-targeting applications. Colloids Surf A Physicochem Eng Asp 481:51–62
Mallick N, Anwar M, Asfer M, Mehdi SH, Rizvi MMA, Panda AK et al (2016) Chondroitin sulfate-capped super-paramagnetic iron oxide nanoparticles as potential carriers of doxorubicin hydrochloride. Carbohydr Polym 151:546–556
Moghimi SM, Hunter AC, Murray JC (2005) Nanomedicine: current status and future prospects. FASEB J 19(3):311–330
Mohapatra A, Uthaman S, Park IK (2021 Jan) External and internal stimuli-responsive metallic nanotherapeutics for enhanced anticancer therapy. Front Mol Biosci 11(7):437
Moscariello P, Raabe M, Liu W, Bernhardt S, Qi H, Kaiser U et al (2019) Unraveling in vivo brain transport of protein-coated fluorescent nanodiamonds. Small 15(42):1902992
Muthiah M, Park IK, Cho CS (2013) Nanoparticle-mediated delivery of therapeutic genes: focus on miRNA therapeutics. Expert Opin Drug Delivery 10(9):1259–1273
Nanotechnology Task Force Report 2007 | FDA [Internet]. [cited 2022 Sep 28]. Available from: https://www.fda.gov/science-research/nanotechnology-programs-fda/nanotechnology-task-force-report-2007
National Research Council (US) Committee on A Framework for Developing a New Taxonomy of Disease (2011) Toward precision medicine: building a knowledge network for biomedical research and a new taxonomy of disease, pp 1–128
Nicholls FJ, Rotz MW, Ghuman H, MacRenaris KW, Meade TJ, Modo M (2016) DNA–gadolinium–gold nanoparticles for in vivo T1 MR imaging of transplanted human neural stem cells. Biomaterials 77:291–306

Ojha B, Jain VK, Mehra NK, Jain K (2021) Nanotechnology: Introduction and basic concepts. In: Mehra NK, Jain K (eds) Dendrimers in nanomedicine: concept, theory and regulatory perspectives. CRC Press, pp 1–17

Pan C, Liu Y, Zhou M, Wang W, Shi M, Xing M et al (2018) Theranostic pH-sensitive nanoparticles for highly efficient targeted delivery of doxorubicin for breast tumor treatment. Int J Nanomedicine 13:1119–1137

Papachristodoulou A, Signorell RD, Werner B, Brambilla D, Luciani P, Cavusoglu M et al (2019) Chemotherapy sensitization of glioblastoma by focused ultrasound-mediated delivery of therapeutic liposomes. J Control Release 295:130–139

Paszko E, Ehrhardt C, Senge MO, Kelleher DP, Reynolds J, v. (2011) Nanodrug applications in photodynamic therapy. Photodiagn Photodyn Ther 8(1):14–29

Qin JX, Yang XG, Lv CF, Li YZ, Liu KK, Zang JH et al (2021) Nanodiamonds: synthesis, properties, and applications in nanomedicine. Mater Des 210:110091

Rajendrakumar SK, Cherukula K, Park HJ, Uthaman S, Jeong YY, Lee B et al (2018) Dual-stimuli-responsive albumin-polyplex nanoassembly for spatially controlled gene release in metastatic breast cancer. J Controlled Release 276:72–83

Reczyńska K, Marszałek M, Zarzycki A, Reczyński W, Kornaus K, Pamuła E et al (2020) Superparamagnetic iron oxide nanoparticles modified with silica layers as potential agents for lung cancer treatment. Nanomaterials 10(6):1076

Richard C, de Chermont QLM, Scherman D (2008) Nanoparticles for imaging and tumor gene delivery. Tumori 94(2):264–270

Sainz V, Conniot J, Matos AI, Peres C, Zupančič E, Moura L et al (2015) Regulatory aspects on nanomedicines. Biochem Biophys Res Commun 468:504–510

Sarbadhikary P, George BP, Abrahamse H (2021) Recent advances in photosensitizers as multifunctional theranostic agents for imaging-guided photodynamic therapy of cancer. Theranostics 11(18):9054–9088

Sarwal A, Singh G, Singh K, Garg S (2019) Recent interventions for nanotechnology based drug products: insights into the regulatory aspects. Curr Pharm Des 24(43):5219–5228

Shao L, Li Q, Zhao C, Lu J, Li X, Chen L et al (2019) Auto-fluorescent polymer nanotheranostics for self-monitoring of cancer therapy via triple-collaborative strategy. Biomaterials 194:105–116

Shi J, Wang L, Gao J, Liu Y, Zhang J, Ma R et al (2014) A fullerene-based multi-functional nanoplatform for cancer theranostic applications. Biomaterials 35(22):5771–5784

Siafaka PI, Okur NÜ, Karantas ID, Okur ME, Gündoğdu EA (2021) Current update on nanoplatforms as therapeutic and diagnostic tools: a review for the materials used as nanotheranostics and imaging modalities. Asian J Pharm Sci 16(1):24–46

Skupin-Mrugalska P, Sobotta L, Warowicka A, Wereszczynska B, Zalewski T, Gierlich P et al (2018) Theranostic liposomes as a bimodal carrier for magnetic resonance imaging contrast agent and photosensitizer. J Inorg Biochem 180:1–14

Song J, Hu Q, Huang J, Chen T, Ma Z, Shi H (2018) Mr targeted imaging for the expression of tenascin-C in cervical cancer. Br J Radiol 91(1090):20170681

Sun JZ, Sun YC, Sun L (2019) Synthesis of surface modified Fe3O4 super paramagnetic nanoparticles for ultra sound examination and magnetic resonance imaging for cancer treatment. J Photochem Photobiol B 197:111547

Syama S, Mohanan P (2019) Comprehensive application of graphene: emphasis on biomedical concerns. Nano-Micro Lett 11(1):1–31

Taylor ML, Wilson RE, Amrhein KD, Huang X (2022) Gold nanorod-assisted photothermal therapy and improvement strategies. Bioengineering 9(5):200

Vines JB, Yoon JH, Ryu NE, Lim DJ, Park H (2019) Gold nanoparticles for photothermal cancer therapy. Front Chem 7:167

Wahsner J, Gale EM, Rodríguez-Rodríguez A, Caravan P (2019) Chemistry of MRI contrast agents: current challenges and new frontiers. Chem Rev 119(2):957–1057

Wan Q, Xie L, Gao L, Wang Z, Nan X, Lei H et al (2012) Self-assembled magnetic theranostic nanoparticles for highly sensitive MRI of minicircle DNA delivery. Nanoscale 5(2):744–752

Wang AZ, Bagalkot V, Vasilliou CC, Gu F, Alexis F, Zhang L et al (2008) Superparamagnetic iron oxide nanoparticle–aptamer bioconjugates for combined prostate cancer imaging and therapy. ChemMedChem 3(9):1311–1315

Weissleder R (2002) Scaling down imaging: molecular mapping of cancer in mice. Nat Rev Cancer 2(1):11–18

Xiao YD, Paudel R, Liu J, Ma C, Zhang ZS, Zhou SK (2016) MRI contrast agents: classification and application (review). Int J Mol Med 38(5):1319–1326

Xiong C, Lu W, Zhou M, Wen X, Li C (2018) Cisplatin-loaded hollow gold nanoparticles for laser-triggered release. Cancer Nanotechnol 9(1):1–13

Xu HL, Fan ZL, ZhuGe DL, Shen BX, Jin BH, Xiao J et al (2017) Therapeutic supermolecular micelles of vitamin E succinate-grafted ε-polylysine as potential carriers for curcumin: enhancing tumour penetration and improving therapeutic effect on glioma. Colloids Surf B Biointerfaces 158:295–307

Yallapu MM, Foy SP, Jain TK, Labhasetwar V (2010) PEG-functionalized magnetic nanoparticles for drug delivery and magnetic resonance imaging applications. Pharm Res 27(11):2283–2295

Yoon J, Cho SH, Seong H (2017) Multifunctional ultrasmall superparamagnetic iron oxide nanoparticles as a theranostic agent. Colloids Surf A Physicochem Eng Asp 520:892–902

Zhang H, Li J, Hu Y, Shen M, Shi X, Zhang G (2016) Folic acid-targeted iron oxide nanoparticles as contrast agents for magnetic resonance imaging of human ovarian cancer. J Ovarian Res 9(1):19

Zhao B, He YY (2014) Recent advances in the prevention and treatment of skin cancer using photodynamic therapy. Expert Rev Anticancer Ther 10(11):1797–1809. https://doi.org/10.1586/era10154

Zhou LQ, Li P, Cui XW, Dietrich CF (2020) Ultrasound nanotheranostics in fighting cancer: advances and prospects. Cancer Lett 470:204–219

Zhou B, Yin C, Feng Q, Wu Y, Pan X, Liu C et al (2021) Polypyrrole-based nanotheranostic agent for MRI guided photothermal-chemodynamic synergistic cancer therapy. Nanoscale 13(45):19085–19097

Designing of Smartly Functionalized Theranostic Nanomedicines

2

Dheeraj Pandey, Parth Patel, Keerti Jain, and Abha Sharma

Abstract

The consistent advances in the synthesis of nanoparticles (NPs) enable their potential use in the field of treatment and diagnosis. The NPs that are utilized for simultaneous diagnosis and treatment of disease are known as theranostic nanomedicines. The controlled size, shape, surface area, and permeation of NPs enable their superiority over conventional medicines. Functionalized NPs are advantages to achieve the desired effect, local or direct delivery, prolonged drug effects, and carrying off the theranostic property. Functionalization is achieved by altering the properties of NPs through chemical or physical modifications. Numerous functional technologies have been engaged for the modification and functionalization of NPs for theranostic applications. The functionalizations are mainly classified as small molecule functionalization or bio-functionalization. Small molecule functionalization entails the conjugation of drugs, chemical ligands, imaging agents, etc. to the NPs using chemical crosslinking reagents. For chemical functionalization copious chemical approaches such as click reactions, maleimide coupling, amide coupling, etc. have been utilized. Latterly, bio-functionalizations of NPs have drawn extensive debate because of their high biocompatibility, low cost, biodegradability, and eco-friendliness in contrast with their chemical counterparts. Bio-functionalization of NPs involves the use of natural phytochemicals,

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bio-inspired ligands, and the use of natural bio-resources such DNA, RNA, protein enzymes, etc. A brief information about various methods and approaches for the functionalization of NPs has been provided in this chapter.

Keywords

Theranostic · Functionalized nanoparticles · Nanomedicines · Gold nanoparticles · Bio-functionalization · Chemical functionalization · Diagnosis

2.1 Introduction

Nanotechnology is the study committed to design and synthesis of nanosize molecules for various applications such as therapeutic, imaging, target site drug delivery, tissue repair, etc. Nanotechnology is one of the most rapidly extending areas in diverse scientific research domains. The term nanotechnology was first ever coined in 1959 by Nobel physicist Richard Feynman (Drexler 2004). In the past decades, multiple forms of NPs have attracted a lot of attention tremendous in various applications because of their unusual physicochemical and mechanical properties. Unlike small molecules, NPs are capable of carrying wide range of therapeutic, diagnosis, and selectivity agents to the target site (Caruthers et al. 2007; Xie et al. 2010; Sun 2010; Lammers et al. 2010). Even though development of nanoparticle-based therapeutic and imaging NPs into clinical trials is grappling due to toxicity concerns, advancements have been recorded in the last decade. More than 35 imaging or therapeutic NPs are approved by the Food and Drug Administration till date, for clinical trials e.g., CRLX101, Rexin-G, etc. (Thakor and Gambhir 2013).

The combination of therapeutic and diagnosis application within a single nanomedicine dosage form is known as theranostic nanomedicine (Jokerst and Gambhir 2011). Theranostic NPs are utilized for monitoring drug delivery, for imaging drug release and to monitor the efficacy of drugs. Theranostic NPs are employed as contrast agents that can be chased in vivo utilizing minimum of one of the available options of optical imaging technique, e.g., positron emission tomography (PET), magnetic resonance imaging (MRI), computed tomography (CT), fluorescence, or surface-enhanced Raman scattering (SERS). Theranostic NPs comprise of a therapeutic part such as immunotherapeutic agent, chemotherapeutics, or photosensitive molecules along with the contrast agents (Nicolson et al. 2020). Theranostic NPs can be formulated by employing various techniques, e.g., already existing imaging agents such iron oxide nanoparticle (IONPs) quantum dots (QDs) are conjugated with therapeutic agents (e.g., anticancer drugs and photosensitizers). Additionally, encapsulation of both imaging and therapeutic agent in a nanoplatform is also effective (Chen et al. 2014). Theranostic agents make it possible for the fine tune drug dose delivery and simultaneous imaging of the dose reaching target site. Ideal theranostic NPs must reach selectively and rapid to their target. In recent years theranostic applications of NPs have been flourished and explored widely due to

their superiority in simultaneous diagnosis and treatment of disease. Theranostic nanomedicines enable the real-time monitoring of treatment progress and efficacy of the treatment regimen (Kelkar and Reineke 2011). Theranostic nanomedicines are composed of therapeutic drug on one hand along imaging agents on other either conjugated with each other or co-loaded in classical drug delivery systems, such as NPs. This conjugation of the nanoimaging agent along with the therapeutic agent is known as functionalization (Lammers et al. 2005).

2.2 Approaches for Functionalization of Theranostic Nanoparticles

Functionalization of NPs allows inculcation of specific properties of interest to the NPs. Additionally, functionalized NPs have good physical properties, noninvasive characteristics, anti-corrosion, and anti-agglomeration properties (Subbiah et al. 2010). To overcome the toxicity-related issues and enhance the imaging properties, there is a need for modification of surface characters of NPs. Similarly, to enhance the theranostic properties and selectivity of NPs for the biological application, the surface of the NPs is modified, i.e., functionalization of NPs, which includes conjugation of chemicals (small molecules) or biomolecules on to their surface (Herranz et al. 2012; Aravind et al. 2012). It not only improves the image quality, but also enables the treatment of disease. Various budding theranostic nanoplatforms such as carbon nanodots (CDs), superparamagnetic iron oxide nanoparticles (SPIONs), gold nanoparticles (AuNPs), and graphene oxide (GO) are capable of diagnosis and treatment combinedly. Nowadays, surface functionalization has a very beneficial obligation for productively modulating the structures, physicochemical properties, and interface features of these nanoplatforms. This method is comparatively dependable and compliant for the adjustment to achieve both regulatory and physicochemical requirements as a justification for their applications in specific field (Mauro et al. 2021). Surface modification of NPs is a demanding task; as a result the role of chemists comes into picture as they are fully furnished to provide functionality using various synthetic strategies (Boisselier and Astruc 2009). Theranostic NPs can be developed using both organic and inorganic NPs (Xie et al. 2010). NPs can be functionalized using various ligands such as small molecules, fluorescent dyes, polymers, and biomolecules like enzymes, antibodies, DNA, RNA, proteins, carbohydrates, etc. (Fig. 2.1). Many biomolecules or therapeutic drugs can be incorporated on polymeric ligands and even on the surface of NPs by utilizing covalent or non-covalent binding strategies (Moyano and Rotello 2011). As a result, biocompatibility and specific recognition qualities can be achieved in case of biomolecule-conjugated NPs (Rana et al. 2012). Different chemical approaches have been utilized for the functionalization of NPs that can be classified as covalent and non-covalent strategies. Covalent strategies involve amide coupling, click chemistry reaction, thiol coupling, etc., while the non-covalent approaches include ionic coupling and hydrophobic coupling (Conde et al. 2014a). Further in this chapter the different types of functionalizations will be elaborated in brief.

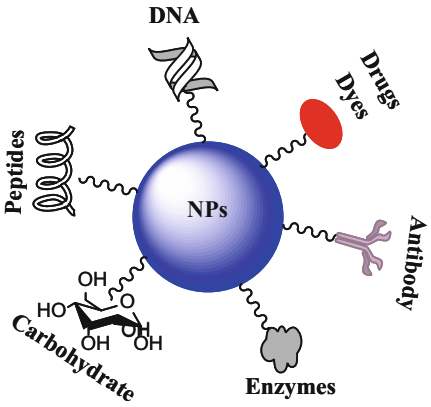

Fig. 2.1 Functionalization of NPs using various biomolecules and small molecule ligands

2.2.1 Functionalization of Nanoparticles Using Small Molecule Ligands

A wide range of small molecule ligands can be conjugated on the surface of NPs that allows the use of NPs in wide-ranging applications such as diagnosis, targeting, intracellular delivery, treatment of disease like cancer, etc. (El-Boubbou et al. 2010). It is also used for the identification of some specific cells; the NPs are conjugated with the ligands that have affinity towards the specific cells (Saha et al. 2011). The surface charge of the NPs is due to the groups present on them that allows the conjugation of molecules on the NPs. The cellular interaction and uptake of small molecules depends on their surface charge, so in general high cellular uptake is expected in case of positively charged molecules as compared to neutral and negatively charged molecules (Nel et al. 2009; Cho et al. 2009). For the incorporation of diagnostic properties on the therapeutic NPs, various fluorescent dyes are incorporated on the surface of NPs. Zhou et al. developed fluorescent molecule conjugated NPs for targeting cancer cells. In this report cyanine structure molecule was conjugated with IONPs, and the ability of the formulated NPs to target cancer cells was observed (Zhou et al. 2019). Zhang et al. developed AuNPs decorated with 5-aminolevulinic acid (ALA) for the diagnosis and photodynamic treatment of cancer (Zhang et al. 2015). Abedi et al. reported the development of silica paramagnetic NPs decorated with cisplatin for its theranostic application in cancer (Abedi et al. 2020). Fabio et al. also fabricated NPs with small molecule; in their report authors developed the mesoporous silica NPs conjugated with ibuprofen and Gd(III) chelates that can act as theranostic probes (Carniato et al. 2015).

2.2.2 Bio-functionalization of Nanoparticles

Use of NPs is creating enthusiasm in biomedicine and biotechnology fields, with preferable prominence in combined clinical diagnostics and therapy (theranostics).

NPs with distinctive, wide-ranging optical properties, easy to be synthesized and simplistic surface chemistry that can be easily functionalized and those lie within appropriate size scale are considered to be ideal NPs. However, functionalization of NPs is required using one of the several available options of biomolecules, such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA), peptides, antibodies oligonucleotides (i.e., ssDNA/RNA, dsDNA/RNA), enzymes, tumor markers, etc., to establish the required bio-functionalities. The conjugation approach is ultimately dependent on numerous factors such as surface chemistry, shape, size, as well as the type of ligands and functional groups to be used for functionalization. In addition, while assessing the conjugation strategy, the theranostic application of the NP conjugate and the type of biological molecule are crucial (Conde et al. 2014a). Further, the most commonly used biofunctional molecules for bio-functionalization of theranostic nanomedicines are as follows.

2.2.2.1 Nucleic Acids

Nucleic acids are complex organic molecules that are present in the living cells; they are long chain polymer formed by monomeric units of nucleotides and function for the storage and expression of genetic information. DNA and RNA are the two main classes of nucleic acids. The structure of DNA was first described by Watson and Crick in the form of two coiled chains each loped around a single axis (Watson and Crick 1953). This helical chain is composed of simple and self-replicating blocks called nucleotides having backbones consisting of sugars and phosphate groups ligated by antiparallel ester bonds. The significance of DNA and RNA within a living system is undeniable. Nucleic acid polymers can also be engaged in specific binding to target due to Watson–Crick base pairing along with their biological function (Fichou and Ferec 2006). DNA when employed as a therapeutic or targeting agent has many advantages that are excellent for biomedical application like high biocompatibility, low cytotoxicity, recognition properties and predictable intermolecular interaction, easy to synthesize specific sequences using enzymatic or chemical process, and optimum half-life in biological fluids. Additionally, DNA structures can undergo selective chemical modifications with anticancer drugs, and contrast agents prove DNA feasible to be designed as theranostic nanomedicine (Kumar et al. 2016). DNA-functionalized NPs (DNA-NPs) were first reported by Mirkin et al. in 1996. The authors described conjugation methods needed for the formulation of nucleic acid NPs (DNA molecules embedded to a thiol end covalently bound to the gold NP surface) (Nishi et al. 1996). Improvement in various attributes like cooperative binding and resistance to nuclease degradation was observed in case of DNA-NP when compared with free DNA. Alternatively, the AuNP core was substituted by other polymeric and inorganic materials such as QDs, proteins, Pd, Ag, Fe_3O_4, nanoshells, and polymers carrying different physicochemical, catalytic, and optical attributes (Zhang et al. 2013). The cellular entry of DNA doesn't require any special transfection agent or any structural modifications when functionalized as DNA NP. Recently, DNA-based multi-modal NPs were evaluated by Pal et al., on mouse model of ovarian and glioblastoma cancers. The mechanism of cancer

imaging and therapy was based on surface-enhanced Raman scattering (SERS) (Pal et al. 2019).

RNA oligomers are used by RNA interference (RNAi) for inhibition of gene expression products by binding to mRNA and cleaving them before their translation into protein. RNA oligomers are designed and produced synthetically for targeting and silencing specific oncogenes to provide cancer therapy. The main challenge in this synthetic RNA therapy is the delivery of RNA to the target site that can be achieved with the help of metallic NPs (Sasaki et al. 2018). An RNA interference (RNAi)-based theranostic NP was evaluated by Jensen et al., in glioblastoma multiforme (GBM) for attenuation of oncogene expression. The evaluated RNA-NP was formulated by binding firmly packed and greatly aligned small interfering RNA (si-RNA) duplexes on AuNP. The formulated NPs permeated in brain penetrating blood–brain barrier (BBB) and accumulated all over the mass of tumor in mouse models of GBM (Jensen et al. 2013). A short portion of DNA or RNA that binds to a specific target is known as aptamer. A theranostic aptamer conjugated Au@Ag/Au NPs was synthesized, and its potential for lung cancer therapy on mouse model was evaluated by Shi et al. The Au@Ag/Au NPs utilized in the formulation of theranostic NPs featured a significant cross-section of absorption (400–1100 nm), so it operated as a fluorescence quencher and optic radiator with increased hyperthermia capacity in comparison with gold nanorods (AuNRs). A target-specific signal alteration mechanism was incorporated that resulted in improvement of imaging contrast, lessen the detection time, and improved the photo thermal therapy (PTT) potency. The authors demonstrated a strong spatiotemporal target activation as well as photodynamic anticancer impact (Shi et al. 2014). From the examples mentioned above, it is clear that nucleic acids can be effectively functionalized for the synthesis of theranostic nanomedicines.

2.2.2.2 Enzymes

Enzymes are mostly utilized for bio-functionalization because of their capabilities in biotechnology and biomedicine, due to their operating friendliness, can be easily separated from the reaction mixture, and can be reused in next reaction cycle, cost-effective as well as they can act as highly specialized protein catalysts. The immobilization of enzymes in NPs mostly decreases diffusion restrictions and/or advances the catalytic actions of enzymes. Enzyme electrode is the main research focus in the field of AuNPs-based biosensors (Conde et al. 2014a). One of the latest examples is of biosensors for glucose. Zhang et al. reported the apparatus modification of a gold electrode using Au-S conjugate with AuNPs; here a cystamine superstructure was adsorbed, so as a result an assembly of amino groups was exposed. The exposed amino groups were further allowed to react with aldehyde groups of periodate oxidized glucose oxidase using Schiff base reaction. The reported NPs were observed to serve as conductivity stages, promote transfer of electrons, and have a minor impact on enzymatic activity. It was also evident that the limit of detection of glucose was lowered due to the improved sensitivity and affinity for glucose by the enzyme (Zhang et al. 2005). Sahoo et al. in another study reported modification of magnetic nanoparticles (MNPs) with N-phosphonomethyl iminodiacetic acid for

encapsulation of urease enzyme. As a result, after modification of MNPs the carboxyl groups were used for immobilization of urease employing carbodiimide coupling. While employing enzyme-dependent biosensors, the primary source of worry is the reutilization of the enzyme. Use of MNPs is advantageous as the product can be isolated using a permanent magnet, as a result reduction in overall cost. It was also reported by the author that the thermal decomposition of urease enzyme was improved, displaying that the use of MNPs is a prominent stuff for enzyme immobilization and storage (Sahoo et al. 2011). Authors Khoshnevisan et al., in order to circumvent the dilemma produced by use of MNPs, reported immobilization of cellulase enzyme on it. The binding of enzyme was confirmed using FT-IR. It was reported that immobilization is responsible for higher stability of enzyme and thus convinced that the use of MNP in this type of enzyme immobilization may be beneficial (Khoshnevisan et al. 2011).

2.2.2.3 Peptides

Peptides are short chains of monomers of amino acids connected to each other by amide bonds and are composed of less than 50 amino acids. Peptides are found naturally such as in form of hormones or can be synthesized in lab using chemical or biotechnological process. Peptides bear high potential for the bio-functionalization and stabilization of NPs. Peptides can be functionalized on AuNPs, and due to their superiority as a contrast agent, protein–AuNPs are proved to be beneficial in immunohistology. Furthermore, protein–AuNPs are nowadays majorly used for some other applications associated with therapeutics, diagnostics, and theranostics (Conde et al. 2014a). Wang et al. reported that one step surface coating of AuNPs by peptides can yield multi-functional peptide stabilized AuNPs, and their surface characteristics can be individually targeted by proteomics. The authors reported evolution of a basic route for the synthesis of stable AuNPs, with single as well as dual biological functionality. These reported NPs exhibited unique recognition characteristics for the target in absence of any manifestation of particle aggregation or non-specific binding (Chen et al. 2008). The stability acquired by peptide ligands is majorly reliable on their hydrophobicity, length, and charge, and in some instances all these three factors contribute to further improved stability. Further another group, Levy et al., reported CALNN, a pentapeptide ligand which transforms citrate stabilized AuNPs into extremely stable and water-soluble AuNPs that display several chemical characteristics that are comparable with peptides. The formulated peptide-functionalized AuNPs can be lyophilized and stored as powder and again converted into stable aqueous dispersion by dissolving in water for its further use (Levy et al. 2004). Cai et al. developed thiolated arginine-glycine-aspartic acid (RGD) peptide conjugated with QDs, utilized for tumor vasculature targeted imaging (Cai and Chen 2008). Nevertheless, for permeation through the vascular wall, the properties of these RGD peptides should be upgraded. Later Sugahara et al. developed a looping peptide iRGD (internalizing RGD) that contains the cancer-homing RGD motif as well as tissue permeation element. As a result, the tumor-homing RGD sequence allows the selectivity of peptide to the vascular endothelial cells of tumor, along with the tissue permeation sequence activation with the help of protease

which allows its binding to a separate receptor (neuropilin-1) that is responsible for extravasation and improved tissue penetration (Sugahara et al. 2009). Although there exists a remarkable and speedy advancement in methods for functionalization of NP with peptides, comparatively very less information is available about the role of NPs on behaviors of the immune system, as the immune system is accountable for preserving the stability of body and protection from foreign incursion. Bastus et al. reported that peptide AuNPs conjugates demonstrated the inhibition of macrophage proliferation and induction of pro-inflammatory cytokines. Moreover, the role of AuNPs conjugate on macrophage activation demonstrated to be independent of polarity and peptide length, but was found to be dependent on the pattern of peptide at the surface of NPs (Bastus et al. 2009).

2.2.2.4 Carbohydrates

Carbohydrates along with proteins and nucleic acids are integral biomolecules for life. A great deal of data is available regarding the operations, structure, and interactions of proteins, nucleic acids, and carbohydrates. Despite the fact that the interactions of individual carbohydrates are comparatively weak, nature uses polyvalent interactions of the ligand present on cellular surface and their physiological receptors to record biological mechanism including regular tissue growth and repair, cell adhesion, leucocytes trapping, viral/bacterial infection, cancer transfer, and signal transduction. As a result, decrypting interactions of carbohydrate enables the use of carbohydrates for functionalization of NPs and to use them for diagnosis and/or treatment (Penades et al. 2008). Carbohydrates are non-toxic, highly stable, biodegradable, and hydrophilic in nature; as a result the use of carbohydrates is preferred in living system as a therapeutic or diagnosis aid. Additionally, the distinctive chemical, optical, and physical characteristics of the nanocarriers functionalized with carbohydrate carry several advantages such as enhanced water solubility, stability to targeting properties, low toxicity, and biocompatibility. Carbohydrates consist of some reactive groups that are utilized for conjugation along with therapeutics (small organic molecules, peptides, fluorophores) and/or diagnostic agents (sensors, contrast agents) (Adachi et al. 1995). Among all the NPs, gold glyconanoparticles (glycol AuNPs) have sought attention considering their favorable attributes like water solubility and capability of simultaneous diagnosis and treatment. Penades et al. reported glyconanotechnology method utilizing NPs for evaluation of the interaction of carbohydrates with proteins and other carbohydrates. These NPs can be used as potential methods for cell–cell adhesion studies, anti-adhesive therapy, for prevention of invasion from pathogen invasion, and for studying the blood–brain barrier permeability after functionalization with neuropeptides. Thiolation of small carbohydrates, like lactose, glucose, and mannose, can be performed enabling conjugating to AuNPs using ligand exchange technique. These carbohydrate NPs can be utilized as specific colorimetric probes for different metal ions (Penades et al. 2008). Schofield et al. reported the possibility of conjugation of thiol derivatized carbohydrates with gold and silver NPs (Schofield et al. 2006). After the attachment of carbohydrates on the surface of MNPs, it's indeed critical that they should continue to operate biologically. Cell targeting and

enhancement of cellular uptake can be achieved using carbohydrate NPs, e.g., Moros et al. published the functionalization of MNPs using 1-Ethyl-3-(3-dimethylaminopropyl)- carbodiimide (EDC) with monosaccharides like glucose and galactose and a study of their cellular uptake was performed using Vero cells. Even though both the monosaccharides have same chemical formula, the difference is just in the geometric conformation of the hydroxyl group at C-4; broad difference was observed in cell entry patterns. The glucose-MNPs penetrated all over the entire Vero cell; galactose-MNPs endure primarily in the edges of the cell (Moro et al. 2012). Chitosan and hyaluronic acid (HA) are two of highly sophisticated carbohydrate NPs that have been examined extensively in theranostics. HA is a non-sulfated glycosaminoglycan and is negatively charged; it is also called as hyaluronan. It is made up of recurring blocks of N-acetyl-D-glucosamine and D-glucuronic acid linked by beta glycosidic linkages. It is ubiquitously present all-over the body in connective and neural tissue, epithelial tissue, skin, synovial fluid, and vitreous of the eye. The most common approach for the formulation of HA-NPs is the conjugation of hydrophobic moieties, like cholanic acid and ceramide with the HA backbone. One of the most common conjugates is amphiphilic HA-5β-cholanic acid conjugates (HACA) that has been most extensively used as a theranostic in different types of cancer. Besides cancer, HACA-NPs also display promising results in the treatment and diagnosis of atherosclerosis. In the study, high accumulation of dye marked HACA-NPs was observed in atherosclerotic plaques present in the aorta of animal model containing disease, although very low indicative signal was observed in the aortas of healthy animals (Lee et al. 2015). Chitosan is a polysaccharide with linear structure containing D-glucosamine and N-acetyl-D-glucosamine building blocks bound with β-(1–4)- glycosidic linkages, and is positively charged. Yoon et al. encapsulated doxorubicin (DOX) and Bcl-2 small interfering RNA (siRNA) for gene therapy (siRNA) utilizing two distinct formulation methods for synthesis of glycol chitosan-based NPs (GCNPs); additionally imaging agents were conjugated on the surface of NPs. According to this research, authors administered DOX-GCNPs and Bcl-2 siRNA-GCNPs with the moto to resolve the problems related drug resistant tumors by down-regulation of anti-apoptotic defense machinery of cancer cells and simultaneously promoting apoptosis (Yoon et al. 2014).

2.2.2.5 Antibodies

The antigen–antibody detection functions of proteins are important and very useful in biotechnology. Antibodies (Abs) also called as immunoglobulins are some groups of proteins having same structure containing four chains merged in a Y shape. They contain two indistinguishable domains with effector functions (Fc fragment) and two other twinning domains functioning for antigen recognition (Fab fragment). The primary advancement using Abs is that the antigen–antibody recognition fragments are different for various Abs and are highly specific; as a result, different target-specific activities can be achieved using various Abs (Arruebo and Valladares 2009). Nevertheless, conjugation of Abs to the NPs will probably disturb this activity if the antigen binding receptors experience steric blockage due to functionalization. This is the reason: the orientation of Abs is highly important for the production of bioactive

and effective antibody functionalized NPs. Lee et al. reported the orientation-based functionalization of AuNP by initially oxidizing the hydroxy groups present on the Fc region of antibody to aldehyde. The aldehyde in second step was conjugated on amine group of the linker molecule, and as thiol group is present on the other end of linker, the complex formed by conjugation of antibody and linker can be linked with the AuNP forming a gold–sulfur covalent bond. The work presented was highlighted as the orientation of the antibodies is crucial for the achievement of maximum antigen-binding functions (Lee et al. 2014).

The selectivity and specificity of antigen–antibody interaction is used by immunoassays to quantify and detect the presence of analyte in the sample. Antibody conjugated NPs can be utilized to reach the desired properties of the NP on its own for immunoassays. In this context, Putman et al. reported the role of immunolabeled gold as markers at cell surface for human lymphocytes by using atomic force microscopy (AFM). The immunolabeled gold particles can be viewed on the cell surface in the AFM images and demonstrated its function as locator of antigens (Putman et al. 1993). A theranostic nanomedicine was established by Conde et al. for the concurrent detection of tumor cells along with their inhibition. The highly sensitive nanomedicines were formulated for in vivo tumor cell recognition with super capability of targeting specific cancer biomarkers like epidermal growth factor receptor (EGFR) on xenograft tumor models and even on human cancer cells. The reported nanomedicine was formulated by capping AuNPs by a Raman reporter. The capped AuNPs were then entrapped and encapsulated using some large polymers including Cetuximab (Erbitux®) a Food and Drug Administration (FDA) approved conjugate of antibody and a drug. A high Raman signal was demonstrated by these smart surface enhanced Raman scattering (SERS) theranostic nanomedicines in both cancer cells and in xenograft tumors bearing mice model. An extensive inhibition in tumor growth was observed simultaneously with strong Raman detection signals, and hence, the functionalized AuNPs was visualized to be acting as a theranostic nanomedicine (Conde et al. 2014b).

2.3 Methods for Functionalization of Nanoparticles

The physicochemical properties and efficacy of a biomolecule can be altered due to modifications to be done during functionalizations. The biggest hurdle is that the NPs should remain stable in the solution while functionalization is done. Furthermore, many NPs tend to precipitate out of solution due to imbalance between cohesive and repulsive forces. This disadvantage can be countered by using various functionalization methods. Functionalization is the method of conjugation of NPs requiring the involvement of small molecule ligands (drugs), fluorescent dyes, biomolecules such as proteins, Abs, etc., to act as a theranostic nanomedicine. Further chemical functionalization of NPs involves covalent strategy and non-covalent strategy (Conde et al. 2014a).

2.3.1 Covalent Strategies

The functionalization by direct conjugation of molecule of interest on the reactive sites on NPs is carried out using covalent conjugation. Covalent coupling ensures strong and stable binding of the molecule of interest to the surface NPs. The NPs bearing reactive groups like aldehyde can be functionalized with the molecules bearing amine groups like proteins. Even the coupling of molecules containing aldehyde and epoxide group can be performed with the NPs that contain surface amine groups. Apart from the chemical moieties, some of the biomolecules such as oligonucleotides, antibodies, peptides, and carbohydrates need to be modified before functionalization as they do not include highly reactive groups such as aldehydes, e.g., oxidation of hydroxy group of carbohydrates found in several antibodies utilizes periodate to construct aldehyde groups; this generated aldehyde reacts with the amino groups found on the surface of NPs. However, chemical modification of some of the biomolecules may influence their activity; as a result linkers are frequently employed for the functionalization of NPs using biomolecules (Nobs et al. 2004). Some specific bifunctional groups containing linkers can be used that can link to the NPs using one reactive group while the molecule of interest to the other end. Svenson et al. proved that covalent linkers should be preferred over electrostatic interaction during formation of siRNA-poly(D,L-lactic-co-glycolic acid) (PLGA) for the efficient release of siRNA for the application of tumor selective gene silencing (Svenson et al. 2016).

2.3.1.1 Click-Chemistry Reaction

A group of reactions that are high yielding, stereospecific, and modular are known as click-chemistry reactions. Among all the click reactions, copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction (click reaction) has already been acknowledged as an easy and simple way used reaction for functionalization of NPs. The click reaction makes use of azides and alkynes that are extremely dynamic functional groups with limited distribution of extent of reaction. In living cells, they are inert towards the biological molecules and are unreactive. In an organic molecule, the azide groups can be conveniently introduced by both nucleophilic and electrophilic processes. The rate of copper catalyzed reactions is accelerated enormously at about 10^7–10^8 times when compared with the uncatalyzed 1,3-dipolar cycloaddition (Himo et al. 2005). This reaction is tremendously popular as the "click chemistry" and refers to the concept "click" that implies triazole synthesis using an alkyne and azide. The reaction is highly chemoselective as the alkyne and azide are unreactive towards various functional groups assuming the typical moderate reaction settings. The reaction proceeds at room temperature (RT), displaying a great extent of pH and are solvent insensitive. Furthermore, a broad pH range of 4 to 12 is required for the reaction and is insensitive to aqueous conditions (Hein and Fokin 2010). Triazole is the cyclic product formed in a click reaction. The copper catalyst not only accelerates the rate of reaction but also enables the reaction to be performed at RT and imparts regioselectivity, and 1,4 regioisomer is the only product. The Cu(I) is formed in situ by reducing copper(II) with the help of a reducing agent (e.g., reduction of copper

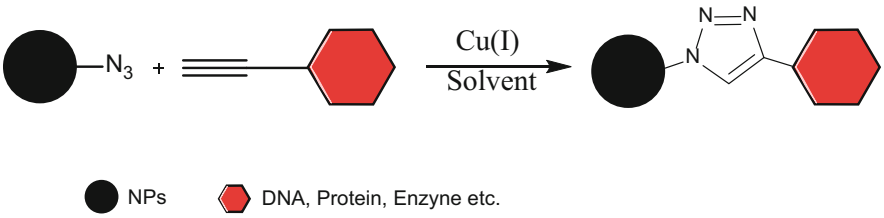

Fig. 2.2 Conjugation of NPs modified as azide and ligand modified as alkyne via CuCAA

sulfate is performed by using sodium ascorbate). Click chemistry is a term used to describe a series of reactions that are quick, easy to employ, easily purified, flexible, regiospecific, and provide maximum yield (Hong et al. 2009). Along with various advantages click reaction has certain limitations. First, the reaction will not proceed if the azide group has very few electrons. Second but the most common disadvantage is alkyne homocoupling. In a click reaction if an alkyne couples with second molecule of alkyne present in the reaction instead of azide, such an instance is known as alkyne homocoupling. Use of sterically hindered base which can stabilize the reactive mediators of homocoupling can minimize the alkyne homocoupling (Hein et al. 2008). CuCAA can be used for conjugation of proteins with AuNPs by addition of the azide or the alkyne group to AuNPs and the second one to the proteins (Fig. 2.2). For example, AuNPs were first conjugated with thiol containing polyethylene glycol (PEG)–carboxylic acid molecules utilizing the thiol side. Further, amide bond was formed using EDC coupling with propargylamine to achieve terminal alkyne, and protein was conjugated with azide on cysteine to have azide modified protein. Then the AuNPs were functionalized using azide-modified protein and alkyne-modified AuNPs in using Cu(I) as catalyst. In another example, process was reversed and the alkyne group was conjugated to the protein while the azide group was introduced to the AuNPs, to achieve similar covalent functionalization (Zhang et al. 2010). Stable protein–AuNP conjugates were formed in both the case; it is clear from the above two examples that the alkyne or azide groups can be conjugated with proteins or AuNPs for functionalization. The activity of both the conjugated proteins remained intact even though both the proteins were enzymes. This result encourages and appreciates the use of CuCAA for functionalization of NPs along with proteins with enzymatic activity. Maltzahn and coworkers reported the functionalization of fluorescent MNPs using a cancer-targeting peptide (Lyp-1) using click chemistry, and the formulated NPs were navigated stably to the systemic circulation, eject into the tumors, and permeate through the interstitial area, and specific binding was observed to receptors on tumor cells (von Maltzahn et al. 2008).

2.3.1.2 EDC Coupling Reaction

The coupling reaction of carboxyl or phosphate group with a primary amine is done using a zero-length crosslinking agent EDC. The EDC initially reacts with carboxyl group of a molecule and forms a highly reactive O-acylisourea intermediate that reacts with amine. Later, N-hydroxysulfosuccinimide (sulfo-NHS) is added to

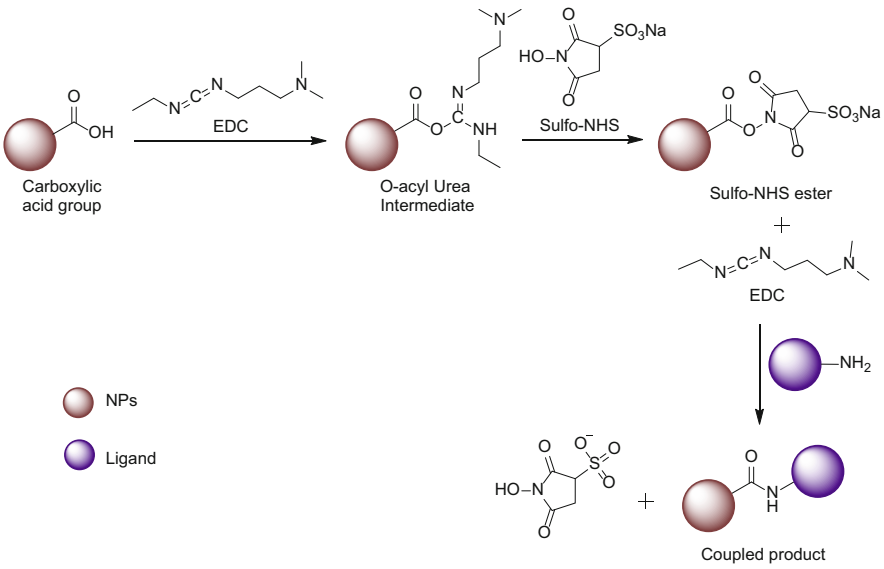

Fig. 2.3 Amide bond formation from carboxylic acid and amine using EDC and Sulfo-NHS

stabilize the amine-reactive intermediate by formation of sulfo-NHS ester that in turn reacts with amine. This sulfo-NHS in next step reacts with amine group and forms a covalent bond (amide bond) between carboxyl and amine groups (Fig. 2.3). The O-acylisourea intermediate formed after reaction of EDC can also react with an amine on another molecule, producing a conjugate of the two molecules that are ligated by a stable amide bond. The EDC coupling reaction has diverse applications, such as amide bond formation between a carboxyl and amine groups of peptides and proteins, and can also be used to tag nucleic acids through reaction at 5-phosphate groups (Grabarek and Gergely 1990). EDC coupling has an advantage of water solubility allowing the functionalization without dissolution in organic solvents. Apart from this, the unreacted starting materials and undesired products can conveniently be removed by gel-filtration or dialysis (Sheehan et al. 1965). The reactive ester formed in EDC coupling undergoes rapid hydrolysis in aqueous solution; as a result the coupling reaction is to be worked out quickly; controlling the pH of reaction can also help in preventing hydrolysis as the hydrolysis is pH dependent. Sulfo-NHS and N-hydroxysuccinimide (NHS) are used in order to enhance the stability of the active ester (Jang and Keng 2008). The EDC coupling enables the functionalization of most of the molecules such as antibodies, DNA, enzymes, fluoroprobes, etc. on the surface of NPs without a prior modification. Dhar et al. reported quick intracellular penetration of AuNPs targeting the delivery and activation of platinum(IV) prodrug to the cancerous cells in lungs. The functionalization of this AuNPs was carried out using thiol containing oligonucleotides bearing a dodecyl amine at the end that was conjugated with a platinum(IV) compound capable of binding to an amine functionalized DNA-AuNP surface by amide bond

formation. The formulated NPs demonstrated potent anticancer activity in HeLa cells. The disadvantage of EDC coupling is if both amine and carboxylate groups are present on the same molecule there are chances of self-polymerization and the efficacy may be lost. This kind of self-polymerization can be minimized by eliminating extra EDC prior to addition of the molecule in solution of NPs (Dhar et al. 2009).

2.3.1.3 Maleimide Coupling

Primary amines can be conjugated to thiols using maleimide coupling (Michael et al. 1992). The modification of sulfhydryl groups by using maleimide has been mostly reported in literature. A highly stable 3-thiosuccinimidyl ether linkage is generated in reactions containing sulfhydryl groups and occurs at pH of 6.5–7.5. The limitation of maleimide coupling is that the maleimide ring is susceptible to hydrolysis in aqueous medium to an unreactive cismaleamic acid derivative if the reaction is operated for a longer period of time or at pH >8. However, a large number of biomolecules bearing thiol and amine groups can be used for functionalization of NPs. Sometimes the maleimide coupling may proceed towards the non-specific bonds and crosslinking between the NPs and biomolecule due to presence of several other thiol groups on a single biomolecule (Wold 1977). Molecules such as DNA, chemotherapeutic agents, peptides, antibodies, and dyes can be conjugated to NPs using maleimide coupling. The coupling of Abs with NPs is depicted in Fig. 2.4. A controlled of immobilization of AuNPs on the surface of living cells was reported by Ba et al. The NPs were chemically bound to phospholipids in liposomes by maleimide thiol reaction, in order to retain the optothermal and sensing capabilities of the original colloidal nanoparticle (Ba et al. 2010). In fact, peptides, DNA, antibodies, and proteins can be coupled with QDs using maleimide coupling. The solitary nanocrystals were encapsulated in DNA conjugated phospholipid block-copolymer micelles, and in order to address the biocompatibility issues, their fluorescent functions were evaluated. Similarly, investigators reported the functionalization of MNPs utilizing the maleimide coupling reaction with DNA, PEG, or even drugs such as chlorotoxin. In case of antibodies, maleimide coupling can be used with amine or thiol groups present on the surface of NPs. The maleimide

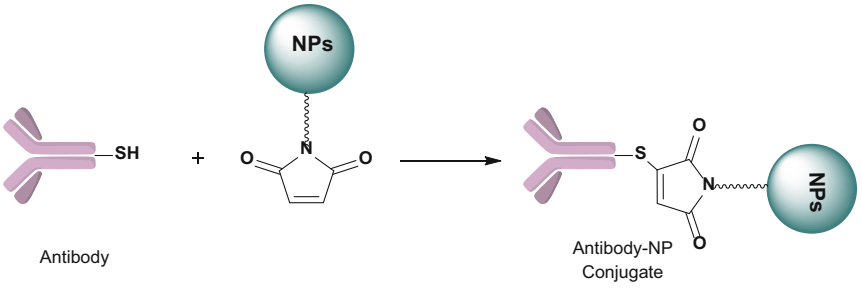

Fig. 2.4 Coupling of an antibody with nanoparticle using maleimide coupling

coupling of amine group of NPs enables an ordered attachment between thiol groups of the Ab because the orientation is important for activity in case of Abs (Kuhn et al. 2006).

2.3.2 Non-covalent Strategies

Non-covalent interactions such as electrostatic, hydrophobic, and affinity interactions can be involved in the formation of drug NPs conjugate. Affinity-based receptor ligand interaction is believed to be utilized in con-covalent functionalization of NPs. Non-covalent interactions have several advantages over covalent interactions, that is, neither the molecule nor the NPs need to be altered chemically. In case of hydrophobic or electrostatic interactions, ease of functionalization is another advantage; speed of interaction is higher as compared to covalent interactions. However, while considering the disadvantages functionalization is less stable, the number of bound molecules and their orientation is difficult to control and has very low reproducibility when compared to covalent methods. Agudelo and coworkers reported the functionalization of chitosan NPs with tRNA molecules using non-covalent electrostatic interactions and hydrogen bond involving G–C and A–U base pairs (Agudelo et al. 2016). Li et al. functionalized cadmium telluride (CdTe) QDs with lysozyme binding DNA through electrostatic interactions for luminescence detection of lysozyme (Li et al. 2014).

2.3.2.1 Ionic Interaction

The most simple and uncomplicated method for functionalization of NPs with other molecules is ionic interaction. Conjugation can be successfully achieved between NPs and polymers, biological molecules carrying opposite charges, or even between various opposite charged NPs or even between the oppositely charged biomolecules and NPs (Fig. 2.5) (Liu et al. 2012). The rate of ionic interaction

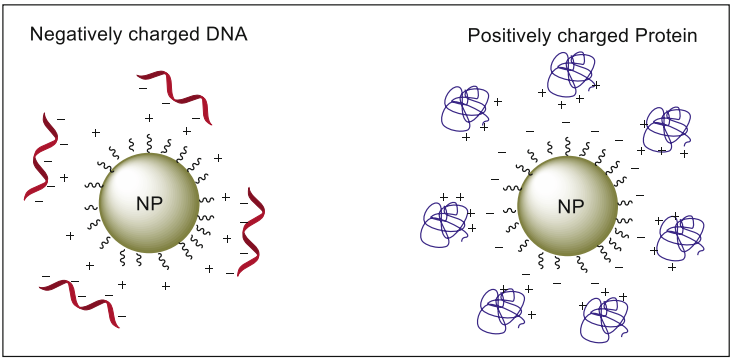

Fig. 2.5 Coupling of negatively charged DNA with positively charged NP (left) and coupling of positively charged proteins with negatively charged NP (right)

in multipunctual (multiple point) binding depends upon the number of charges present on the desired molecules and NPs. As a result, during the conjugation of complex biological molecules like proteins or Abs, their isoelectric point must be taken care of as the net charges of any peptide depend on isoelectric point. Some of the proteins like serum albumin cause stabilization of NPs by prevention of aggregation so ionic coupling is most widely used to functionalize NPs with proteins. However, to enhance the cellular influx of proteins by some tumor cells, proteins are adsorbed to NPs using ionic interactions strategy (Brewer et al. 2005). Ionic interactions were also used by negatively charged HA for their self-assembly on the surface of positively charged QDs. A novel and very simple strategy for electrostatic coupling method was developed by Bhang et al., for easy functionalization of QD with HA that have cancer treating potency to be used in diagnostic and therapeutic applications (Bhang et al. 2009). Functionalization of negatively charged siRNA with the positive charge of quaternary ammonium group (R_4N^+) using ionic interaction was reported by Conde et al. Two opposite charges, i.e., the negatively charged siRNA backbone (via phosphate groups) and positively charged quaternary ammonium groups, led to ionic interaction, and the binding of siRNA with the AuNPs was ensured (Conde et al. 2012).

2.3.2.2 Hydrophobic Coupling

The lipophilic drugs are conjugated with NPs using hydrophobic interactions, and once the NPs reach the target cell, the drug gets released inside the cell. Controlled release drug profile of a month can be achieved by adsorbing docetaxel on the surface of oleic acid that surrounds the hydrophobic MNPs before their encapsulation into a polymeric vesicle (Wahajuddin 2012). Encapsulation of drugs can also be performed by adsorbing them on cyclodextrins or hydrophobic polymers (Fig. 2.6). Using similar approach some NPs can be functionalized with hydrophobic molecules such as fluorophores. Some hydrophobic NPs were coated with amphiphilic polymers by Foy and coworkers; to these NPs they adsorbed five different types of dyes, and then the biodistribution of MNP was determined in a xenograft breast tumor model (Foy et al. 2010). Further, Kim et al. performed functionalization of AuNPs with water-soluble zwitterionic ligands forming kinetically stabilized complexes using lipophilic drugs and dyes that were prominently distributed into cells. This coupling method enables the formulation of NPs with dual properties of diagnosis and therapy by minimizing the changes on surface. Hydrophobic

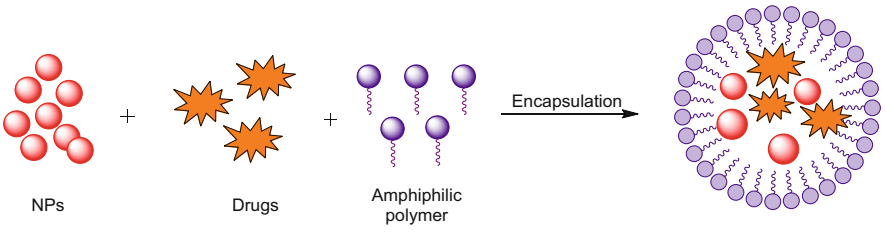

Fig. 2.6 Hydrophobic encapsulation of drugs and NPs in a vesicle of amphiphilic polymer

interactions can also be utilized for the conjugation of some proteins and Abs to the NP surface (Kim et al. 2009). The disadvantage related to protein and Abs is that they can denature which leads to loss of their biological function. The activity of proteins and Abs can probably be lost because of the hydrophobic interactions with NPs due to modification in their native structure as a result of their inner surface being exposed (Shemetov et al. 2012).

2.4 Conclusion and Future Perspectives

This book chapter summarizes the recent development in the field of nanotheranostics which includes various approaches, coupling methods, and different types of theranostic NPs for various bio-applications. The conjugation reactions for biomolecules should be carefully designed and operated as the biomolecule's functions need to be preserved, e.g., proteins. The reaction used for functionalization of NPs is well-established and can be utilized based on the requirement of functionalization. Conjugation of molecules with NPs opens doors for various types of applications that can be an area of extensive research in the near future. Also, the functionalization of some theranostic NPs like SPIONs, AuNPs, MNPs, CDs, etc., may enhance the imaging power or therapeutic potential or both related to the NPs. The modification of NPs can be performed using biomolecules that can be beneficial for enhancement of biocompatibility. It is expected that the strategies involved for functionalization of theranostic nanomedicines may revolutionize in the near future and theranostic nanomedicines will be largely applied in the form of ultrasensitive and effective nanotheranostics in the field of diagnosis and treatment.

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References

Abedi M, Abolmaali S, Abedanzadeh M, Farjadian F, Samani S, Tamaddon AM (2020) Core–shell imidazoline–functionalized mesoporous silica superparamagnetic hybrid nanoparticles as a potential theranostic agent for controlled delivery of platinum (II) compound. Int J Nanomedicine 15:2617

Adachi N, Maruyama A, Ishihara T, Akaike T (1995) Cellular distribution of polymer particles bearing various densities of carbohydrate ligands. J Biomater Sci Polym Ed 6(5):463–479

Agudelo D, Kreplak L, Tajmir-Riahi HA (2016) tRNA conjugation with chitosan nanoparticles: an AFM imaging study. Int J Biol Macromol 85:150–156

Aravind A, Jeyamohan P, Nair R, Veeranarayanan S, Nagaoka Y, Yoshida Y, Kumar DS (2012) AS1411 aptamer tagged PLGA-lecithin-PEG nanoparticles for tumor cell targeting and drug delivery. Biotechnol Bioeng 109(11):2920–2931

Arruebo M, Valladares M (2009) Antibody conjugated nanoparticles for biomedical applications. J Nanomater 2009(1):439387

Ba H, Rodriguez-Fernandez J, Stefani F, Feldmann D (2010) Immobilization of gold nanoparticles on living cell membranes upon controlled lipid binding. Nano Lett 10(8):3006–3012
Bastus NG, Sanchez-Tillo E, Pujals S, Farrera C, Lopez C, Giralt E, Puntes V (2009) Homogeneous conjugation of peptides onto gold nanoparticles enhances macrophage response. ACS Nano 3(6):1335–1344
Bhang SH, Won N, Lee TJ, Jin H, Nam J, Park J, Kim S (2009) Hyaluronic acid − quantum dot conjugates for in vivo lymphatic vessel imaging. ACS Nano 3(6):1389–1398
Boisselier E, Astruc D (2009) Gold nanoparticles in nanomedicine: preparations, imaging, diagnostics, therapies and toxicity. Chem Soc Rev 38(6):1759–1782
Brewer SH, Glomm WR, Johnson MC, Knag M, Franzen S (2005) Probing BSA binding to citrate-coated gold nanoparticles and surfaces. Langmuir 21(20):9303–9307
Cai W, Chen X (2008) Preparation of peptide-conjugated quantum dots for tumor vasculature-targeted imaging. Nat Protoc 3(1):89–96
Carniato F, Munoz UM, Tei L, Botta M (2015) Selective functionalization of mesoporous silica nanoparticles with ibuprofen and Gd(III) chelates: a new probe for potential theranostic applications. Dalton Trans 44(41):17927–17931
Caruthers SD, Wickline SA, Lanza GM (2007) Nanotechnological applications in medicine. Curr Opin Biotechnol 18(1):26–30
Chen CL, Zhang P, Rosi NL (2008) A new peptide-based method for the design and synthesis of nanoparticle superstructures: construction of highly ordered gold nanoparticle double helices. J Am Chem Soc 130(41):13555–13557
Chen F, Ehlerding EB, Cai W (2014) Theranostic nanoparticles. J Nucl Med 55(12):1919–1922
Cho EC, Xie J, Wurm P, Xia Y (2009) Understanding the role of surface charges in cellular adsorption versus internalization by selectively removing gold nanoparticles on the cell surface with a I2/KI etchant. Nano Lett 9(3):1080–1084
Conde J, Ambrosone A, Sanz V, Hernandez Y, Marchesano V, Tian F, de la Fuente JM (2012) Design of multifunctional gold nanoparticles for in vitro and in vivo gene silencing. ACS Nano 6:8316–8324
Conde J, Dias JT, Grazu V, Moros M, Baptista P, de la Fuente JM (2014a) Revisiting 30 years of biofunctionalization and surface chemistry of inorganic nanoparticles for nanomedicine. Front Chem 2:48
Conde J, Bao C, Cui D, Baptista P, Tian F (2014b) Antibody–drug gold nanoantennas with Raman spectroscopic fingerprints for in vivo tumour theranostics. J Control Release 183:87–93
Dhar S, Daniel WL, Giljohann DA, Mirkin C, Lippard SJ (2009) Polyvalent oligonucleotide gold nanoparticle conjugates as delivery vehicles for platinum (IV) warheads. J Am Chem Soc 131(41):14652–14653
Drexler KE (2004) Nanotechnology: from Feynman to funding. Bull Sci Technol Soc 24(1):21–27
El-Boubbou K, Zhu DC, Vasileiou C, Borhan B, Prosperi D, Li W, Huang X (2010) Magnetic glyco-nanoparticles: a tool to detect, differentiate, and unlock the glyco-codes of cancer via magnetic resonance imaging. J Am Chem Soc 132(12):4490–4499
Fichou Y, Ferec C (2006) The potential of oligonucleotides for therapeutic applications. Trends Biotechnol 24(12):563–570
Foy SP, Manthe RL, Foy ST, Dimitrijevic S, Krishnamurthy N, Labhasetwar V (2010) Optical imaging and magnetic field targeting of magnetic nanoparticles in tumors. ACS Nano 4(9): 5217–5224
Grabarek Z, Gergely J (1990) Zero-length crosslinking procedure with the use of active esters. Anal Biochem 185(1):131–135
Hein JE, Fokin VV (2010) Copper-catalyzed azide-alkyne cycloaddition (CuAAC) and beyond: new reactivity of copper(I) acetylides Chem. Soc Rev 39:1302–1315
Hein CD, Liu X, Wang D (2008) Click chemistry, a powerful tool for pharmaceutical sciences. Pharm Res 25(10):2216–2230
Herranz F, Pellico J, Ruiz-Cabello J (2012) Covalent functionalization of magnetic nanoparticles for biomedical imaging. Resonance 4:6

Himo F, Lovell T, Hilgraf R, Rostovtsev VV, Noodleman L, Sharpless K, Fokin VV (2005) Copper (I)-catalyzed synthesis of azoles. DFT study predicts unprecedented reactivity and intermediates. J Am Chem Soc 127(1):210–216

Hong V, Presolski SI, Ma C, Finn MG (2009) Analysis and optimization of copper-catalyzed azide–alkyne cycloaddition for bioconjugation. Angew Chem Int Ed 48(52):9879–9883

Jang LS, Keng HK (2008) Modified fabrication process of protein chips using a short-chain self-assembled monolayer. Biomed Microdevice 10(2):203–211

Jensen SA, Day ES, Ko CH, Hurley LA, Luciano JP, Kouri F, Stegh AH (2013) Spherical nucleic acid nanoparticle conjugates as an RNAi-based therapy for glioblastoma. Sci Transl Med 5(209):152

Jokerst JV, Gambhir SS (2011) Molecular imaging with theranostic nanoparticles. Acc Chem Res 44(10):1050–1060

Kelkar S, Reineke TM (2011) Theranostics: combining imaging and therapy. Bioconjug Chem 22(10):1879–1903

Khoshnevisan K, Bordbar AK, Zare D, Davoodi D, Noruzi M, Barkhi M, Tabatabaei M (2011) Immobilization of cellulase enzyme on superparamagnetic nanoparticles and determination of its activity and stability. Chem Eng J 171(2):669–673

Kim CK, Ghosh P, Pagliuca C, Zhu ZJ, Menichetti S, Rotello VM (2009) Entrapment of hydrophobic drugs in nanoparticle monolayers with efficient release into cancer cells. J Am Chem Soc 131(4):1360–1361

Kuhn SJ, Finch SK, Hallahan D, E & Giorgio T. D. (2006) Proteolytic surface functionalization enhances in vitro magnetic nanoparticle mobility through extracellular matrix. Nano Lett 6(2): 306–312

Kumar V, Palazzolo S, Bayda S, Corona G, Toffoli G, Rizzolio F (2016) DNA nanotechnology for cancer therapy. Theranostics 6(5):710

Lammers T, Kuhnlein R, Kissel M, Subr V, Etrych T, Pola R, Peschke P (2005) Effect of physicochemical modification on the biodistribution and tumor accumulation of HPMA copolymers. J Control Release 110(1):103–118

Lammers T, Kiessling F, Hennink WE, Storm G (2010) Nanotheranostics and image-guided drug delivery: current concepts and future directions. Mol Pharm 7(6):1899–1912

Lee IH, Lee JM, Jung Y (2014) Controlled protein embedment onto Au/Ag core–shell nanoparticles for immuno-labeling of nanosilver surface. ACS Appl Mater Interfaces 6:7659–7664

Lee GY, Kim JH, Choi KY, Yoon HY, Kim K, Kwo IC, Kim IS (2015) Hyaluronic acid nanoparticles for active targeting atherosclerosis. Biomaterials 53:341–348

Levy R, Thanh NT, Doty RC, Hussain I, Nichols RJ, Schiffrin DJ (2004) Rational and combinatorial design of peptide capping ligands for gold nanoparticles. J Am Chem Soc 126:10076–10084

Li S, Gao Z, Shao N (2014) Non-covalent conjugation of CdTe QDs with lysozyme binding DNA for fluorescent sensing of lysozyme in complex biological sample. Talanta 129:86–92

Liu Y, Han X, He L, Yin Y (2012) Thermoresponsive assembly of charged gold nanoparticles and their reversible tuning of plasmon coupling. Angew Chem Int Ed 51(26):6373–6377

Mauro N, Utzeri MA, Varvara P, Cavallaro G (2021) Functionalization of metal and carbon nanoparticles with potential in cancer theranostics. Molecules 26(11):3085

Michael CM, Lazarou Y, Papagiannakopoulos P (1992) Infrared multiphoton dissociation of tetramethylsilane: formation of electronically excited trimethylsilyl radical. Chem Phys Lett 194(4–6):415–422

Moro M, Hernaez B, Garet E, Dias JT, Saez B, Grazu V, de la Fuente JM (2012) Monosaccharides versus PEG-functionalized NPs: influence in the cellular uptake. ACS Nano 6(2):1565–1577

Moyano DF, Rotello VM (2011) Nano meets biology: structure and function at the nanoparticle interface. Langmuir 27(17):10376–10385

Nel AE, Mädler L, Velegol D, Xia T, Hoek E, Somasundaran P, Thompson M (2009) Understanding biophysicochemical interactions at the nano–bio interface. Nat Mater 8(7):543–557

Nicolson F, Ali A, Kircher M, Pal S (2020) DNA nanostructures and DNA-functionalized nanoparticles for cancer theranostics. Adv Sci 7(23):2001669

Nishi N, Tsuruga T, Hira T, Murata M, Sato D, Sasaki T, Takahashi H (1996) Use of salmon shirako DNA in biomaterials. Nature 382:607

Nobs L, Buchegger F, Gurny R, Allemann E (2004) Current methods for attaching targeting ligands to liposomes and nanoparticles. J Pharm Sci 93(8):1980–1992

Pal S, Ray A, Andreou C, Zhou Y, Rakshit T, Wlodarczyk M, Kircher MF (2019) DNA-enabled rational design of fluorescence-Raman bimodal nanoprobes for cancer imaging and therapy. Nat Commun 10(1):1–13

Penades S, Barrientos AG, Clavel C, Martínez-Avila O, Alcantara D (2008) Multifunctional glyconanoparticles: applications in biology and biomedicine. Nanomater Appl Med Biol 1760:636–651

Putman CA, de Grooth BG, Hansma PK, van Hulst NF, Greve J (1993) Immunogold labels: cell-surface markers in atomic force microscopy. Ultramicroscopy 48(1–2):177–182

Rana S, Bajaj A, Mout R, Rotello VM (2012) Monolayer coated gold nanoparticles for delivery applications. Adv Drug Deliv Rev 64(2):200–216

Saha K, Bajaj A, Duncan B, Rotello VM (2011) Beauty is skin deep: a surface monolayer perspective on nanoparticle interactions with cells and bio-macromolecules. Small 7(14): 1903–1918

Sahoo B, Sahu S, Pramanik P (2011) A novel method for the immobilization of urease on phosphonate grafted iron oxide nanoparticle. J Mol Catal B Enzym 69(3–4):95–102

Sasaki HM, Tadakuma H, Tomari Y (2018) Single-molecule analysis for RISC assembly and target cleavage. Argonaute Proteins 1680:145–164

Schofield CL, Haines AH, Field R, Russell DA (2006) Silver and gold glyconanoparticles for colorimetric bioassays. Langmuir 22(15):6707–6711

Sheehan JC, Presto J, Cruickshank PA (1965) A rapid synthesis of oligopeptide derivatives without isolation of intermediates. J Am Chem Soc 87(11):2492–2493

Shemetov AA, Nabiev I, Sukhanova A (2012) Molecular interaction of proteins and peptides with nanoparticles. ACS Nano 6:4585–4602

Shi H, Ye X, He X, Wang K, Cui W, He D, Jia X (2014) Au@ Ag/Au nanoparticles assembled with activatable aptamer probes as smart "nano-doctors" for image-guided cancer thermotherapy. Nanoscale 6(15):8754–8761

Subbiah R, Veerapandian M, Yun KS (2010) Nanoparticles: functionalization and multifunctional applications in biomedical sciences. Curr Med Chem 17:4559–4577

Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Girard O, Ruoslahti E (2009) Tissue-penetrating delivery of compounds and nanoparticles into tumors. Cancer Cell 16(6): 510–520

Sun D (2010) Nanotheranostics: integration of imaging and targeted drug delivery. Mol Pharm 7(6): 1879–1879

Svenson S, Case RI, Cole RO, Hwang J, Kabir SR, Lazarus D, Eliasof S (2016) Tumor selective silencing using an RNAi-conjugated polymeric nanopharmaceutical. Mol Pharm 13(3):737–747

Thakor AS, Gambhir SS (2013) Nanooncology: the future of cancer diagnosis and therapy. CA Cancer J Clin 63(6):395–418

von Maltzahn G, Ren Y, Park JH, Min DH, Kotamraju VR, Jayakumar J, Bhatia SN (2008) In vivo tumor cell targeting with "click" nanoparticles. Bioconjug Chem 19(8):1570–1578

Wahajuddin SA (2012) Superparamagnetic iron oxide nanoparticles: magnetic nanoplatforms as drug carriers. Int J Nanomedicine 7:3445

Watson JD, Crick FHC (1953) Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. Nature 171:737

Wold F (1977) Affinity labeling—an overview. Methods Enzymol 46:3–14

Xie J, Lee S, Chen X (2010) Nanoparticle-based theranostic agents. Adv Drug Deliv Rev 62(11): 1064–1079

Yoon HY, Son S, Lee SJ, You DG, Yhee JY, Park J, Pomper MG (2014) Glycol chitosan nanoparticles as specialized cancer therapeutic vehicles: sequential delivery of doxorubicin and Bcl-2 siRNA. Sci Rep 4(1):1–12

Zhang S, Wang N, Yu H, Niu Y, Sun C (2005) Covalent attachment of glucose oxidase to an Au electrode modified with gold nanoparticles for use as glucose biosensor. Bioelectrochemistry 67(1):15–22

Zhang M-X, Huang B-H, Sun X-Y, Pang D-W (2010) Clickable gold nanoparticles as the building block of nanobioprobes. Langmuir 26:10171–10176

Zhang Y, Lu F, Yager KG, van der Lelie D, Gang O (2013) A general strategy for the DNA-mediated self-assembly of this article is licensed under a creative commons attribution 3.0 Unported Licence. Functional nanoparticles into heterogeneous systems. Nat Nanotechnol 8(11):865–872

Zhang Z, Wang S, Xu H, Wang B, Yao C (2015) Role of 5-aminolevulinic acid-conjugated gold nanoparticles for photodynamic therapy of cancer. J Biomed Opt 20(5):051043

Zhou L, Wu Y, Luo Y, Li H, Meng X, Liu C, Cai L (2019) Mitochondria-localized self-reporting small-molecule-decorated theranostic agents for cancer-organelle transporting and imaging. ACS Appl Bio Mater 2(11):5164–5173

Theranostic Applications of Functionalized Vesicular Carriers

3

Theranostic Applications of Functionalized Vesicular Carriers (Liposomes, Niosomes, Virosomes, Ethosomes, Phytosomes)

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Abstract

Metallic nanoparticles with therapeutic as well as diagnostic components have recently gained enormous attention in pharmaceutical research. The emergence of nanotechnology has put diagnosis and therapy closer to each other. By applying nanotechnology, many nanovesicular systems can be loaded with diagnostic and therapeutic moieties, called theranostic functionalized vesicular carriers, allowing the early detection of pathology and a targeted treatment. Targeted nanoparticles (NPs), nanovesicles (NVs), liposomes (LPs), and nanoclays for combined diagnosis and therapy have been extensively studied by researchers in the recent past. Diseases like breast cancer, hepatocellular carcinoma, neurodegenerative diseases, and cardiovascular diseases have been studied for the potential application of vesicular theranostic agents. Though the application of nanovesicle-based theranostic carriers is gaining attention, certain metallic nanovesicular system has been reported to be complicated in terms of designing, stability, and control of drug release, manufacturing at large scale, and has also been reported to have toxicity concern owing to their nanosizing, thus causing behavioral,

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physiological, and metabolic complications. In spite of limitations, numerous publications and patents are evident in last decades, indicating the acceptance of nanocarrier-based theranostic regime as an emerging science. Here in the present study, we have accumulated all the relevant information on theranostic attributes of functionalized vesicular carriers and also highlighted the limitations of its application to provide a window of further scientific exploration.

Keywords

Theranostic agents · Nanoparticles · Nanovesicles · Liposomes · Cancer · Neurodegenerative diseases

3.1 Introduction

The role of biological membrane in the survival of an organism is crucial. The rational of such realization has been explored in the creation of artificial system with the potency to mimic the cell membrane and the function as well. Such artificial membrane bound vesicular complex, when used for diverse purpose, is termed as functionalized vesicular carriers (Gai et al. 2020). Such carrier vesicle has been developed with the synthetic amphiphils with designed vesicular architecture (Lombardo et al. 2015). The assembly of synthetic amphiphils are non-covalent in nature which allows the complex to become more efficacious and provides more robust functional attributes in molecular recognition and catalysis (Sebaaly et al. 2019). In broader spectrum, such artificial vesicles are categorized under exosomes.

Nanotechnology has made extraordinary progress in the recent years which is attributed to their application in multidisciplinary fields such as pharmaceuticals, cosmetic industries, robotics, informatics, water treatment, chemical industry, etc. (Sahoo et al. 2007). Nanotechnology is being extensively applied in the pharmaceutical field because of their unique characteristics, extremely small size, and ability to carry and deliver drug in a controlled manner (Bajwa et al. 2017). Apart from this they also provide safety, efficacy, and stability in the formulation.

In the last few decades nanotechnology-based drug delivery system has been extensively studied which includes liposomes (Jahangir et al. 2020), ethosomes (Yetisgin et al. 2020), nanoemulsions (Imam et al. 2021), nanoparticles (Bin-Jumah et al. 2020), niosomes (Mohanty et al. 2020), phytosomes (Jahangir et al. 2020), etc. Most of them have been studied for their unique ability to enhance the bioavailability and efficacy of the drug without compromising the safety profile of the drug (Caruthers et al. 2007). Among these nanocarrier systems, vesicular carriers are now attracting researchers for the potency to load both hydrophilic and lipophilic drugs. Depending on their composition, vesicular systems can be classified in different ways. Liposomes are one of the first vesicular systems studied and were composed of lipids. With the advent of technology, novel vesicular systems were developed using both polymers and lipids and were known as hybrid vesicles. These hybrid vesicular systems were able to combine the advantage of both the

components and limit the drawbacks of developing vesicular carrier from either lipid or polymer only. Vesicular systems made of lipids only have been reported with low stability while vesicles made of polymers showed biocompatibility issues (Le Meins et al. 2013) which were overcome by developing hybrid vesicles.

Extracellular vesicles-based theranostic nanoplatforms are slowly getting attraction for their potential clinical application. Extracellular vesicles are nanosized membrane made up of lipids which are secreted from all types of cells. They can be loaded with proteins, nucleic acids, and lipids from cell. As emerging nanoparticles with therapeutic potential, they are now considered promising biomarkers. However, their application must pass through complex procedures of extraction, isolation, and analysis before being developed as nano-extracellular vesicle-based theranostic agents (Xing et al. 2020).

The emergence of nanotechnology not only created impact on the delivery system but also has pulled together diagnosis and therapy closer to each other. Theranostic agents are capable of providing diagnosis along with specific therapy at the same time. The unique ability of co-delivering imaging as well as therapeutic agent has made them an emerging field of science. Theranostic agents are now being extensively studied for their potential application in heterogeneous diseases like cancer, tuberculosis, diabetes, etc. (Gilani et al. 2020). Conventional therapies have their own limitations in treating and diagnosing heterogeneous diseases. Developing nanocarrier-based theranostic agents has drawn separate attention in the recent past. It is an extension of conventional theranostic agent and is more focused on co-delivery and providing imaging not before or after the treatment but also during the entire period of therapy.

3.2 Functionalized Nanovesicular Carriers

Vesicular carriers contain drug and imaging agent dissolved, encapsulated, or linked to the structure within the particle range of 10–100 nm; however with some vesicles they may even reach larger sizes (Jeevanandam et al. 2018). Small vesicular sizes provide the advantage of increasing the bioavailability of drug with low solubility and also assist in specific targeting of sites. This further reduces the amount of drug needed to be incorporated for desired action which eventually reduces toxic effects (Manconi et al. 2016). Nano-vesicular systems also improve organoleptic properties for orally administered drug, reduce dose frequency, and prevent incompatibility between the encapsulated materials in the formulation (Mei et al. 2013). For topical application nanovesicular carriers enhance skin penetration of active constituents and also reduces possible irritation (Pradhan et al. 2018).

Vesicular carriers stand out among nanocarrier systems because of their versatility to carry out and ability to trap a variety of active substances and to produce prolonged therapeutic effect as shown in Fig. 3.1 (Kazi et al. 2010). Vesicular carriers have lamellar structure made up of amphiphilic molecules forming bilayer immersed in an aqueous medium (Kumar and Rao 2012).

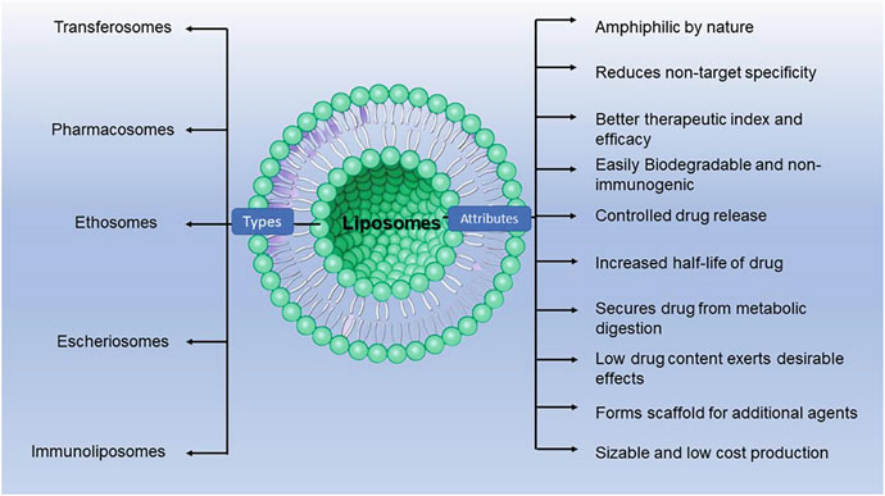

Fig. 3.1 Different theranostic vesicles with their application

Vesicular carriers can encapsulate both lipophilic molecules inside the bilayer and hydrophilic molecules either in the aqueous medium present between the bilayer or outside the vesicle thus providing them the ability to carry a variety of active constituents. These vesicular carriers are classified based on their composition which strongly influence size, elasticity, charge, thermodynamic property, lamellarity, etc. (Kumar and Rajeshwarrao 2011). Liposomes are one of the first vesicular systems developed and studied. They are simple vesicles and composed of one or more lipid bilayer capable of holding lipophilic and hydrophilic molecules of varied sizes (Fan et al. 2018). Liposomes were widely successful as vesicular carrier because of their biomimetic character due to their structure being analogous to biological systems. Thus, they provided enhanced biocompatibility, biodegradability, ease of preparation, and low toxicity (Pai et al. 2019). Despite wide acceptance, liposomes had a major drawback of chemical and physical stability because of the lipid layer being susceptible to oxidative degradation, premature leakage of encapsulated material, high cost of material, and variable degree of purity of phospholipids (Cerqueira-Coutinho et al. 2016).

Niosomes are other vesicular carriers which were first studied for application in cosmetic industry (Pham et al. 2012). Niosomes are composed of nonionic surfactants and have structures comparable to lipid vesicles obtained by similar methods. However, they possess the advantage of greater stability and low cost over liposomes. Liposomes possess low permeability which limits their application for topical administration; these limitations promoted the research for the development of other vesicular carriers like ethosomes, transferosomes, invasomes, etc. Ethosomes are composed of lipids in association with small quantity of ethanol which acts as permeation enhancer (Touitou et al. 2000). Transferosomes are composed of both lipids and nonionic surfactants which increase the flexibility of

the nanocarrier system (Uchino et al. 2014). Invasomes in addition contain terpenes which increase lipid permeation and diminish lipid packing (Shah et al. 2015).

Polymersomes are another class of artificial vesicular carrier which are composed of amphiphilic polymers which can rearrange themselves forming a bilayer. They have greater stability and versatility than liposomes; however their compatibility with biological system is very limited (Bhosale et al. 2017).

3.3 Liposomes

Liposomes are artificial spherical vesicular carrier composed of phospholipids and cholesterol. Liposomes are developed by drying organic solvent-soluble lipids, dispersing the lipid in aqueous solution and purifying the developed vesicular carrier. Liposomes of less than 100 nm size are usually adopted for systemic application (Lee and Im 2019). Extrusion or sonication method is used to produce small-sized liposomes. They show hydrophobic characteristics in their lipid bilayers, but they display hydrophilic characteristics in their vesicles, making them capable of encapsulating both hydrophilic and hydrophobic drug. Liposomal drugs show enhanced efficacy and reduced toxicity (Bulbake et al. 2017). They may be used to treat cancers of different types based on these characteristics (Gardikis et al. 2010), hepatitis (Bovier 2008), fungal diseases (Merhav and Mieles 1997), macular degeneration, etc. (Jonas 2002). The PEGylation of liposomes enhances their permeability and retention effect (EPR) by reducing circulation time and enhancing targeting efficiency. PEGylation of liposomes interferes between the hydrophobic interaction of serum protein and liposomes. The adherence of serum protein over liposomes influences the absorption of liposomes by RES (reticuloendothelial system) like the spleen and liver thus resulting in stealth effects. However, PEGylated liposomes show accelerated blood clearance for which anti-PEG IgM antibody is held responsible (Im et al. 2016).

Due to the ease with which different imaging functions can be loaded into liposomes, liposomes are also widely used for imaging and theranostic applications. For example, to achieve optical imaging, hydrophobic fluorescence dye is usually incorporated into the lipid bilayer of liposome. The hydrophilic area of liposomes can also be loaded with quantum dots (Mukthavaram et al. 2011). In vivo SPECT imaging or PET imaging can be performed by radio-labeling the liposomes. Studies have shown that radio-labeled liposomes can be developed by adding chelators to the hydrophilic regions of liposomes which is followed by incubating it with the radioisotopes (Petersen et al. 2016). In addition to the lipid-conjugated bifunctional chelator, liposomes can also be radio-labeled by incorporating the chelator into the liposome bilayer for further radio-labeling (Seo et al. 2008).

An innovative liposomal formulation of a cascade-activated prodrug and multifunctional theranostic liposomes for tumor-sensitive combination therapy was developed recently by Dai et al. Due to the presence of a post-treatment phototoxic effect, photodynamic therapy is limited in its application. By using multimodal imaging to overcome phototoxic effects, the researchers developed a near-IR activated liposome

loaded with chlorine e6 specific to cancer cells. Indocyanine green was loaded as a photothermal agent, and tirapazamine was loaded as hypoxia-activated prodrug. Additionally, cRGD was applied to the surface, followed by conjugation with GdIII to form ICG/TPZ@Ce6-GdIII theranostic liposomes (ITC-GdIII TLs). By means of fluorescence resonance energy transfer, the fluorescence and photodynamic properties of Ce6 were quenched in the ITC-GdIII TLs. There was a noticeable increase in the permeability and retention of ITC-GdIII TLs and an ability to actively target tumor sites with this TL. Irradiation with 660 nm laser was reported to release Ce6 which kills tumor cells by generating cytotoxic singlet oxygen. Further, the photodynamic therapy-induced hypoxia combines to further enhance its antitumor activity (Dai et al. 2019).

In another research Ma et al. developed doxorubicin, folate acid, and conjugated polymer dots loaded liposomes for bioimaging and tumor targeting. The researchers demonstrated higher accumulation of drug in tumor cell for theranostic liposome. Further in vivo study in tumor-bearing mice confirmed significant inhibition by PFBT-Dox-Lip-FA along with negligible in vivo toxicity thus confirming the simultaneous capability of cancer diagnosis and therapy by their developed doxorubicin, folate acid, and conjugated polymer dots loaded liposomes (Ma et al. 2015).

Using PEGylated liposomes, Kaul et al. developed ofloxacin and rifampicin loaded liposomes to fight mycobacterial infections. It was observed that the pharmacokinetics of the developed formulation follow a slow biphasic pattern showing over 19 h of half-life. The maximum organ localization was observed in the kidney, spleen, and liver 24 h after injection. Additional studies (in vivo) demonstrated that infected lesions showed higher uptake in the murine model of tuberculosis infection. The researchers concluded enhanced therapeutic efficacy of liposomal preparation of rifampicin and ofloxacin against murine model of tuberculosis (Kaul et al. 2016).

Theranostic pH-responsive tumor-specific targeting was developed using peptide-modified liposomes containing paclitaxel and superparamagnetic iron oxide nanoparticles by Zheng et al. They studied specific targeting effect, magnetic resonance imaging, and anti-tumor activity of the developed formulation in in vitro and in vivo studies in human breast carcinoma MDA-MB-231 cell models. The researchers reported pH-responsive characteristics of the $H_7K(R_2)_2$ in MDA-MB-231 cell line from in vitro and in vivo studies. Further the theranostic effect of PTX/SPIO-SSL-$H_7K(R_2)_2$ in MDA-MB-231 tumor-bearing model was confirmed through in vivo magnetic resonance imaging and in vivo anti-tumor activity (Zheng et al. 2018).

Oliveira and colleagues further investigated the potential for photodynamic theranostic therapy in cancer and ocular diseases with lipid polymer liposomes loaded with verteporfin. The 5-(6)-carboxyfluorescein moiety was covalently incorporated into the triblock copolymer F127 for their photodynamic application. The thin film hydration technique produces small 100 nm liposomal vesicles by F127 by assistance of unilamellar liposomes. Through fluorescence lifetime assay and Stern-Volmer assay, the heterogenous distribution of verteporfin and 5(6)-carboxyfluorescein was confirmed. The researchers further confirmed the cellular

uptake of verteporfin by time-resolved fluorescence microscopy, and presence of 5 (6)-carboxyfluorescein at membrane level was confirmed by fluorescence microscopy which further justified their theranostic potential in cancer and ocular therapies (de Oliveira et al. 2020).

3.4 Niosomes

Niosomes are bilayered unique nano-lipid structure which are developed by self-aggregation of nonionic surfactant. It was first introduced for its cosmetic application by the L'Oréal company. Further investigation and studies lead to its application as drug delivery devices in 1980. Research in the last two decades were successful in incorporating various proteins, genes, and therapeutic molecules into niosomes for their potential delivery (Aparajay and Dev 2022). Niosomes are usually characterized based on their size and number of vesicular lipid bilayer. Vesicles are characterized into unilamellar vesicles which are further classified as small (10–100 nm), large unilamellar vesicles (100–250 nm), and multilamellar vesicles (100–1000 nm) based on their size ranges (Khoee and Yaghoobian 2017). Some other types of niosomes which have clinical application are proniosome, aspasome, bola niosome, discome, polyhedral niosome, etc. Proniosomes are dry powdered water-soluble formulations and are coated with surfactants. At the time of use, these formulations are rehydrated which helps in overcoming the limitations of aggregation of particles, fusion of vesicular structure, and leaking of therapeutic moieties (Aparajay and Dev 2022). Aspasomes are used for transdermal delivery of drug. They are multi-layered vesicles formulated using cholesterol, diacetyl phosphate (negatively charged lipid), and ascorbyl palmitate. They reduce the effect of reactive oxygen species during transdermal delivery of drug (Gopinath et al. 2004). Bola niosomes are prepared using Bola C_{16} surfactants along with cholesterol and span80. Bola niosomes are capable of enhancing the permeability of drug during transdermal delivery (Paolino et al. 2008). Discomes are multilamellar vesicles prepared using low amount of cholesterol with Solulan C_{24}. These formulations are used for ocular delivery and to produce sustained release action (Aparajay and Dev 2022). Polyhedral niosomes do not have a fixed shape and can entrap water-soluble particles. Cholesterol is not used in their preparation and are replaced with polyoxyethylene cholesteryl ether or hexadecyl diglycerol ether (Aparajay and Dev 2022).

Niosomes possess several advantages such as non-immunogenic, biodegradable, and biologically inert characteristic and possess low toxicity. Cholesterol provides rigidity and permeability and also assists in controlled and sustained release of drugs. They are capable of delivering both hydrophilic and hydrophobic drugs. However, sometime the application of niosomes is associated with drug leakage and certain minor toxicity (Gharbavi et al. 2018).

Pereira and associates developed and studied a pH-sensitive pHLIP coated niosomes as an effective nanotheranostic agent for cancer. pH-sensitive niosomes having diameter of 80–90 nm were formulated using Span20 (nonionic surfactant), cholesterol, and pH (low) insertion peptide conjugated either with DSPE lipids

pyrene, a hydrophobic fluorescent dye. A new formulation was developed by the said group that could sense the extracellular acidity of cancerous cells. Their study conducted in mice carrying tumors found niosomes coated with fluorescent labeled (R18) pHLIP accumulating primarily in tumors, with minimal accumulation in the liver, kidneys, and other tissues. They further concluded that the pHLIP coated niosomes exhibited two- to threefold enhanced uptake in tumor cells compared with non-targeting PEG polymer coated niosomes. Further study demonstrated uniform biodistribution in tumor cells and long circulation time (Pereira et al. 2016).

In another research Yang and associates developed theranostic niosomes for the effective delivery of siRNA/microRNA encapsulated with indocyanine green. Span80 (nonionic surfactant) and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) were used to develop the formulation to effectively deliver the siRNA/microRNA to human mesenchymal stem cells. The researchers further reported that the developed siRNA/microRNA encapsulated niosomes were effectively delivered to hMSCs, and specific inhibition of miR-138 was achieved. It was further reported that "iSPN exhibited off/on activatable fluorescence upon cellular internalization resulting in efficient NIR labeling and the capability to dynamically monitor stem cells in mice" (Yang et al. 2018).

A generic theranostic platform has been developed by Demir and associates that combines two therapies with an active targeting approach. To do this, they used gold nanoparticles (AuNPs) and folic acid (FA) to study the encapsulation of gold nanoparticles with protoporphyrin IX (PpIX) in niosome vesicles (AuNP-PpIX-FA). The developed formulation showed high encapsulating efficiency. In vivo studies were conducted on "folate receptor positive (FR(+)) human cervical cancer cell line (HeLa) and FR negative (FR(-)) human alveolar type-II (A549) like cell line" and was demonstrated that upon combining radiotherapy and photodynamic therapy the cellular uptake by FR (+) HeLa cells was positive concluding the advantage of all-in-one approach of developed theranostic platform (Demir et al. 2018).

In a similar study by Nowroozi and associates developed theranostic niosomes by co-loading doxorubicin and Ag_2S quantum dots as direct intratumoral injection for spontaneous drug delivery and bioimaging for tumor cells (Nowroozi et al. 2018). The researchers demonstrated that Tween 60, Span 60, or Tween 80 produced strong and detectable fluorescence signals. Among them Span 60 showed highest anti-tumor efficacy despite Tween 60 showing highest accumulation. Direct intratumoral injection of theranostic niosomes could be a potential tool for multimodal imaging and therapy (Nowroozi et al. 2018).

Alemi et al. combined paclitaxel and curcumin in a novel cationic PEGylated niosomal formulation which was developed as a model for enhanced transfection efficiency and stability in MCF-7 cell line. The optimized formulation showed high entrapment efficiency, spherical shape, positive zeta potential, and sustained release. The researchers further demonstrated threefold decrease in curcumin and paclitaxel concentration in MCF-7 cell line upon nano-niosomal administration. It was further concluded that the curcumin and paclitaxel in free or niosomal form are less toxic to MCF-10A human normal cells compared to MCF-7 cells. Thus, combination

therapy of paclitaxel and curcumin loaded in PEGylated niosomal delivery could be a promising tool to achieve enhanced effectiveness in breast cancer treatment (Alemi et al. 2018).

3.5 Exosomes

Exosomes are a class of homogenous extracellular vesicles of size ranging between 30 and 150 nm (Ailuno et al. 2020). Exosomes are derived from the membranes of endosomes (Colombo et al. 2014) having high levels of tetraspanins and heat shock proteins in their proteome along with a specific protein of the parent cell. Tumor cells release high amounts of extracellular vesicles (vesicles), and several studies suggest the involvement of exosomes in the formation and progression of tumor (Abak et al. 2018). Many other studies highlighted the involvement of tumor-derived exosomes in metastatic process (Hood et al. 2011). Exosomes possess many characteristics like size in the nano-range, biocompatibility, non-toxicity, and targeting ability which are suitable for drug delivery (Lim and Kim 2019). In many terms they are similar to small unilamellar liposomes such as similar size range and ability to carry both hydrophilic and hydrophobic molecules. Furthermore, their specific protein composition and asymmetrical lipid distribution supports their homing ability (Antimisiaris et al. 2018). However, there are many glitches such as heterogenous structure, low production yield, difficulties in drug loading, scalability, and cost-effectiveness that affect clinical translation of exosomes into a potential delivery system (Butreddy et al. 2021). To overcome the drawbacks of naturally derived exosomes, bioinspired exosome-like vesicles are being developed. The artificial exosome mimetic system is also called hybrid exosomes which are obtained by the fusion of exosomal and liposomal membranes (Sato et al. 2016). Many researchers have developed exosomes mimetic system for its potential application in cancer treatment (Lim and Kim 2019) loaded with doxorubicin (Ye et al. 2020), paclitaxel (Kim et al. 2021a), gemcitabine (Haney et al. 2020), aspirin (Tran et al. 2019), imperialine (Lin et al. 2019), several miRNA (Kobayashi et al. 2020) and mRNA molecules (Forterre et al. 2020), tumor necrosis factor-α (Zhuang et al. 2020), and recombinant methioninase (Xin et al. 2021). Apart from being an active therapeutic carrier, exosomes have also been loaded with diagnostic agents to achieve active and passive diagnosis. Exosomes are exploited as active diagnostic tools by loading them with nanoparticulate compounds which are detectable using different imaging techniques such as computed tomography (CT), optimal fluorescence, positron emission tomography, and magnetic resonance imaging for diagnosis of difficult to reach cancer such as brain tumors (Shang et al. 2017). On the contrary passive diagnostic application involves natural derived tumor exosomes which act as cancer prognostic and diagnostic biomarkers (van der Meel et al. 2014) since their abundance in blood allows for easy detection in small plasma or serum sample (Shao et al. 2015). By analyzing the genomic and proteomic profile of these exosomes, it is possible to detect the different types and stages or tumor (Kosaka et al. 2010).

Through the secretory and endocytic pathways of eukaryotic cells, exosomes allow proteins and lipids to travel between compartments (Abels and Breakefield 2016). Vesicles such as these serve as vehicles for signaling molecules to communicate between cells and can carry other functional molecules such as nucleic acids, proteins, and lipids to perform specific functions (Yáñez-Mó et al. 2015). Having been identified as physiological nucleic acid vectors in humans and others, these vesicles have been investigated to deliver DNA, RNA, and other cargoes as a therapeutic strategy (Wiklander et al. 2019; Jeyaram et al. 2020).

Gene therapy is another potential use of theranostic vesicles because they are able to release nucleic acids or proteins. Having demonstrated great potential for applications in the field of diagnosis and treatment of diseases, vesicles have been developed as vectors for the delivery of drugs and contrast agents (Walker et al. 2019). Moreover, such vesicles are natural nanocarriers produced by living cells for intercellular communication (Lamichhane et al. 2015). Exosomal vesicles are also natural carriers for the exchange of information, matter, and energy between cells, including molecular transport between cells (Villata et al. 2020). Cell-to-cell communication relies on the transport of biomolecules by extracellular vesicles, while synthetic drug delivery systems can overcome biological barriers by using extracellular vesicles (Meng et al. 2020). The RNA transfer mechanism within exosomes has been identified as one of their therapeutic potentials, making them good RNA delivery vehicles (Elsharkasy et al. 2020). Cell-free therapeutics for solid organ injury are being developed using vesicles derived from mesenchymal stem cells (MSCs) (Zhao et al. 2020).

As a new delivery system, niosomes (nonionic surfactant vesicles) have the ability to enhance the solubility and stability of natural pharmaceutical molecules. Drug carriers composed of nonionic surfactant vesicles or niosomes, which require a two-layered chemical structure consisting mainly of nonionic surfactants and lipid compounds (cholesterol or soy phosphatidylcholine) dissolved in water (Kumar and Rajeshwarrao 2011). Nonionic surfactant vesicles can be obtained using various approaches that affect the composition and properties of the drug, the amount of cholesterol, the structure, type and amount of surfactant (Dan 2017). Polymers are thus interesting in part because they are structurally similar to phospholipid vesicles (liposomes), which are important components of biological systems in which they behave as phospholipid vesicles of membranes within cells. They have also been extensively studied in a range of fields (Akbarzadeh et al. 2013), such as drug and gene delivery (Torchilin et al. 2003). Polymers consist of a membrane and an aqueous core, similar to phospholipid vesicles, allowing for the incorporation of hydrophobic and hydrophilic molecules, resulting in our understanding of multiple properties (Bozzuto and Molinari 2015). An ideal biosensing environment can be provided by vesicles that contain reaction components within a selectively permeable membrane (Vallejo et al. 2017). The integration of vesicle membranes with diverse biological properties and modular delivery vectors is a favorable and efficient method for creating engineered Vesicles for the regulation of inflammation (Patra et al. 2018). A possible explanation for MSC-EV's biological effects, such as reducing tissue damage, may be found in its physical, biochemical, or structural

properties (Driscoll et al. 2021). It is both genetically and protein manipulative of parental cells that provides an opportunity for MSC-Vesicles to be used as a delivery platform for therapeutics, as well as for the selective loading of MSC-Vesicles after isolation (Tang et al. 2019). Synthetic vesicles have been used in several studies to study vesicle biology (Sedgwick and D'Souza-Schorey 2018). Microparticles and vesicles are such example where, polymer assemblies can encapsulate and release the drugs in a controlled manner, and also preserve drugs from premature degradation, have been extensively studied as carriers to facilitate drug release (Simone et al. 2008).

As possible vectors for delivering drugs to the CNS, niosomes targeting folic acid and transferrin have been designed (Gharbavi et al. 2018). In addition, nucleic acids also have the functions of targeting (aptamer), programmability, drug loading and immunomodulation, which will significantly improve the prospects for the use of functionalized Vesicles (Zhou and Rossi 2017). Another study reports that EV protonation to create a pH gradient across EV membranes can be used to increase the loading of vesicles with nucleic acid cargo, in particular miRNA (miRNA), small interfering RNA (siRNA), and single-stranded DNA (ssDNA) (Jeyaram et al. 2020). Several results are suggesting that intravenous loading based on a pH gradient may be a promising approach to obtain functional therapeutic effects of such miRNA release (O'Brien et al. 2020). A growing body of evidence indicates that cow's milk-derived Vesicles may be a viable alternative source for obtaining large numbers of biocompatible vesicles (Tan et al. 2021).

3.6 Disease-Based Study of Theranostic Vesicular Carriers

Theranostic applications utilize a variety of nanopreparations, including liposomes, inorganic nanoparticles, protein-drug nanocomposites, polymeric nanoparticles, and micelles (Cole and Holland 2015). Among the most widely used nanoparticles for drug delivery in nanoplatforms are liposome-based nanoparticles, which deliver small peptides, nucleic acids, and proteins (Gao et al. 2023). The roles of Vesicles are numerous and may play an important role in cancer biology (Jabalee et al. 2018). In the rapidly developing field of cancer nanomedicine, there is the potential to improve the accuracy and toxicity of diagnostics, as well as improve drug delivery (Alshehri et al. 2020). A recent research topic has focused on Vesicles associated with cancer regulatory processes and their potential therapeutic value (Kogure et al. 2020). In addition, the nanoplatform itself can be further engineered to deliver cytotoxic drugs to cancer cells using controlled release properties (Yao et al. 2020, p. 1). Furthermore, targeting nanoparticles as monoclonal antibody-based drug delivery systems is one of the main approaches for CRC treatment currently in preclinical development (Gulbake et al. 2016). External control of therapeutic delivery using nanoparticles further enhances tumor-specific effects and reduces off-target effects of often painful anticancer therapies (Senapati et al. 2018). It has been reported that liposomes combined with drugs and imaging agents can evaluate the effect of cancer treatment (Olusanya et al. 2018). In order to better utilize

liposomal therapeutics, multiple targeting and imaging agents as well as multiple drugs have been included in liposome formulations to enable these products to deliver drugs efficiently to diseased tissues (Sercombe et al. 2015). Further, externally modulated theranostic nanoparticles are rapidly developing and have been approved for cancer therapy in multiple preclinical models (Urban et al. 2013). Several reviews have discussed the therapeutic prospects of Vesicles in skin diseases and aging related complications (Shao et al. 2020). A number of recent studies have revealed that functionalized vesicles play an important role in skin aging and a variety of skin disorders, including psoriasis, lupus erythematosus, vitiligo, and wound healing (Kim et al. 2021b). Studies have highlighted the advantages of Vesicles as potential biomarkers for pathological diagnosis of acute and chronic skin malfunction which also attracted a lot of attention for their theranostic use (Kim et al. 2021b). There is an urgent need to develop multifunctional and multimodal theranostic methods that use vesicular drug delivery systems to simultaneously visualize the etiology of COVID-19 (Satija et al. 2020). Various drugs, such as chloroquine, could be delivered via vesicles with a theranostic strategy for the development of novel COVID-19 treatment regimens as a futuristic treatment method (Satija et al. 2020). Nucleoside analogues are a class of drugs of particular interest for targeted aptamer-based therapies (Li et al. 2020). As a co-administration tool for personalized theranostics that combine image-guided diagnostic, prognostic, and therapeutic effects, vesicular drug delivery systems are increasingly used (Singh et al. 2019).

The development of exosome-based nanotheranostic strategies has received tremendous attention to improve therapeutic interventions and disease prognosis. In order to develop exosome-based therapeutic systems, it is essential to understand how exosomes interact with recipient cells and what happens to them in vivo (Siafaka et al. 2021). In addition, these theranostic techniques will enable monitoring and evaluation of viral cell interactions using clinical imaging, independently or in association with other antiviral agents, as a form of co-administration (Rojas and Thorne 2012). Through theranostic nanomedicine, targeted therapy is then provided through multi-receptor blocking, reducing systemic toxicity through reducing the amount of chemotherapeutic drugs in systemic circulation, and providing chemotherapeutic agents customized to tumors, improving the therapeutic effect (Zhao et al. 2018).

3.7 Vesicular Carrier System as Imaging Agents

Personalized theranostics are increasingly using vesicular drug delivery systems as co-administration tools to combine image-guided diagnostic, prognostic, and therapeutic effects (Nogueira 2020). Pharmacosomal approaches can circumvent many of the inherent limitations of various classical forms of vesicular drug delivery, such as drug incorporation, leakage from the vector, or insufficient shelf life (Kaur et al. 2004). In another study, rather than using phospholipids in the vesicular drug delivery system, nonionic surfactants were used due to the firm conditions necessary

to work with liposomes in a cryogenic atmosphere (Yingchoncharoen et al. 2016; Bartelds et al. 2018). Recent study has shown to stabilize therapeutic compounds, improve their biodistribution, work with hydrophilic and hydrophobic drugs, and be biocompatible and biodegradable (Mitchell et al. 2021). Liposomes are used in the pharmaceutical and cosmetic industries to transport various molecules and are one of the best-studied drug delivery systems (Alavi et al. 2017). Due to its good penetration and flexibility, Liposomes can be used to effectively administer NSAIDs such as ibuprofen and diclofenac (Marmon et al. 2021). In this respect, ethosomes, the other forms of vesicle carriers for drug delivery, are notable (Verma and Pathak 2010).

On the other hand, the unique carrying capacity of exosomes has recently been explored as a delivery vehicle for anticancer drugs and imaging agents (Srivastava et al. 2022). There have been numerous studies on functional nanoparticles as drug carriers, and they have been reformulated to deliver imaging agents as part of their delivery systems (Janib et al. 2010). Metal nanoparticles have been used as imaging contrast agents (Thangam et al. 2021), laser therapy (Fekrazad et al. 2016), optical biosensors (Malekzad et al. 2017), and drug delivery vehicles (Patra et al. 2018). A growing number of medical applications of metal nanoparticles, including bioimaging, biosensors, targeted/sustained drug release, hyperthermia, and photoablation therapy, have been investigated in recent years (Cardoso et al. 2018).

Due to their stability, metal nanoparticles make great platforms for drug delivery and contrast media delivery, some of which can be visualized directly and some of which can be magnetically guided (Sun et al. 2008). Nanoparticles can also influence the fate of these encapsulated drugs as they provide controlled release kinetics, increased bioavailability, improved pharmacokinetics, and reduce side effects as well as enhance patient compliance (Rizvi and Saleh 2018). By targeting the delivery of drugs or imaging agents to specific locations, efficiency can be greatly increased (De Jong and Borm 2008). The introduction of new drugs may also allow targeted drug delivery using vectors or chemical derivatization to deliver drugs directly to a specific target cell type, or localizing the action of the drug by spatially positioning controlled release systems (Tiwari et al. 2012). A bilayer vesicle can also reduce the cytotoxicity of many potent therapeutic agents when they are used in site avoidance or site-specific treatments (Pandey et al. 2021). Recent studies have shown that the vesicles structure can be efficiently loaded with covalent or non-covalent drugs and imaging agents, enabling targeted strategies, prolonging systemic circulation, and increasing tumor accumulation (Edis et al. 2021). Further, lipid vesicles have found applications in membrane biology, immunology, genetic engineering, and therapy (He et al. 2018). Cell membrane-based vesicles are natural drug carriers that have been used in various systems. With regard to vesicles and multifunctional silica nanoparticles, membrane protein retention and nanoscale structure make vesicles more suitable for drug delivery to a specific location than a whole cell as a drug carrier (Zavaleta et al. 2018). A number of recent studies have investigated the use of microsponges, solid lipid nanoparticles, and nanostructured lipid carriers as DDS carriers/vesicles (Naseri et al. 2015). For example, carrier erythrocytes (erythrocytes) are widely used for the encapsulation or binding of low-molecular-weight drugs, nucleic acids, proteins, and NPs to treat systemic diseases due to their long existence

and high biocompatibility (Muzykantov 2010). Apart from these, there are few other functionalized vesicles reported, namely, emulsomes, enzymosomes, virosomes, ethosomes, pharmacosomes, transferosomes, sphingosomes, etc., which have potential for target-specific activities. Details of which have been presented in Table 3.1.

As a futuristic approach, several medications, such as chloroquine, could be delivered vesicularly and theranostically in order to develop novel COVID-19 treatments (Satija et al. 2020). In addition, formulation strategies are discussed here to improve the therapeutic capacity of vesicular delivery systems, including their incorporation into environmentally sensitive dispersants such as hydrogels, ionic liquids, and deep eutectic solvents (Pedro et al. 2019).

3.8 Toxicity Concerns Related to Nanovesicular Carrier Systems

Interestingly, nanoparticles are used in the pharmaceutical sciences to reduce toxicity and side effects of drugs; scientists have realized that the delivery system itself can pose a risk to patients (Khan et al. 2019). Nanoparticles are becoming more popular for the purpose of addressing these issues, particularly in cancer treatment and other diseases where selective drug delivery has proved to be a major challenge, avoiding negative effects on healthy organs (Blanco et al. 2015). Currently, nanoparticles are widely used to deliver drugs, polypeptides, proteins, vaccines, nucleic acids, genes, etc. (Hong et al. 2020). In addition to increasing therapeutic efficacy and reducing side effects, polymer nanoparticles possess the ability to respond to stimuli in order to enhance drug delivery systems (Adepu and Ramakrishna 2021). Using nanoparticles for targeted delivery and controlled release of therapeutic agents, nanoparticle drug delivery systems provide targeted delivery and controlled release of therapeutic agents (De Jong and Borm 2008). There have been several studies that demonstrate traditional dosage forms are clearly more toxic and less effective than nanoparticle-based delivery systems (Li et al. 2019). As a prophylactic measure against oxidative damage associated with neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and Wilson's disease, nanoparticle-based drug delivery systems are under development (Kabanov and Gendelman 2007). As evidenced by the numerous drugs and delivery systems based on nanoparticles in use today, nanoparticles have had a major impact on the treatment of cancer, HIV/AIDS, and eye and respiratory disorders (Chang et al. 2015). A variety of nanotechnologies and nanocarrier-based drug delivery systems are being used to enhance the effectiveness of therapeutic agents. The most commonly used nanosystems in the treatment of degenerative diseases are liposomes, polymer nanoparticles, polymer micelles, protein nanoparticles, inorganic nanoparticles, carbon nanotubes, polymer conjugates, hybrid nanoparticles with solid lipid nanoparticles, nanoparticles, and dendrimers, which have been extensively used in (Chowdhury et al. 2017). The use of cytotoxic, chemosensitizing, and small interfering RNA (miRNA) drugs, as well as nanosystems, such as nanoclays, scaffolds, or nanotubes, has been used for many purposes, including drug loading,

Table 3.1 Different types of functionalized vesicles

S. no.	Vesicular systems	Advantages	Disadvantage	Reported usage	References
1	Emulsomes	Higher target specificity, lower quantity able to provide desired effect, limiting dose-dependent adverse effects	Comparatively costly in small scale production	Anticancer: Methotrexate loaded emulsomes have been reported with potential for delivery of methotrexate. Higher bioavailability is added advantage	Paliwal et al. (2009)
				Antifungal: Amphotericin B loaded emulsomes showed promising antileishmanial activity	Gupta and Vyas (2007)
				Antifungal: Amphotericin B loaded emulsomes showed promising macrophage targeting and functional execution. Moreover it provides sustained release and functional efficacy at lower concentration	Gupta et al. (2007)
				Antiviral: Zidovudine loaded emulsomes showed promising hepatic binding specificity. It also provides sustained release and functional efficacy at lower concentration	Vyas et al. (2006)
2	Enzymosomes	Increased stability, reduction in toxicity of the encapsulated agent, and improved pharmacokinetic effect (increase circulation time)	High production cost, leakage, and fusion of encapsulated drug molecules	Antioxidant: Antioxidant loaded enzymosomes extend their half-life and also increase their activity and stability	Skólmowska and Kmieć (2011)
				Anti-hyperuricemic: Uricase alkaline enzymosomes provide better thermal, hypothermal, acid-	Zhou et al. (2016)

(continued)

Table 3.1 (continued)

S. no.	Vesicular systems	Advantages	Disadvantage	Reported usage	References
				base, and proteolytic stabilities, in vitro and in vivo kinetic characteristics, and uric acid lowering effects	
				Anti-arthritis: Having potential to exert therapeutic effect for arthritis and provides sustained release of the enzyme	Gaspar et al. (2007)
				Anti-hepatic ischemia-reperfusion injury: Enzymosomes mediated therapy is more effective than conventional liposomes in reducing liver ischemia-reperfusion injury and this may be due to a short therapeutic window	Marcelino et al. (2017)
3	Virosomes	Biodegradable, biocompatible, nontoxic, promote fusion activity in endosomal pathway, and protect drugs against degradation	Limited bioactives can be delivered	Vaccine adjuvant and carrier system	Moser et al. (2013)
				Antigen and DNA delivery	Daemen et al. (2005)
				Cancer immunotherapy	Adamina et al. (2006)
4	Ethosomes	Delivery of large molecules (peptides, protein molecules) is possible	Skin irritation due to excipients and enhancers of drug delivery systems	Skin delivery formulations	Paiva-Santos et al. (2021)
				Treatment of acne vulgaris	Ansari et al. (2021)
				Drug delivery	Mbah et al. (2014)

5	Pharmacosomes	Suitable for incorporating both hydrophilic and lipophilic drugs, no need of following the tedious, time-consuming step for removing the free, unentrapped drug from the formulation. Oral, topical, extra- or intravascular delivery is possible	Required covalent bonding to protect the leakage of drugs on storage, undergo fusion and aggregation, as well as chemical hydrolysis	Delivery of aceclofenac	Semalty et al. (2010)
				Autoimmune diseases	Neupane et al. (2020)
				Anti-inflammatory response via curcumin nanocarriers	Upadhyay et al. (2022)
6	Transferosomes	It can accommodate drug molecules with wide range of solubility	Expensive and chemically unstable because of their predisposition to oxidative degradation	Delivery of plant bioactives	Sahu and Mohapatra (2021)
				Therapy against diabetes and hypertension	Ramkanth et al. (2021)
				Treatment of erectile dysfunction	Ali et al. (2015)
				Antibacterial activity	Maji et al. (2021)
7	Sphingosomes	Flexibility to couple with site-specific ligands to achieve active targeting, improve pharmacokinetic effect, and increase efficacy and stability	High production cost	Treatment for vesicular urokinase	Erdoğan et al. (2005)
				Treatment for acute lymphocytic leukemia	Thomas et al. (2006)

target cell uptake, bioassays, and imaging (Miele et al. 2012). Despite encouraging advances in materials science for using inorganic nanoparticles in bionanotechnology, the use of such materials in vivo is still limited by issues of toxicity, biodistribution, and bioaccumulation (Mohammadpour et al. 2019). Organic solvent residues and polymer toxicity are two potential problems related to polymeric nanoparticles. Inorganic metal nanoparticle systems can cause problems when they interact with biosystems. A significant number of the particles can remain in the body after treatment, leaving a potential residue that may cause toxicity (Jeevanandam et al. 2018).

In contrast, there is limited knowledge about nanoparticles' toxic effects and where they are found in biological systems (Khan and Shanker 2015). Nanoparticle toxicity can be studied from inhalation toxicity in order to determine hazards from nanoparticles (De Jong and Borm 2008). A nanoparticle can be inhaled, swallowed, or penetrated through the skin (Gupta and Xie 2018). Although some other studies have demonstrated that nanoparticles can reduce the toxicity of included drugs, it may not always be possible to differentiate between the toxicity of the drug and the nanoparticle (Bakand and Hayes 2016). To ensure that nanoparticles for gene-assisted cell therapy are safe, they must be evaluated for in vivo toxicity first. This is not the case for nanoparticles for drug delivery (Savage et al. 2019). Recent developments have used iron oxide nanoparticles for improved intratumoral drug delivery while reducing systemic toxicity and providing image-guided drug delivery (Zhu et al. 2017; Hu et al. 2018). Despite reported toxicity, it was also evident that multifunctional delivery systems based on graphene oxide can act on cancer tissues without causing systemic toxicity in B16 tumor-bearing mice (Sharma and Mondal 2020). Furthermore, another report indicated that nanoparticles may form blood clots when used as vectors for thrombolytic drugs being used to treat vascular thrombosis (Shen et al. 2021).

3.9 Conclusion and Future Perspective

Theranostic vesicles provide the potential for image-guided therapy, thereby providing a personalized approach to nanomedicine; nanoparticles allow you to localize bioactive agents to specific target sites and prevent them from deteriorating. Theranostic NPs may revolutionize the treatment of disease by combining diagnostics with the therapeutic potential of NPs. Nanomedicine offers the promise of theranostic treatment in vivo, but there are several challenges to be met at the preclinical and clinical stages. A theranostic nanomedicine must be developed that can be commercialized and regulated so that it can be used by patients in clinics after it leaves research labs. Further, research efforts are required to explore these concepts on an accessible nanomedicine clinical platform in order to develop versatile and intelligent theranostics applications. As nanotechnology has evolved rapidly in recent years, nanoparticles have been developed that can simultaneously monitor drug delivery, deliver drugs, and evaluate therapeutic efficacy all from a single nanoparticle. Polymeric nanoparticles offer simplicity and versatility for the

development of soft materials for nanomedicine applications due to their facile synthesis and versatility. Besides serving as carriers, nanoparticles' optical properties enable their use in photon-triggered therapies, such as photothermal therapy and photodynamic therapy. By means of photoacoustic imaging, plasmonic nanoparticles can convert absorbed light energy into heat. If these surface-modified nanoparticles were capable of targeting cancer cells, they could only destroy cancerous cells. A key feature of metal nanoparticles is that they possess unusual physical, chemical, electronic, and biological properties, allowing them to be used as an alternative strategy for cancer theranostics. B-NPs may become competent and alternative candidates for use in cancer theranostics. In addition to these radiometals, others are discussed which could have potential medical applications, with particular emphasis on their theranostic properties. The theranostic nanomaterials facilitate simultaneous diagnosis and treatment to the cancer patients. The delivery systems like metal and silica nanoparticles, liposomes, dendrimers, quantum dots, and carbon nanotubes, could improve cancer treatment and reduce side effects. Together, theranostics represents a relatively new area of medicine that describes a practical combination of targeted therapy based on the results of specific diagnoses. Although nanotheranostics hold promise, it is probable that global understanding of functionalized vesicle carrier mediated therapeutic and diagnostic tools will need to be improved in order to maximize their clinical potential.

References

Abak A, Abhari A, Rahimzadeh S (2018) Exosomes in cancer: small vesicular transporters for cancer progression and metastasis, biomarkers in cancer therapeutics. PeerJ 6:e4763. https://doi.org/10.7717/peerj.4763

Abels ER, Breakefield XO (2016) Introduction to extracellular vesicles: biogenesis, RNA cargo selection, content, release, and uptake. Cell Mol Neurobiol 36:301–312. https://doi.org/10.1007/s10571-016-0366-z

Adamina M, Guller U, Bracci L et al (2006) Clinical applications of virosomes in cancer immunotherapy. Expert Opin Biol Ther 6:1113–1121. https://doi.org/10.1517/14712598.6.11.1113

Adepu S, Ramakrishna S (2021) Controlled drug delivery systems: current status and future directions. Molecules 26:5905. https://doi.org/10.3390/molecules26195905

Ailuno G, Baldassari S, Lai F et al (2020) Exosomes and extracellular vesicles as emerging theranostic platforms in cancer research. Cell 9:E2569. https://doi.org/10.3390/cells9122569

Akbarzadeh A, Rezaei-Sadabady R, Davaran S et al (2013) Liposome: classification, preparation, and applications. Nanoscale Res Lett 8:102. https://doi.org/10.1186/1556-276X-8-102

Alavi M, Karimi N, Safaei M (2017) Application of various types of liposomes in drug delivery systems. Adv Pharm Bull 7:3–9. https://doi.org/10.15171/apb.2017.002

Alemi A, Zavar Reza J, Haghiralsadat F et al (2018) Paclitaxel and curcumin coadministration in novel cationic PEGylated niosomal formulations exhibit enhanced synergistic antitumor efficacy. J Nanobiotechnol 16:28. https://doi.org/10.1186/s12951-018-0351-4

Ali MFM, Salem HF, Abdelmohsen HF, Attia SK (2015) Preparation and clinical evaluation of nano-transferosomes for treatment of erectile dysfunction. Drug Des Devel Ther 9:2431–2447. https://doi.org/10.2147/DDDT.S81236

Alshehri S, Imam SS, Rizwanullah M et al (2020) Progress of cancer nanotechnology as diagnostics, therapeutics, and theranostics nanomedicine: preclinical promise and translational challenges. Pharmaceutics 13:24. https://doi.org/10.3390/pharmaceutics13010024

Ansari SA, Qadir A, Warsi MH et al (2021) Ethosomes-based gel formulation of karanjin for treatment of acne vulgaris: in vitro investigations and preclinical assessment. 3 Biotech 11:456. https://doi.org/10.1007/s13205-021-02978-3

Antimisiaris SG, Mourtas S, Marazioti A (2018) Exosomes and exosome-inspired vesicles for targeted drug delivery. Pharmaceutics 10:E218. https://doi.org/10.3390/pharmaceutics10040218

Aparajay P, Dev A (2022) Functionalized niosomes as a smart delivery device in cancer and fungal infection. Eur J Pharm Sci 168:106052. https://doi.org/10.1016/j.ejps.2021.106052

Bajwa S, Munawar W, Khan A (2017) Nanotechnology in medicine: innovation to market. Pharmaceutl Bioprocess 5:11–15

Bakand S, Hayes A (2016) Toxicological considerations, toxicity assessment, and risk management of inhaled nanoparticles. Int J Mol Sci 17:E929. https://doi.org/10.3390/ijms17060929

Bartelds R, Nematollahi MH, Pols T et al (2018) Niosomes, an alternative for liposomal delivery. PLoS One 13:e0194179. https://doi.org/10.1371/journal.pone.0194179

Bhosale RR, Gangadharappa HV, Hani U et al (2017) Current perspectives on novel drug delivery systems and therapies for management of prostate cancer: an inclusive review. Curr Drug Targets 18:1233–1249. https://doi.org/10.2174/1389450117666160613103705

Bin-Jumah M, Gilani SJ, Jahangir MA et al (2020) Clarithromycin-loaded ocular chitosan nanoparticle: formulation, optimization, characterization, ocular irritation, and antimicrobial activity. IJN 15:7861–7875. https://doi.org/10.2147/IJN.S269004

Blanco E, Shen H, Ferrari M (2015) Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nat Biotechnol 33:941–951. https://doi.org/10.1038/nbt.3330

Bovier PA (2008) Epaxal: a virosomal vaccine to prevent hepatitis a infection. Expert Rev Vaccines 7:1141–1150. https://doi.org/10.1586/14760584.7.8.1141

Bozzuto G, Molinari A (2015) Liposomes as nanomedical devices. Int J Nanomedicine 10:975–999. https://doi.org/10.2147/IJN.S68861

Bulbake U, Doppalapudi S, Kommineni N, Khan W (2017) Liposomal formulations in clinical use: an updated review. Pharmaceutics 9:E12. https://doi.org/10.3390/pharmaceutics9020012

Butreddy A, Kommineni N, Dudhipala N (2021) Exosomes as naturally occurring vehicles for delivery of biopharmaceuticals: insights from drug delivery to clinical perspectives. Nanomaterials (Basel) 11:1481. https://doi.org/10.3390/nano11061481

Cardoso VF, Francesko A, Ribeiro C et al (2018) Advances in magnetic nanoparticles for biomedical applications. Adv Healthc Mater 7. https://doi.org/10.1002/adhm.201700845

Caruthers SD, Wickline SA, Lanza GM (2007) Nanotechnological applications in medicine. Curr Opin Biotechnol 18:26–30. https://doi.org/10.1016/j.copbio.2007.01.006

Cerqueira-Coutinho C, Dos Santos EP, Mansur CRE (2016) Niosomes as Nano-delivery systems in the pharmaceutical field. Crit Rev Ther Drug Carrier Syst 33:195–212. https://doi.org/10.1615/CritRevTherDrugCarrierSyst.2016016167

Chang EH, Harford JB, Eaton MAW et al (2015) Nanomedicine: past, present and future - a global perspective. Biochem Biophys Res Commun 468:511–517. https://doi.org/10.1016/j.bbrc.2015.10.136

Chowdhury A, Kunjiappan S, Panneerselvam T et al (2017) Nanotechnology and nanocarrier-based approaches on treatment of degenerative diseases. Int Nano Lett 7:91–122. https://doi.org/10.1007/s40089-017-0208-0

Cole JT, Holland NB (2015) Multifunctional nanoparticles for use in theranostic applications. Drug Deliv Transl Res 5:295–309

Colombo M, Raposo G, Théry C (2014) Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu Rev Cell Dev Biol 30:255–289. https://doi.org/10.1146/annurev-cellbio-101512-122326

Daemen T, de Mare A, Bungener L et al (2005) Virosomes for antigen and DNA delivery. Adv Drug Deliv Rev 57:451–463. https://doi.org/10.1016/j.addr.2004.09.005

Dai Y, Wang B, Sun Z et al (2019) Multifunctional theranostic liposomes loaded with a hypoxia-activated prodrug for cascade-activated tumor selective combination therapy. ACS Appl Mater Interfaces 11:39410–39423. https://doi.org/10.1021/acsami.9b11080

Dan N (2017) Core–shell drug carriers: liposomes, polymersomes, and niosomes. In: Andronescu E, Grumezescu AM (eds) Nanostructures for drug delivery. Elsevier, pp 63–105

De Jong WH, Borm PJ (2008) Drug delivery and nanoparticles: applications and hazards. Int J Nanomed 3:133–149

de Oliveira DCS, de Freitas CF, Calori IR et al (2020) Theranostic verteporfin- loaded lipid-polymer liposome for photodynamic applications. J Photochem Photobiol B 212:112039. https://doi.org/10.1016/j.jphotobiol.2020.112039

Demir B, Barlas FB, Gumus ZP et al (2018) Theranostic niosomes as a promising tool for combined therapy and diagnosis: "all-in-one" approach. ACS Appl Nano Mater 1:2827–2835. https://doi.org/10.1021/acsanm.8b00468

Driscoll J, Moirangthem A, Yan IK, Patel T (2021) Fabrication and characterization of a biomaterial based on extracellular-vesicle functionalized graphene oxide. Front Bioeng Biotechnol 9:686510. https://doi.org/10.3389/fbioe.2021.686510

Edis Z, Wang J, Waqas MK et al (2021) Nanocarriers-mediated drug delivery systems for anticancer agents: an overview and perspectives. Int J Nanomedicine 16:1313–1330. https://doi.org/10.2147/IJN.S289443

Elsharkasy OM, Nordin JZ, Hagey DW et al (2020) Extracellular vesicles as drug delivery systems: why and how? Adv Drug Deliv Rev 159:332–343. https://doi.org/10.1016/j.addr.2020.04.004

Erdoğan S, Ozer AY, Bilgili H (2005) In vivo behaviour of vesicular urokinase. Int J Pharm 295:1–6. https://doi.org/10.1016/j.ijpharm.2005.01.021

Fan Y, Chen M, Zhang J et al (2018) Updated progress of nanocarrier-based intranasal drug delivery systems for treatment of brain diseases. Crit Rev Ther Drug Carrier Syst 35:433–467. https://doi.org/10.1615/CritRevTherDrugCarrierSyst.2018024697

Fekrazad R, Naghdi N, Nokhbatolfoghahaei H, Bagheri H (2016) The combination of laser therapy and metal nanoparticles in cancer treatment originated from epithelial tissues: a literature review. J Lasers Med Sci 7:62–75. https://doi.org/10.15171/jlms.2016.13

Forterre AV, Wang J-H, Delcayre A et al (2020) Extracellular vesicle-mediated in vitro transcribed mRNA delivery for treatment of HER2+ breast cancer xenografts in mice by prodrug CB1954 without general toxicity. Mol Cancer Ther 19:858–867. https://doi.org/10.1158/1535-7163.MCT-19-0928

Gai M, Simon J, Lieberwirth I et al (2020) A bio-orthogonal functionalization strategy for site-specific coupling of antibodies on vesicle surfaces after self-assembly. Polym Chem 11:527–540. https://doi.org/10.1039/C9PY01136F

Gao Y, Liu X, Chen N et al (2023) Recent advance of liposome nanoparticles for nucleic acid therapy. Pharmaceutics 15:178

Gardikis K, Tsimplouli C, Dimas K et al (2010) New chimeric advanced drug delivery nano systems (chi-aDDnSs) as doxorubicin carriers. Int J Pharm 402:231–237. https://doi.org/10.1016/j.ijpharm.2010.10.007

Gaspar MM, Boerman OC, Laverman P et al (2007) Enzymosomes with surface-exposed superoxide dismutase: in vivo behaviour and therapeutic activity in a model of adjuvant arthritis. J Control Release 117:186–195. https://doi.org/10.1016/j.jconrel.2006.10.018

Gharbavi M, Amani J, Kheiri-Manjili H et al (2018) Niosome: a promising nanocarrier for natural drug delivery through blood-brain barrier. Adv Pharmacol Sci 2018:e6847971. https://doi.org/10.1155/2018/6847971

Gilani SJ, Beg S, Kala C et al (2020) Chemically nano-engineered theranostics for phytoconstituents as healthcare application. Curr Biochem Eng 6:53–61. https://doi.org/10.2174/2212711906666190723144111

Gopinath D, Ravi D, Rao BR et al (2004) Ascorbyl palmitate vesicles (Aspasomes): formation, characterization and applications. Int J Pharm 271:95–113. https://doi.org/10.1016/j.ijpharm.2003.10.032

Gulbake A, Jain A, Jain A et al (2016) Insight to drug delivery aspects for colorectal cancer. World J Gastroenterol 22:582–599. https://doi.org/10.3748/wjg.v22.i2.582

Gupta R, Xie H (2018) Nanoparticles in daily life: applications, toxicity and regulations. J Environ Pathol Toxicol Oncol 37:209–230. https://doi.org/10.1615/JEnvironPatholToxicolOncol.2018026009

Gupta S, Dube A, Vyas SP (2007) Antileishmanial efficacy of amphotericin B bearing emulsomes against experimental visceral leishmaniasis. J Drug Target 15:437–444. https://doi.org/10.1080/10611860701453836

Gupta S, Vyas SP (2007) Development and characterization of amphotericin B bearing emulsomes for passive and active macrophage targeting. J Drug Target 15:206–217. https://doi.org/10.1080/10611860701195395

Haney MJ, Zhao Y, Jin YS et al (2020) Macrophage-derived extracellular vesicles as drug delivery systems for triple negative breast cancer (TNBC) therapy. J Neuroimmune Pharmacol 15:487–500. https://doi.org/10.1007/s11481-019-09884-9

He C, Zheng S, Luo Y, Wang B (2018) Exosome theranostics: biology and translational medicine. Theranostics 8:237–255. https://doi.org/10.7150/thno.21945

Hong S, Choi DW, Kim HN et al (2020) Protein-based nanoparticles as drug delivery systems. Pharmaceutics 12:604. https://doi.org/10.3390/pharmaceutics12070604

Hood JL, San RS, Wickline SA (2011) Exosomes released by melanoma cells prepare sentinel lymph nodes for tumor metastasis. Cancer Res 71:3792–3801. https://doi.org/10.1158/0008-5472.CAN-10-4455

Hu Y, Mignani S, Majoral J-P et al (2018) Construction of iron oxide nanoparticle-based hybrid platforms for tumor imaging and therapy. Chem Soc Rev 47:1874–1900. https://doi.org/10.1039/c7cs00657h

Im H-J, England CG, Feng L et al (2016) Accelerated blood clearance phenomenon reduces the passive targeting of PEGylated nanoparticles in peripheral arterial disease. ACS Appl Mater Interfaces 8:17955–17963. https://doi.org/10.1021/acsami.6b05840

Imam SS, Jahangir MA, Gilani SJ, et al (2021) Nanoemulsions as delivery vehicle for nutraceuticals and improving food nutrition properties. In: Nanoemulsions in food technology. CRC Press

Jabalee J, Towle R, Garnis C (2018) The role of extracellular vesicles in cancer: cargo, function, and therapeutic implications. Cell 7:93. https://doi.org/10.3390/cells7080093

Jahangir MA, Anand C, Muheem A et al (2020) Nano phytomedicine based delivery system for CNS disease. Curr Drug Metab 21:661–673. https://doi.org/10.2174/1389200221666200523161003

Janib SM, Moses AS, MacKay JA (2010) Imaging and drug delivery using theranostic nanoparticles. Adv Drug Deliv Rev 62:1052–1063. https://doi.org/10.1016/j.addr.2010.08.004

Jeevanandam J, Barhoum A, Chan YS et al (2018) Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. Beilstein J Nanotechnol 9:1050–1074. https://doi.org/10.3762/bjnano.9.98

Jeyaram A, Lamichhane TN, Wang S et al (2020) Enhanced loading of functional miRNA cargo via pH gradient modification of extracellular vesicles. Mol Ther 28:975–985. https://doi.org/10.1016/j.ymthe.2019.12.007

Jonas JB (2002) Verteporfin therapy of subfoveal choroidal neovascularization in age-related macular degeneration. Am J Ophthalmol 133:857. https://doi.org/10.1016/S0002-9394(02)01423-X

Kabanov AV, Gendelman HE (2007) Nanomedicine in the diagnosis and therapy of neurodegenerative disorders. Prog Polym Sci 32:1054–1082. https://doi.org/10.1016/j.progpolymsci.2007.05.014

Kaul A, Chaturvedi S, Attri A et al (2016) Targeted theranostic liposomes: rifampicin and ofloxacin loaded pegylated liposomes for theranostic application in mycobacterial infections. RSC Adv 6:28919–28926. https://doi.org/10.1039/C6RA01135G

Kaur IP, Garg A, Singla AK, Aggarwal D (2004) Vesicular systems in ocular drug delivery: an overview. Int J Pharm 269:1–14. https://doi.org/10.1016/j.ijpharm.2003.09.016

Kazi KM, Mandal AS, Biswas N et al (2010) Niosome: a future of targeted drug delivery systems. J Adv Pharm Technol Res 1:374–380. https://doi.org/10.4103/0110-5558.76435

Khan HA, Shanker R (2015) Toxicity of nanomaterials. Biomed Res Int 2015:e521014. https://doi.org/10.1155/2015/521014

Khan I, Saeed K, Khan I (2019) Nanoparticles: properties, applications and toxicities. Arab J Chem 12:908–931. https://doi.org/10.1016/j.arabjc.2017.05.011

Khoee S, Yaghoobian M (2017) Niosomes: a novel approach in modern drug delivery systems. In: Andronescu E, Grumezescu AM (eds) Nanostructures for drug delivery. Elsevier, pp 207–237

Kim H, Jang H, Cho H et al (2021a) Recent advances in exosome-based drug delivery for cancer therapy. Cancers (Basel) 13:4435. https://doi.org/10.3390/cancers13174435

Kim H, Lee JW, Han G et al (2021b) Extracellular vesicles as potential theranostic platforms for skin diseases and aging. Pharmaceutics 13:760. https://doi.org/10.3390/pharmaceutics13050760

Kobayashi M, Sawada K, Miyamoto M et al (2020) Exploring the potential of engineered exosomes as delivery systems for tumor-suppressor microRNA replacement therapy in ovarian cancer. Biochem Biophys Res Commun 527:153–161. https://doi.org/10.1016/j.bbrc.2020.04.076

Kogure A, Yoshioka Y, Ochiya T (2020) Extracellular vesicles in cancer metastasis: potential as therapeutic targets and materials. Int J Mol Sci 21:4463. https://doi.org/10.3390/ijms21124463

Kosaka N, Iguchi H, Ochiya T (2010) Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. Cancer Sci 101:2087–2092. https://doi.org/10.1111/j.1349-7006.2010.01650.x

Kumar G, Rao P (2012) Ultra deformable niosomes for improved transdermal drug delivery: the future scenario. Asian J Pharmaceut Sci 7

Kumar GP, Rajeshwarrao P (2011) Nonionic surfactant vesicular systems for effective drug delivery—an overview. Acta Pharm Sin B 1:208–219. https://doi.org/10.1016/j.apsb.2011.09.002

Lamichhane TN, Sokic S, Schardt JS et al (2015) Emerging roles for extracellular vesicles in tissue engineering and regenerative medicine. Tissue Eng Part B Rev 21:45–54. https://doi.org/10.1089/ten.teb.2014.0300

Le Meins J-F, Schatz C, Lecommandoux S, Sandre O (2013) Hybrid polymer/lipid vesicles: state of the art and future perspectives. Mater Today 16:397–402. https://doi.org/10.1016/j.mattod.2013.09.002

Lee W, Im H-J (2019) Theranostics based on liposome: looking Back and forward. Nucl Med Mol Imaging 53:242–246. https://doi.org/10.1007/s13139-019-00603-z

Li C, Wang J, Wang Y et al (2019) Recent progress in drug delivery. Acta Pharm Sin B 9:1145–1162. https://doi.org/10.1016/j.apsb.2019.08.003

Li Q, Maier SH, Li P et al (2020) Aptamers: a novel targeted theranostic platform for pancreatic ductal adenocarcinoma. Radiat Oncol 15:189. https://doi.org/10.1186/s13014-020-01624-1

Lim W, Kim H-S (2019) Exosomes as therapeutic vehicles for cancer. Tissue Eng Regen Med 16:213–223. https://doi.org/10.1007/s13770-019-00190-2

Lin Q, Qu M, Zhou B et al (2019) Exosome-like nanoplatform modified with targeting ligand improves anti-cancer and anti-inflammation effects of imperialine. J Control Release 311–312:104–116. https://doi.org/10.1016/j.jconrel.2019.08.037

Lombardo D, Kiselev MA, Magazù S, Calandra P (2015) Amphiphiles self-assembly: basic concepts and future perspectives of supramolecular approaches. Adv Condensed Matter Phys 2015:e151683. https://doi.org/10.1155/2015/151683

Ma M, Lei M, Tan X et al (2015) Theranostic liposomes containing conjugated polymer dots and doxorubicin for bio-imaging and targeted therapeutic delivery. RSC Adv 6:1945–1957. https://doi.org/10.1039/C5RA24485D

Maji R, Omolo CA, Jaglal Y et al (2021) A transferosome-loaded bigel for enhanced transdermal delivery and antibacterial activity of vancomycin hydrochloride. Int J Pharm 607:120990. https://doi.org/10.1016/j.ijpharm.2021.120990

Malekzad H, Zangabad PS, Mirshekari H et al (2017) Noble metal nanoparticles in biosensors: recent studies and applications. Nanotechnol Rev 6:301–329. https://doi.org/10.1515/ntrev-2016-0014

Manconi M, Marongiu F, Castangia I et al (2016) Polymer-associated liposomes for the oral delivery of grape pomace extract. Colloids Surf B Biointerfaces 146:910–917. https://doi.org/10.1016/j.colsurfb.2016.07.043

Marcelino P, Marinho HS, Campos MC et al (2017) Therapeutic activity of superoxide dismutase-containing enzymosomes on rat liver ischaemia-reperfusion injury followed by magnetic resonance microscopy. Eur J Pharm Sci 109:464–471. https://doi.org/10.1016/j.ejps.2017.09.008

Marmon P, Owen SF, Margiotta-Casaluci L (2021) Pharmacology-informed prediction of the risk posed to fish by mixtures of non-steroidal anti-inflammatory drugs (NSAIDs) in the environment. Environ Int 146:106222. https://doi.org/10.1016/j.envint.2020.106222

Mbah CC, Builders PF, Attama AA (2014) Nanovesicular carriers as alternative drug delivery systems: ethosomes in focus. Expert Opin Drug Deliv 11:45–59. https://doi.org/10.1517/17425247.2013.860130

Mei L, Zhang Z, Zhao L et al (2013) Pharmaceutical nanotechnology for oral delivery of anticancer drugs. Adv Drug Deliv Rev 65:880–890. https://doi.org/10.1016/j.addr.2012.11.005

Meng W, He C, Hao Y et al (2020) Prospects and challenges of extracellular vesicle-based drug delivery system: considering cell source. Drug Deliv 27:585–598. https://doi.org/10.1080/10717544.2020.1748758

Merhav H, Mieles L (1997) Amphotericin B lipid complex in the treatment of invasive fungal infections in liver transplant patients. Transplant Proc 29:2670–2674. https://doi.org/10.1016/s0041-1345(97)00551-4

Miele E, Spinelli GP, Miele E et al (2012) Nanoparticle-based delivery of small interfering RNA: challenges for cancer therapy. Int J Nanomedicine 7:3637–3657. https://doi.org/10.2147/IJN.S23696

Mitchell MJ, Billingsley MM, Haley RM et al (2021) Engineering precision nanoparticles for drug delivery. Nat Rev Drug Discov 20:101–124. https://doi.org/10.1038/s41573-020-0090-8

Mohammadpour R, Dobrovolskaia MA, Cheney DL et al (2019) Subchronic and chronic toxicity evaluation of inorganic nanoparticles for delivery applications. Adv Drug Deliv Rev 144:112–132. https://doi.org/10.1016/j.addr.2019.07.006

Mohanty D, Rani MJ, Haque MA et al (2020) Preparation and evaluation of transdermal naproxen niosomes: formulation optimization to preclinical anti-inflammatory assessment on murine model. J Liposome Res 30:377–387. https://doi.org/10.1080/08982104.2019.1652646

Moser C, Müller M, Kaeser MD et al (2013) Influenza virosomes as vaccine adjuvant and carrier system. Expert Rev Vaccines 12:779–791. https://doi.org/10.1586/14760584.2013.811195

Mukthavaram R, Wrasidlo W, Hall D et al (2011) Assembly and targeting of liposomal nanoparticles encapsulating quantum dots. Bioconjug Chem 22:1638–1644. https://doi.org/10.1021/bc200201e

Muzykantov VR (2010) Drug delivery by red blood cells: vascular carriers designed by mother nature. Expert Opin Drug Deliv 7:403–427. https://doi.org/10.1517/17425241003610633

Naseri N, Valizadeh H, Zakeri-Milani P (2015) Solid lipid nanoparticles and nanostructured lipid carriers: structure, preparation and application. Adv Pharm Bull 5:305–313. https://doi.org/10.15171/apb.2015.043

Neupane YR, Mahtab A, Siddiqui L et al (2020) Biocompatible nanovesicular drug delivery systems with targeting potential for autoimmune diseases. Curr Pharm Des 26:5488–5502. https://doi.org/10.2174/1381612826666200523174108

Nogueira MS (2020) Optical theranostics and treatment dosimetry for COVID-19 lung complications: towards increasing the survival rate of vulnerable populations. Photodiagn Photodyn Ther 31:101892. https://doi.org/10.1016/j.pdpdt.2020.101892

Nowroozi F, Dadashzadeh S, Soleimanjahi H et al (2018) Theranostic niosomes for direct intratumoral injection: marked enhancement in tumor retention and anticancer efficacy. Nanomedicine (Lond) 13:2201–2219. https://doi.org/10.2217/nnm-2018-0091

O'Brien K, Breyne K, Ughetto S et al (2020) RNA delivery by extracellular vesicles in mammalian cells and its applications. Nat Rev Mol Cell Biol 21:585–606. https://doi.org/10.1038/s41580-020-0251-y

Olusanya TOB, Haj Ahmad RR, Ibegbu DM et al (2018) Liposomal drug delivery systems and anticancer drugs. Molecules 23:907. https://doi.org/10.3390/molecules23040907

Pai DR, Venkatesh MP, Kumar TMP (2019) Current developments in therapeutic drug targeting for the management of rheumatoid arthritis: an emerging paradigm. Crit Rev Ther Drug Carrier Syst 36:485–536. https://doi.org/10.1615/CritRevTherDrugCarrierSyst.2019025729

Paiva-Santos AC, Silva AL, Guerra C et al (2021) Ethosomes as Nanocarriers for the development of skin delivery formulations. Pharm Res 38:947–970. https://doi.org/10.1007/s11095-021-03053-5

Paliwal R, Paliwal SR, Mishra N et al (2009) Engineered chylomicron mimicking carrier emulsome for lymph targeted oral delivery of methotrexate. Int J Pharm 380:181–188. https://doi.org/10.1016/j.ijpharm.2009.06.026

Pandey M, Choudhury H, Gorain B et al (2021) Site-specific vesicular drug delivery system for skin cancer: a novel approach for targeting. Gels 7:218. https://doi.org/10.3390/gels7040218

Paolino D, Cosco D, Muzzalupo R et al (2008) Innovative bola-surfactant niosomes as topical delivery systems of 5-fluorouracil for the treatment of skin cancer. Int J Pharm 353:233–242. https://doi.org/10.1016/j.ijpharm.2007.11.037

Patra JK, Das G, Fraceto LF et al (2018) Nano based drug delivery systems: recent developments and future prospects. J Nanobiotechnol 16:71. https://doi.org/10.1186/s12951-018-0392-8

Pedro SN, Freire MG, Freire CSR, Silvestre AJD (2019) Deep eutectic solvents comprising active pharmaceutical ingredients in the development of drug delivery systems. Expert Opin Drug Deliv 16:497–506. https://doi.org/10.1080/17425247.2019.1604680

Pereira MC, Pianella M, Wei D et al (2016) pH-sensitive pHLIP® coated niosomes. Mol Membr Biol 33:51–63. https://doi.org/10.1080/09687688.2017.1342969

Petersen AL, Henriksen JR, Binderup T et al (2016) In vivo evaluation of PEGylated ^{64}Cu-liposomes with theranostic and radiotherapeutic potential using micro PET/CT. Eur J Nucl Med Mol Imaging 43:941–952. https://doi.org/10.1007/s00259-015-3272-6

Pham TT, Jaafar-Maalej C, Charcosset C, Fessi H (2012) Liposome and niosome preparation using a membrane contactor for scale-up. Colloids Surf B Biointerfaces 94:15–21. https://doi.org/10.1016/j.colsurfb.2011.12.036

Pradhan M, Srivastava S, Singh D et al (2018) Perspectives of lipid-based drug carrier systems for transdermal delivery. Crit Rev Ther Drug Carrier Syst 35:331–367. https://doi.org/10.1615/CritRevTherDrugCarrierSyst.2018020856

Ramkanth S, Anitha P, Gayathri R et al (2021) Formulation and design optimization of nano-transferosomes using pioglitazone and eprosartan mesylate for concomitant therapy against diabetes and hypertension. Eur J Pharm Sci 162:105811. https://doi.org/10.1016/j.ejps.2021.105811

Rizvi SAA, Saleh AM (2018) Applications of nanoparticle systems in drug delivery technology. Saudi Pharm J 26:64–70. https://doi.org/10.1016/j.jsps.2017.10.012

Rojas JJ, Thorne SH (2012) Theranostic potential of oncolytic vaccinia virus. Theranostics 2:363–373. https://doi.org/10.7150/thno.3724

Sahoo SK, Parveen S, Panda JJ (2007) The present and future of nanotechnology in human health care. Nanomedicine 3:20–31. https://doi.org/10.1016/j.nano.2006.11.008

Sahu AN, Mohapatra D (2021) Nanovesicular transferosomes for the topical delivery of plant bioactives. Nanomedicine (Lond) 16:2491–2495. https://doi.org/10.2217/nnm-2021-0316

Satija S, Mehta M, Sharma M et al (2020) Vesicular drug-delivery systems as theranostics in COVID-19. Future Med Chem 12:1607. https://doi.org/10.4155/fmc-2020-0149

Sato YT, Umezaki K, Sawada S et al (2016) Engineering hybrid exosomes by membrane fusion with liposomes. Sci Rep 6:21933. https://doi.org/10.1038/srep21933
Savage DT, Hilt JZ, Dziubla TD (2019) In vitro methods for assessing nanoparticle toxicity. Methods Mol Biol 1894:1–29. https://doi.org/10.1007/978-1-4939-8916-4_1
Sebaaly C, Greige-Gerges H, Charcosset C (2019) Lipid membrane models for biomembrane properties' investigation. In: Basile A, Charcosset C (eds) Current trends and future developments on (bio-) membranes. Elsevier, pp 311–340
Sedgwick AE, D'Souza-Schorey C (2018) The biology of extracellular microvesicles. Traffic 19: 319–327. https://doi.org/10.1111/tra.12558
Semalty A, Semalty M, Rawat BS et al (2010) Development and evaluation of pharmacosomes of aceclofenac. Indian J Pharm Sci 72:576–581. https://doi.org/10.4103/0250-474X.78523
Senapati S, Mahanta AK, Kumar S, Maiti P (2018) Controlled drug delivery vehicles for cancer treatment and their performance. Signal Transduct Target Ther 3:7. https://doi.org/10.1038/s41392-017-0004-3
Seo JW, Zhang H, Kukis DL et al (2008) A novel method to label preformed liposomes with 64Cu for positron emission tomography (PET) imaging. Bioconjug Chem 19:2577–2584. https://doi.org/10.1021/bc8002937
Sercombe L, Veerati T, Moheimani F et al (2015) Advances and challenges of liposome assisted drug delivery. Front Pharmacol 6:286. https://doi.org/10.3389/fphar.2015.00286
Shah SM, Ashtikar M, Jain AS et al (2015) LeciPlex, invasomes, and liposomes: a skin penetration study. Int J Pharm 490:391–403. https://doi.org/10.1016/j.ijpharm.2015.05.042
Shang M, Ji JS, Song C et al (2017) Extracellular vesicles: a brief overview and its role in precision medicine. Methods Mol Biol 1660:1–14. https://doi.org/10.1007/978-1-4939-7253-1_1
Shao H, Chung J, Lee K et al (2015) Chip-based analysis of exosomal mRNA mediating drug resistance in glioblastoma. Nat Commun 6:6999. https://doi.org/10.1038/ncomms7999
Shao S, Fang H, Li Q, Wang G (2020) Extracellular vesicles in inflammatory skin disorders: from pathophysiology to treatment. Theranostics 10:9937–9955. https://doi.org/10.7150/thno.45488
Sharma H, Mondal S (2020) Functionalized graphene oxide for chemotherapeutic drug delivery and cancer treatment: a promising material in nanomedicine. Int J Mol Sci 21:6280. https://doi.org/10.3390/ijms21176280
Shen M, Wang Y, Hu F et al (2021) Thrombolytic agents: nanocarriers in targeted release. Molecules 26:6776. https://doi.org/10.3390/molecules26226776
Siafaka PI, Okur NÜ, Karantas ID et al (2021) Current update on nanoplatforms as therapeutic and diagnostic tools: a review for the materials used as nanotheranostics and imaging modalities. Asian J Pharmaceut Sci 16:24–46. https://doi.org/10.1016/j.ajps.2020.03.003
Simone EA, Dziubla TD, Muzykantov VR (2008) Polymeric carriers: role of geometry in drug delivery. Expert Opin Drug Deliv 5:1283–1300. https://doi.org/10.1517/17425240802567846
Singh AP, Biswas A, Shukla A, Maiti P (2019) Targeted therapy in chronic diseases using nanomaterial-based drug delivery vehicles. Signal Transduct Target Ther 4:33. https://doi.org/10.1038/s41392-019-0068-3
Skólmowska M, Kmieć M (2011) Antioxidant enzymosomes - properties and application. Postepy Hig Med Dosw (Online) 65:640–644. https://doi.org/10.5604/17322693.962163
Srivastava A, Rathore S, Munshi A, Ramesh R (2022) Organically derived exosomes as carriers of anticancer drugs and imaging agents for cancer treatment. Semin Cancer Biol S1044-579X(22) 00047–5. https://doi.org/10.1016/j.semcancer.2022.02.020
Sun C, Lee JSH, Zhang M (2008) Magnetic nanoparticles in MR imaging and drug delivery. Adv Drug Deliv Rev 60:1252–1265. https://doi.org/10.1016/j.addr.2008.03.018
Tan X-H, Fang D, Xu Y-D et al (2021) Skimmed bovine milk-derived extracellular vesicles isolated via "salting-out": characterizations and potential functions as nanocarriers. Front Nutr 8: 769223. https://doi.org/10.3389/fnut.2021.769223
Tang T-T, Lv L-L, Lan H-Y, Liu B-C (2019) Extracellular vesicles: opportunities and challenges for the treatment of renal diseases. Front Physiol 10:226. https://doi.org/10.3389/fphys.2019.00226

Thangam R, Paulmurugan R, Kang H (2021) Functionalized nanomaterials as tailored theranostic agents in brain imaging. Nanomaterials (Basel) 12:18. https://doi.org/10.3390/nano12010018

Thomas DA, Sarris AH, Cortes J et al (2006) Phase II study of sphingosomal vincristine in patients with recurrent or refractory adult acute lymphocytic leukemia. Cancer 106:120–127. https://doi.org/10.1002/cncr.21595

Tiwari G, Tiwari R, Sriwastawa B et al (2012) Drug delivery systems: an updated review. Int J Pharm Investig 2:2–11. https://doi.org/10.4103/2230-973X.96920

Torchilin PV, Torchilin VP, Torchilin V, Weissig V (2003) Liposomes: a practical approach. Oxford University Press, Oxford

Touitou E, Dayan N, Bergelson L et al (2000) Ethosomes - novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J Control Release 65:403–418. https://doi.org/10.1016/s0168-3659(99)00222-9

Tran PHL, Wang T, Yin W et al (2019) Aspirin-loaded nanoexosomes as cancer therapeutics. Int J Pharm 572:118786. https://doi.org/10.1016/j.ijpharm.2019.118786

Uchino T, Lefeber F, Gooris G, Bouwstra J (2014) Characterization and skin permeation of ketoprofen-loaded vesicular systems. Eur J Pharm Biopharm 86:156–166. https://doi.org/10.1016/j.ejpb.2013.02.009

Upadhyay P, Singh D, Upadhyay S (2022) Vesicular approach review on nanocarriers bearing curcumin and applications. Recent Adv Drug Deliv Formul 16:256. https://doi.org/10.2174/2667387816666220404092415

Urban C, Urban AS, Charron H, Joshi A (2013) Externally modulated theranostic nanoparticles. Transl Cancer Res 2:292–308. https://doi.org/10.3978/j.issn.2218-676X.2013.08.05

Vallejo D, Lee S-H, Lee A (2017) Functionalized vesicles by microfluidic device. Methods Mol Biol 1572:489–510. https://doi.org/10.1007/978-1-4939-6911-1_31

van der Meel R, Krawczyk-Durka M, van Solinge WW, Schiffelers RM (2014) Toward routine detection of extracellular vesicles in clinical samples. Int J Lab Hematol 36:244–253. https://doi.org/10.1111/ijlh.12247

Verma P, Pathak K (2010) Therapeutic and cosmeceutical potential of ethosomes: an overview. J Adv Pharm Technol Res 1:274–282. https://doi.org/10.4103/0110-5558.72415

Villata S, Canta M, Cauda V (2020) EVs and bioengineering: from cellular products to engineered nanomachines. Int J Mol Sci 21:6048. https://doi.org/10.3390/ijms21176048

Vyas SP, Subhedar R, Jain S (2006) Development and characterization of emulsomes for sustained and targeted delivery of an antiviral agent to liver. J Pharm Pharmacol 58:321–326. https://doi.org/10.1211/jpp.58.3.0005

Walker S, Busatto S, Pham A et al (2019) Extracellular vesicle-based drug delivery systems for cancer treatment. Theranostics 9:8001–8017. https://doi.org/10.7150/thno.37097

Wiklander OPB, Brennan MÁ, Lötvall J et al (2019) Advances in therapeutic applications of extracellular vesicles. Sci Transl Med 11:eaav8521. https://doi.org/10.1126/scitranslmed.aav8521

Xin L, Yuan Y-W, Liu C et al (2021) Preparation of internalizing RGD-modified recombinant methioninase exosome active targeting vector and antitumor effect evaluation. Dig Dis Sci 66:1045–1053. https://doi.org/10.1007/s10620-020-06262-x

Xing Y, Cheng Z, Wang R et al (2020) Analysis of extracellular vesicles as emerging theranostic nanoplatforms. Coord Chem Rev 424:213506. https://doi.org/10.1016/j.ccr.2020.213506

Yáñez-Mó M, Siljander PR-M, Andreu Z et al (2015) Biological properties of extracellular vesicles and their physiological functions. J Extracell Vesicles 4. https://doi.org/10.3402/jev.v4.27066

Yang C, Gao S, Song P et al (2018) Theranostic niosomes for efficient siRNA/microRNA delivery and activatable near-infrared fluorescent tracking of stem cells. ACS Appl Mater Interfaces 10:19494–19503. https://doi.org/10.1021/acsami.8b05513

Yao Y, Zhou Y, Liu L et al (2020) Nanoparticle-based drug delivery in cancer therapy and its role in overcoming drug resistance. Front Mol Biosci 7

Ye H, Wang K, Lu Q et al (2020) Nanosponges of circulating tumor-derived exosomes for breast cancer metastasis inhibition. Biomaterials 242:119932. https://doi.org/10.1016/j.biomaterials.2020.119932

Yetisgin AA, Cetinel S, Zuvin M et al (2020) Therapeutic nanoparticles and their targeted delivery applications. Molecules 25:E2193. https://doi.org/10.3390/molecules25092193

Yingchoncharoen P, Kalinowski DS, Richardson DR (2016) Lipid-based drug delivery systems in cancer therapy: what is available and what is yet to come. Pharmacol Rev 68:701–787. https://doi.org/10.1124/pr.115.012070

Zavaleta C, Ho D, Chung EJ (2018) Theranostic nanoparticles for tracking and monitoring disease state. SLAS Technol 23:281–293. https://doi.org/10.1177/2472630317738699

Zhao AG, Shah K, Cromer B, Sumer H (2020) Mesenchymal stem cell-derived extracellular vesicles and their therapeutic potential. Stem Cells Int 2020:8825771. https://doi.org/10.1155/2020/8825771

Zhao C-Y, Cheng R, Yang Z, Tian Z-M (2018) Nanotechnology for cancer therapy based on chemotherapy. Molecules 23:826. https://doi.org/10.3390/molecules23040826

Zheng X-C, Ren W, Zhang S et al (2018) The theranostic efficiency of tumor-specific, pH-responsive, peptide-modified, liposome-containing paclitaxel and superparamagnetic iron oxide nanoparticles. Int J Nanomedicine 13:1495–1504. https://doi.org/10.2147/IJN.S157082

Zhou J, Rossi J (2017) Aptamers as targeted therapeutics: current potential and challenges. Nat Rev Drug Discov 16:181–202. https://doi.org/10.1038/nrd.2016.199

Zhou Y, Zhang M, He D et al (2016) Uricase alkaline enzymosomes with enhanced stabilities and anti-hyperuricemia effects induced by favorable microenvironmental changes. Sci Rep 7:20136. https://doi.org/10.1038/srep20136

Zhu L, Zhou Z, Mao H, Yang L (2017) Magnetic nanoparticles for precision oncology: theranostic magnetic iron oxide nanoparticles for image-guided and targeted cancer therapy. Nanomedicine (Lond) 12:73–87. https://doi.org/10.2217/nnm-2016-0316

Zhuang M, Chen X, Du D et al (2020) SPION decorated exosome delivery of TNF-α to cancer cell membranes through magnetism. Nanoscale 12:173–188. https://doi.org/10.1039/c9nr05865f

Theranostic Applications of Functionalized Polymeric Nanoparticles

4

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Abstract

Nanoparticles offer a potential alternative for drug delivery with a promising targeting efficiency attributed to their size and tailor-made functional groups. Further nanoparticles are also showing their candidature to be explored for theranostic potential in various research investigations to treat and diagnose different diseases including cancer, infectious diseases, and neurodegenerative disorders. Polymers, metals, and lipids are used to prepare different nanoparticles, i.e., polymeric, metallic, and lipidic nanoparticles, respectively. Availability of vast number of polymers with different polymeric architecture, ease of synthesis and surface modification make polymeric nanoparticle an attractive nano-scaffold for various therapeutic, imaging, and theranostic applications. In this chapter we have reviewed polymeric nanoparticles, their composition, and polymers used to develop nanoparticles with emphasis on internal and external stimuli sensitive delivery and theranostic applications of functionalized polymeric nanoparticles.

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Keywords

Polymers · Drug delivery · Diagnosis · Imaging agent · Stimuli responsive delivery

4.1 Introduction

The emergence of diseases and disorders has made it more important than ever to treat the condition and restore the patient's health. Conventional dosage forms have a number of drawbacks that prevent them from achieving steady-state drug concentration and drug targeting, such as multiple administrations of the therapeutic agent with a shorter half-life, lessened patient compliance, and increased toxicity. This restriction calls for the creation of functionalized and targeted medication delivery systems that improve the limitations of conventional preparation. Due to their sustained release property, site specificity, and high patient compliance, functionalized and targeted therapeutic delivery is used for the treatment of disease (Suthar et al. 2022; Patel et al. 2023).

Functionalized therapeutic nanomedicines are established for the diagnosis as well as treatment of different ailments. It offers a high level of drug delivery and transportation to the intended site for therapy and diagnosis. The physicochemical properties of the nano-formulation, such as size, shape, and the type of ligand attached for identifying and activating the receptor, extend the cellular and subcellular reach of the functionalized nano-formulation. As a result, developing nano-formulation requires very precise processes and control of the physicochemical properties. A variety of active drugs, such as imaging agents, proteins, peptides, and anticancer drugs may be given using several types of nano-formulation, including polymeric, lipid, and metals. Due to their structure's flexibility and ease of modification, polymeric nanocarriers have drawn a lot of attention. They can also be transported to the desired location for action or response in response to environmental or physiological stimuli. Functionalization of polymeric nanocarriers can reduce sensitivity in biological environments when exposed to various stimuli like temperature, radiation, UV light, magnetic fields, oxidation, reduction, and enzymatic conditions, which may directly influence payload on NPs, biodistribution, biodegradability, stability, and biocompatibility of the therapeutic agent (Feng et al. 2014; Dong et al. 2014).

The therapeutic use, route of administration, and target site all influence the structure of the polymeric NPs. Polymeric NPs are typically administered intravenously, although they can also be administered orally, topically, or mucosally. In those circumstances, NPs must be provided by smart excipients and be able to target different body parts. Functionalized polymeric NPs have been developed to support in treating and diagnosing diseases such as cancer, viral infections, cardiovascular illness, lung disease, and urinary tract infections. Advanced polymeric NPs with variable size, shape, and charge are made possible by polymerization (O'Reilly et al. 2006). The vast surface area of polymeric NPs allows for the attachment of many

ligands or functional groups. Polymeric NPs can quickly connect to capillaries and enter the target region. Because it has a longer clearance period than a typical dosage form, a smaller amount of the drug can still have good therapeutic effects while reducing toxicity.

Numerous researchers have reported that functionalized polymeric nanoparticles improve the efficacy of various medicinal treatments. Saroj and its affiliates developed functionalized, pH-sensitive polymeric NPs of etoposide to treat cancer. At blood pH, PNs showed a significantly higher release. It was significantly more hazardous to PC-3 and LNCaP, two types of prostate cancer cells, than pure etoposide (Saroj and Rajput 2018). Utilizing poly-lactic acid polymer and polyethylene glycol to adorn the surface, Patil and his collaborators created the functionalized polymeric NPs of paclitaxel. Biotin and folic acid functionalized the NPs, which were then verified by an NMR investigation. The ligand significantly increased NP accumulation at the mouse tumor site (Patil et al. 2009). For the purpose of addressing liver cancer cells, Zhu et al. developed the polydopamine functionalized NPs loaded with docetaxel using TPGS and polylactic acid polymer. The liver cancer cell line HepG2 was significantly taken up by the NPs. It also reduces the size of hepatoma tumors in nude mice much more than nonfunctionalized NPs (Zhu et al. 2016).

4.2 Composition

There are various types of polymer used for the development of functionalized NPs which are discussed below.

(a) Polyethylene Glycol Transferrin

Transferrin is a blood plasma glycoprotein containing iron bonds. Transferrin maintains the free iron level in biological fluids. Transferrin was established to preserve the character of PEGylation as well as allow NPs to reach the tumor site after recombinant human necrosis factor-alpha. This combination mainly exhibited preferential specificity for target tumor cells. Gan and his team formulated transferrin conjugate polylactic acid-TPGS pegylated NPs for brain delivery using Coumarin 6 as imaging or docetaxel as therapeutic agents. The conjugated NPs significantly crossed the blood-brain barrier and showed a significantly lower IC_{50} than unconjugated NPs (Gan and Feng 2010). Li et al. formulated the transferrin-coupled polyethylene glycol for gene delivery (PDNA). It exhibited prolonged drug release after an initial burst release (30%). It also showed significantly greater enhancement in binding to K562 cells than non-targeted NPs (Li et al. 2003). Wang et al. formulated the transferrin and folate conjugate Pluronic/poly (lactic acid) NPs loaded with paclitaxel. It showed an initial fast release, followed by a slow release over time. It was found to be toxic to cancer cells and penetrated cells better than non-targeted NPs (Wang et al. 2022).

b. Gelatin

Gelatin is a water-soluble polymer obtained from the chemical degradation of collagen. It is biocompatible and biodegradable. It did not cause any side effects after administration into the body. It provides the slow release of drugs (Manna et al. 2016). Chen et al. used photothermal therapy for breast cancer cells using folic acid-functionalized gelatin and gold nanoparticles. It showed high photothermal conversion power and killed the breast cancer cells under near-infrared laser radiation. Subcutaneous implantation of NPs showed excellent photothermal potential against breast cancer cells (Chen et al. 2020).

c. Alginate

It is an alginate-based natural, biocompatible, and biodegradable polymer used as a carrier of various drugs. It is a co-polymer of (1, 4) linked β-D mannuronate and α-l-glucuronate. It is water soluble in nature and mostly used for the preparation of nanoparticles, microparticles, hydrogels, etc. It is also used for the preparation of functionalized polymeric NPs (Bagre et al. 2013). Sahatsapan et al. formulated the *Garcinia mangostana* L extract loaded alginate NPs. The prepared NPs were further functionalized with catechol for intravesical chemotherapy. It exhibited high drug load, sustained release, and high stability. It accumulated in bladder tissue and MB49 cells at a higher rate than pure *Garcinia mangostana* L. extract (Sahatsapan et al. 2020). Fan et al. formulated doxorubicin-loaded sodium alginate NPs which are functionalized with adipic acid dihydrazide. It exhibited significantly higher release in the tumor environment at pH 5 than at pH 6.5. It also showed significantly high cellular uptake into Hela cells overexpressing CD44 and improved cell toxicity (Fan et al. 2016). Zhang and his colleagues formulated 5-fluorouracil-loaded sodium alginate NPs and functionalized them with graphene oxide for colon cancer. It showed the 5-fluorouracil properly released into the colon in a sustained manner. It was also observed that tumor growth significantly decreased with pure 5-fluorouracil. It also exhibited a controlled loading dose as well as improved bioavailability (Zhang et al. 2017a, b, c). Xie et al. developed graphene oxide functionalized sodium alginate NPs for the delivery of peptide protamine sulfate for doxorubicin delivery in another study. It showed that NPs were stable in various pH conditions as well as inhibited protein interaction. It also showed site-specific action against MCF-7 cells and higher cytotoxicity than plain doxorubicin (Xie et al. 2018).

d. Poly (Lactic-co-Glycolic) Acid

It is a synthetic biodegradable and biocompatible polymer that easily hydrolyzes into the body. It is water-insoluble and dissolved in various organic solvents like acetone, methanol, tetrahydrofuran, and ethyl acetate. PLGA is broadly applicable for the development of NPs, microspheres, and microcapsules (Table 4.1).

Table 4.1 Theranostic polymers and their applications

Polymers	Drug	Application	References
Poly(amido amine)	Paclitaxel/ Fluorophore	Cancer therapy and imaging	Xin et al. (2010)
Dextran	Radiolabeling	Cancer imaging	Hudecz et al. (1995)
PLA-PEG Poly(lactic acid)-poly(ethylene glycol)	Paclitaxel	Cancer therapy	Chen et al. (2009)
PCL-PEG Polycaprolactone poly (ethylene glycol)	Docetaxel	Cancer therapy	Li et al. (2012)
PAA-PEG Poly(aspartic acid)-poly (ethylene glycol)	Platinum	Cancer therapy	Raut et al. (2010)
Hyaluronic acid	Paclitaxel	Cancer therapy	Singer et al. (2005)
Poly(lactic-co-glycolic acid)	Docetaxel	Cancer therapy	Zarabi et al. (2009)
Poly(ethylene glycol)	Camptothecin	Cancer therapy	Zhu et al. (2011)
PEI, PEG, and folic acid	SPIONs	Breast cancer	Huang et al. (2017)
Graphene oxide	SPIONS	Metastatic breast cancer	Yang et al. (2012)

4.3 Types of Theranostic Polymers

Theranostic polymeric nanomaterial has become very popular and has been used as a carrier for the diagnosis and treatment of cancer. Polymeric NPs have been developed to enhance the efficacy of drugs. The polymeric nanomaterial provides a long circulation time, leading to increased beneficial effects as well as reduced side effects (Jain and Zhong 2022; Greco and Vicent 2009). Functionalized polymeric NPs can load the drug for the target area and regulate the release of the drug at a personalized dose and time, which may enhance the therapeutic responses and minimize the side effects. The stimuli-responsive polymeric NPs are capable of controlling the release of therapeutic agents in disease areas (Ke et al. 2019) and can be used for the cure of various tumors (Fleige et al. 2012; Yu et al. 2014a, b). The responsive system can be classified into two types, i.e., internal as well as external stimuli. The pH, enzyme, redox potential, and hypoxia are the internal stimuli. However, light, magnetic field, temperature, ultrasound, and radiation are external stimuli (Karimi et al. 2016). On applying stimuli, the physiochemical characteristics of polymeric NPs change (permeability, hydrophilicity, or hydrophobicity), leading to imaging or therapeutic agent release to the target site.

4.3.1 Internal Stimuli

(a) pH-Responsive Polymers

The pH-sensitive polymeric material is very interesting and commonly used to design responsive polymeric NPs. Due to the fast metabolism, the high amount of lactic acid secreted by cancer cells may give a toxic effect on the nearest tissue at pH 5.7–6.9. There are various pH stimuli-responsive polymeric NPs which have been reported by the researcher for the treatment of cancer cell (Kanamala et al. 2016). Chang et al. formulated poly[(D, L-lactide)-co-glycolide]-PEG-poly[(D, L-lactide) coglycolide] polymeric micelle and functionalized with N-bochistidine loaded with doxorubicin. The N-bochistidine increases the biodegradability and biocompatibility of the micelles. The release of DOX at pH 6.2 in the cancer environment than pH 7.4 in the normal tissue increases the therapeutic activity (Chang et al. 2010). Polymeric NPs can increase intracellular drug delivery and reduce the release of drug in the extracellular space. Hu et al. developed the pH-sensitive doxorubicin-loaded polymeric micelles. The micelles decrease the tumor cell due to the high accumulation of micelles (Hu et al. 2012). Yu et al. formulated the polymeric micelles of curcumin using pH-sensitive co-polymer methoxy poly (ethylene glycol)-poly(lactide)-poly (β-amino ester) polymeric formulation. The polymeric micelles exhibited high drug loading and remain stable at 37 °C. It showed significantly high cellular uptake in breast cancer cells when the pH fell from 7.4 to 5.5 and particle size decreases from 171 to 22 nm. It also showed a longer circulation time than simple micelles and deposited into tumors with high fluorescent intensity and 65.6% inhibition of tumor (Yu et al. 2014a, b). In another research, Zhao et al. formulated the mixed micelles using different polymers loaded with doxorubicin. The micelles showed significantly improved cytotoxicity of the tumor by binding of the ligand with the membrane and penetrated by endocytosis to the tumor and releasing the doxorubicin (Zhao et al. 2010). Xiong and associates formulated the pH-sensitive polymeric micelles loaded siRNA and doxorubicin using poly (ethylene oxide)-blockpoly (ε-caprolactone) biodegradable polymer. The doxorubicin was significantly released in an acid environment through hydrazone linkage. The siRNA inhibited Pgp expression and doxorubicin easily penetrated into MDA-MB-435 tumor models (Xiong and Lavasanifar 2011). Further, Ling et al. formulated polymeric NPs using pH-responsive ligands. The formulation showed higher therapeutic activity in heterogeneous drug resistance tumors (Ling et al. 2014).

(b) Redox-Responsive Polymers

The redox potential is a new technique to regulate drug release from polymeric NPs. The redox potential developed between healthy and tumor tissues as well as between intracellular and extracellular areas (Zhang et al. 2017). The glutathione tripeptide (γ-glutamyl-cysteinylglycine) (GSH) concentration is higher (4 times) in cancer cells than in normal cells (Thambi et al. 2016). However, the GSH level in intracellular (2–10 mM) is approximately 100–1000 times higher than in extracellular (2–10 μM) (Han et al. 2017). Wang et al. formulated the polyanhydride copolymer comprising

disulfide bonds among the hydrophobic and hydrophilic sections. The copolymer self-assembled into a core-shell structure and GHS activated the micelles for disarrangement. The micelles showed significantly high embarrassment of tumor cells in mice due to the fast penetration of the therapeutic agent. The redox stimulating micelles showed improved therapeutics effect in solid tumors than non-redox micelles and prevented MDR resistance and enhanced overall anticancer activity (Wang et al. 2021). Liu and his group formulated the redox-responsive doxorubicin prodrug micelles using dextran-poly (ethylene imine) copolymers and bind through a disulfide link. The polymeric micelles exhibited 100–140 nm size and fast drug release under the influence of an intracellular redox environment. The micelles improved the deposition of doxorubicin in MCF-7/ADR cells. The MTT result showed prodrug micelles significant antitumor activity than pure doxorubicin. So redox-sensitive formulation could be a remarkable delivery to overcoming MDR (Liu et al. 2013).

Han and his group formulated the polymeric NPs of doxorubicin using hyaluronic acid (HA)-polycaprolactone block polymer via disulfide linkage. The NPs significantly reduce the drug release under pH 7.4 and increased in existence of GSH bonds in the cytoplasm. It exhibited significantly higher therapeutic activity in cancer cells than non-crosslink polymer and free drugs (Han et al. 2015). Chiang et al. formulated the redox-sensitive micelles of camptothecin for the cure of selective cancer. The micelles released the camptothecin in tumor cells by activating reactive oxygen species and GSH. The reacting oxygen species stimulating diethyl sulfide of micelles causes swelling and GSH to promote the breaking of co-polymer and release of the drug into cancer cells (Chiang et al. 2015).

c. Enzymatic-Responsive Polymers

Enzyme-stimulating polymers were used as a drug carrier for the treatment of cancer (Mu et al. 2018). The enzymic is more efficient and faster than other stimuli. Various types of enzymes like proteases and phosphatases have been detected as biomarkers for the treatment and diagnosis of various diseases (He et al. 2016). The metalloproteinases (MMPs) enzyme is used to stimuli of enzyme-responsive systems in cancer theranostics. The MMPs are zinc-dependent endopeptidases accountable for the deprivation of extracellular matrix protein as well as regulated the bioactive substance on cells (Khokha et al. 2013). The expression of this enzyme is highly expressed in tumor cells than in normal cells and indorses tumor metastasis. As per the level of expression level variation, the MMPs are stimulators, and different nano-carrier systems are reported for diverse uses (Gallo et al. 2014; Callmann et al. 2015). Zhu et al. formulated the MMP2-sensitive polymeric micelles loaded with siRNA and hydrophobic drug using a PEG-ppPEI-PE copolymer. It exhibited good stability and easily penetrated cancer cells by enhancing the permeation effect and MMP2 activation (Zhu et al. 2014).

4.3.2 External Stimuli

(a) Light-Triggered Polymers

Light is the most generally employed stimulator for polymeric NPs to release the drug for treatment of any disease. The photothermal and photodynamic therapy (PTT and PDT) mostly used as light source for stimulation. The PTT is applied as a light-sensitive material that alters the light energy to heat and increases the temperature and activated NPs around tumor cell as well as cell death (Liu et al. 2019a, b). The PTT permits the precise dose of radiation to reduce the side effect of the normal cell around the cancer cell. PTT can also be used in combination with other therapy such as chemotherapy, surgery, and radiotherapy as a synergistic effect to increase the therapeutic effect (Liu et al. 2014; Yong et al. 2015). Bagheri and his research team developed light-sensitive pyrene-containing nanoparticles. The pyrene moieties stimulated the hydrophilic and hydrophobic transition of block polymer and fragment of the NPs, and then PTT stimulate the therapeutic compound for release into tumor cells (Bagheri et al. 2019). PDT is also an important light source for stimulating light-sensitive material. It is used at a definite wavelength, to produce the cytotoxic ROS and oxidize cellular macromolecules as well as stimulate tumor cell removal (Lucky et al. 2015). Light-stimulating polymeric NPs also use photo-induced drug release irradiated by external light. The mechanism involved a light-stimulating chemical effect, reducing hydrophobicity and photothermal effect (Son et al. 2019).

(b) Temperature-Responsive Polymers

Temperature is also usually applied as external inducer to stimulate the thermosensitive nanomaterial for releasing of the drug at desired site. The temperature-sensitive material can respond to changes in the temperature and destabilize the structure or change the aqueous solubility and release the drug to the target area (Karimi et al. 2016). The drug can be simply incorporated into the polymers at LCST and released at the desired site after applying external temperature. Choi and associates formulated the temperature-responsive polymeric NPs using pluronic/polyethyleneimine. The NPs showed swelling/deswelling behavior at 24–37 °C and PS size decreased from 330 to 100 nm, so free diffusion of encapsulated drug takes place due to high porosity (Choi et al. 2006). Goodall and his team formulated the thermosensitive polymeric NPs using *N*-isopropyl acrylamide (NIPAM) and decorated with scFv antibody targeting epidermal growth factor receptor (EGFR) expressed in the cancer cell. It significantly binds the MDA MB-468 cancer cells (Goodall et al. 2015). Similarly, Zeighamian et al. developed the curcumin loaded polymeric NPs using poly (N-isopropylacrylamide-co-methacrylic acid) (PNIPAAm–MAA) for MCF-7 breast cancer cells. NPs showed significantly high cytotoxicity against MCF-7 cells than pure curcumin (Zeighamian et al. 2015).

(c) Magnetic-Responsive

Magnetic-responsive formulation contains the magnetite or maghemite core, so it is called the superparamagnetic iron oxide NPs. By applying of the magnetic filled

NPs increases the accumulation of drug at the site of action. The magnetic-responsive system has the property of diagnosis in a single formulation and therapy so-called theranostic (Yildiz and Yildiz 2015). The magnetic polymeric NPs must be stable and biocompatible as well as functionalized in various uses. Basuki and associates formulated the α-D-mannose-functionalized diblock PEG-glycopolymer and coated with magnetic NPs for the lung cancer. It exhibited significantly increased cellular uptake into lung cancer cell (Basuki et al. 2014). In another study, Jaidev et al. formulated the fluorescent IONPs and gemcitabine encapsulated NPs using PLGA polymer and decorated them with HEGF antibodies for pancreatic cancer. It significantly enhanced tumor retention and inhibited the growth of tumors (Jaidev et al. 2015).

4.4 Theranostic Applications of Functionalized Polymeric Nanoparticles

Polymers are large molecules made up of several repeating subunits. These macromolecules polymers have been successfully used for carrier of a variety of therapeutic agents to the body. Due to their electro-optical and photoluminescence characteristic, these types are quite interesting (Pecher and Mecking 2010) for drug delivery. Biodegradable NPs offer an advantage over non-biodegradable NPs such as surface-to-mass ratio, quantum characteristics, biodegradability, decreased toxicity, and capacity to adsorb and transport other molecules into body (Wu et al. 2020) (Table 4.2).

4.4.1 Application in Bioimaging

In comparison to inorganic nanomaterials, polymeric NPs have advantages like biocompatibility, stability, biodegradability, and inexpensive (Abelha et al. 2020). Smart polymers, or stimulus-responsive polymers, are extremely effective that adapt

Table 4.2 List of polymeric delivery systems under clinical trials (Chang et al. 2021)

S. no	Polymer	Drug	Formulation	Clinical phase
1	Poly(L-glutamic acid)	Paclitaxel	XYOTAX (CT-2103)	II
2	Poly(L-glutamic acid)	Camptothecin	CT2106	II
3	Cyclodextrin-PEG	Docetaxel	CRLX301	I/IIa
4	Polyethylene glycol	Cisplatin	SPI-77	II
5	Pluronic R P-61 and F-127 block copolymers	Doxorubicin	SP1049C	II
6	Polyethylene glycol	Irinotecan	NKTR-102 4-arm	I/II

to their surroundings. In addition to light intensity and wavelength, responsive polymers can also be sensitive to electrical and magnetic fields, humidity, chemical compounds, temperature, and pH levels. These materials may react in a variety of ways, such as changing their transparency or color, turning into water conductive materials, or changing their shape. Usually, only very slight environmental modifications are required to cause a polymer's properties to alter (Chatterjee and Hui 2019). Bioimaging is a non-invasive technique for tracking biological behavior over time that doesn't interfere with normal life processes like movement and respiration while also making it easy to capture the 3D structure of the specimen. It helps multicellular organisms' tissues and studies of subcellular structure to be connected (Xu 2018).

4.4.2 Optical Imaging

Most commonly used imaging technique is optical imaging for diagnosis of disease (Liu et al. 2019a, b). Due to the exceptionally low tissue absorption, the optical imaging systems use near-infrared (NIR), i.e., 700–1000 nm (Carr et al. 2018). Fluorescent NIR probes like cyanine compounds are low molecular weight organic molecules with exceptional optical properties which are used (Nagamani et al. 2019) for imaging. Cyanine were added to various polymer compositions in an effort to increase bioavailability and durability in order to alleviate these restrictions like NIR fluorescent probes insertion in lipooligosaccharides (Wang et al. 2019), water-soluble carboxylated N-acylated poly (amino ester)-based polymers (Mahmoud et al. 2019), and polymer micelles have all been reported (Shao et al. 2019). Yang and his research group developed a self-assembled polymer nanocarrier for imaging and anticancer therapy (Yang et al. 2020). Their nanocarrier is comprised of PEG that has hydrophobic poly (ortho ester) and hydrophilic poly (glycidyl methacrylate) modified with ethylenediamine (PEG-g-p(GEDA-co-DMDEA)).

4.4.3 Ultrasound Imaging

In ultrasound imaging technique, the sound waves transferred to the body at a frequency of 2 MHz or higher for diagnosis. It is a cheap, non-invasive, efficient, and real-time imaging technique (Liu et al. 2019a, b). This strategy is widely used in the cancer treatment and received the greatest attention. Yang et al. described multifunctional PLGA nanobubbles as theranostic agents and doxorubicin for breast cancer (Yang et al. 2015). MCF-7 cancer cells that had been treated with doxorubicin and P-gp siRNA as a platform for performing cellular ultrasound imaging. In another study, nanobubble-paclitaxel loaded liposomes prepared for ultrasound-sensitive drug and ultrasound imaging were recorded (Prabhakar and Banerjee 2019).

4.4.4 Magnetic Resonance Imaging

MRI contrast agents (CAs) can modify the relaxation periods of protons in various organs on contact with external magnetic field (Luk and Zhang 2014). The low molecular weight complexes are unable to produce accurate MRI images of the tumor. It has shown significant promise to solve these drawbacks to encapsulate contrast agent with polymeric NPs especially smart polymers that respond to tumor-specific stimuli (Vijayan and Muthu 2017; Hu 2020). There are different types of formulations reported for MRI imaging like nanogels, polymersomes, and micelles. Aouidat and his team explained the gold core-shell NPs (Gd(@AuNPs) are a major component of the new Gd (III)-biopolymer-Au (III) complex. They demonstrated the advantages of Gd@AuNPs for treating the hepatocytes in the liver. It provides a potent cellular uptake of Gd@NPs and conserving a T1 contrast inside cells that allows consistent in vivo detection using T1-weighted MR imaging.

4.4.5 Photoacoustic Imaging

This is a visualization method and also known as photoacoustic imaging, and was just recently discovered. This technique generates localized heat and thermoelastic stress waves when tissues absorb a brief light pulse effect (Valluru and Willmann 2016). Lyu and associates developed an intraparticle molecular orbital engineering technique to increase the effectiveness of polymeric NPs for phototherapy and photoacoustic illumination for cancer therapy. They showed that it can be used with theranostic nanoagents to produce superior photoacoustic imaging (Lyu et al. 2016).

4.4.6 X-Ray Computed Tomography

The visualization method known as photoacoustic imaging was just recently discovered. This technique generates localized heat and thermoelastic stress waves when tissues absorb a brief light pulse effect. The delivery system made of polymers is the best choice for addressing biocompatibility and biodegradability issues (Zhou et al. 2016; Zhang et al. 2017a, b, c). Lyu et al. (2016) developed an intraparticle molecular orbital engineering technique to increase the effectiveness of polymeric NPs for cancer therapy. They showed that it can be used with theranostic nanoagents to produce superior photoacoustic imaging.

4.4.7 Radionuclide Imaging

Numerous investigations have concentrated on the development of polymeric nanovehicles with the radionuclide imaging (Di Mauro et al. 2015). Sun and his research team described a polymeric carrier using a hydrophobic poly (FTS) block, a

hydrophilic poly (oligo (ethylene glycol) (POEG) block, and a central block of poly (4-vinylbenzyl azide). They developed a farnesylthiosalicylate-based, triblock copolymer. Radio-labeled PTX/POVF nano-micelles were rapidly absorbed and slowly excreted from tumor tissues in the mice using PET imaging (Sun et al. 2018). In another study, Le Goas et al. (2019) formulated the hybrid poly (methacrylic acid)-grafted gold NPs to enhance systemic absorption in tumor-bearing mice. This research examined a nanomedicine technique for lowering the dose of radioiodine to accomplish RT imaging.

4.4.8 Radioactive Polymeric Nanoparticles for Imaging and Therapy

In addition to providing functional and molecular imaging, radioactive nanoparticles (RNPs) are also helpful for diagnosing since theranostic applications. The radioactive polymeric NPs can be developed by using one of two approaches for achieving the imaging goals. This approach is adaptable and can be used in functionalization chemistry to add different radioelements into ligands on the surface of NPs. RNPs based on polymeric NPs have many benefits over a large number of NPs that have undergone preclinical and clinical testing. In contrast to conventional radionuclide therapy, RNPs have a higher payload capacity for radionuclides that can be employed for non-invasive imaging and/or therapy. Concentration of radioactive material used for nuclear imaging or radiotherapy depends upon types of radioactive material (El-Say and El-Sawy 2017). By boosting the accumulation of the diseased tissue target areas through passive or active targeting, the RNPs have the potential to significantly enhance therapies and diagnostics. It was observed that DTPA-derivatized liposomes and radiolabeled micelles have been frequently utilized to study the biodistribution studies (Psimadas et al. 2012). In addition, they did not exhibit high intestinal excretion 12 h after injection but showed high accumulation of NPs in the liver and spleen due to high radioactivity levels in healthy Lewis rats (Psimadas et al. 2012; Lim et al. 2016). In a different study, the ^{111}In-labeled polymeric NPs with a radiosensitizer based on ruthenium were found to be targeted therapeutic effects in tumor that overexpress EGFR (Ng et al. 2014; Gill et al. 2018). Nano radiopharmaceuticals based on 99mTc and rhenium-186 have become crucial tools for the diagnosis and treatment of several illnesses or dysfunctions of organs and systems (Hua et al. 2015; Costa et al. 2019). A nicotinic acid (HYNIC)-type ligand system was utilized to label with 99mTc during the radiolabeling process, which was carried out via a direct labeling strategy (Kovacs et al. 2014). Direct irradiation of NPs, direct labeling, utilizing radioactive species as raw materials, and indirect labeling using radioactive species as raw materials are the ways to produce polymeric radioactive NPs (Lamb and Holland 2018). The fundamental issue with direct irradiation of NPs, especially polymeric NPs, is the heating and damage to the nanostructure that the high c-radiation background and irradiation process induce (Haume et al. 2016; Lamb and Holland 2018).

4.4.9 Application in Infectious Diseases

Pathogens like viruses, bacteria, fungus, and parasites can cause infectious diseases. These microbes quickly reproduce and alter homeostasis. A rise in the totality and morbidity index is highly correlated with the occurrence of various diseases (Jain et al. 2015a, b; Chauhan et al. 2022; Juneja et al. 2022; Bagre et al. 2022). By utilizing radio-labeled chitosan-leukocytes, Fairclough and associates developed the radio-labeled chitosan-leukocytes for enhancement of diagnosis of inflammatory process (Fairclough et al. 2016). The ^{89}Zr and ^{64}Cu were used for radiolabeling NPs. The findings indicated that compared to ^{64}Cu-chitosan NPs, the ^{89}Zr-chitosan NPs displayed a reduced efflux (Fairclough et al. 2016) of therapeutic agents. Santos et al. formulated the betamethasone and dexamethasone PLA (poly-lactic acid) NPs labeled with 99mTc and reported an in vivo model of *Staphylococcus aureus* infection and inflammation. The 99mTc-PLA NPs-betamethasone showed accumulation at the *S. aureus* inflammation site, demonstrating the potential application of this technology for the detection of infection and inflammation foci in vivo (Santos et al. 2017). Pterostilbene and crude grape pomace extract loaded in PLGA-NPs were tested by Simonetti and colleagues against a *Candida albicans* biofilm using six coumarin fluorescence probes. These PLGA-NPs showed a substantial suppression of *C. albicans* biofilm (Simonetti et al. 2019). Helal-Neto et al. developed 99mTc labeled ethambutol NPs using PCL (poly-caprolactone) polymer. The outcomes demonstrated that these NPs have a theranostic impact on the *Mycobacterium bovis* strain both in vitro and in vivo (Helal-Neto et al. 2019).

4.5 Conclusion

Over last few decades, significant advancements in application of nanomedicine have been witnessed to diagnose and treat different diseases and ailments including cancer by exploiting phenomena of targeting delivery based on enhanced permeability and retention (EPR) effect, pH-responsive delivery, or ligand-based targeting. Nanoparticles made of polymers and metals have been explored by scientists for therapeutic, imaging, and theranostic application. Although metal-based nanoparticles have shown various applications in the field of biomedical sciences including therapeutics and diagnostics as well as in the fields like electronics due to their unique physicochemical properties, yet use of metal-based nanoparticles is limited by colloidal instability, related toxicity issues, and non-specific interactions with biological systems. In contrast, polymeric nanoparticles offer advantages of versatility, biocompatibility and biodegradability, colloidal stability, and site specificity which could be further improved by surface functionalization. Furthermore nano-hybrids or nano-conjugates of polymer and metallic nanoparticles may be designed as a multifunctional nanomedicine to circumvent challenges of each other and to potentiate the drug delivery efficiency as well as for simultaneous delivery of imaging and/or therapeutic agents in nanotheranostics.

References

Abelha TF, Dreiss CA, Green M, Dailey LA (2020) Conjugated polymers as nanoparticle probes for fluorescence and photoacoustic imaging. J Mater Chem B 8:592–606

Bagheri A, Boyer C, Lim M (2019) Synthesis of light-responsive pyrene-based polymer nanoparticles via polymerization-induced self-assembly. Macromol Rapid Commun 40: e1800510

Bagre AP, Jain K, Jain NK (2013) Alginate coated chitosan core shell nanoparticles for oral delivery of enoxaparin: in vitro and in vivo assessment. Int J Pharm 456(1):31–40. https://doi.org/10.1016/j.ijpharm.2013.08.037

Bagre A, Patel PR, Naqvi S, Jain K (2022) Chapter 1 - Emerging concerns of infectious diseases and drug delivery challenges. In: Nanotheranostics for treatment and diagnosis of infectious diseases: developments in microbiology. Elsevier, pp 1–23

Basuki JS, Esser L, Duong HTT, Zhang Q, Wilson P, Whittaker MR, Haddleton DM, Boyer C, Davis TP (2014) Magnetic nanoparticles with diblock glycopolymer shells give lectin concentration-dependent MRI signals and selective cell uptake. Chem Sci 5:715–726

Callmann CE, Barback CV, Thompson MP, Hall DJ, Mattrey RF, Gianneschi NC (2015) Therapeutic enzyme-responsive nanoparticles for targeted delivery and accumulation in tumors. Adv Mater 27:4611–4615

Carr JA, Franke D, Caram JR, Perkinson CF, Saif M, Askoxylakis V, Datta M, Fukumura D, Jain RK, Bawendi MG (2018) Shortwave infrared fluorescence imaging with the clinically approved near-infrared dye indocyanine green. Proc Natl Acad Sci U S A 115:4465–4470

Chang G, Li C, Lu W, Ding J (2010) N-Boc-histidine-capped PLGA-PEGPLGA as a smart polymer for drug delivery sensitive to tumor extracellular pH. Macromol Biosci 10:1248–1256

Chang D, Ma Y, Xu X, Xie J, Ju S (2021) Stimuli-responsive polymeric nanoplatforms for cancer therapy. Front Bioeng Biotechnol 9:707319

Chatterjee S, Hui C-L (2019) Review of stimuli-responsive polymers in drug delivery and textile application. Molecules 24:2547

Chauhan S, Jain K, Naqvi S (2022) Chapter 8 - Dendrimers and its theranostic applications in infectious diseases. In: Nanotheranostics for treatment and diagnosis of infectious diseases: developments in microbiology. Elsevier, pp 199–228

Chen J, Zeng F, Wu SZ, Su J, Tong Z (2009) Photoreversible fluorescent modulation of nanoparticles via one-step miniemulsion polymerization. Small 5:970–978

Chen H, Wang X, Sutrisno L, Zeng T, Kawazoe N, Yang Y, Chen G (2020) Folic acid-functionalized composite scaffolds of gelatin and gold nanoparticles for photothermal ablation of breast cancer cells. Front Bioeng Biotechnol 8:589905

Chiang YT, Yen YW, Lo CL (2015) Reactive oxygen species and glutathione dual redox-responsive micelles for selective cytotoxicity of cancer. Biomaterials 61:150–161

Choi SH, Lee SH, Park TG (2006) Temperature-sensitive pluronic/poly(ethylenimine) nanocapsules for thermally triggered disruption of intracellular endosomal compartment. Biomacromolecules 7:1864–1870

Costa B, Ilem-Ozdemir D, Santos-Oliveira R (2019) Technetium-99m € metastable radiochemistry for pharmaceutical applications: old chemistry for new products. J Coord Chem 72:1759–1784

Di Mauro PP, Goómez-Vallejo V, Baz Maldonado Z, Llop Roig J, Borroós S (2015) Novel 18F labeling strategy for polyester-based NPs for in vivo PET-CT imaging. Bioconjug Chem 26: 582–592

Dong J, Zhang R, Wu H, Zhan X, Yang H, Zhu S, Wang G (2014) Polymer nanoparticles for controlled release stimulated by visible light and pH. Macromol Rapid Commun 35(14): 1255–1259

Dos Santos SN, Dos Reis SRR, Pinto SR et al (2017) Anti-inflammatory/ infection PLA nanoparticles labeled with technetium 99m for in vivo imaging. J Nanopart Res 19:345

El-Say KM, El-Sawy H (2017) Polymeric nanoparticles: promising platform for drug delivery. Int J Pharm 528:675–679

Fairclough M, Prenant C, Ellis B et al (2016) A new technique for the radiolabelling of mixed leukocytes with zirconium-89 for inflammation imaging with positron emission tomography. J Labelled Comp Radiopharm 59:270–276
Fan L, Ge H, Zou S, Xiao Y, Wen H, Li Y, Feng H, Nie M (2016) Sodium alginate conjugated graphene oxide as a new carrier for drug delivery system. Int J Biol Macromol 93:582–590
Feng N, Dong J, Han G, Wang G (2014) Polymer nanoparticles based on pyrene-functionalized poly(acrylic acid) for controlled release under photo and pH stimulation. Macromol Rapid Commun 35(7):721–726
Fleige E, Quadir MA, Haag R (2012) Stimuli-responsive polymeric nanocarriers for the controlled transport of active compounds: concepts and applications. Adv Drug Deliv Rev 64:866–884
Gallo J, Kamaly N, Lavdas I, Stevens E, Nguyen QD, WylezinskaArridge M et al (2014) CXCR4-targeted and MMP-responsive iron oxide nanoparticles for enhanced magnetic resonance imaging. Angew Chem Int Ed Engl 53:9550–9554
Gan CW, Feng SS (2010) Transferrin-conjugated nanoparticles of poly(lactide)-D-alpha-tocopheryl polyethylene glycol succinate diblock copolymer for targeted drug delivery across the blood-brain barrier. Biomaterials 31(30):7748–7757
Gill MR, Menon JU, Jarman P et al (2018) 111In-labelled polymeric nanoparticles incorporating a ruthenium-based radiosensitizer for EGFR-targeted combination therapy in oesophageal cancer cells. Nanoscale 10(22):10596–10608
Goodall S, Howard CB, Jones ML, Munro T, Jia Z, Monteiro MJ, Mahler S (2015) An EGFR targeting nanoparticle self assembled from a thermoresponsive polymer. J Chem Technol Biotechnol 90:1222–1229
Greco F, Vicent MJ (2009) Combination therapy: opportunities and challenges for polymer-drug conjugates as anticancer nanomedicines. Adv Drug Deliv Rev 61:1203–1213
Han HS, Thambi T, Choi KY, Son S, Ko H, Lee MC et al (2015) Bioreducible shell-cross-linked hyaluronic acid nanoparticles for tumor-targeted drug delivery. Biomacromolecules 16:447–456
Han L, Zhang XY, Wang YL, Li X, Yang XH, Huang M et al (2017) Redox-responsive theranostic nanoplatforms based on inorganic nanomaterials. J Control Release 259:40–52
Haume K, Rosa S, Grellet S et al (2016) Gold nanoparticles for cancer radiotherapy: a review. Cancer Nanotechnol 7:8
He H, Sun L, Ye J, Liu E, Chen S, Liang Q et al (2016) Enzyme-triggered, cell penetrating peptide-mediated delivery of anti-tumor agents. J Control Release 240:67–76
Helal-Neto E, Pinto SR, Portilho FL et al (2019) Development and biological evaluation of a new nanotheranostic for tuberculosis. Drug Deliv Transl Res 9:97–105
Hu H (2020) Recent advances of bioresponsive nano-sized contrast agents for ultra-high-field magnetic resonance imaging. Front Chem 8:203
Hu FQ, Zhang YY, You J, Yuan H, Du YZ (2012) pH triggered doxorubicin delivery of PEGylated glycolipid conjugate micelles for tumor targeting therapy. Mol Pharm 9:2469–2478
Hua J, Dobrucki LW, Sadeghi MM et al (2015) Noninvasive imaging of angiogenesis with a 99mTc-labeled peptide targeted at alphavbeta3 integrin after murine hindlimb ischemia. Circulation 111:3255–3260
Huang Y, Mao K, Zhang B, Zhao Y (2017) Superparamagnetic iron oxide nanoparticles conjugated with folic acid for dual target-specific drug delivery and MRI in cancer theranostics. Mater Sci Eng C 70:763–771
Hudecz F (1995) Design of synthetic branched-chain polypeptides as carriers for bioactive molecules. Anti-Cancer Drugs 6:171–193
Jaidev LR, Krishnan UM, Sethuraman S (2015) Gemcitabine loaded biodegradable PLGA nanospheres for in vitro pancreatic cancer therapy. Mater Sci Eng C Mater Biol Appl 47:40–47
Jain K, Zhong J (2022) Theranostic applications of nanomaterials. Curr Pharm Des 28(2):77. https://doi.org/10.2174/1381612828022112231501 53

Jain K, Verma AK, Mishra PR, Jain NK (2015a) Characterization and evaluation of amphotericin B loaded MDP conjugated poly(propylene imine) dendrimers. Nanomedicine 11(3):705–713. https://doi.org/10.1016/j.nano.2014.11.008

Jain K, Verma AK, Mishra PR, Jain NK (2015b) Surface-engineered dendrimeric nanoconjugates for macrophage-targeted delivery of amphotericin B: formulation development and in vitro and in vivo evaluation. Antimicrob Agents Chemother 59(5):2479–2487. https://doi.org/10.1128/aac.04213-14

Juneja M, Suthar T, Pardhi VP, Ahmad J, Jain K (2022) Emerging trends and promises of nanoemulsions in therapeutics of infectious diseases. Nanomedicine (Lond). 17(11):793–812. https://doi.org/10.2217/nnm-2022-0006

Kanamala M, Wilson WR, Yang M, Palmer BD, Wu Z (2016) Mechanisms and biomaterials in pH-responsive tumour targeted drug delivery: a review. Biomaterials 85:152–167

Karimi M, Ghasemi A, Sahandi Zangabad P, Rahighi R, Moosavi Basri SM, Mirshekari H et al (2016) Smart micro/nanoparticles in stimulus responsive drug/gene delivery systems. Chem Soc Rev 45:1457–1501

Ke W, Li J, Mohammed F, Wang Y, Tou K, Liu X et al (2019) Therapeutic polymersome nanoreactors with tumor-specific activable cascade reactions for cooperative cancer therapy. ACS Nano 13:2357–2369

Khokha R, Murthy A, Weiss A (2013) Metalloproteinases and their natural inhibitors in inflammation and immunity. Nat Rev Immunol 13:649–665

Kovacs L, Tassano M, Cabrera M et al (2014) Labeling polyamidoamine (PAMAM) dendrimers with technetium-99m via Hydrazinonicotinamide (HYNIC). Curr Radiopharm 7:115–122

Lamb JR, Holland JP (2018) Advanced methods for radiolabeling multimodality nanomedicines for SPECT/MRI and PET/MRI. J Nucl Med 59:382–389

Le Goas M, Paquet M, Paquirissamy A, Guglielmi J, Compin C, Thariat J, Vassaux G, Geertsen V, Humbert O, Renault J-P (2019) Improving 131I radioiodine therapy by hybrid polymer-grafted gold nanoparticles. Int J Nanomedicine 14:7933

Li Y, Ogris M, Wagner E, Pelisek J, Rüffer M (2003) Nanoparticles bearing polyethyleneglycol-coupled transferrin as gene carriers: preparation and in vitro evaluation. Int J Pharm 259(1–2): 93–101

Li M, Kim HS, Tian L, Yu MK, Jon S, Moon WK (2012) Comparison of two ultrasmall superparamagnetic iron oxides on cytotoxicity and MR imaging of tumors. Theranostics 2: 76–85

Lim YH, Tiemann KM, Hunstad DA et al (2016) Polymeric nanoparticles in development for treatment of pulmonary infectious diseases. Wiley Interdiscip Rev Nanomed Nanobiotechnol 8: 842–871

Ling D, Park W, Park SJ, Lu Y, Kim KS, Hackett MJ et al (2014) Multifunctional tumor pH-sensitive self-assembled nanoparticles for bimodal imaging and treatment of resistant heterogeneous tumors. J Am Chem Soc 136:5647–5655

Liu P, Shi B, Yue C, Gao G, Li P, Yi H (2013) Dextran-based redoxresponsive doxorubicin prodrug micelles for overcoming multidrug resistance. Polym Chem 4:5793–5799

Liu T, Wang C, Gu X, Gong H, Cheng L, Shi X (2014) Drug delivery with PEGylated MoS2 nanosheets for combined photothermal and chemotherapy of cancer. Adv Mater 26:3433–3440

Liu Y, Bhattarai P, Dai Z, Chen X (2019a) Photothermal therapy and photoacoustic imaging via nanotheranostics in fighting cancer. Chem Soc Rev 48:2053–2108

Liu JB, Merton DA, Forsberg F, Goldberg BB (2019b) Contrast-enhanced ultrasound imaging. In: Diagnostic ultrasound. CRC, Boca Raton, pp 51–74

Lucky SS, Soo KC, Zhang Y (2015) Nanoparticles in photodynamic therapy. Chem Rev 115:1990–2042

Luk BT, Zhang L (2014) Current advances in polymer-based nanotheranostics for cancer treatment and diagnosis. ACS Appl Mater Interfaces 6:21859–21873

Lyu Y, Fang Y, Miao Q, Zhen X, Ding D, Pu K (2016) Intraparticle molecular orbital engineering of semiconducting polymer nanoparticles as amplified theranostics for in vivo photoacoustic imaging and photothermal therapy. ACS Nano 10:4472–4481

Mahmoud AM, De Jongh PA, Briere S, Chen M, Nowell CJ, Johnston AP, Davis TP, Haddleton DM, Kempe K (2019) Carboxylated Cy5-labeled comb polymers passively diffuse the cell membrane and target mitochondria. ACS Appl Mater Interfaces 11:31302–31310

Manna S, Lakshmi US, Racharla M, Sinha P, Kanthal LK, Kumar SPN (2016) Bioadhesive HPMC gel containing gelatin nanoparticles for intravaginal delivery of tenofovir. J Appl Pharm Sci 6: 22–29

Mu J, Lin J, Huang P, Chen X (2018) Development of endogenous enzyme-responsive nanomaterials for theranostics. Chem Soc Rev 47:5554–5573

Nagamani N, Lakshmanan S, Govindaraj D, Ramamoorthy C, Ramalakshmi N, Antony SA (2019) Selective and efficient detection of picric acid among other nitroaromatics by NIR fluorescent cyanine chemosensors. Spectrochim Acta Part A Mol Biomol Spectrosc 207:321–327

Ng QK, Olariu CI, Yaffee M (2014) Indium-111 labeled gold nanoparticles for in-vivo molecular targeting. Biomaterials 35:7050–7057

O'Reilly RK, Hawker CJ, Wooley KL (2006) Cross-linked block copolymer micelles: functional nanostructures of great potential and versatility. Chem Soc Rev 35(11):1068–1083

Patel P, Kumar K, Jain VK, Popli H, Yadav AK, Jain K (2023) Nanotheranostics for diagnosis and treatment of breast cancer. Curr Pharm Des 29(10):732–747. https://doi.org/10.2174/1381612829666230329122911

Patil YB, Toti US, Khdair A, Ma L, Panyam J (2009) Single-step surface functionalization of polymeric nanoparticles for targeted drug delivery. Biomaterials 30(5):859–866

Pecher J, Mecking S (2010) Nanoparticles of conjugated polymers. Chem Rev 110:6260–6279

Prabhakar A, Banerjee R (2019) Nanobubble liposome complexes for diagnostic imaging and ultrasound-triggered drug delivery in cancers: a theranostic approach. ACS Omega 4:15567–15580

Psimadas D, Georgoulias P, Valotassiou V, Loudos G (2012) Molecular nanomedicine towards cancer: 111In-labeled nanoparticles. J Pharm Sci 101:2271–2280

Raut SL, Kirthivasan B, Bommana MM, Squillante E, Sadoqi M (2010) The formulation, characterization and in vivo evaluation of a magnetic carrier for brain delivery of NIR dye. Nanotechnology 21:395102

Sahatsapan N, Ngawhirunpat T, Rojanarata T, Opanasopit P, Patrojanasophon P (2020) Catechol-functionalized alginate nanoparticles as mucoadhesive carriers for Intravesical chemotherapy. AAPS PharmSciTech 21(6):212

Saroj S, Rajput SJ (2018) Tailor-made pH-sensitive polyacrylic acid functionalized mesoporous silica nanoparticles for efficient and controlled delivery of anti-cancer drug etoposide. Drug Dev Ind Pharm 44(7):1198–1211

Shao C, Xiao F, Guo H, Yu J, Jin D, Wu C, Xi L, Tian L (2019) Utilizing polymer micelle to control dye J-aggregation and enhance its theranostic capability. iScience 22:229–239

Simonetti G, Palocci C, Valletta A (2019) Anti-candida biofilm activity of pterostilbene or crude extract from non-fermented grape pomace entrapped in biopolymeric nanoparticles. Molecules 24:2070

Singer JW, Shaffer S, Baker B, Bernareggi A, Stromatt S, Nienstedt D et al (2005) Paclitaxel poliglumex (XYOTAX; CT-2103): an intracellularly targeted taxane. Anti-Cancer Drugs 16: 243–254

Son J, Yi G, Yoo J, Park C, Koo H, Choi HS (2019) Light-responsive nanomedicine for biophotonic imaging and targeted therapy. Adv Drug Deliv Rev 138:133–147

Sun J, Sun L, Li J, Xu J, Wan Z, Ouyang Z, Liang L, Li S, Zeng D (2018) A multi-functional polymeric carrier for simultaneous positron emission tomography imaging and combination therapy. Acta Biomater 75:312–322

Suthar T, Jain VK, Popli H, Jain K (2022) 12 - Nanoemulsions as effective carriers for targeting brain tumors. In: Kumar L, Pathak YY (eds) Nanocarriers for drug-targeting brain tumors. Elsevier, pp 347–363

Thambi T, Park JH, Lee DS (2016) Stimuli-responsive polymersomes for cancer therapy. Biomater Sci 4:55–69

Valluru KS, Willmann JK (2016) Clinical photoacoustic imaging of cancer. Ultrasonography 35: 267

Vijayan VM, Muthu J (2017) Polymeric nanocarriers for cancer theranostics. Polym Adv Technol 28:1572–1582

Wang T-C, Cochet F, Facchini FA, Zaffaroni L, Serba C, Pascal S, Andraud C, Sala A, Di Lorenzo F, Maury O (2019) Synthesis of the new cyanine-labeled bacterial lipooligosaccharides for intracellular imaging and in vitro microscopy studies. Bioconjug Chem 30:1649–1657

Wang R, Yang H, Khan AR, Yang X, Xu J, Ji J (2021) Redoxresponsive hyaluronic acid-based nanoparticles for targeted photodynamic therapy/chemotherapy against breast cancer. J Colloid Interface Sci 598:213–228

Wang QX, Chen X, Li ZL, Gong YC, Xiong XY (2022) Transferrin/folate dual-targeting Pluronic F127/poly(lactic acid) polymersomes for effective anticancer drug delivery. J Biomater Sci Polym Ed 33(9):1140–1156

Wu S, Helal-Neto E, Matos APDS et al (2020) Radioactive polymeric nanoparticles for biomedical application. Drug Deliv 27(1):1544–1561

Xie M, Zhang F, Liu L, Zhang Y, Li Y, Li H, Xie J (2018) Surface modification of graphene oxide nanosheets by protamine sulfate/sodium alginate for anti-cancer drug delivery application. Appl Surf Sci 440:853–860

Xin HL, Chen LC, Gu JJ, Ren XQ, Wei Z, Luo JQ et al (2010) Enhanced anti-glioblastoma efficacy by PTX-loaded PEGylated poly(epsilon-caprolactone) nanoparticles: in vitro and in vivo evaluation. Int J Pharm 402:238–234

Xiong XB, Lavasanifar A (2011) Traceable multifunctional micellar nanocarriers for cancer-targeted co-delivery of MDR-1 siRNA and doxorubicin. ACS Nano 5:5202–5213. https://doi.org/10.1021/nn2013707

Xu Y (2018) Synthesis of small-molecule fluorescent probe and polymers for bioapplication: bioimaging and enzyme stabilization. MSc thesis, University of South Carolina, Columbia

Yang K, Hu L, Ma X, Ye S, Cheng L, Shi X, Li C, Li Y, Liu Z (2012) Multimodal imaging guided photothermal therapy using functionalized graphene nanosheets anchored with magnetic nanoparticles. Adv Mater 24:1868–1872

Yang H, Deng L, Li T, Shen X, Yan J, Zuo L, Wu C, Liu Y (2015) Multifunctional PLGA nanobubbles as theranostic agents: combining doxorubicin and P-gp siRNA co-delivery into human breast cancer cells and ultrasound cellular imaging. J Biomed Nanotechnol 11:2124–2136

Yang X, An J, Luo Z, Yang R, Yan S, Liu D-E, Fu H, Gao H (2020) A cyanine-based polymeric nanoplatform with microenvironment-driven cascaded responsiveness for imaging-guided chemo-photothermal combination anticancer therapy. J Mater Chem B 8:2115–2122

Yildiz I, Yildiz SB (2015) Applications of thermoresponsive magnetic nanoparticles. J Nanomater 2015:12

Yong Y, Cheng X, Bao T, Zu M, Yan L, Yin W (2015) Tungsten sulfide quantum dots as multifunctional nanotheranostics for in vivo dualmodal image-guided photothermal/radiotherapy synergistic therapy. ACS Nano 9:12451–12463

Yu J, Chu X, Hou Y (2014a) Stimuli-responsive cancer therapy based on nanoparticles. Chem Commun 50:11614–11630

Yu Y, Zhang X, Qiu L (2014b) The anti-tumor efficacy of curcumin when delivered by size/charge-changing multistage polymeric micelles based on amphiphilic poly(beta-amino ester) derivates. Biomaterials 35:3467–3479

Zarabi B, Borgman MP, Zhuo JC, Gullapalli R, Ghandehari H (2009) Noninvasive monitoring of HPMA copolymer-RGDfK conjugates by magnetic resonance imaging. Pharm Res 26:1121–1129

Zeighamian V, Darabi M, Akbarzadeh A, Rahmati-Yamchi M, Zarghami N, Badrzadeh F, Salehi R, Tabatabaei Mirakabad FS, Taheri-Anganeh M (2015) PNIPAAm-MAA nanoparticles as delivery vehicles for curcumin against MCF-7 breast cancer cells. Artif Cells Nanomed Biotechnol 44:735–742

Zhang X, Han L, Liu M, Wang K, Tao L, Wan Q (2017a) Recent progress and advances in redox-responsive polymers as controlled delivery nanoplatforms. Mater Chem Front 1:807–822

Zhang B, Yan Y, Shen Q, Ma D, Huang L, Cai X, Tan S (2017b) A colon targeted drug delivery system based on alginate modificated graphene oxide for colorectal liver metastasis. Mater Sci Eng C 79:185–190

Zhang J, Yang C, Zhang R, Chen R, Zhang Z, Zhang W, Peng SH, Chen X, Liu G, Hsu CS (2017c) Biocompatible D–a semiconducting polymer nanoparticle with light-harvesting unit for highly effective photoacoustic imaging guided photothermal therapy. Adv Funct Mater 27:1605094

Zhao H, Duong HH, Yung LY (2010) Folate-conjugated polymer micelles with pH-triggered drug release properties. Macromol Rapid Commun 31:1163–1169

Zhou W, Zheng S, Schultz JW, Rath NP, Mirica LM (2016) Aromatic cyanoalkylation through double C–H activation mediated by Ni (III). J Am Chem Soc 138:5777–5780

Zhu L, Xie J, Swierczewska M, Zhang F, Quan Q, Ma Y et al (2011) Real-time video imaging of protease expression in vivo. Theranostics. 1:18–27

Zhu L, Perche F, Wang T, Torchilin VP (2014) Matrix metalloproteinase 2-sensitive multifunctional polymeric micelles for tumor-specific co-delivery of siRNA and hydrophobic drugs. Biomaterials 35:4213–4222

Zhu D, Tao W, Zhang H, Liu G, Wang T, Zhang L, Zeng X, Mei L (2016) Docetaxel (DTX)-loaded polydopamine-modified TPGS-PLA nanoparticles as a targeted drug delivery system for the treatment of liver cancer. Acta Biomater 30:144–154

Functionalized Metallic Nanoparticles: Theranostic Applications

5

Kapil D. Patel, Anup Kumar Patel, Prasad Sawadkar, Bineta Singh, and Adam W. Perriman

Abstract

Metallic nanoparticles such as magnetic (iron, manganese, cobalt, chromium, and nickel), gold, silver, and transition element-based nanoparticles have been extensively studied for the development of new generation cancer therapeutic and diagnostic tools. These metallic nanoparticles are easy to synthesize, surface functionalize, and also possess unique physical and chemical properties which are suitable for theranostic applications. The role of metallic nanoparticles as nanoimaging (fluorescence imaging, magnetic resonance imaging) has been potentially used in detection and diagnosis of cancer at early stage. A multifunctional targeted drug delivery using intrinsic stimuli-responsive (pH, thermal) or extrinsic such as light, heat, and ultrasound has endowed a huge nanoplatform for their therapeutic potential for simultaneous detection (diagnostic) and treatment (therapeutic) of cancer. Moreover, the new development of theranostic hybrid multifunctional nanocarriers has greatly expanded their application in nanomedicines through combining the suitable imaging modalities agents with chemotherapeutic drugs to treat cancer with better, more precise, and minimally invasive approaches. This chapter provides the basic characteristics of

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multifunctional metallic nanoparticles and their nanotechnological application in theranostics. Finally, we will highlight the future perspective of multifunctional metallic nanoparticles in clinical and nanotechnological cancer research.

Keywords

Metal nanoparticles · Multifunctional · Theranostic · Cancer · Drug delivery

5.1 Introduction

Cancer is a complex and major disease which causes millions of deaths every year across the world. It is very difficult to find the proper treatment for the cancer mainly due to the heterogeneity of the tumors, which varies from person-to-person. There have been developed several approaches to treat the cancer; however none of the approach is satisfactory. Therefore, a thoughtful combinatory approach of diagnosis and multi-layer chemotherapy, which is called theranostic, involving using smart nanocarriers has been developed and tested. Theranostics is a combination of therapeutics (thera) plus diagnostics (nostics), which is often used to describe the combinatory approach of using radioactive agent to identify (diagnose) and deliver the chemotherapeutic drug to treat the tumor or cancer. Recently, multifunctional nanoparticles with great potential to trace the tumor as well as efficient delivery of drug molecules under the external stimuli have been designed and applied for the cancer treatment. Hence, multifunctional nanoparticles have a great role in recent advancements to target, diagnose, monitor, and control drug delivery in cancer therapy.

Nanoparticles (NPs) have great advantages over micro- or macroparticles due to large surface-to-volume ratio, varying shape and size, surface tunability, and controllable physicochemical properties, which play an important role in design of multifunctional nanoparticles. A multifunctional nanoparticle is a combination of several properties such as high drug loading capacity, effective delivery, precise targeting, tumor imaging, and controlled delivery under internal environment or external stimuli. Therefore, metallic nanoparticles (MNPs) are one of best multifunctional nanoparticles to serve as theranostics nanocarriers.

In the past three decades, MNPs have been engineered in various forms including zero dimension (0D; quantum dots), one dimension (1D; nanotubes, nanorods, nanowires), two dimension (2D; nanosheets, nanoplates), and three dimension (3D; nanosphere, nanoparticles, and hybrid structures), which have attracted great interest as potential multifunctional nanoparticles for theranostics. There has been a rapid increase in the metal-based nanoparticles as multifunctional nanoparticles for design, formulation, and characterizations for targeted theranostics nanomedicine applications. Several metallic multifunctional nanoparticles have been synthesized and utilized for theranostics purposes; however magnetic nanoparticles (FeNPs), gold nanoparticles (AuNPs), silver nanoparticles (AgNPs), and transition metal nanoparticles or up-conversion nanoparticles (UCNPs) have attracted the most

attention. Therefore, in this chapter, we will focus on the design, development, surface functionalization, and applications for imaging and drug delivery of these multifunctional nanoparticles.

Advance and effective diagnosis of cancer requires various types of imaging tools to investigate and understand the characteristics and propagation of biological process at the cellular and subcellular levels. Molecular imaging is one of the effective and powerful tools which can help to detect and diagnose the early stage of cancer metastasis through exploiting the specific molecular contrast or probe agents. The early and precise diagnosis of cancer endows the maximum opportunity to evaluate the method and approach for the treatment. There are various imaging modalities available for early detection of cancer which include optical imaging, two-photon imaging, ultrasound (US), magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), and single photon emission computed tomography (SPECT).

Controlled and targeted drug delivery is an essential characteristic of multifunctional nanoparticles. Metal-based multifunctional nanoparticles have shown the potential for not only imaging and controlled drug delivery but also conjugation with ligand assembly for ligand mediated targeted and stimuli-responsive (pH, temperature, US, light). Different metallic nanoparticles have been successfully produced and applied for multifunctional theranostic approach. Moreover, these nanoparticles have been used in combined system of chemotherapy, photothermal therapy (PTT), and photodynamic therapy (PDT), and endowed great potential for efficient and safe carriers in cancer therapy. Bearing these essential characteristics in mind, we aim to provide the detailed role of multifunctional metallic nanoparticles in theranostics. The schematic photograph of multifunctional metal nanoparticles with varying shape, size, and surface chemistry is presented in Fig. 5.1.

5.2 Metal Nanoparticles

Metal nanoparticles such as iron oxide, gold, silver, and UCNPs have attracted significant interests in their functionalization to achieve multifunctionality. The "top-down" and "bottom-up" techniques are utilized to synthesize the metallic NPs including AuNPs too (Fig. 5.2). The top-down process entails starting with bulk material and shattering into small particles of size new nanometers to 100 nm using various approaches. The bottom-up method creates nanoparticles from the atomic level up. Schematic photograph depicts the top-down and bottom-up techniques' basic steps in Fig. 5.2. To achieve drug loading through surface chemistry modification (functionalization), and imaging by decorating with contrast agents, and improved biocompatibility to overcome the challenges such as internalization in cell, biodistribution, saturation solubility, and glueyness to the cell membrane. The only significant challenge with metal-based nanoparticles is dissolution rate in body, which can cause the risk of long-term side effect, if not cleared from the body in a timely manner. Therefore, surface functionalization of metal nanoparticles endows not only the opportunity to improve the therapeutic efficacy, imaging potential, and

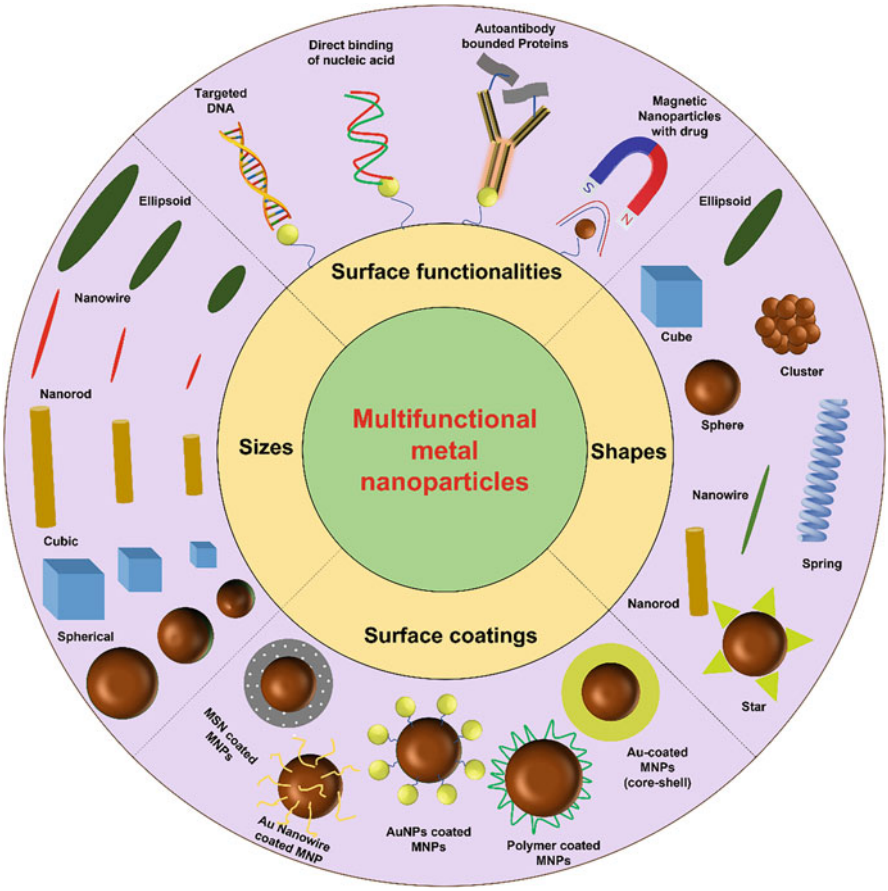

Fig. 5.1 Schematic illustration of multifunctional metal nanoparticles with different sizes, shapes, surface functionalities, and surface coatings for theranostics applications

biocompatibility, but also helps to address the biodistribution and reduces the risk of adverse effect. However, metal nanoparticles still required more clinical trial data with only few examples having been accepted for clinical use.

5.2.1 Magnetic Nanoparticles

In the last three decades, numerous metal nanoparticles including magnetic nanoparticles (MNPs) in different phase such as iron oxide, magnetite, and maghemite, have been designed and developed for various biomedical applications. The fabrication and engineering of MNPs have drawn huge attention due to their unique physical (magnetic, electric, optical, ultrasound, thermal), physicochemical (surface chemistry, shape, size, and biocompatibility), and in vivo biodistribution.

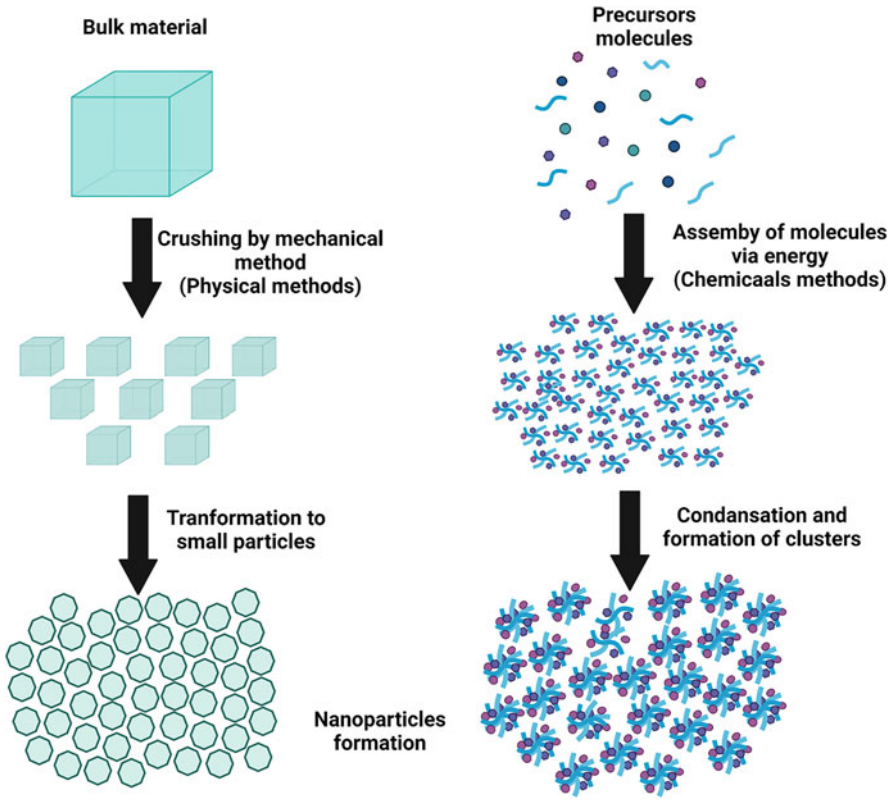

Fig. 5.2 Schematic illustration of top-down and bottom-up approaches involved in the process of nanoparticles. The top-down strategy involved crushing the bulk materials into small particles and then transformation into nanoparticles. Bottom-up approach involves various precursor molecules and assembled into very small particles via chemical reactions, and further stabilized into nanoparticles through condensation and clusters formation

Moreover, MNPs of varying geometry, particularly shape, size, and nanostructures, have been produced by several processing approaches including sol-gel, hydrothermal, thermal decomposition, sonolysis, pyrolysis, and electrochemical deposition (Yang et al. 2022). The modification of MNPs with multifunctional properties has been crucial in consideration of bio-safety, efficiency, and targeted delivery in nanomedicine. The engineering of MNPs was mostly focused to control over nanostructures of different shape, size, and surface modification via chemical reaction, coating, and conjugation with targeting biomolecules (i.e., ligand and peptides). The engineering of MNPs endowed high dispersibility, improved bioavailability, and excellent delivery and facilitated the on-demand theranostics applications. Furthermore, the physicochemical properties of MNPs can greatly impact the expected outcome; therefore, to control both physical and chemical properties of MNPs, various types of synthesis methods have been developed (Neamtu et al.

2018; Zhu et al. 2018). Therefore, surface chemistry is a very important parameter for effective drug loading and magnetic property for MRI in external stimuli through magnetic field.

Iron oxide nanoparticles, particularly, magnetite (Fe_3O_4) have been widely explored for delivery (drug, gene, growth factors) and MRI imaging in cancer therapy (Singh et al. 2014a), magnetic stimulation and differentiation of stem cells in tissue regeneration (Lee et al. 2021; Singh et al. 2014b), and macrophage polarization for immunomodulation (Kim et al. 2022). Many researchers have investigated the metal nanoparticles and confirmed the promising role in drug delivery with various stimuli-responsive approaches (Corem-Salkmon et al. 2011). Specifically, iron oxide nanoparticles have shown tremendous potential as the surface tunability provides biocompatibility, biodegradability, and minimal invasiveness towards normal cells and also enabled high drug delivery in cancer as well as ocular delivery system (Giannaccini et al. 2014; Raju et al. 2011; Patel et al. 2022). Therefore, It is worth to emphasize that the cellular internalization and cytotoxicity are dependent on physicochemical properties of MNPs, and also the type of cells, cell lines, and condition of cell culture (Shang et al. 2014; Park et al. 2011). MNPs have also potential to track the nanoparticles in vivo using MRI technique (Raju et al. 2012). Moreover, chemical functional groups could be a wide range of molecules, such as antibodies, amines, biotin, carboxyl, and streptavidin, and other functional groups which can attach via disulfide cross-linkers and effectively influence the role in the theranostics application (Yanai et al. 2012; Häfeli 2004).

5.2.2 Gold Nanoparticles

Gold is an element which has been used for a variety of biomedical applications. Gold nanoparticles (AuNPs) have been synthesized in various shapes (spherical, cubic, prism, octahedral, nanorods, nanowires) and applied for numerous applications including nanomedicine, electronics, sensing, aeronautics, and smart devices (Chithrani et al. 2006; Xie et al. 2017). In nanomedicine, AuNPs are utilized for antibacterial activities, biosensing, drug delivery, photothermal and photodynamic therapy, and imaging. The unique features and multifunctionality of AuNPs have been widely investigated and studied in nanomedicine. The versatile synthesis approach with tunable size, shape, and geometry has also widened the application in nano-optics, biosensors, electronics, and theranostics. The AuNPs can easily assemble with proteins, oligonucleotides, and antibodies and bioconjugate with various functional groups and compose suitable multifunctional metal nanoparticles for theranostics applications (Yeh et al. 2012). Moreover, the potentials of AuNPs to attach to analytes such as solvent, microbores, and biomolecules can tailor the physicochemical properties for specific application. This tailoring could be useful for surface plasmon resonance, redox chemistry, and increased signal detection in various spectroscopic tests. Moreover, AuNPs also endowed the possibility for pharmacological and targeted moieties to adhere or interact on the surface due to

high reactivity. These characteristics of AuNPs are extremely beneficial for targeted drug delivery, PTT, PDT, and cancer theranostics.

There are many techniques (laser ablation, ion sputtering, light irradiation (UV/NIR), and aerosol technology) to synthesize the AuNPs, whereas the bottom-up approach involves reducing Au^{3+} to Au^{0}. The fabrication of AuNPs basically involves two steps: first, reduction method, in which the gold precursor is typically an aqueous gold salt solution, is reduced to AuNPs in the first step (Bansal et al. 2020). A specific agent helps to stabilize NPs in the next stage. The covering agents prohibit metallic nanoparticles forming aggregates. In general, precise control over shape, size, and dispersion is a critical aspect in determining the efficiency of a synthesis technique. Turkevich method is one of the most popular ways to make spherical gold NPs with particle size of 1–2 nm. The basic idea behind this process is to convert gold ions (Au^{3+}) to gold atoms using various reducing agents, for example, amino acids, ascorbic acid, UV light, and citrate (Au^{0}). AuNPs are stabilized using various capping/stabilizing agents. In the recent improvement of the basic process, researchers have been able to broaden the size of particles in the range of 16–147 nm generated using this technology. The second approach is seed-mediated development method for making rod-shaped AuNPs. The main idea behind this process is to first reduce gold salts to make seed particles (nuclei), and $NaBH_4$ was utilized as a reducing agent. These particles are then transferred to a metallic salt along with a moderate reducing agent (ascorbic acid) to prevent additional nucleation and expedite the creation of rod-like AuNPs. The seeds and the concentration of reductants influence the geometry, size, and shape of AuNPs. Moreover, the surface chemistry, charge, and polarity can significantly influence the ligand coupling and physicochemical properties in theranostics (Pissuwan et al. 2011; Rai et al. 2015).

5.2.3 Silver Nanoparticles

Silver nanoparticles (AgNPs) have been extensively explored for numerous applications in nanomedicine including antibacterial, therapeutic, and food-containing applications for years (Xu et al. 2020). It has been reported that the ancient Greeks, Romans, and Indians (Late Harappan era) have used clay, and silver metal pots for drinking water store, food pot, wine to avoid spoilage and food and water storage for antibacterial preservations (Cobb 2018; Ray et al. 2016). Hippocrates used silver nanoparticles in treating wounds and ulcers, whereas silver nitrate was utilized for instrument disinfection as well as wound healing. In the early nineteenth century, AgNPs were applied for burn and wound infection care, and antibiotics applications, as well as for catalysis, energy and data storage, and sensing mainly because of nanosized and surface reactivity (surface to volume ratio). Similar to gold nanoparticles, AgNPs have been extensively explored for biomedicine due to their unique physicochemical, tunable optical, properties. Depending on the requirement of properties, AuNPs have been synthesized by numerous approaches through physical, chemical, and biological routes (Khodashenas and Ghorbani 2019;

Mathivanan et al. 2019). In nanomedicine, AgNPs have been used for antimicrobial, wound repair, antiangiogenic activity, burn healing, anticancer therapy, and as biosensors mainly due to their release of silver ions (Ag^+), production of reactive oxygen species (ROS) which caused destruction of bacterial/cell membrane.

AgNPs have antiangiogenic properties and are quite effective in suppressing retinal endothelial cell survival (Gurunathan et al. 2009). Other areas of AgNPs use are during burn injuries healing as an adjunctive agent which supports the wound healing via simultaneous reduction of inflammation (Fong and Wood 2006). Several studies have been performed to understand the antibacterial activities of AgNPs, salt, and composites, but still the mechanism is partially understood (Sintubin et al. 2011; Tang et al. 2013). Sondi et al. studied the AgNPs antibacterial activity and confirmed the bacterial membrane damage which leads to cell death (Sondi and Salopek-Sondi 2004). McQuillan et al. have investigated AgNPs interaction with outer and inner walls of bacteria and explained the Ag^+ role in bacterial transcriptional response (McQuillan et al. 2012). Moreover, positively charged metal ions interacted to the negatively charged membrane of bacteria and subsequently blocked the electron transfer, and translocation of ATPase is not upregulated (O'Reilly et al. 2005). Numerous researches have revealed that the AgNPs are in nanomedicine, but microparticles and high concentrations can cause significant cytotoxicity as well as genotoxicity both in vivo and in vitro.

AgNPs are documented for a wide spectrum of bactericidal efficacy for microbes including bacteria and viruses, for example, *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*; fungi, such as *Candida albicans*; and viruses, such as human immunodeficiency virus (HIV) and hepatitis B virus (HBV), respectively. The protective mechanism of NPs considers various products containing AgNPs, for example, wound healing, dressings, scaffold development and creams, and solvents. The release of silver ions (Ag+) from AgNPs sometimes enhances the cytotoxicity when exceeding dose, concentration, and time of exposure (Khodashenas and Ghorbani 2019; Singh et al. 2012; Yin et al. 2017; Yang et al. 2020). AgNPs have been used for drug delivery as well as imaging contrast agent in cancer theranostics (Li et al. 2018).

5.2.4 Up-conversion Nanoparticles

In the recent past few years, lanthanide-doped up-conversion nanoparticles (UCNPs) have gained significant attention due to their unique optical property of up-converting near infrared (NIR) light into high-energy ultra-violet (UV) or visible (Vis) light for photoactivation. The up-conversion of light is an optical phenomenon in which low energy light (NIR) is absorbed by the material and under the excitation, it emits the high energy light (UV or Vis). UCNPs have been extensively used for stimuli-responsive drug delivery as well as imaging contrast agents. The light-activated drug delivery is a potential stimuli-responsive method to control the drug release. The light activation is considered as one of the most potential strategies due to its control over spatiotemporal precision and minimal invasiveness.

Photoreactors, which usually require high energy UV light to initiate their potential role in biomedical application and cause significant tissue damage, can be replaced by UCNPS. Thus, NIR light activated UCNPs are better for cellular and biological tissue and also able to achieve deep tissue penetration (He et al. 2015). However, energy associated with NIR light is not enough to activate the process of photolysis of photoactivable caged molecules. There are many advantages of UCNPs such as weak background signal, long lifetime of luminescence, and high photostability.

Recently, several works based on UCNPs have reported on the NIR light activated drug delivery in cancer therapy (Zhao et al. 2014; Fedoryshin et al. 2014; Gnanasammandhan et al. 2016; Yang et al. 2013). For example, Lui et al. have demonstrated the photo-mediated drug release from mesoporous silica-coated UCNPs with mesopores functionalized by azobenzene molecules. The anticancer drug doxorubicin (DOX) was released from the pore via azobenzene molecules photoisomerization when the NIR laser light irradiated on the mesoporous silica nanoparticles. The release of the DOX was regulated by the *trans-cis* photoisomerization of azobenzene molecules (Liu et al. 2013). Zhao et al. have reported the NIR photo-regulated 7-amino-coumarin delivery in living tumor tissue by use of yolk-shell structured mesoporous silica-coated UCNPs nanocage (Zhao et al. 2014). Many light-mediated drug delivery systems are designed and developed based on photosensitive molecules such as o-nitrobenzyl (Yang et al. 2013; Cui et al. 2015; Yang et al. 2012), azobenzene (Li et al. 2014), and coumarin (Lin et al. 2013; Lin et al. 2012). The UCNPs-based stimuli-responsive drug delivery systems can also be integrated with tracking agents to monitor the drug release and can externally control the drug dose for effective chemotherapy.

5.3 Functionalization of Metal Nanoparticles

In the past few decades, metal nanoparticles have been extensively studied for synthesis and surface functionalization. The synthesis of metal nanoparticles is mainly focused to varying shape, size, pore size, and uniform size distribution; however, surface functionalization with chemistry modifications is mainly focused on improving the biocompatibility, high therapeutic loading and target-specific delivery, stimuli-responsive delivery, and imaging applications. In this section, we focused to highlight the important methods of functionalization and its advantages and limitations in theranostics.

5.3.1 Surface Coating with Mesoporous Silica Nanoparticles

Silica or mesoporous silica (MSN) shell coatings on metal nanoparticles have been tried for many years. The main driver for coating the metal nanoparticles with MSN is to provide the drug loading ability, improvement in dispersibility, and biocompatibility. Moreover, the MSN shell also allowed the further modifications of silica-coated metal nanoparticles with fluorescence molecules, as well as targeting agents.

The main advantage of MSN is tunable porosity, and large surface area which endow high mount of active agents loading, tailoring capping systems has also been developed which can control the drug release via internal or external stimuli (Singh et al. 2019; Qian et al. 2009). Engineered MSN coated metal nanoparticles can be designed to accelerate the stimuli-responsive chemotherapeutic drug release in controlled manner. Furthermore, MSN can be utilized to promote the biocompatibility by means of enhanced dispersion and interfacial interaction with cell membrane.

Silica-coated magnetic nanoparticles have been successfully applied for drug delivery and as an imaging contrast agent for magnetic resonance imaging (MRI), which has been approved by the US Food and Drug Administration (FDA) for treatment of patients suffering from deficiency of iron (Anselmo and Mitragotri 2016). There are several methods for preparation of silica or mesoporous for various biomedical applications (Singh et al. 2012; Yin et al. 2017; Yang et al. 2020). Singh et al. have developed biocompatible magnetic nanoparticles with varying silica shell coating using sol-gel method (Singh et al. 2012). Recently, Keshavarz et al. have developed mesoporous silica-coated magnetic nanocomposites for pH-responsive controlled drug delivery for hyperthermia treatment (Keshavarz et al. 2020). Fang et al. have developed zinc-doped Fe_3O_4 core and mesoporous silica nanoparticles for enhanced MRI, and tumor targeted drug delivery applications (Fang et al. 2020).

MNPs can be coated using ligand exchange, grafting, adsorption, as well as in situ coatings. These coating may be in the form of surface covering of NPs or micelles encapsulations. Usually, the glycol family includes polyethylene glycol (PEG) and their derivates, polysaccharides, polyvinylpyrrolidone (PVP), chitosan, dextran, and polyethyleneimine (PEI), which are extensively used (Fig. 5.3a) (Jin et al. 2014). PEG and PEI collectively provide long-range stability to the NPs in the bloodstream, whereas PLGA gives rise to the hydrophilicity and provides a compartment to the cytoplasmic. MNPs encapsulated polymer micelle nanoparticles can be produce in various sizes, and shapes and used as stimuli-responsive delivery carrier in the solution depending on the temperatures, pH, polymer conc., and ionic strength (Fig. 5.3b).

5.3.2 Functionalization with Stimuli-Responsive Polymer

Functionalization of material nanoparticles with stimuli-responsive polymers is an established approach to achieve multifunctional metal nanoparticle. Stimuli-responsive polymer-coated iron oxide nanoparticles have emerged as an interesting candidate for the controlled drug delivery nanocarriers in theranostics. The polymer functionalization of metal nanoparticles can reduce the cytotoxicity and improve the biodistribution in both in vitro and in vivo. The polymer coatings on metal nanoparticles can be constructed in such a way that it can be responsive towards selective response such as temperature, pH, enzymes, redox environment, magnetic field, and light. A schematic illustration of polymer-coated metal nanoparticles

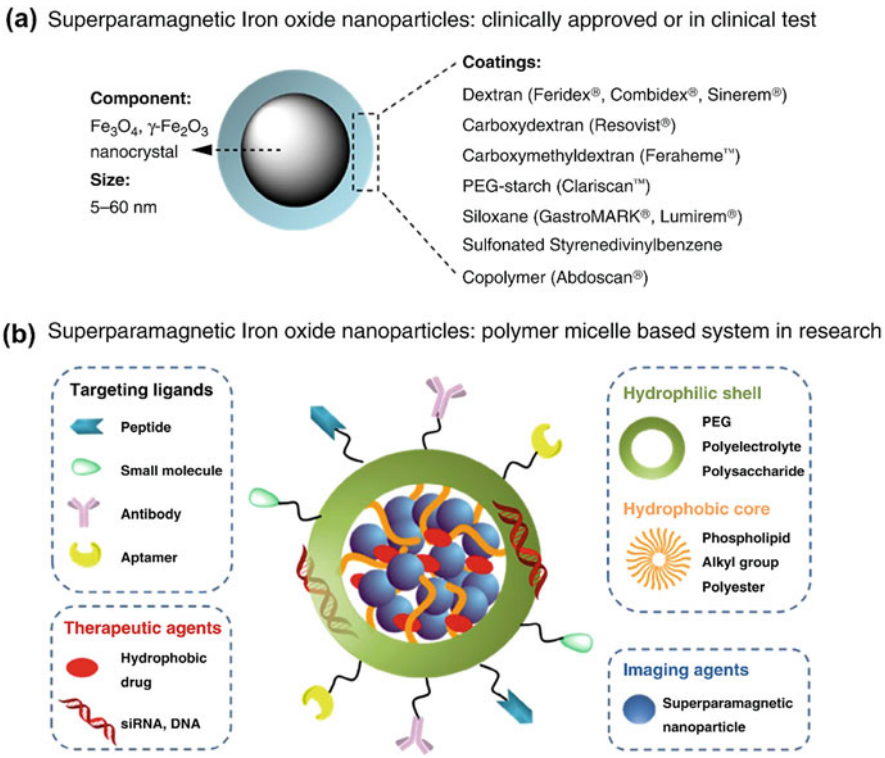

Fig. 5.3 Schematic photographs illustrate the polymer coating and various biomolecules, therapeutic agents, and imaging agents' combination in/on surface of magnetic nanoparticles in polymer micelles nano system cancer theranostics. (**a**) Biocompatible polymer-coated magnetic nanoparticles (clinically approved or in clinical test NPs); for each kind of coating material, commercial products are listed aside. (**b**) Amphiphilic polymers to form a micelle structure with hydrophilic shell and hydrophobic core (Jin et al. 2014). Reproduced with permission from Rongrong Jin, Bingbing Lin, Danyang Li, and Hua Ai. Superparamagnetic iron oxide nanoparticles for MR imaging and therapy: design considerations and clinical applications. Current Opinion in Pharmacology 2014, 18; 18–27. https://doi.org/10.1016/j.coph.2014.08.002; @2014 Elsevier Ltd

functionalization with stimuli-responsive factors is elaborated as multifunctional metal nanoparticles (Fig. 5.4).

Polymer functionalization of metal nanoparticles improved physiochemical property and subsequently role in nanomedicine. Numerous natural (gelatin, chitosan, dextran, silk fibroin, collagen) and synthetic (poly(ethylene glycol) (PEG), poly (ethyleneimine) (PEI), polymethylmethacrylate (PMMA), polyacrylamide (PMMA) polydopamine (PDA), and poly(N-isopropylacrylamide) (PNIPAM)) polymers have been utilized for metal nanoparticles surface functionalization. Particularly, thermo-responsive polymers such as gelatin (Gaihre et al. 2009) and PNIPAM (Walker et al. 2020) have been extensively used for metal surface modifications. Apart from thermo-responsive delivery system, pH-responsive

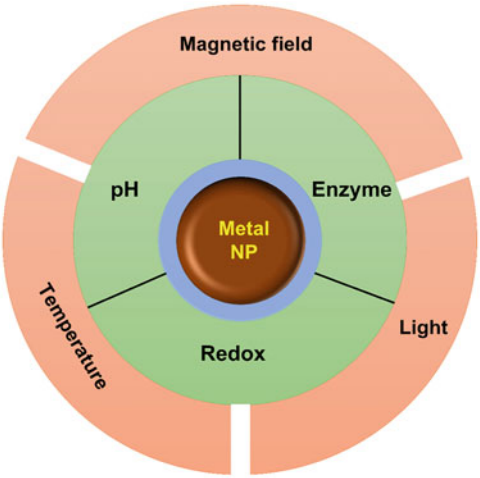

Fig. 5.4 Schematic illustration of several kinds of stimuli-responsive (pH, enzyme, redox, magnetic field, light, and temperature) polymer functionalized multifunctional metal nanoparticles

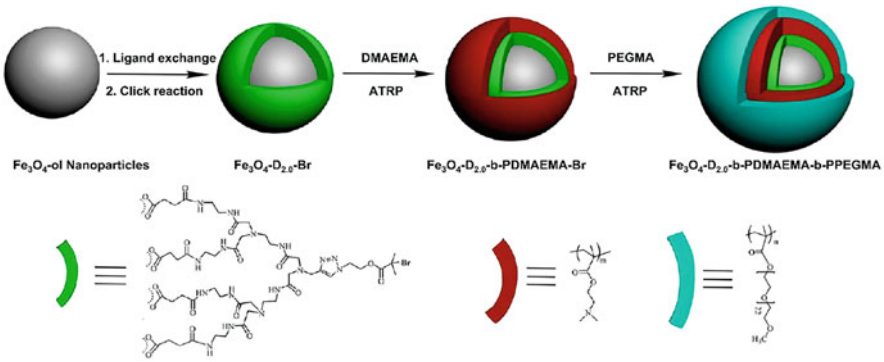

Fig. 5.5 Schematic illustration of surface modification steps of superparamagnetic Fe_3O_4 nanoparticles (He et al. 2012). Reproduced with permission from Xiaohua He, Xiaomeng Wu, Xin Cai, Shaoliang Lin, Meiran Xie, Xinyuan Zhu, and Deyue Yan. Functionalization of magnetic nanoparticles with dendritic-linear-brush-like triblock copolymers and their drug release properties. Langmuir 2012, 28; 11929–11938. https://doi.org/10.1021/la302546m; @2012 American Chemical Society

nanocarriers have also shown potential role in drug delivery applications. In cancer microenvironment, the irregular hypoxia and rapid angiogenesis can create lack of oxygen and nutrients which generates acid, and hence pH of the tumor environments become acidic compared to the normal tissue environment (Webb et al. 2011). He et al. have developed pH-responsive polymer (dendritic-linear-brush-like triblock copolymer) functionalized magnetic nanoparticles for cancer drug delivery (He et al. 2012). In this processing they used poly(amidoamine)-b-poly(2-(dimethylamino)-ethyl methacrylate)-b-poly(poly(ethylene glycol) methyl ether methacrylate) (PAMAM-b-PDMAEMA-b-PPEGMA) water-soluble dendritic copolymer for MNPs coating. A sequential strategy for polymer coating on MNPs surface is demonstrated in the schematic illustration (Fig. 5.5). The anticancer drug (DOX)

release at varying pHs (pH 4.7, 7.4, 11.0) in range of acidic, neutral, and basic condition was studied. They found that the drug release in acidic buffer solution was faster than both neutral and basic conditions. Li et al. have designed and synthesized the multifunctional magnetic nanoparticles coated with silica and then functionalized PAA polymer shell as a pH-responsive drug delivery and cellular imaging nanocarriers (Li et al. 2013). The anticancer drug DOX was electrostatically interacted with the negative charge surface of PAA functionalized and silica-coated magnetic nanoparticles. The DOX release from the multifunctional nanoparticles was significantly higher in the acidic pH media, and fluorescence imaging was confirmed by confocal laser microscope (CLSM) in cancer cell. Numerous studies with different metal nanoparticles have been explored with pH-responsive polymer for drug delivery in cancer therapy.

5.4 Properties of Metal Nanoparticles

Metal nanoparticles have great importance in the diverse field of biomedicine including imaging, targeting, and theranostics. The physicochemical and biological properties of the metal nanoparticles are mostly depending on the use of reagents and opted procedure for the synthesis. Depending on the requirement of the specific applications, various shape, size, structure, and surface functionalized metal nanoparticles have been synthesized by different methods. Efforts have been made to achieve varying dimensions of metal nanoparticles for biomedical application. However, understanding of fundamental mechanism of growth of different types (shape, morphology, and size) of nanostructure metal nanomaterials is still not very clear. The surface engineering of the metal nanoparticles (*i.e.* functionalization, coatings, nano-clustering) are different form of metal nanoparticles. Among the variety of metal nanoparticles, spherical- and nanorod-shaped metal nanoparticles play an imperious role in theranostics.

5.4.1 Physical Properties

Physical properties such as mechanical, thermal, optical, and electrical are very important to play a crucial role for metal nanoparticle theranostics applications. The physical properties are consisting of a variety of significant properties such as optical (UV adsorption, NIR light penetration, scattering, reflections), mechanical (strength, elastic modulus, ductility, etc.), thermal, electrical, and magnetic (Anu Mary Ealia and Saravanakumar 2017). These above properties are strongly dependent and influenced by large surface energy, abundant surface atoms, and their spatial arrangement. For example, copper nanoparticles smaller than 50 nm are super tough and do not show basic metallic characteristics like ductility and meltability, while superparamagnetism is also exhibited by magnetic materials when sizes reduce. It has been observed that the photovoltaic efficiency of thin films is more compared to the bulk materials. Moreover, small particles also facilitate the

sintering compared to bulk powder of micron or macro sizes. Ionic compounds show a high melting point compared to covalent materials, due to their wide gap of electronegativity. This required more energy to break the bonds. But for nanoparticles, less energy is required to break the bonds, hence showing a low melting point from its bulk form. This is also because of high surface energy and reactivity, ultimately, highly susceptible to oxidation. The phase transition temperature of nanosized particles is also found low with decreasing size. For example, barium titanate shows 130 °C in bulk form, while 75 °C when a size of 120 nm, respectively. The mechanical strength of the nanoparticle is found to increase with decreasing size (along with grain size) of nanomaterials. This depends on the internal perfection (fewer defects) and the perfection of facets in nanostructured materials (nanoparticles, nanorods, or nanowires). Bulk materials, including metals and polymers, consist of a large grain size, compared to nanomaterials; therefore, their mechanical strength and other properties are poorer than nanomaterials. Metal nanoparticles are mechanically very stable and possessed very high Young's modulus. For example, bulk Young's modulus for iron, gold, and silver is ~205 GPa, 79 GPa, and 83 GPa, respectively (Walch and Roos 2020; Guo et al. 2013).

The optical properties of metal nanoparticles are highly depending on the shape and size of the nanoparticles. The optical property of materials changes in two ways, firstly, quantum confinement of the electrons and secondly, surface plasmon resonance. In quantum confinement, when the metal particles' size reduced to below the de-Broglie wavelength, quantum confinement occurs, and level of energy becomes discrete. This leads to the different optical absorption and shows the change in colors. For example, below 10 nm size of nanomaterials, this discrete energy level is found so strong and sharp change in colors observed (Khan et al. 2019). The gold nanorod synthesized from small spherical gold nanoparticles by asymmetric growth in presence of shape controlling surfactant endowed varying color mainly due to the surface plasmon resonance (SPR) effects (Liu and Guyot-Sionnest 2005). The SPR is a unique feature of noble metal nanoparticles which generated strong electromagnetic fields on the nanoparticles surface which consequently enhanced the light absorption and scattering. This enhanced radiative optical property of AuNPs endows extremely advantageous for molecular bioimaging in cancer theranostics (Huang et al. 2006; El-Sayed et al. 2005).

The magnetic behavior of metal nanoparticles is different from its bulk form of materials. These specific properties of NPs make them an ideal candidate for MRI, data storage, targeted drug delivery, and biomedical applications. In the bulk form, particles are in multidomain, and when an external magnetic field is applied, all domains get aligned in the applied direction due to the domain growth mechanism which leads to less required energy for demagnetization (less coercivity), while, for nanoparticles, domains decrease with particle size and multidomain, converted into single-domain particles. When it reaches a critical size, a large magnetic field is required for rotating magnetization. Furthermore reduction in partcle size (*i.e.* below 40 nm), reduces the coercivity due to thermal effects, which cause of the transformation of ferromagnetic to superparamagnetic (Enrico 2020). This is a stable

position under an external magnetic field and is widely used in biomedical applications.

The thermal efficacy of NPs is much more superior compared to their solid bulk as well as their fluid form. This is because a large surface area of nanomaterials provides a better transfer of heat compared to bulk form. Nano metal oxides also act as a good filler for non-conducting polymers to enhance their thermal conductivity (Saleh 2020). For example, a small fraction of nano SiO_2 is sufficient to enhance the conduction of polycarbonate composite. The addition of a carbon-based nanofiller enhances the high thermal conductivity of non-conducting substances. The reason is that the NPs provide a large surface area for conduction and the presence of active atoms at the surface.

5.4.2 Chemical Properties

The chemical property of metal nanoparticles plays a tremendous role in theranostics. The chemical properties of the metal nanoparticles can be divided into two parts. First, the property which is indigenously associated with the particles which is mainly due to their solely atomic/molecular structure, and chemical structure, is known as intrinsic chemical property. Second, the chemical properties which can be achieved by surface functionalization or modifications are known as extrinsic chemical properties. The intrinsic chemical property of metal nanoparticles defines the stability and reactivity in their native state, while extrinsic chemical properties endowed the opportunities to further react with molecules and modify based on the one's requirement.

Multifunctional metal nanoparticles are mostly possessed by its extrinsic chemical properties which are conveniently changed through various chemical reactions. For example, during the synthesis of nanoparticles, many chemical reactions involved various agents and surfactants which are prone to condensations on the surface of the particles in the final stage of the reaction. The ligand exchange method is a very novel approach to change the surface chemistry. Merg et al. have demonstrated the control over surface chemistry and other properties of nanoparticles superstructures via ligand exchange approach (Merg et al. 2017). Drug loading is directly associated with chemical properties of the multifunctional nanoparticles. The conjugation of biomolecules, antibody, drug molecules, and imaging contrast agents to the multifunctional metal nanoparticles is associated to the chemical properties. Thus, chemical properties of multifunctional nanoparticles have a great role in theranostics applications.

5.5 Theranostic Applications of Metal Nanoparticles

There are ongoing clinical efforts to develop personalized medicine for various diseases with diagnostic and therapeutic ability into single delivery agent, and theranostic metal nanoparticles are promising in therapeutic paradigm.

5.5.1 Drug Delivery

In the pharmaceutical industry there are several drugs synthesized but limited to toxicological and therapeutic output. Drug delivery by metal nanoparticles is relatedly an advanced approach but rapidly developing field. In the neurodegeneration, central nervous system (CNS) diseases is very difficult to treat due to the blood-brain barrier, a physical and biochemical wall, makes delivery of drugs challenging, but in nasal administration of Lectins modified polyethylene glycol-polylactide-polyglycolide (PEG-PLGA), nanoparticles have improved drug delivery (Zhang et al. 2014). There is evidence that camptothecin (CPT) analogue, SN38 and conjugated with nanographene oxide (nGO) and branched with polyethylene glycol (PEG) combination was lethal to the cancer cells than irinotecan (Liu et al. 2008). Photocatalytic activity of up-conversion nanoparticles and enhanced permeation and retention (EPR) effect with gold and silver NPs have a significant improvement in the potential cancer therapy (Neha et al. 2021). In addition to this cancer tumors can be marked and visualized using metal NPs antigen-dependent and antigen-independent mechanisms. Moreover, due to their cellular and molecular detection and therapeutic mechanism, metal NPs are extensively investigated as a drug carrier in cancer drug delivery. Along with cancer, metal NPs are also used for HIV/AIDS therapy, ocular disease, and respiratory disease therapy (Anderson et al. 2019). Magnetically actuated NPs have been used for intraocular drug delivery by microrobots and magnetically guided self-rolled microrobots for targeted drug delivery (Nguyen et al. 2021; Kim et al. 2020). The AgNPs have been used to deliver ciprofloxacin, chlorhexidine, and metronidazole for treating periodontitis (Hussein-Al-Ali 2022; Steckiewicz et al. 2022). Furthermore, AuNPs coated with carbohydrate, amino acids, peptides, and proteins have been used for anti-cancer drug delivery and topical drug delivery for treating skin cancer (Khoshnevisan et al. 2018; Krishnan and Mitragotri 2020).

5.5.2 Imaging

Metal NPs are doorways to future imaging technology as the NPs overcome limitations of conventional contrast agents, e.g., by using X-ray CT and gold NPs of 1.9 nm as a contrast agent to image tumor in mice (Hainfeld et al. 2006). Nano radiosensitizers have been developed to deliver radiation therapy with therapeutic precision such as nanoparticles of superparamagnetic iron oxide (SPIO) and ultra-small superparamagnetic iron oxide (USPIO) and are mainly reported for use as imaging agent. Carbon nanotubes (CNTs) have attracted their use in imaging because of their thermal conductivity and tensile strength which indicate the potential for CNT use as field emission devices such as nanoscale transistors, scanning microscopy, and dynamic imaging. Along with iron oxide nanoparticles, single-walled carbon nanotubes (SWCNT) have been used for bioimaging applications.

Dendrimers are well-defined highly, branched molecules and synthesized by "divergent" or "convergent" method. The dendrimer such as Magnevist, a

gadolinium (III)-diethylenetriaminepentaacetic acid (Gd-DTPA) complex used as contrast agent for MRI. Quantum dots (QDs) have unique optical properties and they are fluorescent semiconductor nanocrystals (~1–100 nm), and they possess 10–100 times brightness than conventional dyes. QDs are used as fluorophores for in vivo imaging such as QDS of CdSe/Zns coated with PEG have been used to image lungs in mice (Akerman et al. 2002) and manganese-doped QDs as multimodal targeted probes for pancreatic cancer imaging (Yong 2009). Although imaging with multifunctional metal NPs is promising, there is lack of evidence of long-term safety issue with these multifunctional metal NPs. However magnetic NPs for imaging dopaminergic neurons and mammalian spermatozoa have been developed successfully (Jeong et al. 2016; Vasquez et al. 2016). Silver telluride NPs and silver nanoparticles coated with polymer have been used for X-ray imaging for breast cancer and as MRI contrast agent, respectively (Nieves et al. 2021; Amendola et al. 2021). AuNPs have been developed and functionalized to cross the blood-brain barriers (BBB) to deliver the drug and imaging of CNS and incremental X-ray imaging and proton therapy (Male et al. 2016; Torrisi et al. 2018).

5.6 Conclusions

This chapter highlighted the basic properties of multifunctional metal nanoparticles (MNPs, AuNPs, and AgNPs) and their applications as drug delivery and imaging in cancer therapy. The role of metal nanoparticles in theranostics has changed the diagnostics of cancer much faster and with more accuracy. The new methods of synthesis of these metal nanoparticles have achieved the precise control over particles production and surface functionalization with biocompatible molecules and endow high drug loading. The ability to deliver the drug in multiway under external stimuli such as pH thermal, magnetic field, electric field, light, and ultrasound has provided great hope for the treatment of cancer even at advanced stage. Collectively, the metal nanoparticles are one of the best nanocarriers which can be utilized as a multifunctional nanoparticle for theranostics applications.

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References

Akerman ME et al (2002) Nanocrystal targeting in vivo. Proc Natl Acad Sci U S A 99(20):12617

Amendola V et al (2021) Polymer-coated silver-iron nanoparticles as efficient and biodegradable MRI contrast agents. J Colloid Interface Sci 596:332

Anderson SD et al (2019) Magnetic functionalized nanoparticles for biomedical, drug delivery and imaging applications. Nanoscale Res Lett 14(1):188

Anselmo AC, Mitragotri S (2016) Nanoparticles in the clinic. Bioeng Transl Med 1(1):10
Anu Mary Ealia S, Saravanakumar MP (2017) A review on the classification, characterisation, synthesis of nanoparticles and their application. IOP conference series: materials science and engineering, p 263, 032019
Bansal SA et al (2020) Role of gold nanoparticles in advanced biomedical applications. Nanoscale Adv 2(9):3764
Chithrani BD et al (2006) Determining the size and shape dependence of gold nanoparticle uptake into Mammalian cells. Nano Lett 6(4):662
Cobb M (2018) Black pepper consumption in the Roman Empire. J Econ Soc Hist Orient 61 (4):519–559. https://www.jstor.org/stable/26572314
Corem-Salkmon E et al (2011) Convection-enhanced delivery of methotrexate-loaded maghemite nanoparticles. Int J Nanomedicine 6:1595
Cui L et al (2015) NIR light responsive core–shell nanocontainers for drug delivery. J Mater Chem B 3(35):7046
El-Sayed IH et al (2005) Surface plasmon resonance scattering and absorption of anti-EGFR antibody conjugated gold nanoparticles in cancer diagnostics: applications in oral cancer. Nano Lett 5(5):829
Enrico C (2020) Nanomedicine strategies for chemoresistance breast cancer theranostics. In: Thorat ND, Bauer J (eds) Nanomedicines for breast cancer theranostics. Elsevier, p 175
Fang W et al (2020) MRI enhancement and tumor targeted drug delivery using Zn2+-Doped Fe3O4 core/mesoporous silica shell nanocomposites. ACS Appl Bio Mater 3(3):1690
Fedoryshin LL et al (2014) Near-infrared-triggered anticancer drug release from upconverting nanoparticles. ACS Appl Mater Interfaces 6(16):13600
Fong J, Wood F (2006) Nanocrystalline silver dressings in wound management: a review. Int J Nanomedicine 1(4):441
Gaihre B et al (2009) Gelatin-coated magnetic iron oxide nanoparticles as carrier system: drug loading and in vitro drug release study. Int J Pharm 365(1):180
Giannaccini M et al (2014) Magnetic nanoparticles as intraocular drug delivery system to target retinal pigmented epithelium (RPE). Int J Mol Sci 15:1
Gnanasammandhan MK et al (2016) Near-IR photoactivation using mesoporous silica–coated NaYF4:Yb,Er/Tm upconversion nanoparticles. Nat Protoc 11(4):688
Guo D et al (2013) Mechanical properties of nanoparticles: basics and applications. J Phys D Appl Phys 47(1):013001
Gurunathan S et al (2009) Antiangiogenic properties of silver nanoparticles. Biomaterials 30 (31):6341
Häfeli UO (2004) Magnetically modulated therapeutic systems. Int J Pharm 277(1–2):19
Hainfeld JF et al (2006) Gold nanoparticles: a new X-ray contrast agent. Br J Radiol 79(939):248
He X et al (2012) Functionalization of magnetic nanoparticles with dendritic–linear–brush-like triblock copolymers and their drug release properties. Langmuir 28(32):11929
He S et al (2015) Ultralow-intensity near-infrared light induces drug delivery by upconverting nanoparticles. Chem Commun 51(2):431
Huang X et al (2006) Cancer cell imaging and photothermal therapy in the near-infrared region by using gold nanorods. J Am Chem Soc 128(6):2115
Hussein-Al-Ali SH et al (2022) Preparation and characterisation of ciprofloxacin-loaded silver nanoparticles for drug delivery. IET Nanobiotechnol
Jeong S et al (2016) Integrated magneto–electrochemical sensor for exosome analysis. ACS Nano 10(2):1802
Jin R et al (2014) Superparamagnetic iron oxide nanoparticles for MR imaging and therapy: design considerations and clinical applications. Curr Opin Pharmacol 18:18
Keshavarz H et al (2020) pH-Sensitive magnetite mesoporous silica nanocomposites for controlled drug delivery and hyperthermia. RSC Adv 10(64):39008
Khan I et al (2019) Nanoparticles: properties, applications and toxicities. Arab J Chem 12(7):908

Khodashenas B, Ghorbani HR (2019) Synthesis of silver nanoparticles with different shapes. Arab J Chem 12(8):1823
Khoshnevisan K et al (2018) The promising potentials of capped gold nanoparticles for drug delivery systems. J Drug Target 26(7):525
Kim DI et al (2020) Bilayer hydrogel sheet-type intraocular microrobot for drug delivery and magnetic nanoparticles retrieval. Adv Healthc Mater 9(13):e2000118
Kim Y et al (2022) Manipulating nanoparticle aggregates regulates receptor–ligand binding in macrophages. J Am Chem Soc 144(13):5769
Krishnan V, Mitragotri S (2020) Nanoparticles for topical drug delivery: potential for skin cancer treatment. Adv Drug Deliv Rev 153:87
Lee S et al (2021) Magnetic control and real-time monitoring of stem cell differentiation by the ligand nanoassembly. Small 17(41):2102892
Li L et al (2013) Multifunctional magnetic–fluorescent eccentric-(concentric-Fe3O4@SiO2) @polyacrylic acid core–shell nanocomposites for cell imaging and pH-responsive drug delivery. Nanoscale 5(6):2249
Li M et al (2014) NIR-triggered drug release from switchable rotaxane-functionalized silica-covered Au nanorods. Chem Commun 50(68):9745
Li Y et al (2018) Silver nanoparticles for enhanced cancer theranostics: in vitro and in vivo perspectives. J Biomed Nanotechnol 14(9):1515
Lin Q et al (2012) Target-activated coumarin phototriggers specifically switch on fluorescence and photocleavage upon bonding to thiol-bearing protein. J Am Chem Soc 134(11):5052
Lin Q et al (2013) Highly discriminating photorelease of anticancer drugs based on hypoxia activatable phototrigger conjugated chitosan nanoparticles. Adv Mater 25(14):1981
Liu M, Guyot-Sionnest P (2005) Mechanism of silver(I)-assisted growth of gold nanorods and bipyramids. J Phys Chem B 109(47):22192
Liu Z et al (2008) PEGylated nanographene oxide for delivery of water-insoluble cancer drugs. J Am Chem Soc 130(33):10876
Liu J et al (2013) NIR-triggered anticancer drug delivery by upconverting nanoparticles with integrated azobenzene-modified mesoporous silica. Angew Chem Int Ed 52(16):4375
Male D et al (2016) Gold nanoparticles for imaging and drug transport to the CNS. Int Rev Neurobiol 130:155
Mathivanan K et al (2019) Biologically synthesized silver nanoparticles against pathogenic bacteria: synthesis, calcination and characterization. Biocatal Agric Biotechnol 22:101373
McQuillan JS et al (2012) Silver nanoparticle enhanced silver ion stress response in Escherichia coli K12. Nanotoxicology 6:857
Merg AD et al (2017) Ligand exchange for controlling the surface chemistry and properties of nanoparticle superstructures. Chem Nano Mat 3(10):745
Neamtu M et al (2018) Functionalized magnetic nanoparticles: synthesis, characterization, catalytic application and assessment of toxicity. Sci Rep 8(1):6278
Neha D et al (2021) Metallic nanoparticles as drug delivery system for the treatment of cancer. Expert Opin Drug Deliv 18(9):1261
Nguyen KT et al (2021) A magnetically guided self-rolled microrobot for targeted drug delivery, real-time X-ray imaging, and microrobot retrieval. Adv Healthc Mater 10(6):e2001681
Nieves LM et al (2021) Silver telluride nanoparticles as biocompatible and enhanced contrast agents for X-ray imaging: an in vivo breast cancer screening study. Nanoscale 13(1):163
O'Reilly JP et al (2005) Interfacial pH at an isolated silica−water surface. J Am Chem Soc 127 (6):1632
Park MV et al (2011) The effect of particle size on the cytotoxicity, inflammation, developmental toxicity and genotoxicity of silver nanoparticles. Biomaterials 32(36):9810
Patel KD et al (2022) Recent advances in drug delivery systems for glaucoma treatment. Mater Today Nano 18:100178
Pissuwan D et al (2011) The forthcoming applications of gold nanoparticles in drug and gene delivery systems. J Control Release 149(1):65

Qian HS et al (2009) Mesoporous-silica-coated up-conversion fluorescent nanoparticles for photodynamic therapy. Small 5(20):2285
Rai M et al (2015) Bioactivity of noble metal nanoparticles decorated with biopolymers and their application in drug delivery. Int J Pharm 496(2):159
Raju HB et al (2011) Evaluation of magnetic micro- and nanoparticle toxicity to ocular tissues. PLoS One 6(5):e17452
Raju HB et al (2012) Investigation of nanoparticles using magnetic resonance imaging after intravitreal injection. Clin Exp Ophthalmol 40(1):100
Ray M, Ghosh K, Singh S, Mondal KC (2016) Folk to functional: an explorative overview of rice-based fermented foods and beverages in India. J Ethn Foods 3(1):5–18. https://doi.org/10.1016/j.jef.2016.02.002
Saleh TA (2020) Nanomaterials: classification, properties, and environmental toxicities. Environ Technol Innov 20:101067
Shang L et al (2014) Engineered nanoparticles interacting with cells: size matters. J Nanobiotechnol 12(1):5
Singh RK et al (2012) Biocompatible magnetite nanoparticles with varying silica-coating layer for use in biomedicine: physicochemical and magnetic properties, and cellular compatibility. J Biomed Mater Res A 100A(7):1734
Singh RK et al (2014a) Multifunctional hybrid nanocarrier: magnetic CNTs ensheathed with mesoporous silica for drug delivery and imaging system. ACS Appl Mater Interfaces 6(4):2201
Singh RK et al (2014b) Potential of magnetic nanofiber scaffolds with mechanical and biological properties applicable for bone regeneration. PLoS One 9(4):e91584
Singh RK et al (2019) Combinatory cancer therapeutics with nanoceria-capped mesoporous silica nanocarriers through pH-triggered drug release and redox activity. ACS Appl Mater Interfaces 11(1):288
Sintubin L et al (2011) The antibacterial activity of biogenic silver and its mode of action. Appl Microbiol Biotechnol 91(1):153
Sondi I, Salopek-Sondi B (2004) Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface Sci 275(1):177
Steckiewicz KP et al (2022) Silver nanoparticles as chlorhexidine and metronidazole drug delivery platforms: their potential use in treating periodontitis. Int J Nanomedicine 17:495
Tang J et al (2013) Graphene oxide-silver nanocomposite as a highly effective antibacterial agent with species-specific mechanisms. ACS Appl Mater Interfaces 5(9):3867
Torrisi L et al (2018) Gold nanoparticles by laser ablation for X-ray imaging and protontherapy improvements. Recent Pat Nanotechnol 12(1):59
Vasquez ES et al (2016) Bioluminescent magnetic nanoparticles as potential imaging agents for mammalian spermatozoa. J Nanobiotechnol 14:20
Walch EBT, Roos C (2020) Measurement of the mechanical properties of silver and enamel thick films using nanoindentation. Int J Appl Glas Sci 11(1):195
Walker M et al (2020) Magnetically triggered release of entrapped bioactive proteins from thermally responsive polymer-coated iron oxide nanoparticles for stem-cell proliferation. ACS Appl Nano Mater 3(6):5008
Webb BA et al (2011) Dysregulated pH: a perfect storm for cancer progression. Nat Rev Cancer 11(9):671
Xie X et al (2017) The effect of shape on cellular uptake of gold nanoparticles in the forms of stars, rods, and triangles. Sci Rep 7(1):3827
Xu L et al (2020) Silver nanoparticles: synthesis, medical applications and biosafety. Theranostics 10(20):8996
Yanai A et al (2012) Focused magnetic stem cell targeting to the retina using superparamagnetic iron oxide nanoparticles. Cell Transplant 21(6):1137
Yang Y et al (2012) In vitro and in vivo uncaging and bioluminescence imaging by using photocaged upconversion nanoparticles. Angew Chem Int Ed 51(13):3125

Yang Y et al (2013) NIR photoresponsive crosslinked upconverting nanocarriers toward selective intracellular drug release. Small 9(17):2937
Yang H et al (2020) Developing a general method for encapsulation of metal oxide nanoparticles in mesoporous silica shell by unraveling its formation mechanism. Microporous Mesoporous Mater 305:110381
Yang L et al (2022) Harnessing the therapeutic potential of extracellular vesicles for biomedical applications using multifunctional magnetic nanomaterials. Small 18(13):2104783
Yeh Y-C et al (2012) Gold nanoparticles: preparation, properties, and applications in bionanotechnology. Nanoscale 4(6):1871
Yin NQ et al (2017) Preparation and study of a mesoporous silica-coated Fe3O4 photothermal nanoprobe. RSC Adv 7(15):9123
Yong KT (2009) Mn-doped near-infrared quantum dots as multimodal targeted probes for pancreatic cancer imaging. Nanotechnology 20(1):015102
Zhang C et al (2014) Intranasal nanoparticles of basic fibroblast growth factor for brain delivery to treat Alzheimer's disease. Int J Pharm 461(1–2):192
Zhao L et al (2014) ear-Infrared photoregulated drug release in living tumor tissue via yolk-shell upconversion nanocages. Adv Funct Mater 24(3):363
Zhu K et al (2018) Magnetic nanomaterials: chemical design, synthesis, and potential applications. Acc Chem Res 51(2):404

Functionalized Lipidic Nanoparticles: Smartly Engineered Lipidic Theragnostic Nanomedicines

6

Namrata Gautam, Harish Vishkarma, Debopriya Dutta, Muskan Goyal, Lubna Siddiqui, and Sushama Talegaonkar

Abstract

The concept of theranostics revolutionized with the inception of surface engineering of nanolipidic carriers. In the modern era of precision medicine, the surface-functionalized lipidic nanotheranostic is found to be very promising for the diagnosis, therapy, and prognosis of many life-threatening diseases like cancer. The bimodal approach of diagnosis with treatment has paved the way for significant cost-effective therapy with splendid results. The current research updates and study findings are highly encouraging for the usage of nanolipidic theranostics in swift treatment of various inflammatory diseases. Moreover, in the present manuscript, an insight into smartly engineered nanolipidic theranostics is provided. Approaches for surface functionalization and outcomes are the highlight of the chapter. The chapter provides a comprehensive discussion on the impact of physicochemical attributes of nanolipidic theranostics on their in vivo performance (biofate). Additionally, the chapter brings forth the challenges and remedies for clinical translation of nanolipid-based theranostics.

Keywords

Functionalization · Nano-engineered · Theranostics · PEGylation · Ligands · Nanolipidic

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6.1 Introduction

In the current era of precision medicine, dose individualization and targeted drug delivery can easily be manifested by multidisciplinary smartly engineered nanotheranostics. The concept of theranostics was coined decades before with the usage of radionuclides of iodine and iron in the diagnosis and therapy of cancer. However, from the time of its inception till date, it is a field of profound attention among researchers due to its remarkable potential in the prognosis and treatment of several inflammatory diseases. With the emergence of nanosystems in therapeutics and diagnostics, a novel amalgamation of nanotheranostics has evolved. This bimodal approach has augmented a distinct class of nanoformulations with diverse applications, i.e., identification of target receptor (based on real-time diagnosis), monitoring the disease progression, treatment by dose individualization, image/radio/electric/magnetic-guided drug delivery, and pharmacogenomics. Advancement in nanomaterials and their special attributes of electrical, magnetic, optical, thermal, and pH sensitivity have been well explored in designing of nanotheranostics. Furthermore, to device such systems, surface engineering has also been spurred on. It enables tailoring of nanotheranostics for desired attributes and confers a subset of targeted tunable systems with enhanced biocompatibility. Global market share of theranostics comprises enormous nano-compounds for treatment of neurodegenerative diseases (Alzheimer's, Parkinson's, Epilepsy, and Huntington's), cardiovascular diseases (atherosclerosis, ischemia, hypertension, myocardial infarction, and thromboembolism), life-threatening tumors (breast, lung, brain, pancreatic, and colon), and fatal autoimmune disorders. Extensive research is continuously being done for such nanoformulations in preclinical and clinical stages (Nanoformulations in Human Health 2020).

Earlier, theranostics were designed with stimuli-responsive agents only. However, the emergence of new classes of nanolipidic carriers and active targeting with ligands has promoted them to the next level. The basic model of theranostics was designed based on various endogenous (pH, enzymes, hypoxia, etc.) and exogenous (temperature, light, ultrasound, and magnetic field) stimuli. Furthermore, imaging agents were also exploited to determine the pharmacodynamics of nanostructures in real-time. Radiation-based techniques like X-ray imaging, gamma scintigraphy, ultrasound imaging (US), magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), and digital radiography were utilized for photodynamic, photoacoustic, and photo thermal therapy. However novel nanotheranostics are designed on the basis of their physicochemical properties including their optical, magnetic, thermal, and radioactive properties. The primitive nanotheranostics were only limited to inorganic nanoparticles of metallic compounds encompassing an array of bio-actives for therapeutic payload. Usually ferric oxide-based, magnetically modulated systems were the first choice in targeting and therapy of tumors. Despite magnificent applications, inorganic theranostics have certain limitations in vivo, for instance extreme hydrophobicity, immunogenicity, bio-incompatibility, improper biodistribution, and toxicity upon accumulation. Consequently, bio-inspired nanomaterials (lipidic and polymeric systems), capable of

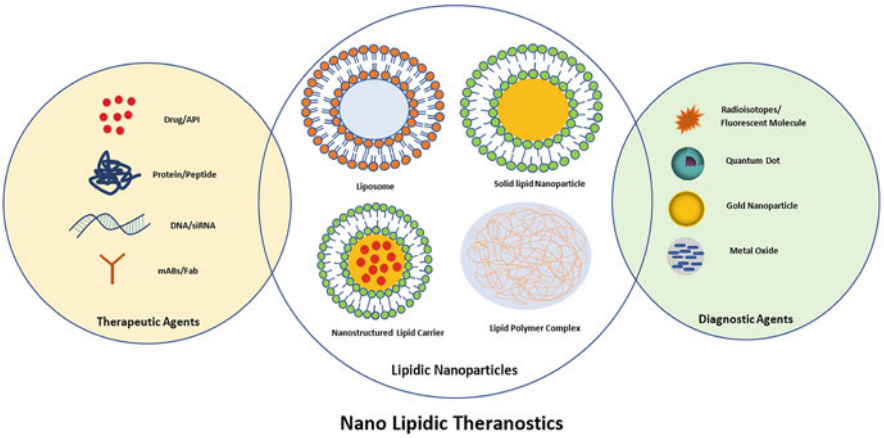

Fig. 6.1 Nanolipidic theranostics

circumventing hurdles associated with inorganic/metallic nanotheranostics, are continuously gaining popularity. Among various subsets of NPDDS, lipidic systems have evolved as the most functional carriers for theranostics due to their comparable characteristics like high drug loading and controlled and site-specific targeting. Various lipid-based nanocarriers like liposomes, micelles, SLNs, NLCs, and peptide drug conjugates have been extensively investigated for their potential as theranostics. Few examples of such nanolipidic theranostic carriers are schematized in Fig. 6.1.

6.1.1 Overview on Smartly Engineered Nanolipid-Based Theranostics

Sophisticated surface engineering techniques have fostered the manipulation and programing of such nanocarriers as per requirement of the biological system. Various categories of ligands peptides, enzymes, polymers, saccharides, antibodies, etc., along with contrast agents have been explored for simultaneous imaging and therapy by well-controlled formulations which are amenable to cross barriers and deliver the biomedicine at their target site. Lipidic nanoconstructs of theranostics are perfect candidates for targeting and early detection of carcinomas. This is the time at which a relevant therapy can be initiated and thereby potential toxicities of cancer therapy can be avoided. Such nanolipidic systems are profoundly designed to penetrate at the tumor site and determine its actual status (benign or malignant). Furthermore, the co-encapsulated drug delivered at tumor site will decide the efficacy of cancer therapy. Upon monitoring the in vivo fate of drug and diagnostic or drug cum diagnostic, disease progression and effectiveness of provided therapy can be accessed. Due to proven superiority of nanolipidic carriers over polymeric and inorganic nanoparticles, formulation of lipidic nanotheranostics is in latest trend.

Therefore, the combinatorial approach of diagnosis and treatment with nanolipids could offer a next level cancer therapy with highly efficacious clinical outcomes. Depending upon lipids selected various vesicular or micellar nanolipidic carriers can be formulated. In general, the most exploited nanolipid carriers for theranostics are liposomes, SLN(s), NLCs, and lipid-drug conjugates. However, nanoemulsion is also the carrier of choice for certain category of drug/diagnostic. Generally, in nanoemulsions the diagnostic/contrast agent and drug are coencapsulated in oil globules, whereas in liposomes partitioning of imaging agent and drug is variable; both of them could be entrapped in aqueous core or in lipid bilayer. Interestingly, in the case of SLNs, it is reported that both drug and contrasting agents are embedded in solidified lipid matrix. However, electron microscopy of NLCs reveals that in these lipidic nanoconstructs, drug and imaging agent are abruptly distributed in oil globules as well as solidified lipid matrix. Therefore, formulation of optimized and stable nanolipidic theranostics with desirable characteristics of size, shape, charge, and polydispersibility requires expertise in field of nanomedicine. Despite of several challenges, this combinatorial approach of simultaneous imaging (diagnosis), targeting (active or passive by surface functionalization), and delivering nanomedicine via nanolipidic nanocarriers is quite worthy and seems to be a new paradigm in the treatment of various diseases including tumors (Bukhari et al. 2021).

Briefly, the reported mechanisms involved in surface functionalization are deposition, encapsulation, and incorporation (based on physical, chemical, or mechanical interaction between ligand and nanocarrier system). In addition, synthetic and natural biomaterials or polymers are coated on the surface or conjugated during fabrication for functionalization. Nevertheless, there are notable hurdles in the surface functionalization of lipidic theranostics and thus their clinical translation to nanomedicines. For instance, besides huge research on nanomaterials, only a few selective categories of compounds are capable of inherent imaging along with therapeutic properties. In addition, the associated toxicity of imaging/guiding/conducting material limits their usage. Storage stability and inconsistent in vivo performance also pose restrictions in designing these nanosystems. Furthermore, hurdles in regulatory approvals impede their clinical translation.

For designing a surface-functionalized theranostic system, thorough screening should be done before selection of drug/diagnostic agent and excipients. The prime decisive variables in formulation of nanotheranostics are nanomaterial for carrier, carrier-drug loading potential, physiochemical properties of drug/imaging agent, and the formulation method adopted. However, it is quite challenging to modulate the properties of diagnostic agent for therapeutic purpose. For instance, radio-contrast or imaging agents are well known to bind faster to target receptor with rapid systemic clearance, whereas for catering therapeutic needs, they are required to stay longer in circulation for their maximum uptake. Here it comes the role of nano-engineered-carrier systems. Thus, to integrate theranostics with nanosystems, one must have sound knowledge of nanomaterials and their inherent physiochemical properties. Additionally, functionalization by passive or active means may harness and potentiate their utilization for widespread applications. Precisely surface engineering has led to attain the following key characteristics in theranostics: (a) identification of

targeted cells/tissues/genetic-material and optimal receptor-interaction, (b) minimal drug degradation during entire residence of drug inside the body, (c) required circulation time for effective treatment with lesser dose, (d) biocompatibility and biodegradability (avoidance of possible toxicities), and (f) ease of fabrication, i.e., industrial applicability. Besides the above-stated advantages, nanotheranostics offer promising potential of high payload for diagnostic or therapeutic agent or both and thus can be opted for a safer and radicalized therapy precluding the redundant treatment models.

In the present chapter, an insight of nanolipidic theranostics carriers is provided, covering the latest trends in surface functionalization (for active and passive targeting) approaches and in vivo biofate of nanotheranostics. The chapter reviews on surface functionalization of nanolipidic carriers (liposomes, SLNs, NLCs, and drug lipid conjugates) for theranostic applications and provides a comprehensive update in the field of nanolipid-based theranostics loaded with drug and diagnostic/biomarker/contrast agent. The chapter also highlights various nanolipid-based theranostics.

6.1.2 Surface Functionalization Approaches for Programmed Theranostics

Researchers have proposed that selective targeting of potent drugs curtails the problems associated with conventional therapies and hence efficient drug delivery of therapeutic agents can be achieved. In the past, the non-specific biodistribution of drugs was very common due to biological barriers, binding with cellular proteins, metabolizing enzymes, undesired uptake, and drug efflux pumps. Also, these challenges addressed further complications and led to the accumulation of drugs at a non-target site and potential toxicities of vital organs. For attaining therapeutic concentration in the requisite body compartment, large doses of the drug were being administered (Riaz et al. 2017). However, current strategies of engineering nanocarriers have resolved these problems to a certain extent. In this sequel of potential targeting, various approaches and strategies have emerged. Guyon, L et al. reviewed various approaches to surface functionalization, purification, and characterization of engineered nanolipidic carriers (Guyon et al. 2021). Kim, CH et al. (2017) explained various approaches of active targeting with ligands, passive functionalization techniques, and stimuli-sensitive systems for efficient targeting of tumor cells (Jeon et al. 2021). They also explained the role of functionalization in increasing biocompatibility, circulation time, and the fate of functionalized nanolipidic systems in vivo. Some of the well-known strategies of surface functionalization and targeting are explained below and illustrated in Fig. 6.2.

6.1.2.1 PEGylation

Passive targeting refers to the recruitment of drug or drug-loaded carriers at the target site without any specific interaction with cells/molecules. Instead of any coupling or chemical interaction of ligands, physicochemical, pharmacological,

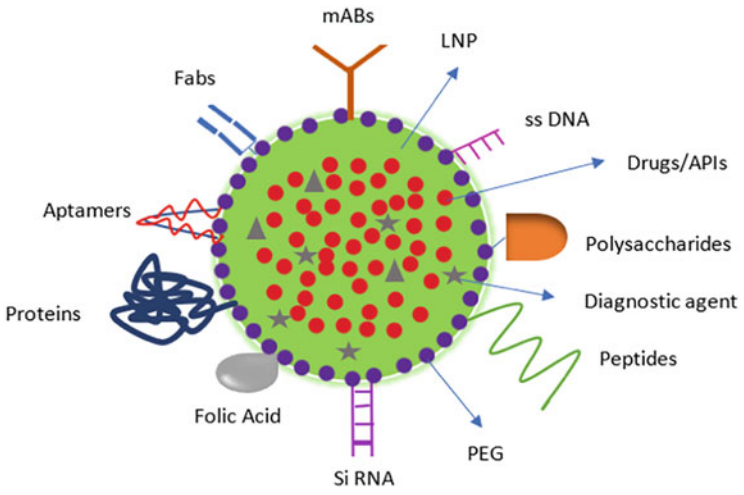

Fig. 6.2 Surface functionalization of theranostics

pathophysiological, and anatomical features of the diseased organ are driving factors of accumulation of drug-loaded carriers at the target site. Several examples of such nanolipidic carriers have been reported in the literature for targeting tumor, malaria, fungal diseases, and so on (Attia et al. 2019). Various physiochemical or pathological circumstances are evaluated as approaches to design nanolipidic systems, which trigger the accumulation and release of drugs at the target site. For instance, permeability and leakiness of membranes at tumor site, pH, temperature, shape, charge, and size are a few criteria that affect the biodistribution, pharmacokinetics, and the toxicity profile of the developed nanocarrier. Surface chemistry and charge impart a key role in passive targeting too. It is well known that hydrophobic or charged carriers undergo rapid opsonization by the MPS. Therefore, surface modifications are recommended to turn their surface more hydrophilic and neutral or slightly anionic. Additionally, PEG-like water-soluble polymers are grafted on the nanocarrier surface either by coating or physical adsorption. PEGylation is one of the most exploited techniques of passive targeting wherein PEG is either coated or conjugated at the surface of nanolipidic system. PEG is a non-ionic hydrophilic polymer with huge applications in pharmaceutical formulations. Due to its highest biocompatibility, it is a suitable candidate for passive targeting of nanolipidic systems. PEGylation is generally done either during the synthesis of nanoparticles (covalent bonding) or surface adsorption/coating after formulation development.

Furthermore, two different configurations of PEG have been reported, i.e., low density or mushroom configuration and a high-density or brush-type configuration, which somehow impact the efficacy of PEGylation. Upon ingress into the bloodstream, PEG functionalized nanolipidic carriers interact with surrounding biomolecules (proteins). However, it has been reported that long polymeric chains of PEG repel and retard the adsorption of proteins on their surface, and thus

recognition and opsonization are avoided. Consequently, PEGylated nanolipidic carriers attain a prolonged retention time in blood circulation and impart a sustained-release effect with maximal bioavailability. Despite huge applications, in some specific cases, PEGylation leads to hindrance for crossing some biological membranes like endosomal layers and thus poor cytosolic delivery. Jeon M and his colleagues prepared PEGylated dual-layered Au-liposome as theranostic for effective tumor targeting and photothermal therapy. They reported that surface functionalization with PEG improves the in vivo stability of gold liposomes and radioisotope labeling enables in vivo imaging. The results confirmed higher tumor targeting and better tumor growth inhibition rate (3.9-fold higher) than non-functionalized liposomes (Jeon et al. 2021). Xiong, H et al. developed dendrimer-based lipid nanoparticles (DLNP) containing PEGylated BODIPY dyes for tumor imaging and systemic mRNA delivery in vivo (Xiong et al. 2020). The theranostic containing both PEGylated BODIPY dyes (PBD) and mRNA was a pH-responsive system with reported 5- to 35-fold more efficient mRNA delivery in vivo. Petersen, AL et al. prepared and evaluated PEGylated Cu-liposomes with theranostic and radio therapeutic potential in tumors. They reported a higher accumulation of radionuclide in tumor cells due to PEGylation and thus serving the purpose of theranostic (Petersen et al. 2016).

6.1.2.2 Ligand Functionalized Nanolipidic Theranostics

All nanolipidic systems exhibit certain challenges and limitations. For instance, liposomes show immunogenicity and require refrigeration for storage. SLNs have comparatively low entrapment efficiency and burst release at their target site. The toxicity of quantum dots carrying liposomes is a major issue to be addressed (Salahpour Anarjan 2019). Therefore, nanolipidic systems should be properly evaluated and optimized before targeting. Despite several advantages of passive targeting, it is noteworthy that the benefits of active targeting are incomparable. Potential merits include selective targeting of biomolecules, genes, and theranostics to target cell/tissue/nucleus without any undesirable wastage of drug in the surrounding region (Yoo et al. 2019; Moosavian and Sahebkar 2019). Thus several classes of potent drugs, cytotoxic drugs, and diagnostics can be accumulated in the desired compartment of the body via active targeting and thereby increased efficiency in minimal dose. Active targeting involves surface engineering of nanolipidic carriers with specific ligands having significant receptor interaction potential. Consequently, deeper penetration of carriers at the target site is obtained to release the drug in adequate therapeutic concentration. Three decades ago, this approach was firstly proposed for liposomes decorated with antibodies on their surface. However upon advancement of diagnostics, biomarkers, and imaging techniques as well as identification of more receptors, the ease of designing and selection of ligands for active targeting became more convenient. Kumar R and his team prepared surface-functionalized nanolipid-drug conjugate-based theranostics of quercetin to target breast cancer. Surface functionalization was done with N-acetyl-D-glucosamine,

which has a strong affinity toward breast cancer cells (MCF-7). The reported results confirmed the higher recruitment of carriers in tumor cells, a high payload of drug, and a more cytotoxic effect of quercetin as compared to free quercetin (Kumar et al. 2022). Moosavian, SA et al. (2019) reviewed over aptamer-functionalized liposome for tumor targeting and disclosed potential targeting receptors and corresponding aptamer ligands for them. Synthesis of aptamers is challenging and noneconomical; despite of that, because of its efficient targeting, a number of aptamers are in clinical trial phase (Moosavian and Sahebkar 2019). Recently a theranostic has been developed by Flores, NG et al. (2021) in the form of functionalized liposome of erythrocruorin. The system comprises liposome complex with EfEc (Eisenia fetida erythrocruorin) and PEG meant for treatment and diagnosis of blood diseases. They reported that, due to oxygen carrying and hemoglobin binding capacity, erythrocruorin can serve the purpose of RBCs in emergency; additionally due to its photo-luminescent properties, it can be used for the localization of proteins, cell, tissues, veins, and arteries (García-Flores et al. 2021). Zafar, A et al. (2022) worked on functionalization of erythromycin loaded nanostructured lipid carriers (NLCs) by transforming them into an in situ gel preparation in presence of Carbopol 940 and chitosan. The study concluded that gel transformed NLCs could be an alternative mode of therapy in bacterial conjunctivitis (Zafar et al. 2022). Neves, A et al. (2021) targeted the brain delivery of neuroprotective curcumin by surface-functionalized NLCs. For this, firstly curcumin-loaded NLCs were prepared, and thereafter surface functionalization was done with transferrin for effective permeation of these carriers through BBB. Furthermore, they concluded that functionalization with transferrin does not interfere with morphology, average particle size, polydispersity index, and zeta potential of NLCs upon 3 months of storage. However, upon longer storage it has been reported that the entrapment efficiency of curcumin in functionalized nanoparticles declines gradually due to steric hindrance as well as competition between transferrin and curcumin molecules (Neves et al. 2021). Therefore, it is recommended to utilize freshly functionalized nanoparticles for therapeutic purposes. In one more study, NLCs of etoposide were prepared for lymphoma targeting, functionalized by multiple ligands. The drug-loaded NLCs were decorated with D-a-tocopheryl succinate (TOS), hyaluronic acid (HA), and cell-penetrating peptide transcription activator (TAT) and prepared different conjugates like (HATOS/TATTOS-ETP-NLCs). The functionalized NLCs showed significantly high transfection efficiency along with cytotoxicity with respect to non-functionalized carriers in lymphoma cells-bearing mice model (Wang et al. 2017). Conclusively it is evident that various classes of ligands have been identified to target not only tumors but also several other diseases like bone disorders, skin problems, brain targeting, parasitic diseases, systemic disorders, etc. Some of the examples of ligands which are in trend for surface modulation include glycoproteins, antibodies and their fragments, peptides, aptamers, enzymes, saccharide ligands, and internalizing ligands, i.e., biotin, folic acid, and transferrin.

6.2 Nanolipidic Carriers as Theranostic Systems

A gradual escalation in nanotechnology from the very first type of solid lipid nanopellets to surface-functionalized NLC has led to a new era of nanolipidic carriers for delivery of theranostics. Over the decades, researchers worked upon designing of nanolipidic systems based upon optimization of several formulation factors including ease of formulation, avoidance of organic solvents, and minimal toxicity. The primary proposed structure of nanolipidic systems possesses subsets of spherical structures with a mandate of presence of at least one lipid bilayer jacketing around an aqueous compartment. Later on, attributes of self-assemblage, bioavailability, biocompatibility, high pay loads, and most importantly tunability of physiochemical attributes to adapt and modulate the biological parameters were explored. In the current arena of nanotechnology, the extremely exploited lipid-based carriers for nanotheranostics are liposomes, lipid-drug conjugates, nanostructured lipid carriers, and solid lipid nanoparticles, as schematized in Fig. 6.3.

6.2.1 Liposomes

In the current era, functionalized liposomes are the best chosen carrier systems for delivery of vaccines, genetic materials, biomedicines, and theranostics. The basic structure of liposomes comprises phospholipids, which can be transformed into unilamellar or multilamellar vesicular structures. Tunability to alter the particle size, surface charge, lipid composition, number of lamellae, and surface engineering enables them to load all the three classes of drugs as per BCS system. Furthermore, their non-immunogenicity, biocompatibility, and ease of surface functionalization allow them to be heavily exploited as carriers for nanomedicine. However, it has been observed that they are also victim of RES system; therefore surface coating and modulations are the best way to bypass their engulfment in macrophasic systems and thus enhance their circulation time. Tremendous research has already been done on liposomal surface functionalization by both active and passive means. For instance, stealth/PEGylated liposomes, immunoliposomes, theranostic liposomes, peptide-based liposomes, and stimuli-sensitive liposomes (temperature, pH, magnetic field) are some of the examples of functionalized liposomes designed for selective target receptors. Liposomes as theranostics are of great importance and gaining popularity day by day. They are system of choice in detection and treatment of cancers due to their biocompatibility and non-immunogenicity. Recent research update over theranostic liposomes has been enlisted in Table 6.1. Panikar, SS et al. prepared methylene blue-based theranostic liposomes for photodynamic therapy of breast cancer. Methylene blue (MB) was coencapsulated with upconverting nanoparticles (UCNPs) of imaging agents in liposomes for diagnosing and targeting breast cancer cells. The UCNPs were added as energy source for the photosensitization of methylene blue and thus selective tracking or bioimaging of HER 2 cells. The liposomes were surface-functionalized anti-HER2 peptides for selective targeting. The results suggested a significant decline in the cell viability by 83% by liposomes

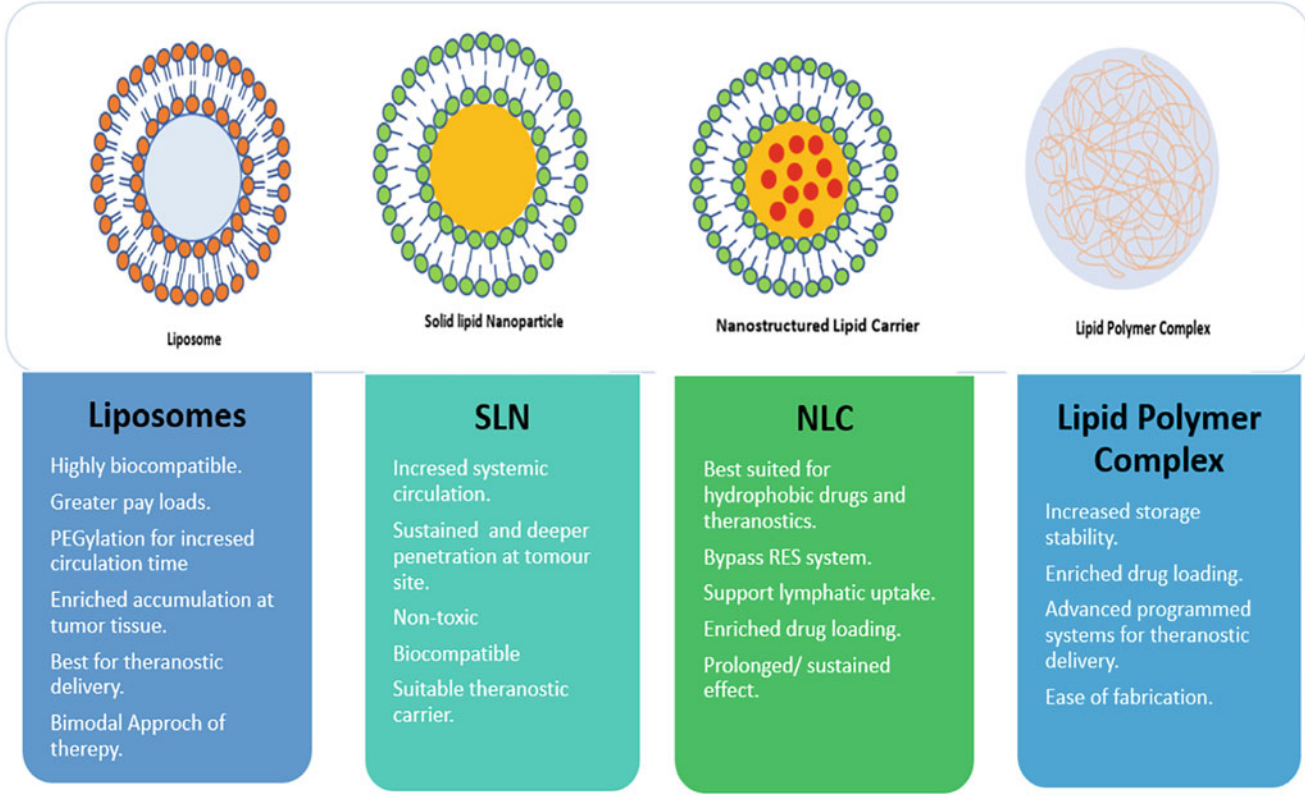

Fig. 6.3 Lipidic nanocarriers as theranostic systems

Table 6.1 Liposomal theranostics

Lipid nanocarrier	Disease	Therapeutic agent	Diagnostic agent	Experimental model	References
Liposome-loaded microbubbles	Rheumatoid arthritis	Dexamethasone	Liposome-loaded microbubbles and ultrasound beam	Murine collagen induced arthritis mouse model	Deprez et al. (2022)
Hydroxyapatite-coated liposomes	Osteoporosis	Bupivacaine	Quantum dots	Rat model	Lopes et al. (2021)
iRGD peptide modified liposome	Hepatocellular carcinoma	Hydroxycamptothecin	Photoacoustic and ultrasound beam	Subcutaneous xenograft model of SK-Hep1, 3D Multicellular Tumor Sphere (MCTS) Model	Li et al. (2021)
PEGylated liposomes	Alzheimer	Benzothiazole	2-(4-aminophenyl) benzothiazolyl-decorated liposomes	In vitro hCMEC/D3 blood-brain barrier (BBB) model	Mourtas et al. (2020)
Transferrin-decorated liposome	Brain cancer	Docetaxel	Glutathione reduced gold nanoparticles	C6 glioma cell line, Albino rat model	Sonkar et al. (2021)
PEGylated liposomal layer gold liposome	Tumor	Laser irradiation to provoke killing of tumor cells via hyperthermia mechanism	Gold nanoparticles and radioisotope labeling (^{64}Cu)	Orthotopic breast cancer mouse model	Jeon et al. (2021)
Anti-CD44 antibodies conjugated liposomes	CD44 receptor/ cancer cell	Curcumin	Carbon dots	U-87MG and HaCaT cell lines	Demir et al. (2020)
Folate liposome	Liver cancer	Doxorubicin	Gd^{3+}-texaphyrin	Liver cancer mouse model, HepG2, NIH3T3, KB and CT26 cell lines	Lee et al. (2016)

(continued)

Table 6.1 (continued)

Lipid nanocarrier	Disease	Therapeutic agent	Diagnostic agent	Experimental model	References
AS1411 aptamer-modified liposomes	Renal carcinoma	Paclitaxel	Manganese oxide nano-contrast agent (enhance MRI effect)	Colon cancer-bearing mouse model	Li et al. (2019)
Fe_3O_4-modified liposomes	Parkinson	Resveratrol	Externally applied magnetic resonance	Parkinson induced rat model	Wang et al. (2018)

as compared to free methylene blue (Panikar et al. 2021). Sonju, JJ and his colleagues reviewed over peptide-functionalized liposomes for anticancer agents and concluded that peptide-functionalized liposomes show synergism in treatment of cancer in comparison to either of them given alone (Sonju et al. 2021). Teixeira, S et al. (2022) studied over albumin-functionalized liposomes and reported that albumin over liposomal carriers facilitates encapsulation of hydrophobic drugs and their selective targeting to cancer cells and hence avoids premature drug release, reduced systemic toxicity, and effective against multidrug-resistant tumors (Teixeira et al. 2022). Thus surface modulations are recommended not for targeting only; however they play commendable job for enhanced bioavailability and biocompatibility.

6.2.2 Solid Lipid Nanoparticles (SLN)

Among various nanolipidic systems, SLN have been extensively exploited due to their comparative beneficial attributes of highest drug loading, avoidance to dose dumping, stability, and targeting efficiency. Albuquerque, J et al. prepared and evaluated anti-CD64 antibody surface-functionalized SLNS of methotrexate and iron oxide for selective targeting of macrophage in treatment of rheumatoid arthritis. They co-encapsulated both Methotrexate (MTX) and super paramagnetic iron oxide nanoparticles inside the SLNs to serve the purpose of theranostics (Albuquerque et al. 2015). Furthermore, Grillone et al. 2015 prepared sorafenib SLN-based magnetic theranostics for treatment of hepatocellular and renal carcinoma. Coencapsulation of sorafenib and iron oxide nanoparticles was done in solid lipid matrix. They concluded that the theranostic is quite efficient to localize enormous amount of sorafenib SLNs and inhibit cancer cell proliferation by its cytotoxic action. A research study has reported specific targeting of red blood cells of parasites upon functionalization with heparin. In addition, the study revealed that heparinized SLNs show superior in vitro activities than free chloroquine on parasites. It has been observed that SLNs are meant for prolong drug release and better candidates for chronic therapy (Muga et al. 2018). However, besides their huge applications, some demerits are also there, like lower payload of drug/diagnostic, dose dumping due to polymorphic transition of lipids when stored for longer durations, and higher water content. In order to meet these challenges, specifically to deal with poor drug loading, newer modified lipids and lipidic systems are introduced. For instance, nanostructured lipid carriers (NLC) and lipid–drug conjugates (LDC) are the exaggerated versions of SLNs. Some of the SLNs-based theranostics are listed in Table 6.2.

6.2.3 Lipid Nanoparticles (LNPs) or Lipid–Drug Conjugates

Another class of nanolipidic systems includes lipid nanoparticles (LNPs) which actually mimic liposomes instead of SLNS and best suited for delivery of theranostics. However besides huge resemblance to liposomes, they also exhibit

Table 6.2 Theranostics as solid lipid nanoparticles and nanolipid–drug conjugates

Lipid nanocarrier	Disease	Therapeutic agent	Diagnostic agent	Experimental model	References
Lipid–Polymer NPs (LiPoNs)	Cancer	Irinotecan	Gd-DTPA/Atto 633	U87 MG cancers cells	Roffo et al. (2022)
Lipid nanocomposite vehicle	Melanoma	Doxorubicin	Fe_3O_4 nanoparticles	Mouse melanoma tumor model	García-Hevia et al. (2022)
Magnetic nanostructure-stabilized lipid nanocapsules (MLNCs)	Cancer and autoimmune diseases	Paclitaxel and curcumin	Oleic acid-coated hydrophobic $Zn0.2Mn0.8Fe_2O_4$ nanoparticles	The HepG2 liver cancer cell line and J774 murine macrophage cells	Nandwana et al. (2018)
Porphyrin-grafted lipid (PGL) NPs	Tumor	Doxorubicin	Dipalmitoyl-sn-glycero-3-phosphocholine (DSPC)	Xenograft mouse model further	Hameed et al. (2018)
Lipid-poly-(hypoxic radiosensitized polyprodrug) nanoparticles	Malignant glioma	Doxorubicin	MIs-based polyprodrug (P-(MIs)n)	Glioma-bearing ICR mice	Hua et al. (2018)
Lipid micellar nanoparticles	Cancer	Paclitaxel (PTX)	Quantum dots (QDs)	Mice bearing EGFR-positive LS174T tumor xenografts	Kang et al. (2018)
Self-assembled lipid nanoparticles (SALNs)	Bleeding	Heparin	Iron oxide nanoparticle	CaCo-2, a cell line	Truzzi et al. (2017)
Multifunctional crgd-IR-780 SLNs	Cancer	cRGD-IR-780	cRGD-IR-780	Female athymic nude mice	Kuang et al. (2017)
SLN	Rheumatoid arthritis	Methotrexate	Superparamagnetic iron oxide nanoparticles (SPIONs)	THP-1 Cells lines	Albuquerque et al. (2015)
SLN	Atherosclerosis	Prostacycline (PGI2) Or α-tocopherol	Iron oxide nanoparticles clusters	Human volunteers' blood	Oumzil et al. (2016)

SLN	Orthotropic prostate cancer	Hpps(NIR)	siRNA	Orthotropic prostate cancer rat model	Lin et al. (2014)
Anti-CD64-conjugated SLNs	Rheumatoid arthritis	Methotrexate	Superparamagnetic iron oxide nanoparticles (SPIONs)	THP-1 cells lines	Albuquerque and Costa (2014)
SLN	Cancer	Paclitaxel/ siRNA	Quantum dots	A59 cancer cells	Bae et al. (2013)

contrasting features like formation of micellar structure around the solid core instead of lamellae. In addition, several morphological modifications are feasible upon modulation of synthetic parameters too. Generally, four subsets of lipids are incorporated in such lipidic systems: (**a**) cationic (ionizable) lipids that are meant to complex with genetic material (anionic) for their endosomal escape; (**b**) phospholipids which maintain integrity of nanoconstruct; (**c**) cholesterol, required for attaining transition and transformation of various shapes and thus enables membrane fusion and stability; and (**d**) PEGylated lipids which are specifically attached to surface for functionalization to avoid RES systems and hence increased circulation time (Pinelli et al. 2020). Due to their simplicity of formulation development and tunability, these are selected as ideal carrier for gene targeting at cellular/nuclear level. Moreover, presence of charge idealizes them for interaction with genetic material. It is reported at physiological pH, they are neutral whereas upon entering in acidic endosomal compartments, they undergo ionization and bear charges, which leads to their endosomal escape and facilitates intracellular delivery. Researchers have also worked upon brain targeting of riluzole for amyotrophic lateral sclerosis, a typical neurodegenerative disease with poor prognosis. To permeate across BBB, they formulated lipid–drug conjugate-based theranostics, functionalized with lactoferrin that interacts efficiently with lactoferrin receptors expressed in brain endothelium. The results suggested that the developed lipidic nanocarriers are capable of crossing across tight junctions of brain endothelium and can deliver riluzole in effective concentration with high biocompatibility and stability (Teixeira et al. 2022). Arshad, R et al. (2021) reviewed over various approaches to functionalize nanolipid carriers to target retinoblastoma. They reported that intraocular chemotherapy is quite challenging since crossing ocular barriers to reach site of malignancy requires thorough understanding of distinctive anatomical features of tumorigenic site (Arshad et al. 2021). Further drug spillage, irritation, burning, vision impairment, patient noncompliance due to discomfort, and other limitations restrict ocular targeting and thus inadequate therapy. However functionalized lipidic–drug conjugates could be a better choice as theranostics to meet these challenges with promising drug delivery. Some of such lipid–drug conjugated-based theranostic systems are enlisted in Table 6.2.

6.2.4 Nanostructured Lipid Carriers (NLCs)

A fascinating subset of lipidic nanocarriers incorporates nanostructured lipid carriers, which are none other than an exaltation of solid lipid nanoparticles. The fundamental composition of NLC comprises an admixture of lipids which could be both in solid and liquid states. This distinctive feature enables them for higher drug loading and allows the carrying of both hydrophilic and lipophilic drugs. In addition, these carriers are well adapted to modulate their shape and size according to the physiological environment and thus are highly biocompatible. Also, the stability issues of other lipidic systems have been resolved in NLCs as they are capable of avoiding the formation of crystals upon storage. The ease of surface

functionalization of NLCs is heavily exploited in the delivery of theranostics by different administration routes (oral, rectal, topical, pulmonary, ocular, nasal, nose-to-brain, and parenteral). Sharma, A et al. reviewed over NLCs and discussed various formulation aspects, physiochemical parameters, surface functionalization strategies, and their applications in diverse fields. They concluded that NLC dispersions exhibit unique consistency and hence can be utilized in multiple dosage forms. In addition, these carriers impart a key role in the drug partitioning and distribution at the target region thus producing comparable increased therapeutic effects and minimal side effects (Sharma and Baldi 2018). A team of scientists prepared and evaluated NLCs of docetaxel surface-functionalized with Herceptin for treatment of HER2-positive breast cancer. Formulation optimization included the incorporation of a range of fatty amines; however in the results they concluded that stearyl amines containing NLCs achieved the least particle size, low polydispersibility index, higher drug loading, and improved drug release, and zeta potential in comparison to other fatty amines. Herceptin was chemically conjugated as well as physically coated to NLCs, but particles having chemical conjugation were reported to be more cytotoxic and extended-release profile (Varshosaz et al. 2018). Huang, R et al. worked upon NLC-based theranostics of gambogic acid, functionalized by cell-penetrating peptide for targeting of cancers. They also confirmed that NLCs decorated with peptide ligands exhibit higher penetrability and cytotoxicity (Huang et al. 2018). Tian C and his team formulated NLCs of curcumin surface-functionalized with N-Acetyl-L-cysteine for improved oral delivery. PEG was also conjugated with an amino acid to enhance mucoadhesion as well as penetrability of curcumin NLCs through mucous layers. Upon pharmacokinetic evaluations in rat, the formed NLCs were reported to be efficient DDS with optimal bioavailability, which is a virtue of the degree of surface functionalization (Tian et al. 2017). Some of the NLCs-based theranostics have been enlisted in Table 6.3. Conclusively, NLCs are considered as the most versatile lipidic nanocarriers that can be tuned to different shapes, sizes, charges, and surfaces engineered to deliver various formulations through different routes.

6.3 Biofate of Functionalized Nanolipidic Theranostics

Clinical studies based on in vivo pharmacokinetic models have revealed that despite huge applications, all nanosystems exhibit challenge of being entrapped in the macrophagic system (MPS), ending up in loss of their activity with final accumulation into RES organs like the lung, liver, kidney, and spleen. However, surface engineering of these carriers has sorted out this problem to a great extent by reducing their hydrophobicity, preventing their opsonization and phagocytosis, and thereby increasing their circulation time in blood and thus more effective targeting and accumulation at the desired location. In the last three decades, surface

Table 6.3 Nanostructured lipid-based theranostics (NLCs)

Lipid nanocarrier	Indication	Therapeutic agent	Diagnostic agent	Experimental model	References
NLC	Cancer	Paclitaxel	Quantum dots	Female Kunming mice	Olerile et al. (2017)
NLC	Cancer	Docosahexaenoic acid, Doxorubicin	Tc-99 m	4 T1-Tumor bearing mice model	Fernandes et al. (2018)
PEGylated NLC	Lung tumor	Cys-Arg-Glu-Lys-Ala	DIM-C-pPhC6H5 (DIM-P)	C57BL/6 mice model	Patel et al. (2014)
IR780-AMD-NLCs	Breast cancer	AMD3100 coating for tumor targeting and IR780 for photothermal therapy	Fluorescent probe coumarin 6	BALB/c female mice	Li et al. (2017)
$^{99m}Tc(CO)_3$-PTX-NLC	Cancer	Paclitaxel	$^{99m}Tc(CO)_3^+$	Wistar albino rats	Ucar et al. (2017)
QDNLCs	Melanoma	Camptothecin	Quantum dots	Melanoma cells	Hsu et al. (2013)

functionalization with multivalent ligands has led to witness a novel class of nanolipidic carriers that can be directed for active targeting of the diseased site in a more efficient manner (Lim et al. 2012). On the contrary, there are also several examples of passive targeting (without any specific moiety/ligand at their surface) to scavenge nanolipidic carriers from MPS by the virtue of their physiochemical properties. For instance, tumor cells have enhanced permeability, leaky membranes, and huge retention capability (EPR effect), due to this; tiny lipidic nanocarriers (less than 20 nm in size) can be directly recruited to these cells without any need for surface functionalization (Hua et al. 2018; Hoshyar et al. 2016). Furthermore, targeting RES organs does not require any surface functionalization, since the nanoparticles having hydrophobic surfaces are automatically attracted to MPS, leading to their opsonization and accumulation in the lung, liver, kidney, and spleen. Therefore, likewise, the chemical interaction of ligands and physical modifications of these nanolipidic systems may have important repercussions on their cellular uptake, biodistribution, and elimination from biological systems (Mitchell et al. 2021).

Conclusively, both active targeting (surface modification) and passive targeting (PEGylation, modulation of physiochemical properties, shape, size, surface charge, etc.) are equally important in deciding the pharmacokinetic fate of nanolipidic systems. The major parameters which influence ADME of nanolipidic systems are (both functionalized or not) particle size, surface charge, and hydrophobicity.

6.3.1 Impact of Particle Size

Nanolipidic systems are one of the extremely exploited carriers for deeper penetration into tissues via fine capillaries. It is reported that, particles in size range of 10–15 nm can easily permeate across various epithelial linings. However, to increase blood circulation and retention time, the size must be optimized toward a higher range, i.e., 20 to 200 nm. While aiming to target specific tissue/cells and avoiding nonspecific biodistribution, functionalization is done considering final size, shape, porosity, and surface topography of nanostructure (Huang et al. 2013). Furthermore, the elimination kinetics is also dependent on the size of these nanosystems, for instance, nanocarriers having diameter around 5 nm or less are excreted via renal clearance whereas the larger ones undergo biliary excretion. For brain targeting, it is reported that to cross the tight endothelium junctions, the nanostructures should have their hydrodynamic diameter less than 20 nm (Kang et al. 2015). Hence it can be concluded that, to achieve longer circulation time and desired accumulation at target site, the ideal size range of these nanostructure should be in range of 10–150 nm. In addition to this, it has been observed that mostly nanolipidic carriers exhibit shapes like spherical, cubic, rod-like, or worm-like which also influences their cellular uptake.

6.3.2 Impact of Surface Charge

Nanolipidic structures upon functionalization undergo modulation of their surface charge which is extremely crucial in deciding their interaction with targeted cells/tissues as well as pharmacokinetic fate. Likewise other physiochemical properties, surface charge is also dependent upon composition of nanosystems or the ligand moiety attached. Upon comparing the cellular uptake of these charged carriers, the cationic nanolipids are preferentially taken up by cells rather neutral and anionic ones. In line to this, either positively charged ligands or polymers (for coating the surface of nanolipidic structures) are selected for functionalization. For instance, to obtain cationic nanolipidic systems, chitosan (CS) coating is in trend as due to its outstanding mucoadhesion and penetration in mucosal layers, it is the polymer of choice for targeting mucosal drug delivery. Furthermore, since it is a natural, biocompatible, and biodegradable, it is heavily exploited in nano-engineering. Not only cationic, neutral ligands/polymers like PEG and PLGA can also be preferred for targeting of particular cells/tissues (tumor cells).

6.3.3 Impact of Hydrophobicity

Almost all nanosystems, either lipid-based or inorganic, face the challenge of being entrapped in MPS systems due to their hydrophobicity. Once administered through intravenous route, these nanolipidic systems interact with blood components and adsorb them over their surface. Consequently, surface adsorbed proteins are

recognized as foreign bodies to MPS, resulting in their opsonization and rapid clearance from blood stream and ultimately their inactivation and accumulation in the kidney and liver. Therefore, to achieve uniform biodistribution or specific targeting, scavenging from MPS is requisite. Functionalizing or coating these nanolipidic constructs with hydrophilic polymers can reduce the hydrophobicity to a great extent. A subset of polymers like PEG, polysorbate 80, polyethylene oxide (PEO), poloxamers, and poloxamine can be utilized to serve the purpose. As discussed about PEGylation and passive targeting of nanolipidic carriers, it is quite clear that PEG coated nanosystems are inert to immune system. Also in case of poloxamers, it is reported that they impact on monocyte activation and capable of modulating the innate immunity. Basically, poloxamers are triblock copolymers of polyethylene oxide (PEO) and polypropylene oxide (PPO) wherein hydrophilic chains of PEO are cross-linked with hydrophobic chains of PPO in a format like PEO-PPO-PEO. Upon coating with poloxamers the nanolipidic structures attain a unique configuration to mask their surface and limit interactions with surrounding biomaterials. Moreover, in case of poloxamine-coated nanolipids, an increased systemic circulation time and limited uptake in liver has been reported. In conclusion, utilization of hydrophilic polymers for surface engineering of nanolipidic carriers may prevent their uptake and thereby may increase the circulation time.

It is apparent that surface functionalization of nanolipidic systems (done by either means, i.e., active or passive) leads to increased systemic circulation and retention time. This is how they can be saved from RES uptake before reaching to the target site and therefore also termed stealth systems. In case of passive targeting, the selection of polymer is done on the basis of its nature (extent of hydrophobicity), surface charge density, chain length, and shape. Upon analysis, it has been reported that for minimal recognition and opsonization by MPS system, the optimal molecular weight of polymers should range between 2 and 5 kDa. However, in case of non-engineered nanolipidic systems, upon entering into systemic circulation, plasma proteins like apolipoproteins strongly interact and get adsorbed over their hydrophobic surface, which further leads to activation of cascade of opsonization and phagocytosis in non-target cells. In addition, protein corona formation is also suggested for larger nanolipidic. Various biopolymers (protein rich) have been reported to incline toward adsorption of more plasma protein and thereby influence MPS phagocytic uptake. Hence, it can be deduced that drug delivery by passive targeting or without targeting may lead to non-selective uptake and unnecessary accumulation of drugs in non-target organs, specifically the liver, spleen, and kidney, and thus their toxicity. Consequently, nano-engineered theranostics are indispensable to achieve desired therapeutic/diagnostic action.

6.4 Limitations and Challenges of Nanolipidic Theranostics

Nanolipidic theranostics exhibit paramount potential for combinatorial site-specific delivery of drug and diagnostic and thereby simultaneous diagnosis and treatment in a cost-effective manner. In the past decade, the fabricated nanolipid theranostics

tailored for active or passive targeting were reported for enhanced colloidal stability and satisfactory results in vivo. It is apparent, the basic design of nanotheranostics is comprised of three components, i.e., contrast-agent/diagnostic, drug, and a targeting ligand, and the fabrication involves coencapsulation of drug with imaging agent in a nanolipidic carrier followed by surface decoration. However, the design and development of nanolipidic theranostics involves extensive investigation and evaluation of all critical attributes on overall performance in vivo. The literature presents numerous limitations, right from the fabrication till clinical translation of nanolipidic theranostics. The major challenges are (a) selection and availability of theranostic nanomaterial (very few compounds are available for diagnostic cum therapeutic purpose); (b) poor bio-interaction and incompatibility with biological components (leads to opsonization and protein corona formation and thus engulfed by microphages in RES system); (c) potential inherent toxicity of theranostic compounds (generally metallic oxides or transition metals); (d) comparatively low payloads; (e) poor stability upon storage; (f) inconsistent and irreproducible results in vivo; (f) complex fabrication process; (g) non-economic and poor industrial scalability; and (h) regulatory obstacles for approval due to lack of clear guidelines for nanoformulations.

The above-addressed limitations of nanolipidic theranostics cannot stop them from being the system of choice in the treatment of life-threatening tumors. Efforts are being made continually by research fraternity to eradicate such limitations and move ahead with such smartly programed nanoconstructs. For instance, to minimize the toxicity of theranostic nanomaterials (which is a virtue of its physiochemical properties), the role of solubility, size, and zeta-potential has been extensively investigated, and modifications have been done accordingly. Furthermore, current trend of green synthesis of nanoparticles is best suited for fabrication of eco-friendly, cost-effective nanolipidic theranostics with enhanced biocompatibility and stability. It is observed that an extensive gap exists between regulatory agencies and scientific bodies, which is the major reason for translational failure of nanolipidic theranostics. In order to mitigate these hurdles and bridge this gap, strategies can be made for rigorous optimization and evaluation of formulations on animal models before entering in clinical trial phase. Extensive studies to provide expedient information on clinical implications of lipidic theranostic nanomedicine should be performed in preclinical stage. Furthermore, safety profile of the theranostic formulation, assessed in terms of its immunotoxicity, genotoxicity, and cytotoxicity, should be evaluated to estimate potential risk in human subjects. Currently various research organizations and academic institutes are facilitating the research opportunity on nanomedicine-based drug delivery, employing new technical advancements which may succeed at small scale. The tricky technical issues and critical attributes identified at small scale can be optimized and utilized to pave the way of commercialization on industrial scale. Thus a strong collaboration between pharmaceutical manufactures and research groups could increase the ease of industrial scalability.

6.5 Conclusion and Future Prospects

The chapter presents the most recent research findings in the field of theranostic using lipid-based nanocarriers. The findings of the study are quite encouraging and strongly urge further research into lipidic nanocarriers in the field of theranostics, with the goal of making the outcomes clinically interchangeable. Furthermore, surface functionalization approaches for programmed theranostics have been discussed in depth. This chapter also analyzes the limits and provides potential solutions for the effective development of lipid-based theranostic nanomedicines with improved properties that can be used in clinics. Researchers and doctors can now visualize the fate of nanoparticles, tumor accumulation, and intracellular uptake through lipid theranostics. These technical improvements have had a considerable impact on medical research and treatment planning offering a huge success for customized medicine.

Besides huge research on theranostic nanomaterials, only a few selective categories of compounds are capable of inherent imaging along with therapeutic properties. Many theranostic nanomedicines are commercially available. Lutathera® (Lutetium Lu 177 oxodotreotide) is Peptide Receptor Radionuclide Therapy (PRRT), which is indicated for gastroenteropancreatic, neuroendocrine tumors (GEP–NETs). Nanobiotix containing functionalized hafnium oxide nanoparticles is used for the treatment of locally advanced soft tissue sarcoma markets Hensify® (NBTXR3). AGuIX® is based on polysilone network covered with gadolinium nanoparticles developed by NH TherAguix is used for the treatment of newly diagnosed glioblastoma. Major challenges in the lipidic nanotheranostic field are to have many functionalities incorporated into a single nanoparticle making the system complicated. Experiments in lipid nanotheranostics have so far concentrated on developing methodologies and procedures that can reliably deliver several imaging modalities and merge many capabilities into a single platform.

In the future, practical research demonstrating the utility of imaging functionality will benefit nanotheranostics, especially when evaluating the benefits of multimodal theranostics. The growing paradigm of inherently multifunctional components is predicted to have a significant impact on nanotheranostics in the future. This notion can be used to various theranostic platforms, such as porphyrin polymers and cyanine micelles, in addition to lipid nanomedicine.

References

Albuquerque JP, Costa E (2014) Development of multifunctional solid lipid nanoparticles for theranostic applications in rheumatoid arthritis

Albuquerque J, Moura CC, Sarmento B, Reis S (2015) Solid lipid nanoparticles: a potential multifunctional approach towards rheumatoid arthritis theranostics. Molecules 20(6): 11103–11118. https://doi.org/10.3390/molecules200611103

Arshad R et al (2021) Multi-functionalized nanomaterials and nanoparticles for diagnosis and treatment of retinoblastoma. Biosensors (Basel) 11:4. https://doi.org/10.3390/BIOS11040097

Attia MF, Anton N, Wallyn J, Omran Z, Vandamme TF (2019) An overview of active and passive targeting strategies to improve the nanocarriers efficiency to tumour sites. J Pharm Pharmacol 71(8):1185–1198. https://doi.org/10.1111/jphp.13098

Bae KH, Lee JY, Lee SH, Park TG, Nam YS (2013) Optically traceable solid lipid nanoparticles loaded with sirna and paclitaxel for synergistic chemotherapy with in situ imaging. Adv Healthc Mater 2(4):576–584. https://doi.org/10.1002/adhm.201200338

Bukhari SI et al (2021) Recent progress in lipid nanoparticles for cancer theranostics: opportunity and challenges. Pharmaceutics 13(6). https://doi.org/10.3390/pharmaceutics13060840

Demir B et al (2020) Carbon dots and curcumin-loaded CD44-Targeted liposomes for imaging and tracking cancer chemotherapy: a multi-purpose tool for theranostics. J Drug Deliv Sci Technol 62:102363. https://doi.org/10.1016/j.jddst.2021.102363

Deprez J et al (2022) Evaluation of liposome-loaded microbubbles as a theranostic tool in a murine collagen-induced arthritis model, pp 1–23

Fernandes RS et al (2018) Nanostructured lipid carrier co-loaded with doxorubicin and docosahexaenoic acid as a theranostic agent: evaluation of biodistribution and antitumor activity in experimental model. Mol Imaging Biol 20(3):437–447. https://doi.org/10.1007/s11307-017-1133-3

García-Flores N et al (2021) Functionalized liposomes fabrication and characterization with erythrocruorin for a potential application as a red blood cells substitute. Curr Nanosci 17(6): 910–920. https://doi.org/10.2174/1573413717666210620132628

García-Hevia L et al (2022) Magnetic lipid nanovehicles synergize the controlled thermal release of chemotherapeutics with magnetic ablation while enabling non-invasive monitoring by MRI for melanoma theranostics. Bioactive Mater 8:153–164. https://doi.org/10.1016/j.bioactmat.2021.06.009

Grillone A et al (2015) Active targeting of sorafenib: preparation, characterization, and in vitro testing of drug-loaded magnetic solid lipid nanoparticles. Adv Healthc Mater 4(11):1681–1690. https://doi.org/10.1002/adhm.201500235

Guyon L, Groo AC, Malzert-Fréon A (2021) Relevant physicochemical methods to functionalize, purify, and characterize surface-decorated lipid-based nanocarriers. Mol Pharmaceut 18(1): 44–64. https://doi.org/10.1021/acs.molpharmaceut.0c00857

Hameed S et al (2018) Self-assembly of porphyrin-grafted lipid into nanoparticles encapsulating doxorubicin for synergistic chemo-photodynamic therapy and fluorescence imaging. Theranostics 8(19):5501–5518. https://doi.org/10.7150/thno.27721

Hoshyar N, Gray S, Han H, Bao G (2016) The effect of nanoparticle size on in vivo pharmacokinetics and cellular interaction. Nanomedicine 11(6):673–692. https://doi.org/10.2217/nnm.16.5

Hsu SH, Wen CJ, Al-Suwayeh SA, Huang YJ, Fang JY (2013) Formulation design and evaluation of quantum dot-loaded nanostructured lipid carriers for integrating bioimaging and anticancer therapy. Nanomedicine 8(8):1253–1269. https://doi.org/10.2217/nnm.12.170

Hua L et al (2018) Hypoxia-responsive lipid-poly-(hypoxic radiosensitized polyprodrug) nanoparticles for glioma chemo- and radiotherapy. Theranostics 8(18):5088–5105. https://doi.org/10.7150/thno.26225

Huang X et al (2013) Effect of injection routes on the biodistribution, clearance, and tumor uptake of carbon dots. ACS Nano 7(7):5684–5693. https://doi.org/10.1021/nn401911k

Huang R, Li J, Kebebe D, Wu Y, Zhang B, Liu Z (2018) Cell penetrating peptides functionalized gambogic acid-nanostructured lipid carrier for cancer treatment. Drug Deliv 25(1):757–765. https://doi.org/10.1080/10717544.2018.1446474

Jeon M, Kim G, Lee W, Baek S, Jung HN, Im HJ (2021) Development of theranostic dual-layered Au-liposome for effective tumor targeting and photothermal therapy. J Nanobiotechnol 19(1). https://doi.org/10.1186/s12951-021-01010-3

Kang H, Mintri S, Menon AV, Lee HY, Choi HS, Kim J (2015) Pharmacokinetics, pharmacodynamics and toxicology of theranostic nanoparticles. Nanoscale 45:18848–18862. https://doi.org/10.1039/c5nr05264e

Kang SJ et al (2018) Anti-EGFR lipid micellar nanoparticles co-encapsulating quantum dots and paclitaxel for tumor-targeted theranosis. Nanoscale 10(41):19338–19350. https://doi.org/10.1039/c8nr05099f

Kuang Y et al (2017) Hydrophobic IR-780 dye encapsulated in cRGD-conjugated solid lipid nanoparticles for NIR imaging-guided Photothermal therapy. ACS Appl Mater Interfaces 9(14):12217–12226. https://doi.org/10.1021/acsami.6b16705

Kumar R, Divya MS, Dubey VK, Chandra P (2022) N-acetyl-d-glucosamine decorated nano-lipid-based carriers as theranostics module for targeted anti-cancer drug delivery. Mater Chem Phys 282:125956. https://doi.org/10.1016/j.matchemphys.2022.12595

Lee MH et al (2016) Liposomal texaphyrin theranostics for metastatic liver cancer. J Am Chem Soc 138(50):16380–16387. https://doi.org/10.1021/jacs.6b09713

Li H et al (2017) Dual-function nanostructured lipid carriers to deliver IR780 for breast cancer treatment: anti-metastatic and photothermal anti-tumor therapy. Acta Biomater 53:399–413. https://doi.org/10.1016/j.actbio.2017.01.070

Li J et al (2019) AS1411 aptamer-modified theranostic liposomes co-encapsulating manganese oxide nano-contrast agent and paclitaxel for MRI and therapy of cancer. RSC Adv 9(60): 34837–34846. https://doi.org/10.1039/c9ra06878c

Li H et al (2021) iRGD peptide-mediated liposomal nanoparticles with photoacoustic/ultrasound dual-modality imaging for precision theranostics against hepatocellular carcinoma. Int J Nanomedicine 16:6455–6475. https://doi.org/10.2147/IJN.S325891

Lim SB, Banerjee A, Önyüksel H (2012) Improvement of drug safety by the use of lipid-based nanocarriers. J Control Release 163(1):34–45. https://doi.org/10.1016/j.jconrel.2012.06.002

Lin Q et al (2014) Nanoparticle-enabled, image-guided treatment planning of target specific RNAi therapeutics in an orthotopic prostate cancer model. Small 10(15):3072–3082. https://doi.org/10.1002/smll.201303842

Lopes FF, de Freitas CF, de Paula E, Lourenço SA, Florian M, Cabeça LF (2021) Hydroxyapatite-coated liposomes for the controlled release of quantum dots and bupivacaine. J Mater Res 36(14):3021–3030. https://doi.org/10.1557/s43578-021-00292-5

Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R (2021) Engineering precision nanoparticles for drug delivery. Nat Rev Drug Dis 20(2):101–124. https://doi.org/10.1038/s41573-020-0090-8

Moosavian SA, Sahebkar A (2019) Aptamer-functionalized liposomes for targeted cancer therapy. Cancer Lett 448:144–154. https://doi.org/10.1016/j.canlet.2019.01.045

Mourtas S et al (2020) Liposomes decorated with 2-(4′-Aminophenyl)benzothiazole effectively inhibit Aβ1-42Fibril formation and exhibit in vitro brain-targeting potential. Biomacromolecules 21(12):4685–4698. https://doi.org/10.1021/acs.biomac.0c00811

J. O. Muga, J. W. Gathirwa, M. Tukulula, W. G. Z. O. Jura, "In vitro evaluation of chloroquine-loaded and heparin surface-functionalized solid lipid nanoparticles," Malaria J, 17, 1, 2018, https://doi.org/10.1186/s12936-018-2302-9

Nandwana V et al (2018) Magnetic lipid nanocapsules (MLNCs): self-assembled lipid-based nanoconstruct for non-invasive theranostic applications. J Mater Chem B 6(7):1026–1034. https://doi.org/10.1039/c7tb03160b

Nanoformulations in Human Health (2020) Springer International Publishing. https://doi.org/10.1007/978-3-030-41858-8

Neves AR, van der Putten L, Queiroz JF, Pinheiro M, Reis S (2021) Transferrin-functionalized lipid nanoparticles for curcumin brain delivery. J Biotechnol 331:108–117. https://doi.org/10.1016/j.jbiotec.2021.03.010

Olerile LD et al (2017) Near-infrared mediated quantum dots and paclitaxel co-loaded nanostructured lipid carriers for cancer theragnostic. Colloids Surf B: Biointerfaces 150:121–130. https://doi.org/10.1016/j.colsurfb.2016.11.032

Oumzil K et al (2016) Solid lipid nanoparticles for image-guided therapy of atherosclerosis. Bioconjug Chem 27(3):569–575. https://doi.org/10.1021/acs.bioconjchem.5b00590

Panikar SS et al (2021) Ligand-targeted Theranostic liposomes combining methylene blue attached upconversion nanoparticles for NIR activated bioimaging and photodynamic therapy against HER-2 positive breast cancer. J Lumin 237:118143. https://doi.org/10.1016/j.jlumin.2021.118143

Patel AR, Chougule MB, Lim E, Francis KP, Safe S, Singh M (2014) Theranostic tumor homing nanocarriers for the treatment of lung cancer. Nanomed Nanotechnol Biol Med 10(5):e1053–e1063. https://doi.org/10.1016/j.nano.2013.12.002

Petersen AL et al (2016) In vivo evaluation of PEGylated 64Cu-liposomes with theranostic and radiotherapeutic potential using micro PET/CT. Eur J Nucl Med Mol Imaging 43(5):941–952. https://doi.org/10.1007/s00259-015-3272-6

Pinelli F, Perale G, Rossi F (2020) Coating and functionalization strategies for nanogels and nanoparticles for selective drug delivery. Gels 6(1). https://doi.org/10.3390/gels6010006

Riaz MK, Tyagi D, Yang Z (2017) Surface engineering: incorporation of bioactive compound 111–143. https://doi.org/10.1007/978-981-10-5864-6_6

Roffo F, Ponsiglione AM, Netti PA, Torino E (2022) Coupled hydrodynamic flow focusing (cHFF) to engineer lipid–polymer nanoparticles (LiPoNs) for multimodal imaging and theranostic applications. Biomedicine 10(2):438. https://doi.org/10.3390/biomedicines10020438

Salahpour Anarjan F (2019) Active targeting drug delivery nanocarriers: ligands. Nano-Struct Nano-Objects 19:100370. https://doi.org/10.1016/j.nanoso.2019.100370

Sharma A, Baldi A (2018) Nanostructured lipid carriers: a review. https://doi.org/10.4172/2329-6631.1000191

Sonju JJ, Dahal A, Singh SS, Jois SD (2021) Peptide-functionalized liposomes as therapeutic and diagnostic tools for cancer treatment. Journal of Controlled Release 329:624–644. https://doi.org/10.1016/j.jconrel.2020.09.055

Sonkar R et al (2021) Gold liposomes for brain-targeted drug delivery: formulation and brain distribution kinetics. Mater Sci Eng C 120:1–25. https://doi.org/10.1016/j.msec.2020.111652

Teixeira MI et al (2022) Formulation, characterization, and cytotoxicity evaluation of lactoferrin functionalized lipid nanoparticles for Riluzole delivery to the brain. Pharmaceutics 14(1). https://doi.org/10.3390/pharmaceutics14010185

Tian C et al (2017) N-acetyl-l-cysteine functionalized nanostructured lipid carrier for improving oral bioavailability of curcumin: preparation, in vitro and in vivo evaluations. Drug Deliv 24(1):1605–1616. https://doi.org/10.1080/10717544.2017.1391890

Truzzi E et al (2017) Self-assembled lipid nanoparticles for oral delivery of heparin-coated iron oxide nanoparticles for theranostic purposes. Molecules 22:6. https://doi.org/10.3390/molecules22060963

Ucar E et al (2017) Synthesis, characterization and radiolabeling of folic acid modified nanostructured lipid carriers as a contrast agent and drug delivery system. Appl Radiat Isot 119:72–79. https://doi.org/10.1016/j.apradiso.2016.11.002

Varshosaz J, Davoudi MA, Rasoul-Amini S (2018) Docetaxel-loaded nanostructured lipid carriers functionalized with trastuzumab (Herceptin) for HER2-positive breast cancer cells. J Liposome Res 28(4):285–295. https://doi.org/10.1080/08982104.2017.1370471

Wang H, Sun G, Zhang Z, Ou Y (2017) Transcription activator, hyaluronic acid and tocopheryl succinate multi-functionalized novel lipid carriers encapsulating etoposide for lymphoma therapy. Biomed Pharmacother 91:241–250. https://doi.org/10.1016/j.biopha.2017.04.104

Wang M et al (2018) Magnetic resveratrol liposomes as a new theranostic platform for magnetic resonance imaging guided Parkinson's disease targeting therapy. ACS Sustain Chem Eng 6(12): 17124–17133. https://doi.org/10.1021/acssuschemeng.8b04507

Xiong H, Liu S, Wei T, Cheng Q, Siegwart DJ (2020) Theranostic dendrimer-based lipid nanoparticles containing PEGylated BODIPY dyes for tumor imaging and systemic mRNA delivery in vivo. J Control Release 325:198–205. https://doi.org/10.1016/j.jconrel.2020.06.030

Yoo J, Park C, Yi G, Lee D, Koo H (2019) Active targeting strategies using biological ligands for nanoparticle drug delivery systems. Cancers (Basel) 11(5). https://doi.org/10.3390/cancers11050640

Zafar A et al (2022) Preparation of NLCs-based topical erythromycin gel: in vitro characterization and antibacterial assessment. Gels 8(2). https://doi.org/10.3390/gels8020116

Functionalized Nanoemulsions: Could Be a Promising Approach for Theranostic Applications

7

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Abstract

Theranostics is an evolving field that utilized technologies/approaches to reach a comprehensive diagnosis, in vivo molecular images, and an individualized treatment regimen for better disease management. Recently various researchers made their efforts with smart materials and exploiting nanotechnology to formulate the nano dimension delivery vehicle for the simultaneous loading of imaging and therapeutic agents. Nanoemulsions can greatly increase the efficacy and/or biopharmaceutical performance of loaded therapeutics/imaging agents. In these recent years, nanoemulsions are popularized in drug development due to the demand for developing effective delivery systems of active pharmaceutical ingredients of low aqueous solubility which are utilized in biomedical applications for different purposes. The functionalized nanoemulsions are investigated for diagnosis/imaging, delivery of drug/gene, and its application in photothermal therapy, photodynamic therapy, chemotherapy, etc. Using various techniques, the surface of this nano-scaffolds has been modified according to the application purposes. Many researchers have formulated the functionalized

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nanoemulsion system to simultaneously load the contrast/diagnostics and therapeutic agents for theranostic applications in better management of different disease conditions including various types of cancers. Here, in this chapter, we discussed the basics of the theranostic approach, and the advancement of functionalized nanoemulsion for theranostic application including highlights of contemporary research carried out in this area.

Keywords

Theranostic · Nanoemulsion · Functionalized nanoemulsion · Surface-engineered · Image-guided therapy · Cancer

7.1 Introduction

Nanoemulsions can greatly increase the efficacy and/or biopharmaceutical performance of loaded actives of natural and/or synthetic origin (Ojha et al. 2022; Acosta 2009). It helps to increase the shelf-life of pharmaceutical/nutraceutical/cosmeceutical products by minimizing the particle aggregation and other instability (Juneja et al. 2022; Tadros et al. 2004). The tiny oil droplets of nanoemulsions weakly scatter the light waves which will be helpful to easily incorporate into an optically clear product without hampering its transparency (Wooster et al. 2008; Velikov and Pelan 2008; Mason et al. 2006). It is a point of discussion in the present literature related to the definitive droplet size range of the nanoemulsions. According to Solans and Sole (2012), the droplet size range of the nanoemulsion system is 20 to 500 nm, while McClements and Rao (2011) reported the average radii of the nanoemulsion droplets in the range of 10 to 100 nm and referred them as mini emulsions.

Nanoemulsions can be developed into viscous system at much lower droplet concentrations compared to conventional emulsions (Tadros et al. 2004; Mason et al. 2006; Sonneville-Aubrun et al. 2004). Therefore, it is utilized to design a product with novel textural characteristics. It has better stability for particle aggregation and gravitational separation (Suthar et al. 2022; Tadros et al. 2004). Further, the nanoemulsion system has various advantages compared to conventional emulsions, particularly utilization of this technology in the development of beverages and food products. It is observed that small droplets of nanoemulsion have scattered the light waves weakly. Therefore, actives (color, flavor, preservatives of poorly soluble nature) loaded within nanoemulsion systems are utilized for dispersion into clear or slightly turbid products like fortified soups, sauces, and soft drinks (Wooster et al. 2008; Mason et al. 2006). Usually, nanoemulsions are highly stable to gravitational separation due to the relatively small particle size which means the effects of Brownian motion dominate over gravitational forces (Kumar et al. 2021; McClements 2004). In addition, nanoemulsion-based delivery systems could be utilized to enhance the performance of the loaded components due to the minimum droplet size and the maximum surface-to-volume ratio (Acosta 2009). Two types of

nanoemulsions are widely utilized in pharmaceutical/nutraceutical/cosmeceutical applications.

Oil-in-water (o/w) type of nanoemulsions are vehicles made up of mainly three ingredients of oil, surfactants, and co-surfactants dispersed in an aqueous phase. This type of system can encapsulate and deliver the hydrophobic and/or lipophilic nature of pharmaceutical/nutraceuticals/cosmeceuticals that are comparatively more deleterious in the free form. Various pharmaceutical products (such as Neoral® [cyclosporine A] and Norvir® [ritonavir]) which are approved for clinical use are o/w nanoemulsion-based drug delivery systems which converted into nanoemulsion upon oral administration in the gastric region (Lin and Metters 2006). Water-in-oil (w/o) nanoemulsion is a class of nanosystems that are used to be enhanced, prolonged-release systems for hydrophilic drugs with the oil and interfacial layers acting as release barriers (Lee and Mooney 2012; Wang et al. 2008). It is also explored for drug delivery purposes and utilized for the design of a stable double emulsion system (Yi et al. 2015; Skelhon et al. 2012; Nadin et al. 2014; Rietberg et al. 2012; Khalid et al. 2013; Matos et al. 2014; Piorkowski and McClements 2014). The multi-phase, compartmentalized structure of double emulsions makes them suitable for the design of the delivery vehicle and controlled release of sensitive components to produce stable pharmaceutical/nutraceutical/cosmeceutical products (Rocha-Selmi et al. 2013; Poyato et al. 2013; Giroux et al. 2013).

Nanoemulsions ingredients are widely utilized as an effective delivery system for hydrophobic drugs. These hydrophobic active pharmaceutical ingredients are subjugated in pharmaceutical manufacturing (Lovelyn and Attama 2011; Chen et al. 2011; Gupta 2020), cosmetic formulation development (Hashemnejad et al. 2019), and food processing (McClements and Rao 2011; McClements 2011). In the field of pharmaceutical applications, it is reported that around 40% of currently marketed drugs and 90% of drugs in the developmental phase are lipophilic in nature (Aslam et al. 2022) which is responsible for their low bioavailability and poor absorption efficiency. The o/w nanoemulsion systems have been widely pursued for the delivery of pharmaceutical compounds of poor biopharmaceutical performance (Pardhi et al. 2022; Suthar et al. 2023; Ahmad et al. 2022). The oil droplets of the nano dimension may act as an effective reservoir for increasing the solubility of various hydrophobic drugs and protecting them from deterioration due to environmental factors (such as oxidation, pH, or hydrolysis) (Aslam et al. 2021). In comparison to conventional emulsions of macron dimension, a nanoemulsion has a uniform distribution of nano oil droplets in the aqueous phase that are more resistant to flocculation and coalescence (Aslam et al. 2016). Furthermore, nanoemulsion systems could be surface-engineered/modified as functionalized nanoemulsions for specific purposes and various applications for the delivery of pharmaceuticals, phytopharmaceuticals, and biologicals. The details are discussed in the subsequent section.

7.2 Functionalized Nanoemulsion: Recent Development and Drug Delivery Opportunity

The development of functionalized nanoemulsion mainly meaning is to increase the simple characteristics of the nanoemulsion scaffold for other specific purposes/applications. The recent advancement in the nanoemulsion-based delivery system as a functionalized nanoemulsion revolutionized the field through the theranostic approach which involves the diagnosis and treatment of various vulnerable diseases simultaneously to know the progress of therapy precisely and accurately.

The surface of functionalized nanoemulsion is modified to achieve the goal of therapy by imparting different characteristics to the drug delivery scaffold. This might help in controlled spatiotemporal releases of active medicaments from the drug delivery scaffold. The site-specific release of active medicaments from the functionalized nanoemulsion scaffold was achieved through the conjugation of specific ligands to the surface of the nanoemulsion scaffold. The targeting ligands may consist of peptides, antibodies, or aptamers in nature which are likely to assist in the precise delivery of therapeutic agents/drugs to diseased tissues by recognizing corresponding receptors or antigens (Nirale et al. 2020). The functionalized nanoemulsion is surface-engineered to target the different receptors such as CD44 receptors (Kim and Park 2017; Tinoco et al. 2019), estrogen receptors (Singh et al. 2021; Back et al. 2020), folate receptors (Song et al. 2020; Loureiro et al. 2015), epidermal growth factor receptor (EGFR) (Grapa et al. 2019; Adhikari et al. 2020), and integrin receptors (Choudhury et al. 2019a) for precise delivery of loaded therapeutics in various disease conditions. Further, the surface of the nanoemulsion may functionalize to make the drug delivery scaffold long circulating after parenteral administration through PEGylation. This would be helpful to prevent/minimize the opsonization of drug delivery scaffold inside in vivo system (Yadav and Gupta 2015; Deshpande et al. 2014).

Additionally, the surface of the nanoemulsion may be functionalized to make the drug delivery scaffold mucoadhesive upon intranasal administration for nose-to-brain targeted delivery (Qu et al. 2021). The mucoadhesive characteristics of the surface of the nanoemulsion scaffold would be provided by coating through chitosan (Choudhury et al. 2019b) or other polymeric solutions (Kumbhar et al. 2020; Akhter et al. 2016) of mucoadhesive nature. The surface charge of functionalized nanoemulsion may be modified by inducing positive zeta potential and converted to cationic nanoemulsion to increase ionic interaction of nanoemulsion scaffold to mucus layer of the vaginal region (Smoleński et al. 2021), gastrointestinal region (Pandey et al. 2018), and intranasal region (Chatterjee et al. 2019) for specific purposes. All these modifications in the surface of functionalized nanoemulsion are ultimately helping to improve the biopharmaceutical performance and therapeutic efficacy of the loaded pharmaceuticals, phytopharmaceuticals, and biologicals.

Nowadays, researchers explored the opportunity of functionalized nanoemulsion to design and develop into a nano-scaffold for theranostic applications (illustrated in Fig. 7.1). The details are discussed in the subsequent section.

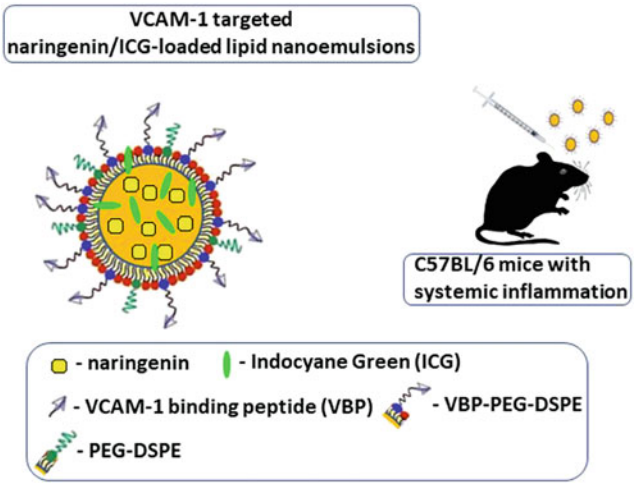

Fig. 7.1 Illustration highlights applicability of functionalized nanoemulsions as theranostic nanoplatforms in inflammation (Reproduced from Fuior et al. (2020), MDPI (2020))

7.3 Functionalized Nanoemulsions Utilized for Theranostic Applications: Contemporary Research

Recently, there is an effort to utilize the functionalized nanoemulsion as an attempt to develop theranostic nanoplatforms which can monitor the therapeutic efficacy of drugs through simultaneous imaging of the disease site that can expedite clinicians to individualized therapeutic decisions in vulnerable diseases like cancers (Suthar et al. 2022; Fang and Zhang 2010). This approach is promising particularly in the case of cancer treatment because of the highly heterogeneous and adaptable disease. The specific types of treatment options need to be chosen depending on the patient's condition and disease progression, particularly to individualize the treatment regimen.

The term "theranostics" may be defined as the combination of therapeutic and diagnostic agents utilized to deliver through a single nanoplatform delivery vehicle like a nanoemulsion. Theranostic nanoemulsions are multifunctional systems developed for specific purposes by virtue of their combined capabilities to deliver diagnostic and therapeutic agents simultaneous (Jokerst and Gambhir 2011). The ideal theranostic system may have the following properties:

- The diagnostic and therapeutic agents should be loaded into a single biocompatible, biodegradable drug delivery system.
- The theranostic system should build up quickly and selectively in targets of interest so that minimum toxicity and efficient delivery is possible.

- The theranostic system should report the biochemical and morphologic characteristics of the disease to be treated.
- The theranostic agent should precisely deliver adequate drugs on demand devoid of damaging healthy tissues.
- The theranostic agent should eliminate easily or metabolized into non-toxic by-products and be safe for human use.

While various theranostic nanoplatforms of organic or inorganic nature have been designed for treatment of cancer/other vulnerable diseases, none has yet satisfied all these criteria (Xie et al. 2010; Lammers et al. 2011). The nanoemulsion developed for theranostic purposes carries large payloads of diagnostic agents inside the core, while the therapeutic agent for the chemotherapy is entrapped inside the scaffold to deliver the drug to the targeted site. By applying this approach, the theranostic nanoemulsion can provide therapeutic protocols that are more specific to individuals and, therefore, more likely to offer an improved prognosis. The use of this technique also allows the many conventionally used diagnostic agent to upgrade to a theranostic agent by simply adding the therapeutic agent because it always required the sufficient accumulation of both diagnostic and therapeutic agents in diseased areas. The nanoemulsion-based theranostic approach is applied in the management of different disease conditions such as the treatment of various cancers including different inflammatory conditions in various diseases, where the accumulation of diagnostic agents on site gives the assurance of targeted delivery.

The theranostic application gives an advantage over the conventional approach for the treatment in that a higher concentration of drug in plasma doesn't guarantee the effective concentration of the therapeutic agent in the disease site. Recently, Fuior et al. designed functionalized nanoemulsion system for theranostic application in the management of inflammatory conditions (Fuior et al. 2020). The developed system was decorated with VCAM-1 targeted ligands. It is loaded with naringenin as an anti-inflammatory therapeutic agent while indocyanine green was utilized to label the developed theranostic system as a near-infrared probe (imaging agent). The results of this investigation were depicted in Fig. 7.2 and signify that the developed system was exploited for specific targeting to the activated endothelium which is utilized for imaging of inflamed vascular areas and improved release of the loaded anti-inflammatory agent (like naringenin).

In the subsequent sub-section, we critically analyzed and reviewed the contemporary research carried out in the area of theranostic nanoemulsion that may simultaneously be helpful to monitor the progress of therapy and treat different disease conditions.

7.3.1 Utilization of Theranostic Nanoemulsion in Different Conditions of Cancers

Macrophages are the specific type of cells which has properties to fight against foreign particles. The specific type of macrophages which expresses

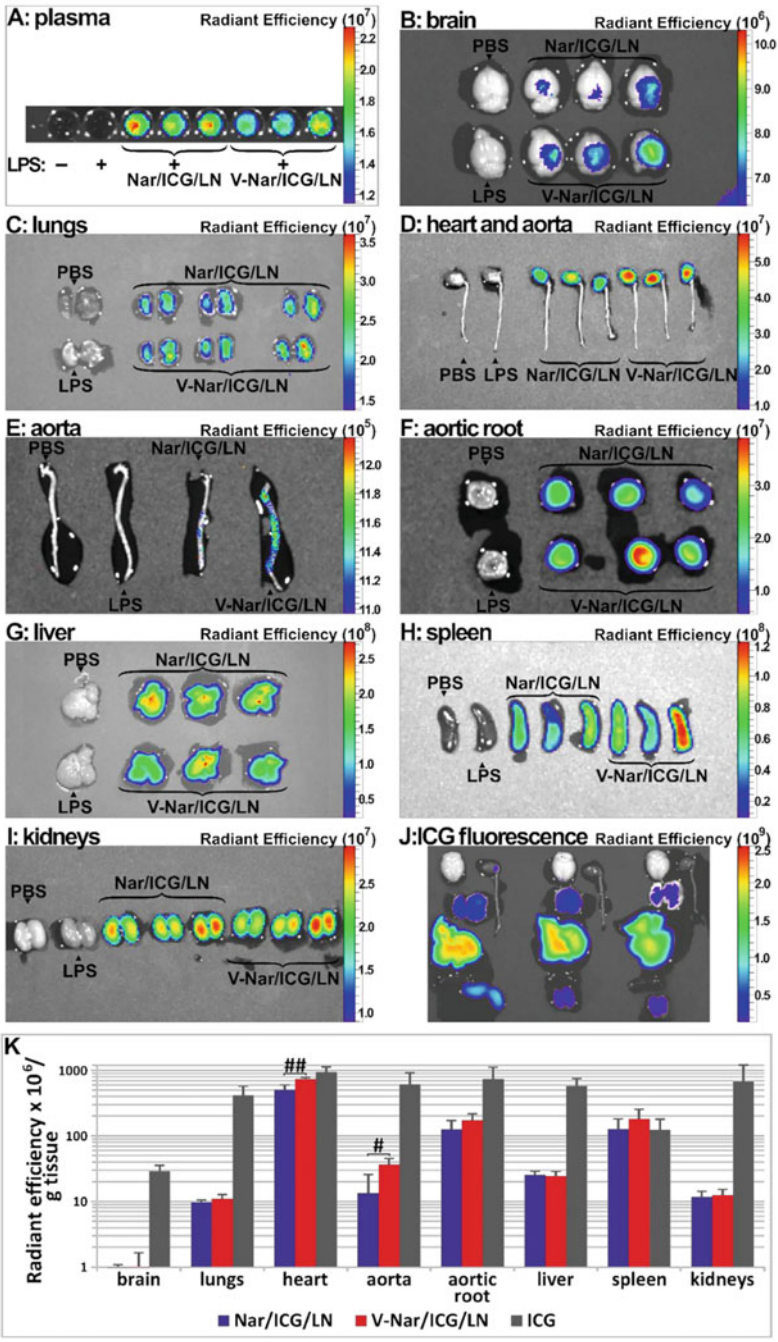

Fig. 7.2 Biodistribution/tissue distribution profile of non-targeted (Nar/ICG/LN) theranostic nanoemulsions, targeted (V-Nar/ICG/LN) theranostic nanoemulsions, free fluorescent near-infrared probe indocyanine green (ICG) in different organs of C57BL/6 mice for effective targeting of the

cyclooxygenase-2 (COX-2) have shown their involvement in various pro-tumor genesis mechanisms and constituted the major portion of the tumor mass. Therefore, macrophages may consider one of the viable targets in cancer therapy. Celecoxib is a selective COX-2 inhibitor and repurposed in anticancer treatment. The low aqueous solubility of celecoxib limits its significant activity against cancer.

Patel et al. formulated celecoxib-loaded theranostic nanoemulsion for intravenous delivery (Patel et al. 2013). These multifunctional nanoemulsions may be imaged by the near-infrared fluorescence and ^{19}F magnetic resonance imaging technique and show equivalent COX-2 enzyme inhibition compared to drug-loaded nanoemulsion system and free drug. Indeed, multifunctional celecoxib-loaded theranostic nanoemulsion may be effective in minimizing systemic exposure of the drug and related adverse effects. They also proposed that developed theranostic nanoemulsions may help in investigating the significance of inhibition of macrophage COX-2 in inflammation-cancer interactions in future study. In another investigation, Yang et al. prepared the nanoemulsion for the co-delivery of paclitaxel and sulforhodamine B (imaging probe) as a theranostic system (Yang et al. 2014). They found that the prepared theranostic emulsion shows increased therapeutic efficacy with increased circulation time and increased imaging efficiency in cancer and meet the imaging requirements in vivo. They suggested the use of this theranostic emulsion for the therapeutic and diagnostic role in the subcutaneous tumor model. For the treatment of ovarian cancer, Patel et al. developed a nanoemulsion that is surface-functionalized with folate and gadolinium (Patel et al. 2016).

Ovarian cancer is a highly common type of cancer among female and exhibited drug resistance (MDR) during the treatment. The prepared theranostic emulsion was supposed to think to resolve these problems by targeting the drug to its desired site. The nanoemulsion was prepared by a high-shear microfluidization process and using GRAS-grade excipients. The particle size of the nanoemulsion was found to be less than 150 nm and exhibited stability in parenteral fluids and plasma. The folate-targeted developed nanoemulsion system exhibited a significant reduction in the IC_{50} value in SKOV3TR cancer cells compared to the free drug (docetaxel). On magnetic resonance imaging (MRI) analysis, folate-targeted developed nanoemulsion system was observed to be accumulated in the cancer site with enhanced contrast in SKOV3 tumor-bearing mice model compared to Magnevist®.

In addition, platinum-based therapeutic systems are the first-line treatments in different cancers including ovarian cancer, but the treatment is limited due to their associated dose-limiting toxicities and resistance. To overcome these limitations Ganta et al. prepared a theranostic nanoemulsion loaded with a cisplatin derivative, myrisplatin, and a pro-apoptotic agent, C_6-ceramide, by applying high-shear microfluidization process (Ganta et al. 2015). The size of the nanoemulsion was noted below 150 nm. The functionalization of the outer surface of the nanoemulsion

Fig. 7.2 (continued) activated endothelium and imaging inflamed vascular areas (Reproduced from Fuior et al. (2020), MDPI (2020))

was done with a receptor (endothelial growth factor receptor—EGFR) binding peptide to enhance the site-specific delivery and gadolinium as a diagnostic agent for image-guided therapy in ovarian cancers. In a pharmacokinetic study, the prepared theranostic nanoemulsion shows prolonged plasma level of platinum in *nu/nu* mice, which further shows reduction in toxicity and improvement in the mean survival time of animals compared to the equivalent cisplatin. The diagnosis utility of the prepared theranostic nanoemulsion in MRI analysis indicates that the developed theranostic system was an effective contrast agent to track the progress of therapy.

In another investigation, for the monitoring of the co-delivery of cancer chemotherapeutics and siRNA gene in a non-invasive real-time manner, Oh et al. formulated a radio-opaque theranostic nanoemulsion system by incorporating paclitaxel and Bcl-2 siRNA for synergistic anticancer effects (Oh et al. 2013). Iodinated poppy seed oil (Lipiodol) was used to dissolve the water-insoluble paclitaxel and Bcl-2 siRNA complexed electrostatically in the design and development of cationic nanoemulsion system. The developed theranostic nanoemulsion system shows the feasibility of imaging when they were utilized in micro-computed tomography analysis of tumor-bearing mouse. This investigation confirms that lipiodol-based nanoemulsion system is utilized as a multifunctional nanosystem for the co-delivery of chemotherapeutic agent and siRNA for theranostic application in cancer. In this context, to understand the improvement in therapeutic efficacy, the theranostic approach was explored by Gianella et al. who simultaneously utilized diagnostic techniques for the detection of the drug at the disease site (Gianella et al. 2011). They prepared o/w nanoemulsion system loaded with iron oxide nanocrystals, fluorescent dye (Cy7), and prednisolone acetate valerate (PAV) for the purpose of MRI, NIRF imaging, and therapeutic agent, respectively. They utilized this multimodal nanoemulsion-based theranostic platform to enable imaging-guided therapy. The developed theranostic system was evaluated in a mouse model bearing colon cancer. MRI and NIRF imaging showed a significant accumulation of developed theranostic systems in the tumors. The profile of tumor growth revealed a potent inhibitory effect in all animals treated with the PAV-loaded theranostic nanoemulsion compared to the animals treated with control nanoemulsions and the free drug. This investigation provides a proof-of-concept that the nanoemulsion system could be a theranostic nanoplatform for the imaging-guided therapy in cancer.

O'Hanlon et al. developed another theranostic nanoemulsion loaded with perfluoropolyether (PFPE) of lipophilic nature (O'Hanlon et al. 2012). The developed formulation system allows monitoring of biodistribution profile of administered nanoemulsion utilizing two imaging (^{19}F-MRI and near-infrared—NIR) modalities. The developed theranostic system has a droplet size of 180 nm and a low polydispersity index (<0.2). The celecoxib was incorporated into the developed formulation at a concentration of 0.2 mg/ml. The developed theranostic system was visible under ^{19}F-NMR and NIR fluorescence spectroscopy and capable to carry the loaded therapeutics with significant potential for cancer imaging and treatment. In another study, Zhang et al. designed a nanomedicine having the ability to clinical translation for photodynamic theranostics, to resolve the extra toxicity of

nanocarriers during its metabolism (Zhang et al. 2019). In their study, investigator concluded that the developed system (all-in-one) is simple in composition and significant theranostic efficacy. So, their formulated nanoemulsions have an intriguing avenue for developing as a theranostic system with clinical translational potential.

Le et al. prepared a theranostic emulsion using polyglycerol (Le Kim et al. 2017). They prepared nanoemulsions using lipoidal as a core oil to dissolve the drug paclitaxel and to serve as a contrasting agent in computer tomography. Linear diblock copolymers are utilized to incorporate the therapeutic and contrast agents. In vitro study was performed in HeLa ovarian cancer cells and imaging was done using Micro-CT. Their study concluded that developed nanoemulsion system acts as an excellent delivery vehicle for cancer therapy and micro-CT imaging of the developed formulation system validates its utility as a contrast agent.

7.3.2 Utilization of Theranostic Nanoemulsion in Inflammatory Conditions of Disease

Macrophages which are present in the white blood cell are the important components of the immune system cascade. These cells are related to engulfing and digestion of pathogens (Mahla et al. 2021). The process is called phagocytosis, which acts to defend the host against infection and injury (Nahrendorf et al. 2020). It is a defense mechanism present in living beings against different types of infections and injuries. These macrophages were first discovered in the year 1884 by a Russian zoologist, Elie Metchinkoff (Zalkind 2001).

Inflammatory condition is a major problem in the curing of many diseases. This inflammation cartel is the progression of various chemical procedures inside the process. Macrophages are one of the cells responsible for inflammation. Targeting of macrophages for the delivery of theranostic system is a promising strategy in the management of inflammatory conditions in various diseases. Patel et al. formulated a nanoemulsion-based theranostic system for the delivery of celecoxib (an anti-inflammatory drug) to macrophages and its optical imaging to observe the changes in inflammation by monitoring the macrophage migration patterns. For the evaluation of the anti-inflammatory activity of the developed theranostic system, inflammation was induced with complete Freund's adjuvant (CFA) in mouse model (Patel et al. 2015). They found a greater accumulation of celecoxib in the inflamed vs. control paw. The histological investigation further confirmed their specific localization in CD68-positive macrophages overexpressing COX-2 compared to the neutrophils.

Furthermore, the contemporary research carried out in this field that highlights the significance of theranostic nanoemulsion for the better prognosis of different conditions of various diseases is summarized in Table 7.1.

Table 7.1 Summary of contemporary research carried out utilizing theranostic nanoemulsion as an approach for better management of different conditions in various diseases

S. no.	Disease condition	Diagnostic agents	Therapeutic agents	In vitro/in vivo model	Outcome	References
1.	Tumor angiogenesis and metastasis	Tyramide conjugated PFPE nanoemulsion	Celecoxib	In vitro toxicity study was performed by Celltiter-Glo luminescence cell viability assay	Avoid systemic exposure to celecoxib and related side effects	Patel et al. (2013)
2.	Multidrug resistance (MDR) in cancer	Sulforhodamine B	Paclitaxel	Human oral epidermoid carcinoma cell line, KB-3–1 and animal imaging in nude mice	Shows increased therapeutic efficacy with improved circulation time and also increased imaging efficiency in both drug-sensitive and drug-resistant cancer	Yang et al. (2014)
3.	Ovarian cancers	Lipidated gadolinium chelate	Dimyrisplatin, dipalmiplatin and distearyplatin, C6-ceramide	HeLa contaminant, KB-WT and KBCR-1000 rich in FR-, were used as in vitro models	Targeted nanoemulsions with di fatty acid platins (FA-NE's) were able to overcome the competition from free FA at ~up to 5000-fold higher concentration than the FA's on the NE	Patel et al. (2016)
4.	Ovarian cancers	Gadolinium	Myrisplatin and pro-apoptotic agent, C_6-ceramide	*nu/nu* mice	Shows less toxicity and prolonged the survival time of the experimental mice as compared to an equivalent cisplatin treatment	Ganta et al. (2015)
5.	Breast adenocarcinoma	Lipiodol	Paclitaxel and Bcl-2 small interfering RNA	MCF7 cancer cells	Inducing significant enhancement in apoptosis and anticancer activity in breast carcinoma	Oh et al. (2013)

(continued)

Table 7.1 (continued)

S. no.	Disease condition	Diagnostic agents	Therapeutic agents	In vitro/in vivo model	Outcome	References
6.	LS174T cancer cells	Iron oxide nanocrystals for MRI and fluorescent dye (Cy7) for NIRF imaging	Prednisolone acetate valerate	Colon cancer mouse model	Potent inhibitory effect observed in all the animals treated with PAV loaded nanoemulsion compared to the control nanoemulsions	Gianella et al. (2011)
7.	Inflammatory condition in cancer	PFPE-tyramide as a ^{19}F MRI tracer	Celecoxib	MTS colorimetric assay in model immune cells, fetal skin dendritic cells (FSDCs)	Formulated theranostic emulsion has significant potential for cancer imaging and treatment	O'Hanlon et al. (2012)
8.	Inflammatory disease	Fluorescent perfluorocarbon (PFC)	Celecoxib	Mouse inflammation model induced with complete Freund's adjuvant	Greater accumulation of celecoxib in the inflamed vs. control paw	Patel et al. (2015)
9.	Breast, ovarian, and lung cancer as well as Kaposi's carcinoma	Perfluoro-15-crown-5-ether (PFCE) was selected as the fluorous imaging agent	Paclitaxel	A549, a human non-small cell lung carcinoma cell line	Stable nanoemulsions that carry payloads of potent chemotherapeutic drugs and 19F-MRI contrast agents are all wrapped into a powerful, theranostic system	Barres et al. (2017)
10.	Chronic pain	MRI agent perfluoro-15-crown-5 ether (PCE)	Celecoxib	Quality-by-Design (QbD)	QbD approach applied for development of theranostic nanoemulsion with high loading of celecoxib	Herneisey et al. (2019)

7.4 Author Opinion and Future Directions

This chapter overviewed the promising approach of functionalized nanoemulsion for theranostic applications. Nanoemulsions are established as a promising delivery systems to enhance the biopharmaceutical performance of hydrophobic drugs. In theranostic applications, it seeks to utilize this delivery vehicle for the inclusion of lipophilic or hydrophilic functional moieties used in various therapeutic processes and same time to integrate the contrasting agents for better diagnosis. Various techniques available to produce and characterize nanoemulsions have been reported by researchers, but some of them have been evaluated to be more suitable than others. It has been generally observed that the technique, which is reproducible within the research laboratories, is not easily feasible to transfer at an industrial scale. While it is also further observed the additional face-up to consequences to release the incorporated imaging and therapeutic agents in the nanoemulsion to the desired target site, even the reported findings have progressed to target specific cancerous cells through driven specific ligands.

A thorough future study is needed to investigate the distribution of theranostic nanoemulsion inside the body and its interaction with the desired target cells. It is always suggested that the incorporation of GRAS-grade ingredients in theranostic nanoemulsion will avoid a further increase in toxicity. It is also suggested an evaluation by continuous verification for the detailed interaction studies between components of theranostic nanoemulsion and possible interaction with the living system. Toxicological exploration of components of the formulation on a short-term and long-term basis is also essential for establishing its safety before administering the theranostic nanoemulsion in human subjects. It is also observed that various multifunctional systems for theranostic applications have been registered for investigating in human beings, but to explore the nanoemulsions for their efficacy in human subjects has to travel a long track. Furthermore, the formulation design of a theranostic nanoemulsion-based delivery system for dual purposes, particularly the utilization of simultaneous treatment and imaging applications exploiting this nano-scaffold, has brought great hope in the treatment of cancer and other diseases.

Conflict of Interest The authors declare no conflict of interest.

References

Acosta E (2009) Bioavailability of nanoparticles in nutrient and nutraceutical delivery. Curr Opin Colloid Interface Sci 14(1):3–15

Adhikari M, Adhikari B, Ghimire B, Baboota S, Choi EH (2020) Cold atmospheric plasma and silymarin nanoemulsion activate autophagy in human melanoma cells. Int J Mol Sci 21(6):1939

Ahmad MZ, Ahmad J, Alasmary MY, Akhter S, Aslam M, Pathak K, Jamil P, Abdullah MM (2022) Nanoemulgel as an approach to improve the biopharmaceutical performance of lipophilic drugs: contemporary research and application. J Drug Deliv Sci Technol 72:103420

Akhter S, Anwar M, Siddiqui MA, Ahmad I, Ahmad J, Ahmad MZ, Bhatnagar A, Ahmad FJ (2016) Improving the topical ocular pharmacokinetics of an immunosuppressant agent with

mucoadhesive nanoemulsions: formulation development, in-vitro and in-vivo studies. Colloids Surf B: Biointerfaces 148:19–29

Aslam M, Aqil M, Ahad A, Najmi AK, Sultana Y, Ali A (2016) Application of Box–Behnken design for preparation of glibenclamide loaded lipid based nanoparticles: optimization, in vitro skin permeation, drug release and in vivo pharmacokinetic study. J Mol Liq 219:897–908

Aslam M, Deeb G, Mirza MA, Ahmad J, Nollet LM (2021) Application of essential oil nanoemulsions in food preservation. In: Nanoemulsions in food technology. CRC Press, pp 229–246

Aslam M, Javed M, Deeb HH, Nicola MK, Mirza M, Alam M, Akhtar M, Waziri A (2022) Lipid nanocarriers for neurotherapeutics: introduction, challenges, blood-brain barrier, and promises of delivery approaches. CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders)

Back PI, Balestrin LA, Fachel FNS, Nemitz MC, Falkembach M, Soares G, da Silva Marques M, Silveira T, Dal Prá M, Horn AP, Braganhol E (2020) Hydrogels containing soybean isoflavone aglycones-rich fraction-loaded nanoemulsions for wound healing treatment–in vitro and in vivo studies. Colloids Surf B: Biointerfaces 196:111301

Barres AR, Wimmer MR, Mecozzi S (2017) Multicompartment theranostic nanoemulsions stabilized by a triphilic semifluorinated block copolymer. Mol Pharm 14(11):3916–3926

Chatterjee B, Gorain B, Mohananaidu K, Sengupta P, Mandal UK, Choudhury H (2019) Targeted drug delivery to the brain via intranasal nanoemulsion: available proof of concept and existing challenges. Int J Pharm 565:258–268

Chen H, Khemtong C, Yang X, Chang X, Gao J (2011) Nanonization strategies for poorly water-soluble drugs. Drug Discov Today 16(7–8):354–360

Choudhury H, Zakaria NFB, Tilang PAB, Tzeyung AS, Pandey M, Chatterjee B, Alhakamy NA, Bhattamishra SK, Kesharwani P, Gorain B, Md S (2019a) Formulation development and evaluation of rotigotine mucoadhesive nanoemulsion for intranasal delivery. J Drug Delivery Sci Technol 54:101301

Choudhury H, Zakaria NFB, Tilang PAB, Tzeyung AS, Pandey M, Chatterjee B, Alhakamy NA, Bhattamishra SK, Kesharwani P, Gorain B, Md S (2019b) Formulation development and evaluation of rotigotine mucoadhesive nanoemulsion for intranasal delivery. J Drug Deliv Sci Technol 54:101301

Deshpande D, Kethireddy S, Gattacceca F, Amiji M (2014) Comparative pharmacokinetics and tissue distribution analysis of systemically administered 17-β-estradiol and its metabolites in vivo delivered using a cationic nanoemulsion or a peptide-modified nanoemulsion system for targeting atherosclerosis. J Control Release 180:117–124

Fang C, Zhang M (2010) Nanoparticle-based theragnostics: integrating diagnostic and therapeutic potentials in nanomedicine. J Control Release 146(1):2

Fuior EV, Mocanu CA, Deleanu M, Voicu G, Anghelache M, Rebleanu D, Simionescu M, Calin M (2020) Evaluation of VCAM-1 targeted naringenin/indocyanine green-loaded lipid nanoemulsions as theranostic nanoplatforms in inflammation. Pharmaceutics 12(11):1066

Ganta S, Singh A, Kulkarni P, Keeler AW, Piroyan A, Sawant RR, Patel NR, Davis B, Ferris C, O'Neal S, Zamboni W (2015) EGFR targeted theranostic nanoemulsion for image-guided ovarian cancer therapy. Pharm Res 32(8):2753–2763

Gianella A, Jarzyna PA, Mani V, Ramachandran S, Calcagno C, Tang J, Kann B, Dijk WJ, Thijssen VL, Griffioen AW, Storm G (2011) Multifunctional nanoemulsion platform for imaging guided therapy evaluated in experimental cancer. ACS Nano 5(6):4422–4433

Giroux HJ, Constantineau S, Fustier P, Champagne CP, St-Gelais D, Lacroix M, Britten M (2013) Cheese fortification using water-in-oil-in-water double emulsions as carrier for water soluble nutrients. Int Dairy J 29(2):107–114

Grapa CM, Mocan T, Gonciar D, Zdrehus C, Mosteanu O, Pop T, Mocan L (2019) Epidermal growth factor receptor and its role in pancreatic cancer treatment mediated by nanoparticles. Int J Nanomedicine 14:9693

Gupta A (2020) Nanoemulsions. In: Nanoparticles for biomedical applications. Elsevier, pp 371–384

Hashemnejad SM, Badruddoza AZM, Zarket B, Ricardo Castaneda C, Doyle PS (2019) Thermoresponsive nanoemulsion-based gel synthesized through a low-energy process. Nat Commun 10(1):1–10

Herneisey M, Liu L, Lambert E, Schmitz N, Loftus S, Janjic JM (2019) Development of theranostic perfluorocarbon nanoemulsions as a model non-opioid pain nanomedicine using a quality by design (QbD) approach. AAPS PharmSciTech 20(2):1–13

Jokerst JV, Gambhir SS (2011) Molecular imaging with theranostic nanoparticles. Acc Chem Res 44(10):1050–1060

Juneja M, Suthar T, Pardhi VP, Ahmad J, Jain K (2022) Emerging trends and promises of nanoemulsions in therapeutics of infectious diseases. Nanomedicine (Lond) 17(11):793–812. https://doi.org/10.2217/nnm-2022-0006

Khalid N, Kobayashi I, Neves MA, Uemura K, Nakajima M (2013) Preparation and characterization of water-in-oil emulsions loaded with high concentration of l-ascorbic acid. LWT-Food Sci Technol 51(2):448–454

Kim JE, Park YJ (2017) Paclitaxel-loaded hyaluronan solid nanoemulsions for enhanced treatment efficacy in ovarian cancer. Int J Nanomedicine 12:645

Kumar D, Yadav S, Ujjwal RR, Jain K (2021) Interfacial characterization of nanoemulsions. In: Ahmad J, Nollet LML (eds) Nanoemulsions in food technology. CRC Press, pp 125–140. eBook ISBN9781003121121

Kumbhar SA, Kokare CR, Shrivastava B, Gorain B, Choudhury H (2020) Preparation, characterization, and optimization of asenapine maleate mucoadhesive nanoemulsion using box-Behnken design: in vitro and in vivo studies for brain targeting. Int J Pharm 586:119499

Lammers T, Aime S, Hennink WE, Storm G, Kiessling F (2011) Theranostic nanomedicine. Acc Chem Res 44(10):1029–1038

Le Kim TH, Jun H, Kim JH, Park K, Kim JS, Nam YS (2017) Lipiodol nanoemulsions stabilized with polyglycerol-polycaprolactone block copolymers for theranostic applications. Biomater Res 21(1):1–10

Lee KY, Mooney DJ (2012) Prog Polym Sci

Lin CC, Metters AT (2006) Hydrogels in controlled release formulations: network design and mathematical modeling. Adv Drug Deliv Rev 58(12–13):1379–1408

Loureiro A, Bernardes GJ, Shimanovich U, Sária MP, Nogueira E, Preto A, Gomes AC, Cavaco-Paulo A (2015) Folic acid-tagged protein nanoemulsions loaded with CORM-2 enhance the survival of mice bearing subcutaneous A20 lymphoma tumors. Nanomedicine 11(5):1077–1083

Lovelyn C, Attama AA (2011) Current state of nanoemulsions in drug delivery. J Biomater Nanobiotechnol 2(05):626

Mahla RS, Kumar A, Tutill HJ, Krishnaji ST, Sathyamoorthy B, Noursadeghi M, Breuer J, Pandey AK, Kumar H (2021) NIX-mediated mitophagy regulate metabolic reprogramming in phagocytic cells during mycobacterial infection. Tuberculosis 126:102046

Mason TG, Wilking JN, Meleson K, Chang CB, Graves SM (2006) Nanoemulsions: formation, structure, and physical properties. J Phys Condens Matter 18(41):R635

Matos M, Gutiérrez G, Coca J, Pazos C (2014) Preparation of water-in-oil-in-water (W1/O/W2) double emulsions containing trans-resveratrol. Colloids Surf A Physicochem Eng Asp 442:69–79

McClements DJ (2004) Food emulsions: principles, practices, and techniques. CRC Press

McClements DJ (2011) Edible nanoemulsions: fabrication, properties, and functional performance. Soft Matter 7(6):2297–2316

McClements DJ, Rao J (2011) Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. Crit Rev Food Sci Nutr 51(4):285–330

Nadin M, Rousseau D, Ghosh S (2014) Fat crystal-stabilized water-in-oil emulsions as controlled release systems. LWT-Food Sci Technol 56(2):248–255

Nahrendorf M, Hoyer FF, Meerwaldt AE, van Leent MM, Senders ML, Calcagno C, Robson PM, Soultanidis G, Pérez-Medina C, Teunissen AJ, Toner YC (2020) Imaging cardiovascular and lung macrophages with the positron emission tomography sensor 64Cu-macrin in mice, rabbits, and pigs. Circ Cardiovas Imaging 13(10):e010586

Nirale P, Paul A, Yadav KS (2020) Nanoemulsions for targeting the neurodegenerative diseases: Alzheimer's, Parkinson's and Prion's. Life Sci 245:117394

O'Hanlon CE, Amede KG, Meredith RO, Janjic JM (2012) NIR-labeled perfluoropolyether nanoemulsions for drug delivery and imaging. J Fluor Chem 137:27–33

Oh MH, Kim JS, Lee JY, Park TG, Nam YS (2013) Radio-opaque theranostic nanoemulsions with synergistic anti-cancer activity of paclitaxel and Bcl-2 siRNA. RSC Adv 3(34):14642–14651

Ojha B, Jain VK, Gupta S, Talegaonkar S, Jain K (2022) Nanoemulgel: a promising novel formulation for treatment of skin ailments. Polym Bull 79:4441–4465. https://doi.org/10.1007/s00289-021-03729-3

Pandey M, Choudhury H, Yeun OC, Yin HM, Lynn TW, Tine CL, Wi NS, Yen KC, Phing CS, Kesharwani P, Bhattamisra SK (2018) Perspectives of nanoemulsion strategies in the improvement of oral, parenteral and transdermal chemotherapy. Curr Pharm Biotechnol 19(4):276–292

Pardhi VP, Suthar T, Sharma A, Jain K (2022) Bedaquiline fumarate microemulsion: formulation optimization, rheological characterization and in vitro studies. Nanomedicine (Lond) 17(21):1529–1546. https://doi.org/10.2217/nnm-2022-0132

Patel SK, Zhang Y, Pollock JA, Janjic JM (2013) Cyclooxgenase-2 inhibiting perfluoropoly (ethylene glycol) ether theranostic nanoemulsions—in vitro study. PLoS One 8(2):e55802

Patel SK, Beaino W, Anderson CJ, Janjic JM (2015) Theranostic nanoemulsions for macrophage COX-2 inhibition in a murine inflammation model. Clin Immunol 160(1):59–70

Patel NR, Piroyan A, Nack AH, Galati CA, McHugh M, Orosz S, Keeler AW, O'Neal S, Zamboni WC, Davis B, Coleman TP (2016) Design, synthesis, and characterization of folate-targeted platinum-loaded theranostic nanoemulsions for therapy and imaging of ovarian cancer. Mol Pharm 13(6):1996–2009

Piorkowski DT, McClements DJ (2014) Beverage emulsions: recent developments in formulation, production, and applications. Food Hydrocoll 42:5–41

Poyato C, Navarro-Blasco I, Calvo MI, Cavero RY, Astiasarán I, Ansorena D (2013) Oxidative stability of O/W and W/O/W emulsions: effect of lipid composition and antioxidant polarity. Food Res Int 51(1):132–140

Qu Y, Li A, Ma L, Iqbal S, Sun X, Ma W, Li C, Zheng D, Xu Z, Zhao Z, Ma D (2021) Nose-to-brain delivery of disulfiram nanoemulsion in situ gel formulation for glioblastoma targeting therapy. Int J Pharm 597:120250

Rietberg MR, Rousseau D, Duizer L (2012) Sensory evaluation of sodium chloride-containing water-in-oil emulsions. J Agric Food Chem 60(16):4005–4011

Rocha-Selmi GA, Bozza FT, Thomazini M, Bolini HM, Fávaro-Trindade CS (2013) Microencapsulation of aspartame by double emulsion followed by complex coacervation to provide protection and prolong sweetness. Food Chem 139(1–4):72–78

Singh TP, Ahmad FJ, Jain GK, Verma N (2021) Formulation development and characterization of nanoemulsion-based gel for topical application of raloxifene hydrochloride. Indian J Pharm Educ Res 55(4):996–1007

Skelhon TS, Grossiord N, Morgan AR, Bon SA (2012) Quiescent water-in-oil Pickering emulsions as a route toward healthier fruit juice infused chocolate confectionary. J Mater Chem 22(36):19289–19295

Smoleński M, Karolewicz B, Gołkowska AM, Nartowski KP, Małolepsza-Jarmołowska K (2021) Emulsion-based multicompartment vaginal drug carriers: from nanoemulsions to nanoemulgels. Int J Mol Sci 22(12):6455

Solans C, Solé I (2012) Nano-emulsions: formation by low-energy methods. Curr Opin Colloid Interface Sci 17(5):246–254

Song B, Wu S, Li W, Chen D, Hu H (2020) Folate modified long circulating nano-emulsion as a promising approach for improving the efficiency of chemotherapy drugs in cancer treatment. Pharm Res 37(12):1–12

Sonneville-Aubrun O, Simonnet JT, L'Alloret F (2004) Nanoemulsions: a new vehicle for skincare products. Adv Colloid Interf Sci 108:145–149

Suthar T, Jain VK, Popli H, Jain K (2022) 12 - Nanoemulsions as effective carriers for targeting brain tumors. In: Kumar L, Pathak YY (eds) Nanocarriers for drug-targeting brain tumors. Elsevier, pp 347–363

Suthar T, Patel P, Singh P, Datusalia AK, Yadav AK, Jain K (2023) Hesperidin microemulsion: Formulation optimization, characterization, and in vitro evaluation. J Drug Deliv Sci Tech 80 (104166):1–12

Tadros T, Izquierdo P, Esquena J, Solans C (2004) Formation and stability of nano-emulsions. Adv Colloid Interf Sci 108:303–318

Tinoco LMDS, Silva FLOD, Ferreira LAM, Leite EA, Carneiro G (2019) Hyaluronic acid-coated nanoemulsions loaded with a hydrophobic ion pair of all-trans retinoic acid for improving the anticancer activity. Braz J Pharm Sci 54

Velikov KP, Pelan E (2008) Colloidal delivery systems for micronutrients and nutraceuticals. Soft Matter 4(10):1964–1980

Wang JJ, Hung CF, Yeh CH, Fang JY (2008) The release and analgesic activities of morphine and its ester prodrug, morphine propionate, formulated by water-in-oil nanoemulsions. J Drug Target 16(4):294–301

Wooster TJ, Golding M, Sanguansri P (2008) Impact of oil type on nanoemulsion formation and Ostwald ripening stability. Langmuir 24(22):12758–12765

Xie J, Lee S, Chen X (2010) Nanoparticle-based theranostic agents. Adv Drug Deliv Rev 62(11): 1064–1079

Yadav S, Gupta S (2015) Development and in vitro characterization of docetaxel-loaded ligand appended solid fat nanoemulsions for potential use in breast cancer therapy. Artif Cells Nanomed Biotechnol 43(2):93–102

Yang X, Wang D, Ma Y, Zhao Q, Fallon JK, Liu D, Xu XE, Wang Y, He Z, Liu F (2014) Theranostic nanoemulsions: codelivery of hydrophobic drug and hydrophilic imaging probe for cancer therapy and imaging. Nanomedicine 9(18):2773–2785

Yi J, Dong W, Zhu Z, Liu N, Ding Y, McClements DJ, Decker EA (2015) Surfactant concentration, antioxidants, and chelators influencing oxidative stability of water-in-walnut oil emulsions. J Am Oil Chem Soc 92(8):1093–1102

Zalkind S (2001) Ilya Mechnikov: his life and work. The Minerva Group

Zhang Y, Bo S, Feng T, Qin X, Wan Y, Jiang S, Li C, Lin J, Wang T, Zhou X, Jiang ZX (2019) A versatile theranostic nanoemulsion for architecture-dependent multimodal imaging and dually augmented photodynamic therapy. Adv Mater 31(21):1806444

Functionalized Dendrimers: Promising Nanocarriers for Theranostic Applications

8

Anchal Pathak, Saba Naqvi, and Keerti Jain

Abstract

"Nanotheranostic" is an integrative approach to achieve diagnosis and therapeutic effect simultaneously, using nanocarriers. Nanotheranostic platform can be engineered to overcome biological barriers, to target the therapeutic agent at the required locus, and to support the monitoring of drug delivery. Dendrimers are well-defined 3D globular nanoarchitectured monodisperse system, pliable to precise size control and surface modification, which could serve as a potential nanotheranostic platform to achieve image-guided therapy, distribution monitoring, and drug targeting. Dendrimers exhibit biodegradable, biocompatible, and stimuli-responsive features that could ensure the anticipated biodistribution and efficacy. In this chapter, the utility of dendrimer as a theranostic nanoplatform embodying diversified category of therapeutic, imaging, and targeting moieties has been explored. Dendrimer-based administration of imaging agents (magnetic resonance imaging, computed tomography, single-photon emission computed tomography) along with its application in chemotherapy, pharmacodynamics therapy, and gene therapy has also been discussed.

Keywords

Dendrimers · Theranostics · Chemotherapy · Computed tomography · Magnetic resonance imaging

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Abbreviations

AuDENPs	Dendrimer-entrapped gold nanoparticles
CNTs	Carbon nanotubes
CT	Computed tomography
DANPs	Dendrimer-assembled nanoparticles
DENPs	Dendrimer-entrapped nanoparticles
DIONPs	Dendrimer-based iron oxide nanoparticles
DOTA	1,4,7,10-Tetraazacyclododecane-N,N′,N′′,N′′′-tetra-acetic acids
DOX	Doxorubicin
DSNPs	Dendrimer-stabilized nanoparticles
DTPA	Asdi-ethylenetriamine-penta-acetic acid
EPR	Enhanced permeation and retention effect
FA	Folic Acid
FMT	Fluorescence molecular tomography
FOI	Fluorescence optical imaging
IONPs	Iron oxide nanoparticles
LHRH	Luteinizing hormone-releasing hormone
MDR	Multidrug resistance
MRI	Magnetic resonance imaging
NIR	Near-infrared radiation
NPs	Nanoparticles
NRs	Nanorods
PAMAM	Polyamidoamine dendrimer
Pc	Phthalocyanine
PDT	Photodynamic therapy
PEG	Polyethylene glycol
PET	Positron emission tomography
PLL	Poly-L-lysine
PPI	Polypropylenimine dendrimer
PTT	Photothermal therapy
RGD	Arg-Gly-Asp
SiNc	Silicon naphthalocyanine
SPECT	Single-photon emission computed tomography
SPIONs	Superparamagnetic iron nanoparticles
TOS	α-Tocopheryl succinate
TPGS	D-α-tocopherol polyethylene glycol 1000 succinate

8.1 Introduction

The word "theranostics" was introduced by Funkhouser in 2002, which unifies the modalities of treatment and diagnostic imaging, comprising the benefits of diagnosis and treatment of the disease simultaneously (Funkhouser 2002). Theranostic nanostructures enable the monitoring of biodistribution and performance of the drug along with the imaging of target tissue. Diseases can be tracked closely and cured at the same time via these "theranostic" nanosystems (McCarthy and Weissleder 2008). The theranostic system utilizes appropriate molecular probe to measure the cellular biological operation noninvasively for disease characterization. Theranostic nanoparticles (NPs) can be engineered suitably to provide a single platform to achieve passive or active targeting, molecular imaging, and stimuli-responsive controlled drug release, simultaneously (Bagre et al. 2022). Although the term was adopted recently, the concept it represents have been well explored till now in the field of molecular biology, immunology, and cancer therapy. The theranostics can also be defined based on the utilization of diagnostic tools in clinical decision-making.

Several nanosystems have been investigated till now to achieve the targeted delivery of theranostics, i.e., liposomes, micelles, nanocrystals, solid lipid NPs, graphene oxide, and dendrimers (Fig. 8.1a) (Jain et al. 2020; Juneja et al. 2022). The engineering of functionalized nanotheranostic is complicated due to the obstacles like inherent toxicity of the nanocarrier components, production cost, nanosystems' stability, and intellectual property control. Theranostics nanosystem contains two different entities, i.e., diagnostic agent and therapeutic component. The

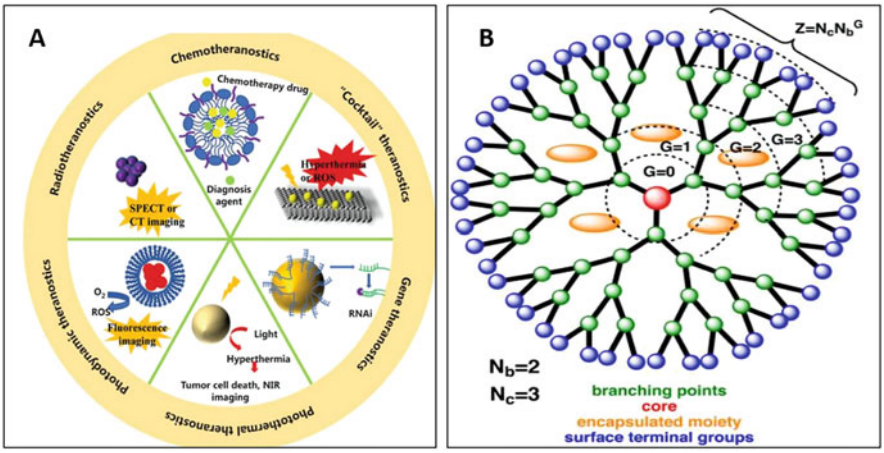

Fig. 8.1 (**a**). Schematic representation of theranostic nanosystems for cancer therapy (Xue et al. 2021). (**b**) Dendrimer with core (red), branching sites (green), surface terminal moieties (blue), and incorporated therapeutic agent. Dotted boundaries signifying generation (G) and N_c indicating the number of branches emerging from the core, while N_b represents the multiplicity of branch cell (Ray et al. 2018)

Table 8.1 Features of diagnostic modalities

Imaging modalities	Probe	Advantages	Disadvantage
FOI	Fluorescent dyes, quantum dots	High sensitivity, no exposure of radiation, provides functional information	Limited tissue penetration and low resolution
CT	Heavy elements (iodine)	High spatial resolution, ability of tissue differentiation	High cost
MRI	Para- and superparamagnetic metals (Mn, Gd)	High-resolution imaging of physiological and anatomical elements	Cannot be used in patients' metallic devices (with pacemakers)
Ultrasound	Gas-filled microbubbles	Noninvasive and easy procedure	Low resolution
Gamma scintigraphy	Radionuclides	Can image biological processes	Radiation and low resolution

diagnostic agent provides rapid, high-fidelity glimpse of precise locus by enhancing the signal-to-noise ratio with respect to adjacent tissues. Depending upon the clearance kinetics of the contrast agent, low or high molecular weight agents are used. The efficient theranostic agent have some give and take between controlled drug release, imaging sensitivity, and targeting accuracy. Various modalities that have been utilized for imaging in theranostics are fluorescence optical imaging (FOI), magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), single-photon emission computed tomography (SPECT), gamma scintigraphy, and ultrasound. Each diagnostic modality has its characteristic feature with relative advantages and disadvantages (Table 8.1) (Saluja et al. 2021).

Dendrimers have emerged as a unique class of 3D nanoarchitectured macromolecule offering surface functionality that can be structured precisely to achieve high degree of molecular uniformity (Fig. 8.1b). Dendrimers may be considered as the ideal vectors for therapeutic agents and imaging modalities as they offer many advantages as compared to other delivery system, i.e., strong surface functionalization, high structural homogeneity, higher cell membrane penetration, higher water solubility with adequate surface functionalization, and high drug loading capacity (Jain 2017; Jain 2018; Gauro et al. 2021a). Drug may interact with the dendrimers via physical encapsulation, electrostatic interactions, or covalent conjugation (Gauro et al. 2021a). However, the translational issues comprising good manufacturing practices and high cost of production are major drawback for dendrimers (Mignani et al. 2021). Yet, the advantages of dendrimers as a versatile delivery system have contributed to its enormous development in the field of nanomedicine for drugs, gene, peptides, and diagnostic agents (Jain and Ahmad 2022).

In this chapter, the utility of dendritic polymer as nanotheranostic platform has been discussed. The dendrimers are well-suited for the theranostic application due to

the (a) presence of modifiable exterior, which can be surface-modified with targeting moieties, (b) improved plasma circulation time and physiochemical properties, and (c) conjugation and encapsulation of contrast and therapeutic agents at predetermined proportions (Bhavana et al. 2021). Further, we will also discuss about dendritic nanoplatform integrating imaging and therapeutic agent and its utility in disease management.

8.2 Dendrimer-Based Molecular Imaging

Various types of dendrimers are available such as polyamidoamine (PAMAM), polypropylenimine (PPI), poly-L-lysine (PLL), glycodendrimer, or metallodendrimer (Gauro et al. 2021b). The surface groups of dendrimers such as carboxyl group, alcohol groups, or amine groups determine the chemical characteristic of dendrimers. The abundance of functional groups over the dendrimers' exterior surface provides optimal attachment sites for targeting ligand for site-specific targeting. The ability to attach both diagnostic and therapeutic agent simultaneously has propelled the utility of dendrimers for the development of nanotheranostic devices. The compliance of dendrimeric contrast agents is appropriate for molecular imaging of target-specific locus (Longmire et al. 2008).

"Molecular imaging" is a noninvasive imaging technique, where the biological processes at the molecular level can be visualized, quantified, and characterized. Molecular imaging methods assists in the estimation of therapeutic response, drug release monitoring, and drug biodistribution quantification (Dasgupta et al. 2020). Figure 8.2 shows the systematic representation of drug release from functionalized nanotheranostic dendrimer in tumor cells. The techniques used for imaging are MRI, CT, PET, SPECT, and FOI. Each technique represents diverse anatomical and molecular imaging information based on the specific attributes it confers, such as sensitivity and specificity. Among these techniques, CT and MRI are utilized to obtain morphological information, while PET and SPECT are used to obtain molecular information (Janib et al. 2010).

In the following sections, dendrimer-based single and multi-model techniques have been focused. The utility of dendrimer-based theranostic techniques to predict the therapeutic response and disease progression imaging has also been discussed.

8.2.1 Dendrimer-Based MRI

MRI is a noninvasive molecular imaging modality, which utilizes strong magnetic field and radio waves. MRI can be used to visualize the anatomical morphology, tumor diagnosis, and treatment monitoring. Pharmacokinetic and biodistribution analysis as well as drug release studies can be estimated by using this technique (Zheng et al. 2019). The uniform orientation of magnetically active nuclei and relaxation in the applied magnetic field generates the signal to create MR images. Specific endogenous contrast image is generated for specific tissues as different

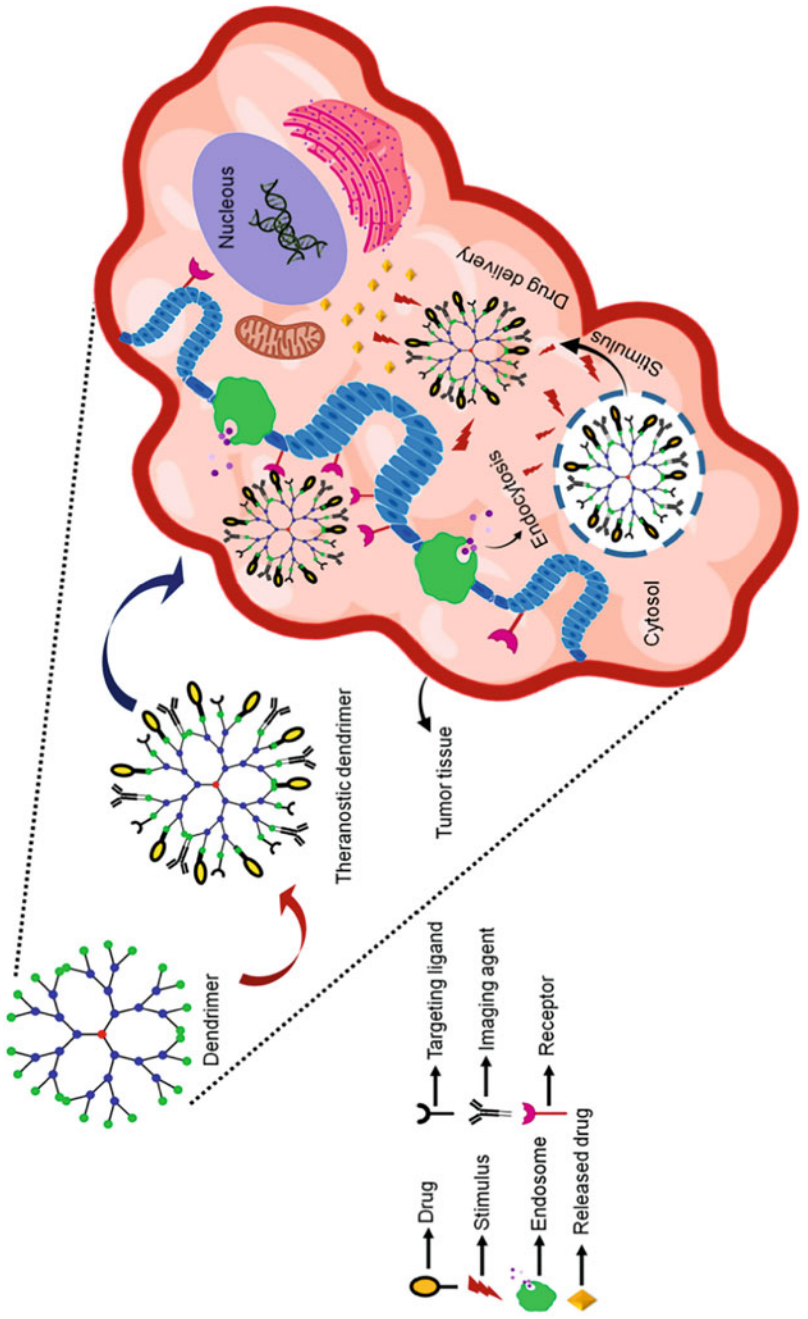

Fig. 8.2 Diagrammatical representation of functionalized nanotheranostic dendrimer (Saluja et al. 2021)

tissue relax at different rates. The time taken for the protons to relax entirely is measured in two ways, i.e., spin-lattice (T1) and spin-spin (T2) relaxation time.

The contrast modalities of MRI are classified as T1 positive, for instance, gadolinium (Gd) chelates, which accelerate the longitudinal relaxation rate and produce brighter image, and T2 negative, which includes superparamagnetic iron that provides darker signal in T2 images by facilitating transverse relaxation rate. Paramagnetic agents such as Gd or manganese can be labeled onto the nanocarrier, while iron oxide nanosystem are innately superparamagnetic that can be tagged over nanocarriers' surface (Shu et al. 2021; Ahmad et al. 2022). The ligand employed in Gd complexes are macrocyclic polyamino carboxylates such as asdi-ethylenetriamine-penta-acetic acid (DTPA) and 1,4,7,10-tetraazacyclododecane-N, N′,N′′,N′′′-tetra-acetic acids (DOTA) (Yan and Zhuo 2001). Superparamagnetic iron nanoparticles (SPIONs) have been explored extensively for imaging contrast enhancement in dendrimer-based MRI.

The longitudinal relaxation of MRI contrast agents determines their efficiency. It was reported that water proton longitudinal relaxation rates, which determines the efficiency of MRI contrast agent, escalate with an increase in dendrimeric generation. The rotational correlation time increases with the increasing molecular mass of dendrimers, which causes increased relaxivity of dendrimers with generation. The terminal functional groups of dendrimers attach covalently with the Gd^{3+} chelates, which upsurges the relaxation rates. Relaxation rates of the contrast agents increases with the increase in the dendrimeric generation and number of terminal functional groups, which leads to improved efficiency (Song et al. 2020). Various dendrimer-based MRI have been mentioned in Table 8.2**.**

To fabricate advanced biocompatible contrast agent, dendrimer-based iron oxide nanoparticles (DIONPs) are being employed for T2-weighted MRI to obtain in vivo diagnostic imaging. The DIONPs can be classified as dendrimer-assembled nanoparticles (DANPs) and dendrimer-stabilized nanoparticles (DSNPs). The DANPs are designed via assembly of dendrimers and pre-developed NPs, which are ruled by electrostatic interactions or covalent bonding. The IONPs can be fabricated and coated with multiple dendrimers simultaneously for stabilization in DSNP. The interaction between dendrimers and NPs plays an important role in stabilization (Strable et al. 2001). Researchers developed DSNP via solgel method, covalent conjugation, and physical absorption to obtain a theranostic nanocarrier for doxorubicin (DOX) delivery. Dendrimers were conjugated with targeting peptides GX1 and RGD to achieve receptor-mediated targeted delivery at vascular endothelial growth factor and αvβ3 integrins, respectively. Further, superparamagnetic IONP coated with dendrigraft of lysine was utilized as an MRI contrast agent. The conjugate showed significant antitumor activity and efficient biodistribution in HepG2 tumor-bearing Balb/c mice (Shen et al. 2017). The varied applications of dendrimeric theranostic nanostructures are attributed to the characteristics such as size flexibility, surface modification, and low toxicity.

Table 8.2 Dendrimer-based MRI contrast agents

MRI contrast agent	Dendrimeric nanoconjugate	Dendrimers	Targeting ligand	Uptake studies	Application	References
Copper (II)	G3.0-Cu	G3.0 phosphorus dendrimer	Ultrasound-targeted microbubble destruction	Mice	Pancreatic cancer	Fan et al. (2019)
DOTA-Gd	RGD-Gd-Au-G5.0-PAMAM	G5.0 PAMAM dendrimer	RGD (Arg-Gly-Asp) peptide for αvβ3 integrins expressing tumor cells	Mice	Lung cancer	Liu et al. (2019)
Manganese (II)	RGD-Au-Mn-G2.0-PAMAM	G2.0 PAMAM dendrimer	RGD peptide	Mice	Brain glioma	Xu et al. (2019)
SPIONs	G4.0-IONPs	G4.0 PAMAM dendrimer	–	MCF_7 and HDF_1 cell line	Breast cancer	Salimi et al. (2018)
SPIONs	SPION-FA-G4.0-PAMAM-curcumin	G4.0 PAMAM dendrimer	Folic acid (FA) to target folate receptors overexpressed over carcinoma cell surface	SKOV3 and HeLa cancer cells	Ovarian and cervical cancer	Luong et al. (2017)
SPIONs	FA-PEG-G3.5-PPI-PTX@IONP	G3.5 PPI dendrimer	FA	Mice	Liver cancer	Chang et al. (2013)
DTPA-Gd	Gd-DTPA-D3.0-PEG-chlorotoxin	G3.0 PLL dendrigraft	Chlorotoxin for MMP-2	Nude mice	Brain tumor	Huang et al. (2011)

8.2.2 Dendrimer-Based CT

CT is another noninvasive molecular diagnostic modality, which exploits specialized X-rays to generate cross-sectional 3D illustrations of organ or tissue with excellent spatial resolution (Hyun and Cho 2019). A contrast agent requires high-electron-density agents such as iodinated molecules and barium sulfate suspension to produce anatomical images of good resolution by increasing imaging area density. However, the drawbacks of these agents include the accumulation at nonspecific targeted site, short imaging duration due to fast clearance of contrast agents, and high dose requirement that may cause renal toxicity (Li et al. 2021). The properties of contrast agent used for CT imaging involves (i) nontoxicity, (ii) good sensitivity even at lower concentration, (iii) targeting at specific site, (iv) higher solubility at physiological medium, (v) complete systemic elimination, and (vi) adequate systemic circulation for imaging (Yeh et al. 2017). The anticipated pharmacokinetic and biodistribution profile conferred by dendrimer could be utilized to fabricate a suitable nanoplatform for CT contrast agents. Dendrimers possess stable highly ordered molecular architecture with low polydispersity index, which makes them an appropriate nanocarrier for contrast agent to achieve targeted therapy, biocompatibility, and imaging (Qiao and Shi 2015).

Dendrimer-entrapped gold nanoparticles (AuDENPs) can be exploited to achieve targeted chemotherapy along with CT imaging of cancers cells. For this purpose, G5.0 PAMAM dendrimer bearing amine as a terminal group was functionalized with polyethylene glycol (PEG)-modified α-tocopheryl succinate (α-TOS), PEGylated FA, and fluorescein isothiocyanate. The conjugated PAMAM dendrimer was further used as a template for the preparation of AuDENPs. The in vitro cell line studies demonstrated that the conjugate generated enhanced CT contrast of cancer cells, where FA receptors were overexpressed and also improved therapeutic efficiency of α-TOS was obtained. The performance of AuDENPs was evaluated for targeted cancer CT imaging in vivo in xenografted U87MG tumor model. After 24 h post-injection of FA-conjugated AuDENPs, the bright tumor CT image was obtained which suggests the FA-mediated active targeting via the theranostic carrier (Zhu et al. 2014). The study suggested that AuDENPs could serve as a versatile nanotheranostic platform to achieve targeted therapy and diagnosis in biological systems.

Scientists developed nanotheranostic system based on dendrimer to obtain CT imaging and targeted chemotherapy. For the preparation of AuDENPs, DOX was first conjugated with the partially acetylated G5.0 PAMAM dendrimer via acid-sensitive cis-aconityl linkage and functionalized with the FA, which were then entrapped within gold nanoparticles (AuNPs). The nanocarrier showed pH-responsive drug release due to the cis-aconityl linkage. The conjugate indicated efficient targeting to FA receptor, which were overexpressed over U87MG glioblastoma cancer cell lines. The CT imaging of the FA receptor-overexpressing carcinoma cells with enhanced contrast sensitivity was obtained (Zhu et al. 2018). The therapeutic delivery system integrated with a targeting ligand and imaging capability

holds a promising potential to achieve chemotherapy and CT imaging of cancer cells simultaneously.

Liu et al. synthesized AuDENPs using G5.0 PAMAM dendrimers to achieve targeted CT imaging of hepatocellular carcinoma cells using lactobionic acid as targeting ligand, which targets asialoglycoprotein receptors. The fluorescein isothiocynate and PEG-coupled lactobionic acid was utilized as a template to develop AuDENPs, where the ligand-conjugated AuDENPs demonstrated exceptional X-ray attenuation activity as compared to the iodine-based CT contrast agents. The developed theranostic nanoprobe showed specific imaging of in vitro human hepatocellular carcinoma cell lines and in vivo xenografted tumor model (Liu et al. 2014). In another study, FA and methotrexate-functionalized G5.0 PAMAM were used as template to design AuDENPs, which were utilized as a nanotheranostic platform for CT imaging and targeted chemotherapy successfully (Zheng et al. 2013). The results suggested that an effective nanotheranostic platform can be developed by decorating the AuNPs with dendrimers and targeting ligands, which obliges them with higher affinity toward overexpressed receptors over carcinoma cell surface that results in the effective CT imaging and chemotherapy simultaneously. Therefore, AuDENPs could serve as an efficient nanotheranostic tool in cancer therapy.

Nanotheranostics with CT imaging uses gold, bismuth, or iodine, which holds high electron density and is reported to overcome shortcomings related to conventional contrast agent. They exhibit prolonged vascular residence time and lower clearance rate and reduce the leakage across the capillary vessels. Dendrimers offer predictable biodistribution and pharmacokinetic profile. Liu et al. created RGD peptide-modified zwitterionic gadolinium (III)-complexed AuDENPs for targeted dual-mode CT/MRI of lung cancer metastasis model. The developed formulation was cytocompatible, displayed specific targeting to αvβ3 integrin-expressing cancer cells, and had good X-ray attenuation property (Liu et al. 2019). In another study, dendrimeric nanotheranostic platform was developed to obtain CT imaging along with the targeted curcumin delivery in chemotherapy. G5.0 PAMAM-based AuDENPs were conjugated with mucin-1 aptamer and loaded with curcumin, where targeted curcumin delivery and CT imaging in colorectal adenocarcinoma were achieved successfully (Alibolandi et al. 2018). Dendrimer-based theranostic system has several benefits over the conventional contrast agents such as prolonged blood circulation time, extended imaging time, desired biocompatibility, facile surface functionalization, and enhanced imaging parameter.

8.2.3 Dendrimer-Based SPECT/PET

Radiopharmaceuticals utilize a radioactive isotope to obtain the image of diseased site noninvasively. Radiopharmaceuticals can be explored to design nanotheranostic platform, as it may offer the advantages of both therapy and diagnosis simultaneously. Generally, metallic radionuclides are used as ionizing radiation source due to its easy availability, rich coordination chemistry, and wider range of nuclear

properties. The SPECT/PET is a radionuclide molecular imaging technique, which provides detailed and quantitative information regarding the disease's pathology and therapy response. These methods can produce high signal-to-noise ratios of the radionuclide due to the exceptional photon tissue-penetrating ability, and therefore the detailed information regarding nanomedicine pharmacokinetics and biodistribution can be obtained (Israel et al. 2019). Dendrimeric nanotheranostics based on radiopharmaceuticals are comprised of (i) a targeting probe, (ii) radiometals (for imaging and treatment), and (iii) bifunctional chelator (Xiao et al. 2020).

SPECT uses a γ-emitting radionuclide isotopes (^{99}mTc, ^{111}In, ^{123}I, and ^{201}Tl), while PET uses a positron-emitting isotopes (^{18}F, ^{11}C, ^{13}N, and ^{15}O). The PET scans offer higher resolution and sensitivity as compared to SPECT; still SPECT is mostly exploited in nanomedicine due to cost-effectiveness and accessibility of longer half-lived isotopes of SPECT. Zhao et al. explored the nanotheranostic capability of ^{131}I-labeled G5.0 PAMAM dendrimer and conjugated with chlorotoxin (targeting ligand), 3-(4′-hydroxyphenyl)propionic acid-OSu (HPAO), and PEG. The in vivo investigations showed excellent SPECT imaging signal intensity and antitumor activity due to the labeling of radioactive ^{131}I in mice glioma model (Zhao et al. 2015). The developed dendrimeric nanotheranostic platform for SPECT imaging and radiotherapy showed good cytocompatibility and organ compatibility in the studies.

Recently, to improve the drawbacks associated with PET/SPECT, i.e., low spatial resolution, multi-modular diagnostic techniques such as PET-CT/SPECT-CT/SPECT-MRI have been introduced to obtain high spatial resolution and desired anatomical distribution of probe (Nolte et al. 2020). AuDENPs using G5.0 PAMAM dendrimer were developed to achieve DOX chemotherapy and to obtain SPECT/CT dual-mode imaging of tumor apoptosis. Dendrimer was conjugated with PEG monomethyl ether, fluorescein isothiocyanate, and PEGylated duramycin for specific targeting at the tumor apoptotic site, where phosphatidylethanolamine was overexpressed. The multifunctionalized dendrimers were utilized as the templates to entrap AuNPs via DOTA chelation and radiolabeled with radionuclide technetium (^{99m}Tc) for SPECT/CT imaging of chemotherapy-induced tumor apoptosis at the targeted locus. SPECT/CT imaging studies suggested that apoptotic C6 cells exposed with the duramycin-dendrimeric AuNPs have higher CT value as compared to the cells treated with the dendrimeric AuNPs, and SPECT images showed brighter image of the targeted site than those of ^{99m}Tc-dendrimeric AuENPs (without targeting ligand). The result of studies suggested that the developed nanotheranostic system was suitable for the monitoring of therapeutic response and confirmed the targeting role of ligand-conjugated AuDENPs for early detection of apoptosis after chemotherapy (Xing et al. 2018). It may be concluded that dendrimers may serve as a viable platform to obtain the molecular imaging of specific target.

8.2.4 Dendrimer-Based FOI

FOI is a noninvasive molecular imaging technique, being explored frequently due to the admirable contrast agent sensitivity and cost-effectiveness. FOI can be utilized to monitor the biodistribution and target site accumulation of nanocarrier. Fluorescence reflectance imaging is one of the FOI techniques based on a planer epi-illumination technique, used to monitor nanotheranostic accumulation in superficial tissues. An advanced tomographic technique, i.e., fluorescence molecular tomography (FMT), has been introduced to overcome the limitations of planer imaging. FMT allocates the nanomedicine accumulation assessment at the non-superficial tissues and represents the better depth resolution. However, drawbacks of this optical imaging technique involve the incorrect signal assignment to precise anatomical locus owing to the strong light absorption by well-perfused organs and scattering of fluorescence signals. To overcome this issue, the hybrid imaging techniques have been introduced to assign accurate accumulation of nanotheranostic to a precise anatomical region, which will enable the assessment of non-superficial tissues (Saluja et al. 2021).

Li et al. designed a theranostic system to achieve chemotherapy and fluorescence imaging, using hybrid nanocarriers, i.e., G5.0 PAMAM dendrimers and blue-emitting carbon dots (CDs) along with chemotherapeutic agent (DOX). Dendrimers were conjugated with RGD peptide and D-α-tocopherol polyethylene glycol 1000 succinate (TPGS) and further complexed with CDs/DOX conjugate via electrostatic interaction. The TPGS overcomes multidrug resistance (MDR) by inhibiting the P-glycoprotein overexpressed over cancer cell surface, which results in the enhanced drug accumulation within the tumor cells. The fluorescence imaging of free CDs and hybrid nanoconjugate was compared using A549 cell line. The results suggested that hybrid nanoconjugate assisted intracellular complex tracking and enabled the excellent fluorescence imaging without quenching due to bright blue luminescence exhibited by CD (Li et al. 2019). The combination of dendrimer/CD nanohybrid could be a promising theranostic nanoplatform to achieve chemotherapy along with the fluorescence imaging of cancer cells. Therefore, dendrimer could serve as functional theranostic system for tumor diagnosis and treatment, where near-infrared (NIR) fluorescence imaging and active tumor targeting can be achieved simultaneously.

8.3 Application of Dendrimer-Based Theranostic System

Theranostic applications of dendrimers is a significant area of research, which involves improved therapeutic agent delivery, diagnostic application, and reduced side effects. Owing to the unique characterizations of dendrimer and their potential to be developed as multifunctional nanotheranostic vector, it can provide treatment and diagnostic platforms for several diseases including cancer. However, clearance and interactions of dendrimer with extra- and intracellular molecules need to be considered. Dendrimers have numerous surface groups to conjugate various molecules such as drug, linker, aptamer, and antibody (Jain et al. 2013; Jain et al.

2014; Jain and Jain 2014; Jain et al. 2010). In the following subsection, application of dendrimer in theranostic-based nanomedicine have been discussed.

8.3.1 Nanotheranostic-Assisted Chemotherapy

One of the major drawbacks of conventional chemotherapy is the resistance of the cancerous cells to the free drug. Often, the traditional chemotherapy is associated with administration at nonspecific site, which causes the systemic toxicity and adverse effect on healthy cells. Dendrimers are a unique class of therapeutic agent carrier, different from conventional polymers, have well-defined 3D nanoarchitectures, and offer the functionalization over its surface through which active targeting can be achieved. Dendrimers have emerged as an excellent noninvasive nanotheranostic functional platform, which can be used for (1) characterization and quantification of biological events involved in the diagnosis of early cancer, (2) tracking cellular events, and (3) stimulation of specific drug delivery to tackle tumor progression (Mignani et al. 2021).

The nanosize and limited dendrimeric cargo space make the dendrimer suitable for diagnostic and imaging application, where fast renal clearance is desirable. Generally, theranostic nanocarriers required three basic components: signal emitter, therapeutic payload, and targeting ligand. Researchers fabricated multifunctional nanocarriers based on 4G, 5G, and 6G PAMAM dendrimer, fixed over polydopamine-coated magnetite NPs (Fe_3O_4), and studied the theranostic application of the nanocarrier for combined chemo- and photothermal therapy (PTT) of liver cancer cells in vitro. The synthesized nanocarriers were nontoxic, exhibited strong photothermal properties, and have competitive contrast properties in MRI. The nanosystem showed synergistic effect in the combined chemotherapy and PTT of liver carcinoma cells at low NPs concentrations (Jędrzak et al. 2019). Therefore, the presence of abundant terminal functional moieties over dendrimer can be functionalized with targeting moiety and imaging modalities to constitute multipurpose theranostic dendrimers.

Zu et al. reported the multifunctional gadolinium-loaded G5.0 PAMAM dendrimer, modified with targeting ligand FA via PEG spacer and encapsulated with DOX within the dendrimeric core. The in vitro cell line studies of the developed dendrimeric nanotheranostic system showed targeted intracellular delivery of DOX in κB carcinoma cells, where FA receptors are overexpressed. Quantitative measurements of the MR signal-to-noise suggested that κB cells treated with the dendrimeric theranostic system enabled MR imaging of FA receptor-overexpressing cancerous cells through FA-mediated active targeting pathway (Zhu et al. 2015a). The multifunctional dendrimer with imaging agents and physically incorporated drug within the core holds enormous potential to be utilized as a theranostic platform, where the imaging and treatment can be achieved simultaneously.

FOI is a noninvasive imaging modality for tumor detection with accuracy, where real-time evidence of tumor margins can be specified and magnitude of cancer span can be determined. Xu and the group developed Pep-1-decorated PEGylated

PAMAM to improve the distribution across blood-brain tumor barrier and homing to glioblastoma cells. The Pep-PEG-PAMAM dendrimeric system showed excellent penetration inside the cancer cells. The in vivo real-time fluorescence imaging with Cy5.5 in U87MG tumor-bearing mice suggested that the accumulation of theranostic system at tumor site was 2.02 times greater as compared to the untargeted group (Jiang et al. 2016).

Luong et al. synthesized polyvalent theranostic nanocarriers based on the superparamagnetic Fe_3O_4 decorated with FA-G4.0 PAMAM dendrimer that encapsulates chemotherapeutic agent 3,4-diflourobenzylidene-curcumin. FA dendrimer and Fe_3O_4 were linked with 3-aminopropyl trimethoxy-silane chain. As compared to nontargeted NPs, the FA-conjugated nanocarrier exhibited rapid cellular uptake in SKOV3 and HeLa cancer cell lines. Also, enhanced MRI contrast was obtained for the targeted carcinoma cells (Luong et al. 2017). Researchers fabricated dendrimeric nanotheranostic system to achieve tumor targeting and CT imaging by using G5.0 PAMAM AuDENPs decorated with fluorescein isothiocyanate, PEGylated TOS, and RGD peptide through PEG chain. α-TOS triggers the proliferation inhibition and carcinoma cell apoptosis, while the RGD rendered the targeting specificity to these nanocarrier toward U87MG cells to enhance the therapeutic efficiency of the nanocarrier. The fabricated AuNP-TOS-RGD dendrimer NPs showed better X-ray absorption properties than Omnipaque (currently used in clinic) (Zhu et al. 2015b). The nanosize, degree of branching, and functionalization of the dendrimers can be controlled through synthetic methods. This tunable architecture linked to the application-related properties, like biocompatibility, stimuli-responsiveness, and self-assembly capability, which are the crucial feature for the chemotherapeutic theranostics.

Dendrimers possess nanometric size, which facilitates the passive and active targeting of the therapeutic agents, where the enhanced permeation and retention effect (EPR) effect promotes accumulation of the dendrimers in the tumor microenvironment through leaky vessels, while the functionalization of dendrimeric surface through the tumor-specific ligands delivers the drug to the targeted cells. The EPR effect also supports the longer circulation and increased therapeutic payload to the tumor tissue (Felder-Flesch 2021). The advanced nanotheranostic multifunctional dendrimeric systems are capable of diagnosing and delivering drug to the cancer site through sustained/controlled release of encapsulated contrast agents and chemotherapeutics (Dendrimer-Based Nanotherapeutics 2021). The multifunctional dendrimeric system facilitates endocytosis and provides a platform to deliver chemotherapeutic agent, avoid MDR, and render an all-in-one single theranostic nanoplatform anchored with targeting ligands for targeted therapy.

8.3.2 Nanotheranostic-Assisted Photothermal Therapy

To improve the cancer treatment outcome, nanocarrier with higher selectivity and good therapeutic efficiency is required along with the minimal invasiveness. PTT triggers the reaction against cancer cells in the presence of light and has brought

predominant attention to achieve specific targeting and effective ablation of the irradiated carcinoma cells in chemotherapy. However, development of multifunctional theranostic nanoplatforms with improved diagnostic sensitivity and photothermal therapeutic efficiency is still a great challenge. Dendrimer-based theranostics have immense potential in cancer phototherapy, as it confers several structural advantages as compared to linear polymers. Dendrimers can be functionalized easily with photoreactive agents and targeting ligands to achieve targeted PTT.

Nava et al. fabricated a dendrimeric system, where AuNPs were incorporated inside FA/bombesin-functionalized and ^{177}Lu-labeled G4.0 PAMAM dendrimers. Bombesin moieties are linked with the fluorescence imaging, while the targeted radiotherapy and PTT can be achieved due to ^{177}Lu-labeling and entrapped AuNPs, respectively. The viability of T47D human breast cancer cells was reduced significantly after the cells were treated with laser irradiation along with the drug-loaded nanosystem. In vivo imaging revealed that ^{177}Lu-dendrimer-AuNP-FA-bombesin retained within the cancer cells up to 96 h followed by intratumoral radiopharmaceutical administration (Mendoza-Nava et al. 2016). The ^{177}Lu-labeled G4.0 PAMAM dendrimers could have application in theranostics due to its combined optical, radioactive, and thermoablative properties. This study depicts the utility of receptor-specific radiopharmaceuticals incorporating dendrimer nanocarrier in targeted radiotherapy and chemotherapy.

AuDENPs have been fabricated and studied for their potential application toward PTT of malignant tissue. For this purpose, G5.0 PAMAM dendrimer-entrapped AuNPs were covalently linked to fluorescein isothiocyanate and surface modified with FA for targeted delivery to tumor cells. The dendrimers showed binding to human epithelial carcinoma cell line κB cells, in vitro, and internalized into lysosomes within 2 h. The dendrimeric nanocarrier represented targeted hyperthermia treatment of cancer through inductive tumor cell heating followed by particle internalization. Dendrimeric AuNP therapeutics was visualized in the targeted cells because of the high-electron-density contrast of the Au atoms (Shi et al. 2007). AuNP represents flexible localized surface plasmon resonance and suitable optical characteristics that make them suitable for imaging and effective for PTT, which could be exploited in cancer therapy along with the dendrimers for targeted delivery.

Ge et al. developed NIR-guided G5.0 PAMAM dendrimer theranostic platform to achieve improved antitumor efficacy by combined chemo-phototherapy. For this purpose, dendrimer was covalently conjugated with a fluorescent targeting agent, i.e., indocyanine green derivative cypate and iRGD (tumor-penetrating peptide). Further, docetaxel was encapsulated within the PAMAM cavity to develop a single agent-based multifunctional nanotheranostic platform, i.e., iRGD-cypate-PAMAM-Docetaxel, to obtain the synergistic effect of phototherapy and chemotherapy. Figure 8.3a represents the schematic synthesis of the nanotheranostic platform with low polydispersity index and average size of 150.67 ± 12.58 nm (Fig. 8.3c). The results suggested that the developed theranostic system could enhance the singlet oxygen species while retaining its fluorescence intensity and heat generation capability followed by NIR irradiation. Figure 8.3b represents the absorption

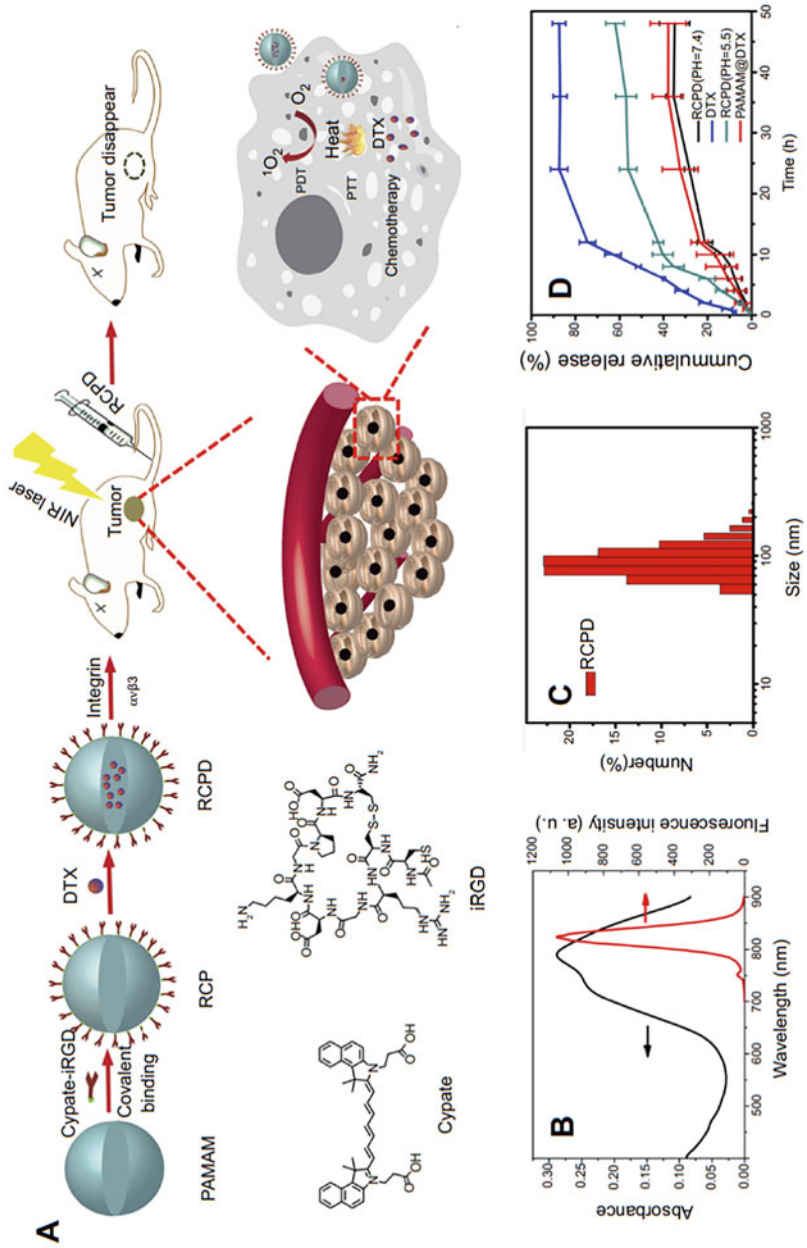

Fig. 8.3 Systematic representation of NIR-guided G5.0 PAMAM dendrimer theranostic platform. (**a**) Synthesis of dendrimeric theranostic nanoplatform. (**b**) UV-visible absorption and fluorescence spectra of iRGD-cypate-PAMAM-Docetaxel. (**c**) Size distribution of the nanotheranostic carrier. (**d**) In vitro drug release studies of drug from the dendrimeric theranostic system (Ge et al. 2019). (DTX=Docetaxel)

spectra, which showed monomer and dimer peak at 790 nm and 730 nm, respectively, while fluorescence spectra exhibited the maximum emission wavelength at 820 nm, which suggests that the dendrimeric system could be utilized for NIR imaging. The in vitro drug release studies demonstrated that the docetaxel was released from the formulation at the faster rate at pH 5.5, while slow release of drug was obtained at the pH 7.4, which suggest that the premature drug release in the systemic circulation can be circumvented, and drug will be released at tumor site (Fig. 8.3d). The in vitro and in vivo studies suggest that the multifunctional theranostic platform can significantly improve the antitumor efficiency (Ge et al. 2019).

Carbon nanotubes (CNTs) exhibit unique physical attributes such as higher absorption capacity in the NIR region than other photothermal agents. The dendrimeric nanohybrids can be developed by conjugating dendrimer with CNTs, which could be further exploited in PTT. Recently, CNT-G4.0-PAMAM-CdS photothermal agent was developed by conjugating PAMAM with CNTs and cadmium sulfide (CdS) nanocarriers. The nanosystem showed photothermal conversion efficiency of 32% under 980 nm NIR laser irradiation than earlier reported Au-based photothermal agents (Ouyang et al. 2021). Dendrimers conjugated with CNTs or quantum dots have unique properties that can be exploited for theranostic application along with thermal ablation for cancer therapy.

8.3.3 Nanotheranostic-Assisted Gene Therapy

Gene therapy is a promising therapeutic approach, which is independent of the utilization of therapeutic drug. Theranostic imaging monitors the expression of gene therapy enzymes, and the therapeutic outcome from the gene therapy can be evaluated. Nanotheranostic imaging applications save the time via assessing therapeutic efficacy while measuring the therapeutic gene expression level. Recently, personalized therapy has been explored widely as promising chemotherapy strategy. With the incorporation of diagnostic imaging agent in the gene therapy, the aftermath of the therapy can be calculated (Sekar and Paulmurugan 2016). Xiong et al. developed mRNA-based dendrimer-lipid nanoparticle nanotheranostic system containing PEGylated BODIPY dyes for mRNA delivery and NIR imaging simultaneously. The result suggested that the developed nanocarrier could mediate luciferase mRNA expression in the tumors and simultaneously illuminate the tumors through the pH-activatable NIR imaging (Xiong et al. 2020).

In RNA interference delivery, which holds great promise in chemotherapeutic application, researchers synthesized gold nanostar stabilized with RGD peptide-modified G3.0 PAMAM dendrimers (Au-RGD-G3NSs). The developed dendrimeric system was used as a gene delivery vector and complexed with siRNA to achieve CT imaging, PTT, and gene therapy simultaneously. The outcomes showed that Au-RGD-G3NSs compact the siRNA to deliver the siRNA within the $\alpha_v\beta_3$ integrin-overexpressed cancerous cells. Followed by NIR and incubation with the Au-RGD-G3NSs/siRNA polyplexes, the viability of U87MG

cancer cells was 20.2%, which is lower than the cells treated with single PTT (44.34% cell viability) or gene therapy (46.9% cell viability). The in vivo studies suggested that Au-RGD-G3NSs/siRNA polyplexes enabled tumor CT imaging and thermal imaging after intratumoral injection (Wei et al. 2016). The study describes the role of dendrimer as a multifunctional nanocarrier for tumor imaging, combinational PTT, and gene therapy.

Recently, researchers explored the utility of dendrimer-functionalized gold nanorods (NRs) to achieve gene therapy, PTT, and CT imaging of the colon cancer, simultaneously. G3.0 PAMAM dendrimer-modified gold NRs were grafted with GX1 peptide (a cyclic 7-mer peptide, CGNSNPKSC), which was utilized as a vector for gene delivery, FAM172A, which controls the apoptosis of colon cancer cells. The results suggested that the polyplex, AuNR-PAMAM-GX1/FAM172A, had tremendous transfection efficiency. The HCT-8 colon cancer cells exposed to AuNR-G3.0-PAMAM-GX1/FAM172A showed viability of 20.45%, under laser irradiation. The in vivo studies showed that the dendrimeric nanosystem can be utilized for combinational tumor thermal imaging, PTT, CT imaging, and gene therapy after tail vein injection (Ye et al. 2021). Therefore, the dendrimer offers a nanotheranostic platform, to exert anticancer activity, and improved diagnostic level of cancer cells.

8.3.4 Nanotheranostic-Assisted Photodynamic Therapy

Photodynamic therapy (PDT) involves the systemic administration of photosensitizers such as porphyrin and phthalocyanine derivatives, followed by photoactivation of photosensitizers at the disease site with the light of specific wavelength. The photosensitizers accumulate intensely in pathological locations, where it excites at the target site by specific light radiating wavelength and generates a series of biochemical reactions leading to the death of diseased cells (Xie et al. 2022). NIR optical properties of phthalocyanine confers enormous potential as theranostic agent, for fluorescence image-guided delivery of therapeutic agent by PDT. Researchers fabricated G4.0 PPI-based theranostic platform to enhance the water solubility, reduce aggregation, and achieve tumor-targeted delivery of phthalocyanine. The phthalocyanine-PPI complex was further surface modified with PEG and luteinizing hormone-releasing hormone (LHRH) peptide to improve biocompatibility and tumor-targeted delivery, respectively. The in vivo studies suggested that LHRH targeted nanocarrier had effective internalization ability within tumor cells. The nanoformulation demonstrated lower cytotoxicity ($IC_{50} = 28$ μg/mL) in dark, whereas the light irradiation on cancerous cells transfected with nanotheranostic agents showed considerable PDT activity ($IC_{50} = 0.9$ μg/mL) due to the excessive release of toxic reactive oxygen species (Taratula et al. 2013). The studies determined the potential of the dendrimeric-based nanosystem as a competent NIR theranostic system.

Dendrimers offer multifunctional theranostic platforms, where NIR fluorescence imaging and phototherapy can be obtained simultaneously for cancer diagnosis and

treatment. A dendrimer-based theranostic nanosystem was developed for NIR fluorescence imaging, with dual PTT and PDT therapeutic mechanism. Silicon naphthalocyanine (SiNc) was developed into biocompatible nanocarrier (SiNc-NC) via SiNc encapsulation within hydrophobic cavity of G5.0 PPI dendrimer followed by surface functionalization with PEG. Encapsulation of SiNc within the dendrimer preserves the NIR fluorescence and PDT and PTT properties of SiNc. Under NIR irradiation, SiNc-NC produces reactive oxygen species and demonstrated heat generation capability required for PDT and PTT, respectively. Studies demonstrated that the PTT mediated by the SiNc can kill the MDR ovarian cancer cells efficiently (Taratula et al. 2015). The PPI dendrimer has hydrophobic cavities, which offer the encapsulation for separate SiNc molecules and thus diminish their aggregation and preserve imaging, PDT, and PTT properties.

8.4 Conclusion

Dendrimers are synthetic polymeric macromolecules with tuneable surface morphology. Various functionalities, i.e., therapeutic, imaging, or targeting, could be functionalized over the dendrimeric surface or encapsulated within the void space of the dendrimers. Dendrimeric nanotheranostic system incorporates therapy (chemotherapy, PDT, PTT) and diagnosis (MRI, CT, SPECT, PET) simultaneously. The judicious dendrimer functionalization for the therapeutic agent, targeting ligand, and the diagnostic agent could deliver the cargo, with negligible toxicity to the targeted locus. The imaging functionality in conjugation allows the drug distribution monitoring, visualization of disease progression, and therapeutic response assessment.

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Conflict of Interest The authors declare no conflict of interest related to this manuscript.

References

Ahmad J, Albarqi HA, Ahmad MZ, Md R, Suthar T, Jain K, Vuddanda PR, Khan MA (2022) Receptor-targeted surface-engineered nanomaterials for breast cancer imaging and Theranostic applications. Crit Rev Ther Drug Carrier Syst 39:1–44. https://doi.org/10.1615/CRITREVTHERDRUGCARRIERSYST.2022040686

Alibolandi M, Hoseini F, Mohammadi M, Ramezani P, Einafshar E, Taghdisi SM, Ramezani M, Abnous K (2018) Curcumin-entrapped MUC-1 aptamer targeted dendrimer-gold hybrid nanostructure as a theranostic system for colon adenocarcinoma. Int J Pharm 549:67–75. https://doi.org/10.1016/j.ijpharm.2018.07.052

Bagre A, Patel PR, Naqvi S, Jain K (2022) Emerging concerns of infectious diseases and drug delivery challenges. In: Nanotheranostics for treatment and diagnosis of infectious diseases, pp 1–23. https://doi.org/10.1016/B978-0-323-91201-3.00013-X

Bhavana V, Pradip T, Jain K, Mehra NK (2021) Dendrimer–guest interaction chemistry and mechanism. Dendrimers in Nanomedicine:171–185. https://doi.org/10.1201/9781003029915-9

Chang Y, Li Y, Meng X, Liu N, Sun D, Liu H, Wang J (2013) Dendrimer functionalized water soluble magnetic iron oxide conjugates as dual imaging probe for tumor targeting and drug delivery. Polym Chem 4:789–794. https://doi.org/10.1039/c2py20740k

Dasgupta A, Biancacci I, Kiessling F, Lammers T (2020) Imaging-assisted anticancer nanotherapy. Theranostics 10:956–967. https://doi.org/10.7150/thno.38288

Dendrimer-Based Nanotherapeutics (2021) https://doi.org/10.1016/c2019-0-03208-6

Fan Y, Zhang J, Shi M, Li D, Lu C, Cao X, Peng C, Mignani S, Majoral JP, Shi X (2019) Poly (amidoamine) dendrimer-coordinated copper(II) complexes as a theranostic nanoplatform for the radiotherapy-enhanced magnetic resonance imaging and chemotherapy of tumors and tumor metastasis. Nano Lett 19:1216–1226. https://doi.org/10.1021/acs.nanolett.8b04757

Felder-Flesch D (2021) Dendrimers as nanomedicine in cancer therapy, in: Dendrimers in nanomedicine, pp. 309–326. https://doi.org/10.1201/9781315364513-13

Funkhouser J (2002) Reinventing pharma: the theranostic revolution. Curr Drug Discov:17–19

Gauro R, Jain K, Jain VK, Mehra NK, Popli H (2021a) Macromolecular architecture and molecular modelling of dendrimers. Dendrimers Nanomed:77–88. https://doi.org/10.1201/9781003029915-5

Gauro R, Nandave M, Jain VK, Jain K (2021b) Advances in dendrimer-mediated targeted drug delivery to the brain. J Nanopart Res 23. https://doi.org/10.1007/s11051-021-05175-8

Ge R, Cao J, Chi J, Han S, Liang Y, Xu L, Liang M, Sun Y (2019) NIR-guided dendritic nanoplatform for improving antitumor efficacy by combining chemo-phototherapy. Int J Nanomedicine 14:4931–4947. https://doi.org/10.2147/IJN.S203171

Huang R, Han L, Li J, Liu S, Shao K, Kuang Y, Hu X, Wang X, Lei H, Jiang C (2011) Chlorotoxin-modified macromolecular contrast agent for MRI tumor diagnosis. Biomaterials 32:5177–5186. https://doi.org/10.1016/j.biomaterials.2011.03.075

Hyun H, Cho CS (2019) Updates in molecular imaging techniques. Tissue Eng Regen Med 16:431–432. https://doi.org/10.1007/s13770-019-00222-x

Israel O, Pellet O, Biassoni L, De Palma D, Estrada-Lobato E, Gnanasegaran G, Kuwert T, la Fougère C, Mariani G, Massalha S, Paez D, Giammarile F (2019) Two decades of SPECT/CT—the coming of age of a technology: an updated review of literature evidence. Eur J Nucl Med Mol Imaging 46:1990–2012. https://doi.org/10.1007/s00259-019-04404-6

Jain A, Jain K, Mehra NK, Jain NK (2013) Lipoproteins tethered dendrimeric nanoconstructs for effective targeting to cancer cells. J Nanopart Res 15. https://doi.org/10.1007/s11051-013-2003-9

Jain K (2017) Dendrimers: smart nanoengineered polymers for bioinspired applications in drug delivery. Biopolym Compos Drug Deliv Biomed Appl:169–220. https://doi.org/10.1016/B978-0-08-101914-6.00007-7

Jain K (2018) Dendrimers: emerging anti-infective nanomedicines. NanoBioMaterials:121–138. https://doi.org/10.1201/9781351138666-6

Jain K, Ahmad J (2022) Nanotheranostics for treatment and diagnosis of infectious diseases, pp 1–391. https://doi.org/10.1016/C2020-0-03819-3

Jain K, Gupta U, Jain NK (2014) Dendronized nanoconjugates of lysine and folate for treatment of cancer. Eur J Pharm Biopharm 87:500–509. https://doi.org/10.1016/j.ejpb.2014.03.015

Jain K, Jain NK (2014) Surface engineered dendrimers as antiangiogenic agent and carrier for anticancer drug: dual attack on cancer. J Nanosci Nanotechnol 14:5075–5087. https://doi.org/10.1166/jnn.2014.8677

Jain K, Kesharwani P, Gupta U, Jain NK (2010) Dendrimer toxicity: let's meet the challenge. Int J Pharm 394:122–142. https://doi.org/10.1016/j.ijpharm.2010.04.027

Jain K, Mehra NK, Jain VK, Jain NK (2020) IPN dendrimers in drug delivery. Interpenetr Polym Netw Biomed Appl:143–181. https://doi.org/10.1007/978-981-15-0283-5_6/COVER

Janib SM, Moses AS, MacKay JA (2010) Imaging and drug delivery using theranostic nanoparticles. Adv Drug Deliv Rev 62:1052–1063. https://doi.org/10.1016/j.addr.2010.08.004

Jędrzak A, Grześkowiak BF, Coy E, Wojnarowicz J, Szutkowski K, Jurga S, Jesionowski T, Mrówczyński R (2019) Dendrimer based theranostic nanostructures for combined chemo- and photothermal therapy of liver cancer cells in vitro. Colloids Surfaces B Biointerfaces 173:698–708. https://doi.org/10.1016/j.colsurfb.2018.10.045

Jiang Y, Lv L, Shi H, Hua Y, Lv W, Wang X, Xin H, Xu Q (2016) PEGylated Polyamidoamine dendrimer conjugated with tumor homing peptide as a potential targeted delivery system for glioma. Colloids Surfaces B Biointerfaces 147:242–249. https://doi.org/10.1016/j.colsurfb.2016.08.002

Juneja M, Suthar T, Pardhi VP, Ahmad J, Jain K (2022) Emerging trends and promises of nanoemulsions in therapeutics of infectious diseases. 17:793–812. https://doi.org/10.2217/nnm-2022-0006

Li D, Fan Y, Shen M, Bányai I, Shi X (2019) Design of dual drug-loaded dendrimer/carbon dot nanohybrids for fluorescence imaging and enhanced chemotherapy of cancer cells. J Mater Chem B 7:277–285. https://doi.org/10.1039/C8TB02723D

Li H, Sun J, Zhu H, Wu H, Zhang H, Gu Z, Luo K (2021) Recent advances in development of dendriticpolymer-based nanomedicines for cancer diagnosis. Wiley Interdiscip Rev Nanomed Nanobiotechnol 13. https://doi.org/10.1002/wnan.1670

Liu H, Wang H, Xu Y, Guo R, Wen S, Huang Y, Liu W, Shen M, Zhao J, Zhang G, Shi X (2014) Lactobionic acid-modified dendrimer-entrapped gold nanoparticles for targeted computed tomography imaging of human hepatocellular carcinoma. ACS Appl Mater Interfaces:6944–6953. https://doi.org/10.1021/am500761x

Liu J, Xiong Z, Zhang J, Peng C, Klajnert-Maculewicz B, Shen M, Shi X (2019) Zwitterionic gadolinium(III)-complexed dendrimer-entrapped gold nanoparticles for enhanced computed tomography/magnetic resonance imaging of lung cancer metastasis. ACS Appl Mater Interfaces 11:15212–15221. https://doi.org/10.1021/acsami.8b21679

Longmire M, Choyke P, Kobayashi H (2008) Dendrimer-based contrast agents for molecular imaging. Curr Top Med Chem 8:1180–1186. https://doi.org/10.2174/156802608785849021

Luong D, Sau S, Kesharwani P, Iyer AK (2017) Polyvalent folate-dendrimer-coated iron oxide theranostic nanoparticles for simultaneous magnetic resonance imaging and precise cancer cell targeting. Biomacromolecules 18:1197–1209. https://doi.org/10.1021/acs.biomac.6b01885

McCarthy JR, Weissleder R (2008) Multifunctional magnetic nanoparticles for targeted imaging and therapy. Adv Drug Deliv Rev 60:1241–1251. https://doi.org/10.1016/j.addr.2008.03.014

Mendoza-Nava H, Ferro-Flores G, Ramírez FDM, Ocampo-García B, Santos-Cuevas C, Aranda-Lara L, Azorín-Vega E, Morales-Avila E, Isaac-Olivé K (2016) 177Lu-dendrimer conjugated to folate and bombesin with gold nanoparticles in the dendritic cavity: a potential theranostic radiopharmaceutical. J Nanomater 2016. https://doi.org/10.1155/2016/1039258

Mignani S, Shi X, Ceña V, Rodrigues J, Tomas H, Majoral JP (2021) Engineered non-invasive functionalized dendrimer/dendron-entrapped/complexed gold nanoparticles as a novel class of theranostic (radio)pharmaceuticals in cancer therapy. J Control Release 332:346–366. https://doi.org/10.1016/j.jconrel.2021.03.003

Nolte T, Gross-Weege N, Schulz V (2020) (Hybrid) SPECT and PET Technologies, in: Recent Results Cancer Res: pp. 111–133. https://doi.org/10.1007/978-3-030-42618-7_3

Ouyang Z, Gao Y, Shen M, Shi X (2021) Dendrimer-based nanohybrids in cancer photomedicine. Mater Today Bio 10. https://doi.org/10.1016/j.mtbio.2021.100111

Qiao Z, Shi X (2015) Dendrimer-based molecular imaging contrast agents. Prog Polym Sci 44:1–27. https://doi.org/10.1016/j.progpolymsci.2014.08.002

Ray S, Li Z, Hsu C, Hwang L, Lin Y, Chou P (2018) Dendrimer- and copolymer-based nanoparticles for magnetic resonance cancer theranostics. Theranostics 8. https://doi.org/10.7150/thno.27828

Salimi M, Sarkar S, Saber R, Delavari H, Alizadeh AM, Mulder HT (2018) Magnetic hyperthermia of breast cancer cells and MRI relaxometry with dendrimer-coated iron-oxide nanoparticles. Cancer Nanotechnol 9. https://doi.org/10.1186/s12645-018-0042-8

Saluja V, Mishra Y, Mishra V, Giri N, Nayak P (2021) Dendrimers based cancer nanotheranostics: an overview. Int J Pharm 600. https://doi.org/10.1016/j.ijpharm.2021.120485

Sekar TV, Paulmurugan R (2016) Theranostic imaging of cancer gene therapy, in: Methods Mol. Biol., pp. 241–254. https://doi.org/10.1007/978-1-4939-3813-1_20

Shen JM, Li XX, Fan LL, Zhou X, Han JM, Jia MK, Wu LF, Zhang XX, Chen J (2017) Heterogeneous dimer peptide-conjugated polylysine dendrimer-Fe3O4 composite as a novel nanoscale molecular probe for early diagnosis and therapy in hepatocellular carcinoma. Int J Nanomedicine 12:1183–1200. https://doi.org/10.2147/IJN.S126887

Shi X, Wang S, Meshinchi S, Van Antwerp ME, Bi X, Lee I, Baker JR (2007) Dendrimer-entrapped gold nanoparticles as a platform for cancer-cell targeting and imaging. Small 3:1245–1252. https://doi.org/10.1002/smll.200700054

Shu G, Chen M, Song J, Xu X, Lu C, Du Y, Xu M, Zhao Z, Zhu M, Fan K, Fan X, Fang S, Tang B, Dai Y, Du Y, Ji J (2021) Sialic acid-engineered mesoporous polydopamine nanoparticles loaded with SPIO and Fe3+ as a novel theranostic agent for T1/T2 dual-mode MRI-guided combined chemo-photothermal treatment of hepatic cancer. Bioact Mater 6:1423–1435. https://doi.org/10.1016/j.bioactmat.2020.10.020

Song C, Shen M, Rodrigues J, Mignani S, Majoral JP, Shi X (2020) Superstructured poly (amidoamine) dendrimer-based nanoconstructs as platforms for cancer nanomedicine: a concise review. Coord Chem Rev 421. https://doi.org/10.1016/j.ccr.2020.213463

Strable E, Bulte JWM, Moskowitz B, Vivekanandan K, Allen M, Douglas T (2001) Synthesis and characterization of soluble iron oxide-dendrimer composites. Chem Mater 13:2201–2209. https://doi.org/10.1021/cm010125i

Taratula O, Schumann C, Duong T, Taylor KL, Taratula O (2015) Dendrimer-encapsulated naphthalocyanine as a single agent-based theranostic nanoplatform for near-infrared fluorescence imaging and combinatorial anticancer phototherapy. Nanoscale 7:3888–3902. https://doi.org/10.1039/c4nr06050d

Taratula O, Schumann C, Naleway MA, Pang AJ, Chon KJ, Taratula O (2013) A multifunctional theranostic platform based on phthalocyanine-loaded dendrimer for image-guided drug delivery and photodynamic therapy. Mol Pharm 10:3946–3958. https://doi.org/10.1021/mp400397t

Wei P, Chen J, Hu Y, Li X, Wang H, Shen M, Shi X (2016) Dendrimer-stabilized gold nanostars as a multifunctional theranostic nanoplatform for CT imaging, photothermal therapy, and gene silencing of Tumors. Adv Healthc Mater 5:3203–3213. https://doi.org/10.1002/adhm.201600923

Xiao T, Li D, Shi X, Shen M (2020) PAMAM dendrimer-based nanodevices for nuclear medicine applications. Macromol Biosci 20. https://doi.org/10.1002/mabi.201900282

Xie L, Ji X, Zhang Q, Wei Y (2022) Curcumin combined with photodynamic therapy, promising therapies for the treatment of cancer. Biomed Pharmacother 146. https://doi.org/10.1016/j.biopha.2021.112567

Xing Y, Zhu J, Zhao L, Xiong Z, Li Y, Wu S, Chand G, Shi X, Zhao J (2018) SPECT/CT imaging of chemotherapy-induced tumor apoptosis using 99mTc-labeled dendrimer-entrapped gold nanoparticles. Drug Deliv 25:1384–1393. https://doi.org/10.1080/10717544.2018.1474968

Xiong H, Liu S, Wei T, Cheng Q, Siegwart DJ (2020) Theranostic dendrimer-based lipid nanoparticles containing PEGylated BODIPY dyes for tumor imaging and systemic mRNA delivery in vivo. J Control Release 325:198–205. https://doi.org/10.1016/j.jconrel.2020.06.030

Xu X, Liu K, Wang Y, Zhang C, Shi M, Wang P, Shen L, Xia J, Ye L, Shi X, Shen M (2019) A multifunctional low-generation dendrimer-based nanoprobe for the targeted dual mode MR/CT imaging of orthotopic brain gliomas. J Mater Chem B 7:3639–3643. https://doi.org/10.1039/c9tb00416e

Xue Y, Gao Y, Meng F, Luo L (2021) Recent progress of nanotechnology-based theranostic systems in cancer treatments. Cancer Biol Med 18:336–351. https://doi.org/10.20892/j.issn.2095-3941.2020.0510

Yan G, Zhuo R (2001) Research progress of magnetic resonance imaging contrast agents. Chin Sci Bull 46:1233–1237. https://doi.org/10.1007/BF03184316

Ye L, Chen Y, Mao J, Lei X, Yang Q, Cui C (2021) Dendrimer-modified gold nanorods as a platform for combinational gene therapy and photothermal therapy of tumors. J Exp Clin Cancer Res 40. https://doi.org/10.1186/s13046-021-02105-3

Yeh BM, FitzGerald PF, Edic PM, Lambert JW, Colborn RE, Marino ME, Evans PM, Roberts JC, Wang ZJ, Wong MJ, Bonitatibus PJ (2017) Opportunities for new CT contrast agents to maximize the diagnostic potential of emerging spectral CT technologies. Adv Drug Deliv Rev 113:201–222. https://doi.org/10.1016/j.addr.2016.09.001

Zhao L, Zhu J, Cheng Y, Xiong Z, Tang Y, Guo L, Shi X, Zhao J (2015) Chlorotoxin-conjugated multifunctional dendrimers labeled with radionuclide 131I for single photon emission computed tomography imaging and radiotherapy of gliomas. ACS Appl Mater Interfaces 7:19798–19808. https://doi.org/10.1021/acsami.5b05836

Zheng F, Xiong W, Sun S, Zhang P, Zhu JJ (2019) Recent advances in drug release monitoring. Nano 8:391–413. https://doi.org/10.1515/nanoph-2018-0219

Zheng L, Zhu J, Shen M, Chen X, Baker JR, Wang SH, Zhang G, Shi X (2013) Targeted cancer cell inhibition using multifunctional dendrimer-entrapped gold nanoparticles. MedChemComm 4:1001–1005. https://doi.org/10.1039/c3md00050h

Zhu J, Fu F, Xiong Z, Shen M, Shi X (2015b) Dendrimer-entrapped gold nanoparticles modified with RGD peptide and alpha-tocopheryl succinate enable targeted theranostics of cancer cells. Colloids Surfaces B Biointerfaces 133:36–42. https://doi.org/10.1016/j.colsurfb.2015.05.040

Zhu J, Wang G, Alves CS, Tomás H, Xiong Z, Shen M, Rodrigues J, Shi X (2018) Multifunctional dendrimer-entrapped gold nanoparticles conjugated with doxorubicin for pH-responsive drug delivery and targeted computed tomography imaging. Langmuir 34:12428–12435. https://doi.org/10.1021/acs.langmuir.8b02901

Zhu J, Xiong Z, Shen M, Shi X (2015a) Encapsulation of doxorubicin within multifunctional gadolinium-loaded dendrimer nanocomplexes for targeted theranostics of cancer cells. RSC Adv 5:30286–30296. https://doi.org/10.1039/c5ra01215e

Zhu J, Zheng L, Wen S, Tang Y, Shen M, Zhang G, Shi X (2014) Targeted cancer theranostics using alpha-tocopheryl succinate-conjugated multifunctional dendrimer-entrapped gold nanoparticles. Biomaterials 35:7635–7646. https://doi.org/10.1016/j.biomaterials.2014.05.046

9 Functionalized Carbon Nanotubes, Graphene Oxide, Fullerenes, and Nanodiamonds: Emerging Theranostic Nanomedicines

Satish Shilpi, Anamika Sahu Gulbake, Sandhya Chouhan, and Pramod Kumar

Abstract

The nanomaterials bear considerable potential as the next generation of therapeutic vehicles that allow the early detection of disease and the simultaneous monitoring and treatment options for targeted drug delivery with reduced toxicity. Carbon-based nanomaterials are in consideration in several clinical areas. It is due to the intrinsic properties, i.e., nanosize, distinctive structural dimensions, and diversified physicochemical properties, which attract the scientific communities for their applications in the drug delivery area as well as for the development of novel diagnostic assays with high specificity and sensitivity. Structurally, these carbon-based nanomaterials exhibit zero-, one-, two-, and three-order dimensions with several valuable features that could be exploited potentially in theranostic nanomedicines. The term "theranostic" was recently implemented for the two-fold beneficial application of the materials simultaneously, as a therapeutic entity and for the diagnostic purpose. The current chapter describes the applications of carbon-based nanomaterials in drug delivery

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and disease diagnosis. The synthesis of carbon-based nanomaterial and functionalization approaches and possible future theranostic applications are also included.

Keywords

Carbon-based nanomaterials · Functionalization · Carbon nanotubes · Graphene oxide · Fullerenes · Nanodiamonds

9.1 Introduction

Nanotechnology is an innovative field of technological development that concerns the material management at a nanometric range. Nanomedicine is generally characterized as nanoscale drug delivery technologies in the field of medicine. Nanomaterials are nanosized compounds that can target the molecular level serving both disease diagnosis and therapy. Thus they are already established as the basis of theranostics (Kim et al. 2017). Nanomaterials based on carbon are getting significant attention by scientists over the past two decades for their unique properties, functionalization, and compatibility (Patel et al. 2020; Li et al. 2019). Carbon is an essential element and is one of the primary building blocks of our body (Sarkar et al. 2020). The carbon-based nanomaterials have been classified as nanotubes, fullerenes, graphene derivatives, nanohorns, nanodiamonds, and nanodots based on their structural geometry obtained by various synthesis processes (Patel et al. 2020).

All the carbon-based nanomaterials can load both the hydrophilic and lipophilic nature of drugs in very high concentrations. Surface modification of carbon-based nanomaterial makes them unique drug delivery systems that can target specific organ or tissue of the body and improve the pharmacokinetic and pharmacodynamic properties and stability of the drugs or other therapeutic molecules. Besides excellent optical properties, carbon-based nanomaterial possesses high surface areas and mechanical and electrical properties. Each carbon nanomaterial has distinct properties and could be used in various biological applications, including biosensing, bioimaging, drug delivery, gene delivery, tissue engineering, fluorescence labeling of cells, diagnosis, and photothermal and photodynamic therapy.

These characteristics make them one of the most promising candidates for theranostic applications in nanomedicine (Gifani et al. 2021; Kim et al. 2017; Patel et al. 2020) and cancer therapy (Maiti et al. 2019). The new development of various carbon-based nanosystems as theranostic specialists and applications of these theranostic in therapeutics and diagnostics in various diseases have been compiled. However, carbon nanotubes (CNTs) are widely studied in various applications, and there is enormous potential in other candidates in the theranostic application field. Thus, the CNTs are the primary focus of the current chapter; however, the application of other carbon-based nanomaterials in theranostic purpose has also been included adequately.

9.2 Carbon-Based Nanomaterials and Theragnosis

Due to the multifunctional characteristics, the carbon-based nanomaterials also serve as potential candidates for image-guided drug/diagnostic molecule delivery platform in case of critical diseases, e.g., cancer, with the following techniques, i.e., positron emission tomography (PET), magnetic resonance (MR), single-photon emission computed tomography (SPECT), fluorescence imaging, bioluminescence, photoacoustic (PA), Raman imaging, and cargo (chemo-/gene therapy) delivery platform. The "theranostic" term was invented in 1998 by John Funkhouser (Theek et al. 2014) for the compound, which can be used for simultaneous application as diagnostic and therapeutic purposes. Thus, it is a portmanteau of therapeutic and diagnostic potency in a single agent (Das et al. 2021), i.e., one radioactive drug to identify (diagnose) and deliver therapy to treat the main tumor or cancer and other diseases (Fig. 9.1) (Theek et al. 2014). In the case of nanotechnology, the amalgamation of diagnostic and therapeutic entities in a single delivery vector using nanotechnology also known as "nanotheranostics" may act as a vital part of the early diagnosis, treatment, and prevention of diseases (Carvalho et al. 2021).

Nanotechnology provides a potential platform for the developing theranostic agents in the form of efficient nanocarrier or drug delivery system by altering the nanoparticle (NP) properties and enabling the conjugation of appropriate drug molecules at their surface. As a result, the interaction of these carriers with targeted sites promotes the physiological response with minimum or no/least toxic effects.

The application of engineering strategies in nanomaterials imparts the multifunctionality to the nanoplatform which enables these platforms for a particular theranostic application (Kim et al. 2017). The primary hypothesis of the development of theranostic nanomaterials is based on optical methods. For example, radioactivity (e.g., 125I, 111In, 18F, and 15O) and fluorescence (e.g., green fluorescent protein, GFP; red fluorescent protein, RFP; Cy5; indocyanine; quantum spot; etc.) properties of these moieties help to screen their biodistribution within the in vitro or in vivo settings. Among all carbon-based nanomaterials, gold NPs have gotten expanding focus recently as a promising theranostic specialist due to their adequate surface plasmonic impacts that consider fluorescence imaging (e.g., fluorescence

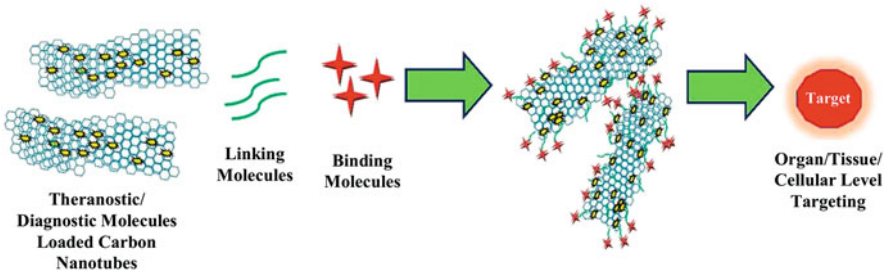

Fig. 9.1 The basic hypothesis of theranostic activity of carbon-based nanomaterials for dual application in diagnosis and treatment of disease simultaneously

tomography and near-infrared (NIR) fluorescence imaging) and photothermal treatments (Mignani et al. 2021; Meher et al. 2018).

9.3 Synthesis and Functionalization of Carbon Nanomaterials

Carbon is a novel component of the occasional table, having the remarkable ability to arrange its four valence electrons in different hybridization states, to be specific sp., sp2, and sp3, prompting areas of strength for both and powerless π-π bonds, and this ability of carbon provides it a capacity to be used in several areas including nanotechnology (Speranza 2021).

In 1985, Rick Smalley and colleagues discovered a new form of carbon. They observed that by vaporizing graphite with lasers, a new molecule made of pure carbon containing 60 carbon atoms could be developed. The researchers named it buckminsterfullerene (fullerene), commonly known as the "buckyball" (Goodarzi et al. 2017). Other allotropes of pure carbon are graphene, graphite, and diamond (Syama and Mohanan 2019).

Graphene is a honeycomb sheet-like carbon monolayer organized in a two-dimensional structure with a trigonal planar lattice. It is a precursor for other carbon nanomaterials, such as carbon nanotubes (CNTs), fullerene, and graphite (Sun et al. 2013).

The synthesis methods for graphene are categorized as bottom-up and top-down approaches shown in Fig. 9.2 (Lee et al. 2019; Kumar et al. 2019; Ajala et al. 2022). Among these methods, the thermal chemical vapor deposition (TCVD) process is the most common and best technique to produce graphene in the presence of copper.

9.3.1 Carbon Nanotubes

Carbon nanotubes (CNTs) are the key promising nanocarrier candidate of carbon family, which was discovered in 1991 by Sumio Ijima (Iijima 1991). CNTs have offered vast applications from early disease diagnostic purposes to targeted drug delivery. The CNTs alone cannot perform their tasks due to agglomeration characteristics, resulting in dispersion problems. Certain toxicity issues in the human body have also been reported using the CNTs. However, some toxicity problem can be resolved by functionalizing the CNTs due to their flexible characteristics, i.e. by introducing carboxyl and amino groups to improve the solubilization. Further chemical linking with other compounds like polymers, biomolecules and inorganic compounds have also been reported in previous literature (Pandey and Dahiya 2016; Raval et al. 2018). CNTs can be further divided into single-walled carbon nanotubes abbreviated as SWCNTs and multiple-walled carbon nanotubes popular as MWCNTs**.**

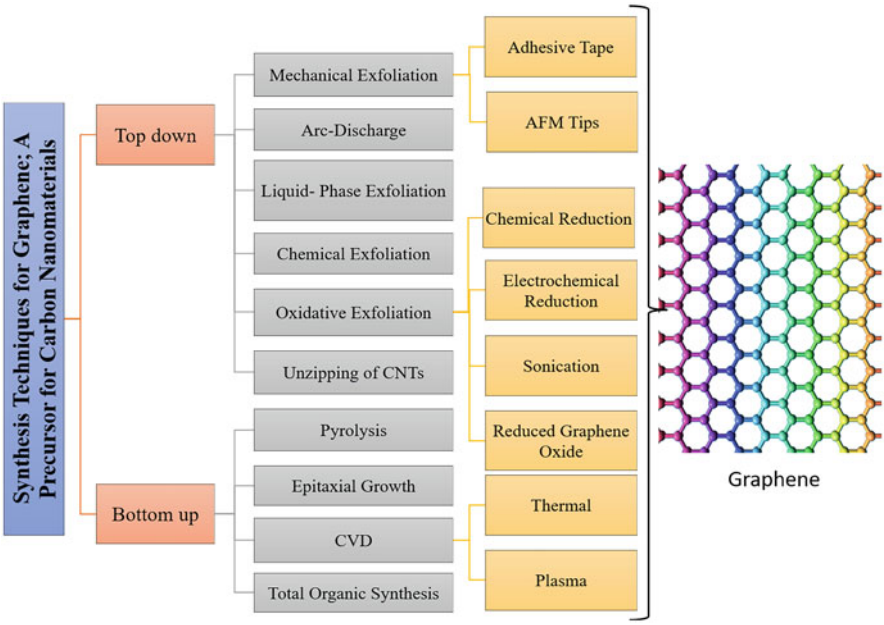

Fig. 9.2 Methodologies for graphene synthesis, a precursor for carbon nanomaterials

9.3.1.1 Single-Walled Carbon Nanotubes (SWCNTs)

Single-walled carbon nanotubes are composed of a single layer of carbon sheet. The diameter of single-walled carbon nanotubes is around 1 nm, and length to diameter ratio is 1000, but the diameter is subjected to change depending upon the temperature. SWCNTs have a 1300 m^2/g surface area that provides high drug loading and bioconjugation ability to the SWCNTs. SWCNTs are preferred over MWCNTs for drug loading because they offer longer blood circulation time; thus the effect of drug can be sustained. One of the characteristic features of SWCNTs is chirality; it means SWCNTs' geometry is nonsuperimposable on their mirror image. SWCNTs show the chirality in zigzag and armchair configurations (Pandey and Dahiya 2016; Raval et al. 2018; Simon et al. 2019).

9.3.1.2 Multiple-Walled Carbon Nanotubes (MWCNTs)

Multiple-walled carbon nanotubes are made up of various layers of carbon sheet rolled around a hollow cylindrical tube, showing the internal diameter between 1 and 3 nm and external diameter around 21–100 nm. Although MWCNTs have distinct physical and chemical properties and are quite strong structurally, they might have small structural defects due to their complex configuration.

MWCNTs are classified into two categories:

- Parchment-like MWCNTs—in which the carbon sheet is rolled up around itself.
- Russian doll MWCNTs—in which the multiple carbon sheets are rolled with concentric structure.

9.3.2 Synthesis of Carbon Nanotubes (CNTs)

Several techniques are developed to fabricate high-quality carbon nanotubes (CNTs). Among them, carbon arc discharge technique, laser ablation technique, and chemical vapor deposition are more common. Carbon arc discharge technique and laser ablation technique are examples of high-temperature (>1700 °C) preparation, while chemical vapor deposition method which is a low-temperature method (<800 °C) is currently used predominant method to substitute previous high-temperature methods (Eatemadi et al. 2014). For the synthesis of MWCNTs, catalytic agents are not required, but for the synthesis of SWCNTs, catalytic agents such as cobalt, nickel, and yttrium are mandatorily required (Pandey and Dahiya 2016; Raval et al. 2018; Simon et al. 2019). Various methods available for the development of carbon nanotubes are described below.

9.3.2.1 Arc Discharge Techniques

This is the oldest method derived in 1991–1992 and the primary technique to fabricate CNTs. The mechanism of this method is the electrical breakdown of a gas to generate plasma at higher temperatures (above 1700 °C) to evaporate carbon atoms providing the expansion of CNTs with minimum structural defects in comparison with other techniques. The carbon atoms are evaporated at high temperatures (>3000 °C) in plasma, where MWCNT and SWCNT are formed (Anzar et al. 2020).

The arc discharge chamber used in this method has one anode and one cathode. The anode is made up of a mixture of graphite powder and catalyst (Salah et al. 2021), while the cathode is filled with a pure graphite rod. Evaporated carbon molecules and metal catalyst particles (cobalt, nickel, and/or iron) are also present in the chamber; the carbon in the negative electrode sublimates because of the high-discharge temperatures. Usually, in an atmosphere of inert gaseous, electrodes remain intact. After the arcing process has started, consequently, direct current is passed. Finally, the temperature of the pressurized chamber is raised to more than 3000 °C.

During this arcing process, electrodes become red hot, and carbon sublimes from the anode, and approximately 50% of the evaporated carbon condense as filamentous carbon product at the cathode tip. Finally, the machine is allowed to be cooled. The CNTs deposited on the chamber walls are collected and proceed for purification. The yield of CNTs depends on various factors, i.e., flow rate, inert gas pressure, and metal concentration.

Advantages

- It is a straightforward and attainable method for the synthesis of carbon nanostructured material.

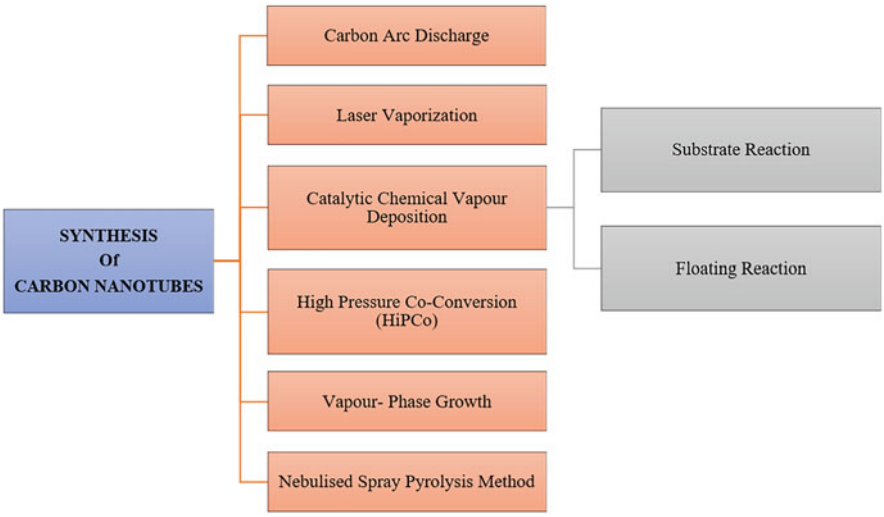

Fig. 9.3 The synthesis methods of carbon nanotubes (CNTs)

- The main advantage of arc discharge method is that synthesized SWCNTs have limited structural defects (Sari et al. 2018).
- It is a low-cost procedure.
- It does not require multiple purifications.

Disadvantages

- It requires high temperature in the fabrication process.
- It requires noble gasses which are very expensive and may increase the cost of production.
- It requires high pressure to seal up the production unit.
- Purification of raw materials is an additional limitation of this method (Jeon et al. 2011) (Fig. 9.3).

9.3.2.2 Laser Ablation Method

With the high-power laser vaporization, laser ablation is a better method for producing SWCNTs. In this method to generate single-walled carbon nanotubes, pure graphite in a quartz tube is heated to vaporize at around 1200°C inside a furnace, in an air atmosphere (Abbasi et al. 2014).

Advantages

- It is a very effective, stable, and effortless method for producing good-quality carbon nanotubes.
- It can produce carbon nanotubes with very few structural defects.
- It gives a higher yield of high purity of SWCNTs (Das et al. 2016).

Disadvantages
- It requires the large raw material.
- This method is only suitable for the production of SWCNTs.

9.3.2.3 Chemical Vapor Deposition

Chemical vapor deposition (CVD) is considered the one standard, most necessary, and suitable process for carbon nanotubes (Eatemadi et al. 2014). CVD further can be classified as catalytic chemical vapor deposition:

(i) Thermal (Vander Wal et al. 2003).
(ii) Plasma enhanced (Iijima 1991).
(iii) Water assisted (Iijima et al. 1992).
(iv) Microwave plasma (Ebbesen and Ajayan 1992).
(v) Radio frequency (Bernholc et al. 1997).
(vi) Hot filament (Dervishi et al. 2009).
(vii) At present catalytic chemical vapor deposition is the usual technique for synthesizing CNTs. In this technique, CNTs expanded on different materials, and the chemical breakdown of a hydrocarbon occurs on a substrate. The mechanism of CNT formulation is similar to arc discharge method.

Advantages
- This method is simple and inexpensive.
- It can be used for both MWCNTs and SWCNTs with high purity and high yield.
- Only low-temperature methods were discussed (Baddour and Briens 2005).

Disadvantages
- Although this method can be used for the production of both MWCNTs and SWCNTs, parameters must be closely watched for the production of SWCNTs.
- Another limitation is that "chamber size limits the substrate size."

9.3.2.4 Synthesis of CNTs by Thermal Decomposition Method

This method is derived from previously described CVD method. In this method, hydrocarbon gas such as ethylene, methane, and acetylene are used as carbon source in the synthesis of CNTs. It is required to maintain 500–900 °C, in quartz tube-containing furnace chamber, radio-frequency (RF) heater heats. A crucible having catalyst nanoparticle-coated substrate is kept inside the quartz tube in the argon gas environment. Then a hydrocarbon gas is supplied into the quartz tube chamber, leading to pyrolysis in which hydrocarbon gas converts into carbon atom vapor. The carbon atoms bind to the substrate and join together by van der Waals force that leads to the formation of multi-walled carbon nanotubes (MWCNTs) on the substrate. To prepare the single-walled carbon nanotubes, iron, cobalt and nickel catalyst nanoparticles can be used (Jagadeesan et al. 2020).

9.3.3 Functionalization of Carbon Nanotubes

While various types of novel nanomaterials have been exploited in tumor theranostics, their distribution, metabolism, and toxicity in organisms have also been a source of concern among researchers. Also, the graphitic inner surface of pristine CNTs exhibits a significant hydrophobic nature; thus there is an aggregation of CNT entities due to van der Waals forces. These aggregates are difficult to break and result in unstable dispersion in most of the solvent including water. To overcome this aggregation problem, purification and functionalization are required for the CNTs. For the purification of CNTs, several physiochemical methods, e.g., acid treatment, oxidation, annealing, ultrasonication, cutting, chromatography, and magnetic forces, could be used. Similarly, the functionalization approach enables the carbon materials with excellent pharmacokinetic behavior, in addition to the less toxic parameters overall with high biocompatibility in vivo.

The functionalization protocols for carbon nanotubes can be classified as either covalent reactions or non-covalent coating by amphiphilic molecules on CNTs (Gulbake et al. 2021; Sahu et al. 2017). For non-covalent functionalization, various non-covalent associations, for instance, π stacking, hydrophobic, and van der Waals collaborations, are the primary factors that have been considered for the functionalization of CNTs to produce the carbon materials with a wide scope in theranostic purpose (Fig. 9.4).

Covalent functionalization can be depicted as a compound attachment onto the sp^2 carbon atoms of the π bond of the CNT skeleton. The fundamental response for

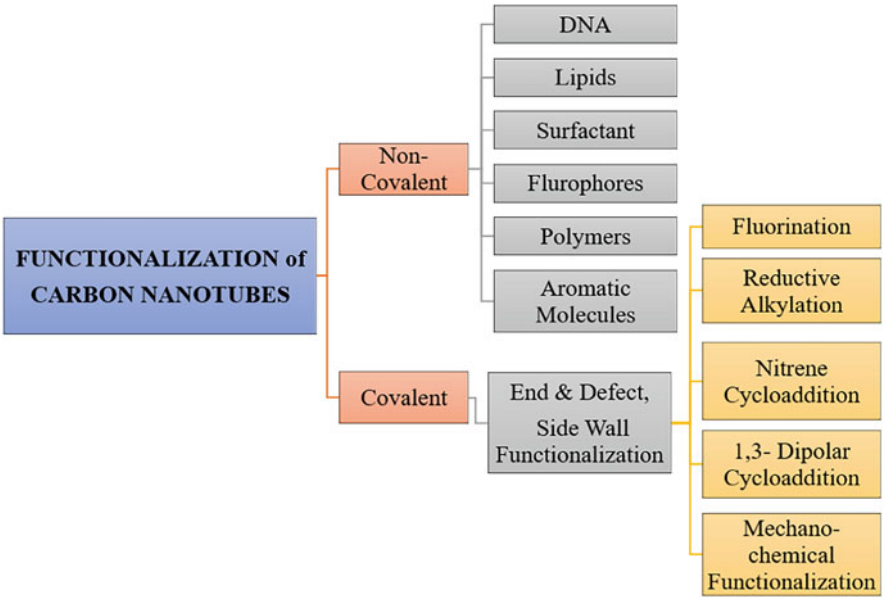

Fig. 9.4 The functionalization strategies of CNTs using several methods

CNT functionalization is oxidation, performed under unequivocally acidic circumstances. There are two fundamental systems for covalently functionalizing nanotubes: a) end and defect change and b) sidewall adjustment. These covalent adjustments emerge from the distinction in reactivity at the nanotube closures and sidewalls (as well as at fundamentally bothered regions). In like manner, each type of functionalization requires unmistakable compound methodologies. The important objectives of functionalizing the carbon material are to enable them as biocompatible with improved encapsulation affinity and high dissolvability, particularly of hydrophilic nature (Rhazouani et al. 2021; Gulbake et al. 2021; Gulbake et al. 2019; Sahu et al. 2017).

The covalently functionalized CNTs have been generally used for drug and gene delivery considering the applications. For this, the conjugation of proteins to CNTs is very critical to produce biocompatible CNTs. This protein can be conjugated in two steps: carboxylation and amidation on multi-walled carbon nanotubes (Liu et al. 2009). For example, it is observed that galactosylated MWCNTs and mannosylated MWCNTs can improve the dispersibility of MWCNTs in aqueous solvents (Jain et al. 2009; Gulbake et al. 2019). For effective intracellular delivery, the drug-loaded conjugated CNTs should show water solubility and biocompatibility, produced on carbon materials via the functionalization (Shi Kam et al. 2004; Kam and Dai 2005).

9.3.4 Synthesis and Functionalization of Graphene Oxide

Graphene oxide (GO), in addition to the graphene quantum dots (GQDs), and reduced graphene oxide (rGO) are newly associated of the graphene family. Their derivatives are also extensively explored in drug delivery, gene delivery (Rhazouani et al. 2021), biosensors, bioimaging, and theranostics (Kumar et al. 2019).

GO is an oxidized derivative of graphene and can act as a precursor of graphene, an excellent two-dimensional member of the carbon allotropes. GO is typically produced by Hummer's method through the oxidative exfoliation of graphite using $KMnO_4/H_2SO_4$. At the same time, rGO can be obtained by treating GO with reducing agents, such as hydrazine, hydrazine hydrate, L-ascorbic acid, etc. GQDs are usually prepared by thermal oxidation of GO or other carbon precursors (Smith et al. 2019). Similar to other carbon materials, the covalent and non-covalent approaches are used to functionalize GO, rGO, and GQDs (Fig. 9.5).

Functionalization of GO can be done using various biocompatible coatings and is also focused on ligands, coupling by covalent methods to improve the dissolvability, biocompatibility, and selectivity of GO (Wang et al. 2013). Some biocompatible and biodegradable macromolecules, like polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), chitosan, etc., have also been utilized to modify the graphene and its subordinates. Moreover, a combination of biomacromolecules, such as deoxyribonucleic acids (DNAs), enzymes, and proteins, has also been reported, which could be potentially utilized to biofunctionalize the graphene and its subordinates (Orsu and Koyyada 2020).

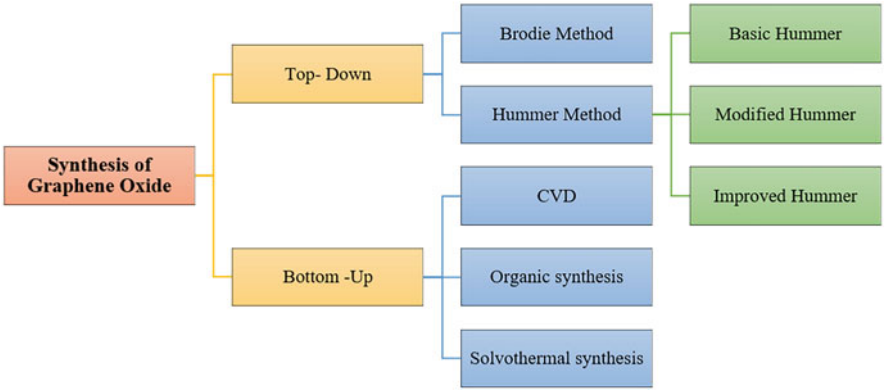

Fig. 9.5 Synthesis methods of graphene oxide

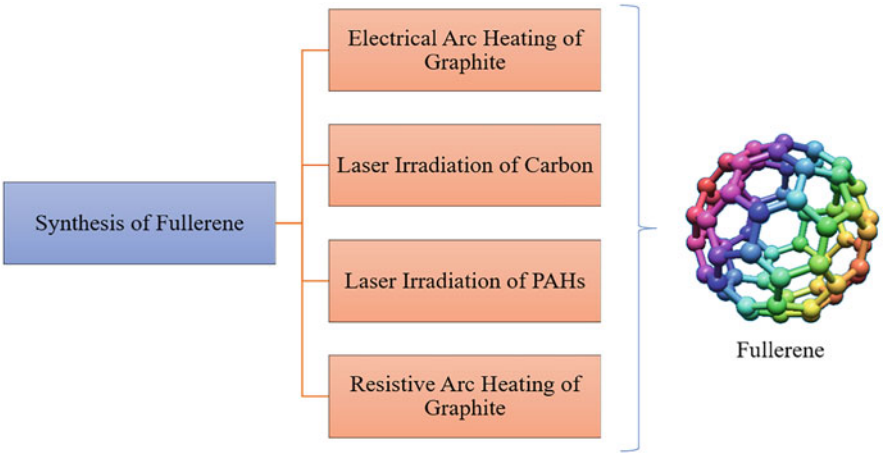

Fig. 9.6 Synthesis methods of fullerene

9.3.5 Synthesis and Functionalization of Fullerene

Fullerene is composed of several homologous carbon isomers with carbon number C60 as a predominant species, and the ration of other carbon is low, e.g., C70, C80, C240, C549, or C720. Thus, fullerene is useful in various scientific fields such as separation and determination of chemical species (Krätschmer et al. 1990). Laser vaporization strategy was the first and foremost method used for the production of fullerenes; however, because of meager yield, the arc heating of graphite and laser irradiation polyaromatic hydrocarbon (PAHs) techniques are utilized to develop the fullerene.

Figure 9.6 represents the brief strategies for the synthesis of fullerenes (Tanzi et al. 2022). Surface functionalization in fullerene is advantageous as it enables the

fullerene to be soluble in both water and natural solvents (Jensen et al. 1996). Chemical change of the fullerene surface might be performed following two distinct techniques: (i) complexation with solubilizing specialist to some degree conceal the fullerene hydrophobic surface and (ii) covalent functionalization of the fullerene surface. Oxygen-based useful gatherings, essentially hydroxyl gatherings, might be connected on the fullerene surface using strong acids at high temperatures. There is an enormous assortment assuming cycloaddition responses used to change the fullerene surface science to produce desired properties (Speranza 2021).

9.3.6 Synthesis and Functionalization of Carbon Nanodiamond

Nanodiamonds (NDs) are allotropes of carbon and perhaps the latest members from the nanocarbon family. They have splendid mechanical and optical properties, huge surface area, high bioconjugation ability, lower harmfulness, and satisfactory biocompatibility, making them especially captivating for various conceivable therapeutic and decisive biomedical applications (Jariwala et al. 2020; Qin et al. 2021). The first method needs conversion of graphite into NDs by applying pressure > 5 GPa in the presence of metal catalyst in a closed chamber. The second process requires detonating carbon precursor and explosives in a closed chamber to synthesize NDs in high pressure, e.g., 20–200 GPa, and high temperature > 1727 °C. And the third method does not need any carbon precursor; however, the used explosives have carbon within their structure (Tinwala and Wairkar 2019). The versatile surface chemistry of NDs resulting from their unique structural characteristics provides an excellent platform for numerous functionalization schemes. A variety of functional groups can be attached to ND surface. The synthesis and purification use numerous oxidative cycles; a large portion of the carbon particles on the outer layer of ND are ended with oxygen moieties like carbonyl or hydroxyl bunches (Fig. 9.7).

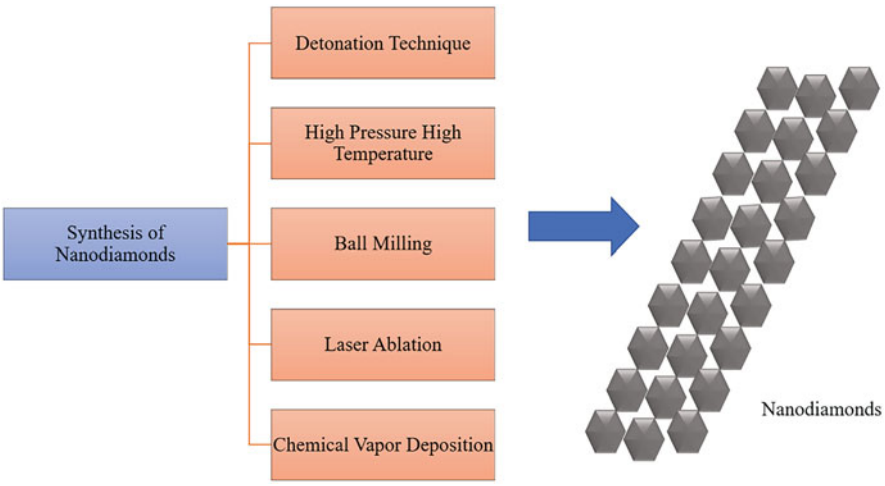

Fig. 9.7 Synthesis methods of carbon nanodiamonds

The surface gatherings on NDs can be adjusted utilizing various types of chemical or physical modifications. NDs because of their low harmfulness and absence of cell obstruction are an ideal nanocarrier for application against various types of malignancy with improved restorative viability and least incidental effects (Speranza 2021; Tinwala and Wairkar 2019).

9.4 Theranostic Application of Carbon-Based Nanocarriers

9.4.1 Carbon Nanotubes

Carbon nanotubes have acquired immense popularity because of their distinct properties, making them suitable for diagnostic purposes and targeted drug delivery. Additionally, one can introduce various biochemical agents on their surface through surface functionalization, which offers various therapeutic benefits (Sanginario et al. 2017; Prajapati et al. 2022; Khademhosseini et al. 2018). Some of the applications of CNTs are as follows.

Carbon nanotubes can be used as a diagnostic tool in different ways such as ultrasound contrast agent, photoacoustic imaging, near-infrared imaging, magnetic resonance imaging, PET/SPECT, in multimodality imaging, photothermal therapy, and photodynamic therapy.

Gu et al. (2018) studied the use of MWCNTs as a new targeted ultrasound-mediated contrast agent delivery for diagnosing prostate cancer. In this study PEG was attached to acidified MWCNTs to improve the solubility and stability in water. Further nucleic acid was attached at the surface to improve the targeting ability and biocompatibility. This nano-ultrasonic imaging has shown good targeting ability in PCa cells (Gu et al. 2018).

Carbon nanotubes have a distinct ability to use the near-infrared radiation and convert it into heat. This unique characteristic of CNTs is responsible for the inclination of researchers toward its use in biomedicine, specifically induction of thermal effect on cells and tissue to be used in near-infrared (NIR) imaging. The NIR diagnosis is based on the principle that tissues and water are transparent at NIR-I (700–900 nm) and NIR-II (1100–1400 nm) wavelengths and the SWCNT molecules get excited simultaneously. This unique feature of CNTs enables them a suitable contrast agent in NIR region.

Pinals et al. (2021) have designed SWCNT-based nanosensors where ACE2 is adsorbed on the surface of SWCNTs which allows the binding of viral s protein with the adsorbed ACE2 (Pinals et al. 2021). The carbon nanotubes can also be used for diagnostic purpose in MRI after functionalization of magnetic substances on their surface. Surface functionalization can either be achieved by filling the hollow cavity of CNTs using magnetic nanostructure or by functionalizing the inert sidewalls. Peci et al. 2015, have prepared surface-functionalized Fe MWCNTs with gadolinium; this system plays double function, i.e., magnetic hyperthermia for cancer therapy in addition to the MRI contrast agent because it has Fe and Gd element which act as heating element and paramagnetic property, respectively (Peci et al. 2015).

Zhao et al. (2021a) have designed a carbon nanotube-based photothermal therapy by coating the SWCNTs and MWCNTs, using peptide lipid or sucrose laurate: the developed bifunctional delivery system has shown a great temperature sensitivity and photothermal performance. In such a system, the release of drug or biomolecule depends on the phase transition of the coated lipid as the coated lipids are temperature sensitive (Zhao et al. 2021a).

In CNTs the drug molecules can be incorporated inside the hollow cavity, while other materials (for functionalization) can be adsorbed on the surface of nanotubes. CNT functionalization can resolve the problem of dispersibility and biocompatibility for the targeting purpose. Kaur et al. (2017) have developed CNT-based targeted drug delivery system for breast cancer therapy. MWCNT surface functionalization was done using FA-PEG bis-amine, which has improved the water solubility, biocompatibility, and pharmacokinetic properties, e.g., circulatory time in blood. After purification the functionalized CNTs were loaded with 5-FU drug. This study concluded that 5-FU-loaded functionalized CNTs effectively target and kill the cancer cell (Kaur et al. 2017).

Hyaluronic acid (HA) is an endogenous targeting ligand that has shown very good targeting efficiency specially in cancer. Using a multifactorial approach, Su et al. 2018, have developed indocyanine green derivative (ICG-COOH) and hyaluronic acid (HA) paclitaxel (PTX) conjugate (HA-PTX). These compounds could be easily used to make a nanomicelle self-assembly that has shown high tumor targeting due to the HA-mediated active targeting paramagnetic resonance-based physical accumulation target site. Consequently, the conjugates could be easily broken using the overexpressed esterase in tumor cells. Ultimately it results in tumor-targeted therapy as well as cell-specific imaging. In this study excellent tumor targeting ability was proved using a series of in vitro and in vivo experiments. Thus, the approach can be milestone step in developing an organic near-infrared dye-based multifunctional delivery system for tumor theranostics (Su et al. 2018). Prajapati et al. (2019) developed gemcitabine-loaded CNTs conjugated with HA and polyethylene glycol to target colon cancer. The gemcitabine release was observed to be faster in acidic pH than at physiological pH with a significant increase in cancer cell cytotoxicity (Prajapati et al. 2019).

Brain drug delivery is a considerable target due to its complex structure, and several studies have been under investigation for the similar purpose. The ligand-receptor-based active targeting also shows high efficacy in brain targeting, e.g., in brain tumor the lipoprotein receptor-related protein (LRP) overexpression is important to target the brain diseases.

Costa et al. (2018) have designed a CNT-based system to deliver drug into the brain for Alzheimer's disease treatment (Costa et al. 2018). Since Alzheimer's disease is a result of accumulation of toxic protein such as amyloid-beta plaque, inflammation, and neurodegeneration, to design an effective brain drug delivery system for Alzheimer's disease, it is necessary to target amyloid-beta. For brain, Pittsburgh compound B (PIB) could be a suitable binding agent for the amyloid-beta, but alone PIB derivative cannot cross the selective blood-brain barrier (Costa et al. 2018). To overcome this problem, CNTs could be a choice of carrier. In this study

two complexes of MWCNTs and PIB were developed, and it was observed that these complexes can successfully deliver the drug to the brain. In a similar study, an angiopep-2(ANG)-targeted chemically functionalized f-MWCNTs were assessed for brain drug delivery. ANG has shown high affinity toward low-density lipoprotein receptor-related protein-1 (LRP1), but alone ANG cannot cross the selective blood-brain barrier. Thus, the application of ANG in conjugated radiolabeled CNT drug delivery platform shows the potential application of CNTs in brain targeting (Kafa et al. 2016).

Functionalized CNTs can also be used for several other applications such as bone repairing and bone targeting. Genady et al. (2020) have developed SWCNT-based system where functionalization was achieved by conjugated polymer containing functional group coated on its surface. This leads to improved dispersibility, which improves the stimulus, responsiveness, and surface adhesion of the complex to the bone. Herein bisphosphonate was used for the targeting purpose which show high binding affinity toward the biomolecule hydroxyapatite found in bone injury and bone disease. The functionalization of SWCNTs with BP could be achieved through covalent and non-covalent functionalization. In this system additionally, Tc-99 m was used for the radiolabeling of SWCNT dispersion, and the biodistribution of both the complexes was confirmed by PA imaging (Genady et al. 2020) (Table 9.1).

9.4.2 Graphene

Graphene is a two-dimensional (2D) carbon nanomaterial with a monoatomic layer of sp2-hybridized carbon molecules coordinated as a honeycomb grid. It was discovered in the year 2002 followed by mechanically exfoliated graphene in 2004. Roy and Jaiswal (2020) synthesized the graphene oxide (GO) and reduced graphene oxide (rGO) (Roy and Jaiswal 2020). Graphene has attracted a lot of attention from the scientific community due to their wider applications (for sensing purpose, as a catalyst, in nanoelectronics, material engineering, energy storage in drug delivery, nucleic acid delivery, phototherapy, bioimaging, and theranostics) and its structural, optical, electrical, and mechanical property enabling graphene to be a superior material than other widely explored carbon and plasmonic nanomaterials (Zare et al. 2021). This important feature makes graphene an attractive delivery platform for theranostic applications. (1) High surface area, (2) functionalization ability, (3) inborn fluorescence property, and (4) optical assimilation in the NIR area empower them to go about as photo agents for photothermal and photodynamic treatments of diseases. Graphene is one of the finest and durable molecule and capable of free form. The in vivo and bioactivity studies suggest that graphene interacts with the cell membranes to enter the cells by endocytosis.

Usman et al. 2018, synthesized a theranostic nanodelivery system (GOTS) made of graphene oxide (GO)—for magnetic resonance imaging (MRI) application where the naturally occurring protocatechuic acid (PA) was used as an anticancer agent. In this system gadolinium (III) nitrate hexahydrate (Gd) was used as a contrast agent, while gold nanoparticles (AuNPs) were used as the second diagnostic agent. The

Table 9.1 Theranostic application of carbon-based nanocarriers

Nanocarriers	Diagnostic material	Therapeutic principal	Imaging technique	Cell line	Theranostic application/findings	Reference
Carbon nanotubes (CNT)						
MWCNT	Gadolinium (Gd)	PTT	Magnetic resonance imaging (MRI)	BxPC-3 (human pancreatic cancer cells)	MWCNT-Gd@PDA nanomaterials were lacking of inherent biological toxicity	Wang et al. (2017)
SWCNT	Evans blue (EB)	PDT and PTT	Fluorescence, PA imaging	SCC-7 cells	The combined phototherapy managed to damage tumor and diminish tumor without recurrence	Xie et al. (2016)
Carboxylic acid-functionalized CNTs (fCNTs)				A375 cells	Cyclic RGD-conjugated CNTs encapsulating an anticancer therapeutic can be a promising platform for treating cancer	Koh et al. (2019)
Fullerene (C_{60})						
C_{60}	Iron oxide nanoparticles (IONP)	PDT, radio-frequency thermal therapy (RTT)	Magnetic resonance imaging (MRI)	B16-F10 mice melanoma cell line	Cancer theranostic application	Shi et al. (2013)
C_{60}	Gold (AU)	DOX, PDT/RTT	X-ray imaging, photodynamic therapy	MCF-7 human breast cancer cell line	Tumor-specific PDT-chemotherapy with X-ray imaging for theranostics	Shi et al. (2016)
Graphene oxide (GO)						
GO	Gold (Au)	PTT	Photoacoustic imaging	SCC7	The tumors irradiated with a laser, and an excellent tumor inhibition was observed without recurrence	Gao et al. (2016)
GO	Hyaluronic acid	Anti-peptide nucleic acid (PNA21)	Fluorescence imaging	MDA-MB-231	A fluorescence-switchable theranostic nanoplatform by HA and GO is capable of both sensitizing oncogenic miR-21 and inhibiting their tumorigenicity	Hwang et al. (2017)

GO	MnOx/TiO2	PTT, SDT, US	Magnetic resonance imaging (MRI)	Murine breast cancer line 4 T1 cells	Nanosonosensitized sonocatalytic tumor eradication via photothermal, sonodynamic, and ultrasound therapy with MRI	Dai et al. (2017)
Nanodiamond (ND)						
ND	PEG	Doxorubicin (DOX)	Fluorescence imaging	Ec-109 cells (human esophageal cancer cell)	Clathrin-/caveolae-mediated endocytosis, pH-responsive drug delivery, selective targeting, and imaging for cancer therapy	Deng et al. (2017)
ND	Mesoporous silica nanoparticles (MSN)		Optical imaging		Bioimaging and drug delivery for theranostic application	Prabhakar et al. (2013)

developed GAGPAu was significantly cytotoxic to the human liver hepatocellular carcinoma cell line (HepG2); however no cytotoxicity was observed in fibroblast cell line (3 T3). The GAGPAu also has better T1 contrast compared to the pure gold (Usman et al. 2018). Thus, GOTS shows good probability of serving as future theranostic platform useful in cancer chemotherapy as well as diagnostic purpose.

The bimodal theranostic nanodelivery system (GAGPAu) using graphene was developed from initial synthesis of GO nanocarrier. It was followed by aqueous doping of Gd^{3+} and protocatechuic acid via hydrogen bonding and physical interactions (GAGPA). The drug release in acidic medium was reported as more than 60% protocatechuic acid which was lesser around 40% in alkaline media, thus showing the effectiveness in cancer or other diseases (Usman et al. 2018).

In another study, GO was used as a nanocarrier for a theranostic purpose using protocatechuic acid and gadolinium/gold. The surface modification of GO with various polymers allows their use in drug delivery, tissue engineering, and imaging techniques (Karki et al. 2020). For example, amine-terminated PEGylated GO was effectively used to deliver high protein due to non-covalent interactions with the PEG-GO surface to the drug. All the above studies have shown high potential of graphene-based materials as drug and gene delivery platform. However, the validation results of in vitro studies in animal models considering the safety, biodistribution, and efficacy are important aspects of developing carbon-based graphene nanomaterials.

According to Lu et al. (2012), developed graphene-based materials have also been assessed in wound healing by formulating graphene in chitosan-PVA nanofibrous scaffolds. Among three compositions, e.g., chitosan-PVA fibers, chitosan-PVA-graphene electrospun fibers, and chitosan fibers, chitosan-PVA-graphene fibers were able to accelerate the wound healing faster than the no scaffold group, to check wound healing affinity in mice and rabbit (Lu et al. 2012).

The large surface area of graphene can adsorb proteins/DNA which might be very useful in several therapeutic applications. For instance, Mahmoudi et al. recently reported the protective role of graphene oxide (GO) and protein-coated GO surfaces in amyloid-beta fibrillation process, which is implicated in various neurodegenerative disorders (Mahmoudi et al. 2012). However, in addition to the wide in vitro characterization of scaffolds, the focus should be on their evaluation in animal studies with respect to their inflammatory responses, biocompatibility, toxicity, and regenerative potential.

9.4.3 Fullerenes

Fullerenes are carbon allotropes that possess distinct characteristics such as shape and size, solubility, and chemical reactivity, which enable fullerenes as a suitable candidate for different applications, e.g., enzyme inhibition, targeted DNA cleavage, free radical scavenging, various imaging applications, radiotherapy, etc. Fullerenes have occurred as C38, C42, and C60; among these C60 has spherical shape, and it has the capability to reorient itself rapidly in the crystalline phase; because of this

property, C60 can enter the cell without altering cell structure; basically C60 fullerenes have a hollow structure, and this hollow structure makes them suitable candidate for imaging and radiotherapy. There are several biomedical applications of fullerenes, e.g., enzyme inhibition, radiotherapy, etc. The fullerene fits well into the HIV protease active site to inhibit the enzyme activity, and this enzyme inhibitory activity against other enzymes can also be manipulated using specific functionalization.

C60 fullerenes have unique antioxidant properties, and these properties could be used for the treatment of liver cirrhosis. The C60 reduces the connective tissue deposition and balances the alkaline phosphate and lactate dehydrogenase and can also help restore the liver function by balancing abnormal conjugated and non-conjugated bilirubin level. Kuznietsova et al. (2020) have designed a water-soluble pristine C60 fullerenes to inhibit liver fibrotic alteration and prevent liver cirrhosis in rats. It is also discovered that C60 fullerenes have anti-fibrotic action by blocking the ATP binding with EGFR and FGFR (Kuznietsova et al. 2020).

Functionalized fullerenes have shown antitumor effect when used in photodynamic therapy, photothermal treatment, radiotherapy, and combination to chemotherapy. Chen et al. 2012, used functionalized fullerenes in tumor targeting and diagnosis. This antitumor activity of fullerene is due to multiple effects such as modulation of oxidative stress, anti-angiogenetic, and immunostimulatory characteristics (Chen et al. 2012).

Li et al. modified trimetallic nitride endohedral fullerenes carboxyl-Gd3N@C80 (an MRI contrast agent) with hydrochalarone and carboxyl moieties and assessed the antioxidative and anti-inflammatory. In this study all derivatives have shown the effective radical (hydroxyl and superoxide anion) scavenging activity. In this study the carboxyl-Gd3N@C80 more efficiently attenuated lipopolysaccharide (LPS)-induced oxidative stress in macrophages in addition to suppression of mRNA expression of nitric oxide synthase and tumor necrosis factor-alpha (TNF-α) and upregulated antioxidative enzyme axis Nrf2 and heme oxygenase-1, possibly via extracellular signal-regulated kinase (ERK) signaling pathways. Thus carboxyl-Gd3N@C80 derivative shows a potential theranostic candidate in inflammation-related diseases (Li et al. 2017).

Reactive oxygen species (ROS) are implicated in the etiology of a wide range of human diseases; thus inhibition of tumor growth by carbon nanomaterials likely relates with typical capacity of scavenging reactive oxygen. Yin et al. 2009, investigated the major physiologically relevant ROS activity using three types of water-soluble fullerenes: C60(C(COOH)(2))(2), C60(OH)(22), and Gd@C(82)(OH)(22). They observed the higher protective role of these molecules by reducing H(2)O(2)-induced oxidative damage, stabilizing the mitochondrial membrane potential, and reducing intracellular ROS production. These compounds also can scavenge all physiologically relevant ROS, e.g., 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), reactive oxygen species (ROS), superoxide radical anion (O(2)(*-)), singlet oxygen, as well as hydroxyl radical (HO(*)), to inhibit lipid peroxidation in vitro. Thus, fullerene derivative can counter the oxidative stress and damage in the etiology and progression of many diseases associated with the role of ROS, for example, cancer,

neurological disorder, and others (Yin et al. 2009). The same research group has assessed the tumor inhibitory activity of endohedral metallofullerenol liposomes composed of bovine liver phosphatidylcholine as a reactive oxygen species scavenger. The developed liposomes [Gd@C82(OH)22]n have a strong inhibitory effect on lipid peroxidation as well as strong ROS scavenging capacity in human lung adenocarcinoma cells or rat brain capillary endothelial cell (EC) culture. Additionally, the [Gd@C82(OH)22]n nanoparticles also inhibit the growth of malignant tumors in vivo (Yin et al. 2008).

UVB-induced diverse harmful effects due to the reactive oxygen species could also be prevented by SHH-F (a type of fullerenols) in keratinocyte culture system. Saitoh et al. 2011, assessed the effect of three fullerenols (C60(OH)6–12: LH-F, C60 (OH)32–347H2O: HH-F, and C60(OH)448H2O: SHH-F) after irradiation of HaCaT cells with UVB. The cell irradiation with UVB resulted in speedy increases in cell injury-associated characteristics, e.g., intracellular oxidative stress levels, cyclobutane pyrimidine dimers formation, and chromatin condensation. These characteristics were downregulated by using the fullerenol SHH-F (Saitoh et al. 2011). Thus, UVB-induced diverse harmful effects could be prevented by SHH-F, which was suggested to exert the cytoprotective effects through intracellular reactive oxygen species scavenging in the keratinocytes.

Zhao et al. (2021b), summarized fullerenes as limited in biomedical function and several fullerene derivatives with solubility manipulation characteristics. The fullerene C_{60} has been used as a powerful free radical scavenger with antioxidant and antibacterial activity with inhibitory effects on cancer cells. Fullerenols also inherit the good properties of fullerenes and are better used in cancer treatment, including loading drug therapy and directly as an anticancer drug (Zhao et al. 2021b). Fullerene derivative polyhydroxylated C60 fullerenol also bears glutamate receptor antagonists and neuroprotective properties. In one of the studies, fullerenol has shown glutamate receptor binding in a dose-dependent manner (Jin et al. 2000).

Fullerenols have shown electron absorption and reduction characteristics; this fact led to the ideas for the treatment of oxidative stress in the repair of peripheral nerve defects. Partha and Conyers 2009, have summarized the physical and chemical properties of fullerene for biomedical applications. It also discusses the approaches, mechanisms, advantages, and various aspects of toxicity in using functionalized fullerenes for biomedical applications particularly in nerve defects (Partha and Conyers 2009).

Fullerene derivatives also have a wide application in cardiovascular diseases. However, the exposure to nanomaterials appears to represent a risk for cardiovascular disorders (CVS); further in vivo validations are required in addition to the cell line studies. Yamawaki and Iwai (2006), studied the direct effects of nanomaterials on endothelial toxicity in human umbilical vein ECs. It was observed that C60(OH) 24 induced cytotoxic morphological changes in a dose-dependent manner like cytosolic vacuole formation and decreased cell density. Fullerene derivative C60 (OH)24 shows good combability in cell culture regarding apoptosis and cellular toxicity. Only the highest dose used, i.e., 100 μg/ml, shows very little toxicity in lactate dehydrogenase assay and cell proliferation assay. At highest dose EC death is

caused by induction of ubiquitin-autophagy cell death pathways (Yamawaki and Iwai 2006).

Gelderman et al. (2008), observed the effect of1–100 microg/mL water-soluble fullerenol C60(OH)24 on human umbilical vein endothelial cell (HUVEC) culture and found that 24-hr treatment of HUVECs with C60(OH)24 at 100 microg/mL increased the cell surface expression of ICAM-1(CD54) and G1 arrest of HUVECs and C60(OH)24 to induce the apoptosis against the untreated cells. This study also demonstrated that fullerenol C60(OH)24 had both pro-inflammatory and pro-apoptotic effects on HUVECs, indicating possible adverse effects (Gelderman et al. 2008). However, in vivo efficacy and efficacy studies are required to show the full effect of fullerene on CVS.

9.4.4 Carbon Nanodiamonds

Diamond powder in nanosize range is also an important member of carbon nanomaterial family for drug delivery purpose. Diamond nanoparticles (size ~5 nm) have distinctive optical and thermal features and a quite large surface area and thus offer wide surface modification opportunities. Several factors, for example, purity, surface chemistry, easy formation of porous structure, ionic composition, and biological toxicity studies, should be considered before utilizing its potential in drug delivery.

The effect of surface modification of carbon nanodiamond can affect adsorption equilibria of drug candidate having different chemical nature. Previously, Zhu et al. 2012, have summarized application and in vitro and in vivo safety data of nanodiamonds in drug delivery field (Zhu et al. 2012). The porous structure form contributes to various desired properties in nanodiamonds like sustained release ability in efficient imaging and drug targeting. The nanodiamonds are synthesized using carbon-containing explosives. The oxygen-deficient explosive mixture containing trinitrotoluene and hexogen is detonated in a closed chamber. Thus, the nanodiamonds are formed at detonation wave in microseconds. Water, air, and CO_2 are generally used as cooling media in the detonation chamber. The percentage yield of nanodiamonds is dependent on the cooling capacity of the cooling medium. On increasing the cooling capacity of media, the percent yield of carbon nanodiamonds increases (Zhu et al. 2012).

Nanodiamonds are relatively safer carbon nanomaterials. Schrand et al. 2007, reported cellular toxicity of nanodiamonds using cell toxicity assay and ATP production assay and observed no significant cell toxicity. In this study, the order of toxicity of nanodiamonds compared to other carbon nanomaterial like carbon black, MWNT, and SWNT in the size range 2–10 nm on neuroblastoma cells and macrophages was reported as minimum, viz., SWNT > MWNT > carbon black > nanodiamonds. The study revealed that the compatibility of oxidized nanodiamonds could be enhanced by vinyltrimethoxysilane functionalization on superparamagnetic Fe_3O_4 nanoparticles. Prepared hybrid nanoparticles have shown strong superparamagnetism and blue photoluminescence. The PrestoBlue assay in MCF-7

cells using up to higher concentrations of 7.2 mg/mL of prepared nanodiamond silanized Ox-ND/Fe_3O_4 indicated excellent biocompatibility (Schrand et al. 2007). Therefore, there is potential hope of therapeutic application of nanodiamond-based carbon nanomaterials.

9.5 Future Outlook

Theranostic delivery systems (TDS) are relatively newer developments and are highly important in biomedical engineering, particularly in anticancer research. To improve the therapeutic effect of cancer chemotherapy, it needs diagnosis with sophisticated molecular imaging techniques, e.g., MRI or CT, which can be significantly improved by using the CNTs. These imaging methods require contrast agents due to the intact poor visibility in subject tissues, which are mostly administered prior to the tests in conventional treatment approach. Thus, repetitive administration of therapeutic agents and diagnostics lead to unnecessary patient incompliance and chance of toxicity due to higher dose of chemotherapeutic agents that may result in severe toxicity to the healthy organs and tissue. Herein, the introduction of TDS using the CNTs is particularly helpful in reducing the repetitive administration and associated toxicity. The TDS can reduce the toxicities of these diagnostic agents and potent therapeutic agents by targeting the cancer cells or diseased tissue. In addition, TDS offers the possibility of monitoring the release of the therapeutic agents at the target sites.

Recently, carbon-based nanomaterials have been used for several other biomedical purposes, e.g., wound healing, stem cell engineering, regenerative medicine, and tissue engineering. These materials provide opportunities for tailoring various functionalities on their flat surfaces with their outstanding mechanical properties, e.g., high surface area, defined structure, high elasticity, strength, flexibility, and surface functionalization. Also, the carbon-based materials can be used in the form of hydrogels, biodegradable films, electrospun fibers and other tissue engineering scaffolds. However, the in vivo biocompatibility and immune activity of these materials need to be explored widely, which have already been improved by functionalization strategies. It is envisaged as safer delivery nanocarriers in the near future for the diagnosis in several clinical purpose.

References

Abbasi E, Aval SF, Akbarzadeh A, Milani M, Nasrabadi HT, Joo SW, Hanifehpour Y, Nejati-Koshki K, Pashaei-Asl R (2014) Dendrimers: synthesis, applications, and properties. Nanoscale Res Lett 9:247

Ajala OJ, Tijani JO, Bankole MT, Abdulkareem AS (2022) A critical review on graphene oxide nanostructured material: properties, synthesis, characterization and application in water and wastewater treatment. Environ Nanotechnol Monitor Manag 18:100673

Anzar N, Hasan R, Tyagi M, Yadav N, Narang J (2020) Carbon nanotube—a review on synthesis, properties and plethora of applications in the field of biomedical science. Sens Int 1:100003

Baddour CE, Briens C (2005) Carbon nanotube synthesis: a review. Int J Chem React Eng 3
Bernholc J, Roland C, Yakobson BI (1997) Nanotubes. Curr Opinion Solid State Mater Sci 2:706–715
Carvalho IC, Mansur AP, Carvalho SM, Mansur HS (2021) Nanotheranostics through mitochondria-targeted delivery with fluorescent peptidomimetic nanohybrids for apoptosis induction of brain cancer cells. Nano 5:213–239
Chen Z, Ma L, Liu Y, Chen C (2012) Applications of functionalized fullerenes in tumor theranostics. Theranostics 2:238–250
Costa PM, Wang JT, Morfin JF, Khanum T, To, W, Sosabowski J, Tóth E, Al-Jamal KT (2018) Functionalised carbon nanotubes enhance brain delivery of amyloid-targeting Pittsburgh compound b (pib)-derived ligands. Nano 2:168–183
Dai C, Zhang S, Liu Z, Wu R, Chen Y (2017) Two-dimensional graphene augments nanosonosensitized sonocatalytic tumor eradication. ACS Nano 11:9467–9480
Das P, Ganguly S, Margel S, Gedanken A (2021) Tailor made magnetic nanolights: fabrication to cancer theranostics applications. Nanoscale Adv 3:6762–6796
Das R, Shahnavaz Z, Ali ME, Islam MM, Abd Hamid SB (2016) Can we optimize arc discharge and laser ablation for well-controlled carbon nanotube synthesis? Nanoscale Res Lett 11:510
Deng X, Yin Z, Lu J, Xia Y, Shao L, Hu Q, Zhou Z, Zhang F, Zhou S, Wu Y, Sheng W, Zeng Y (2017) Two-step assembling of near-infrared "off–on" fluorescent nanohybrids for synchronous tumor imaging and microrna modulation-based therapy. ACS Appl Mater Interfaces 9:3294–3305
Dervishi E, Li Z, Xu Y, Saini V, Biris AR, Lupu D, Biris AS (2009) Carbon nanotubes: synthesis, properties, and applications. Part Sci Technol 27:107–125
Eatemadi A, Daraee H, Karimkhanloo H, Kouhi M, Zarghami N, Akbarzadeh A, Abasi M, Hanifehpour Y, Joo SW (2014) Carbon nanotubes: properties, synthesis, purification, and medical applications. Nanoscale Res Lett 9:393
Ebbesen TW, Ajayan PM (1992) Large-scale synthesis of carbon nanotubes. Nature 358:220–222
Gao Y, Tang P, Zhou H, Zhang W, Yang H, Yan N, Hu G, Mei D, Wang J, Ma D (2016) Graphene oxide catalyzed c−h bond activation: the importance of oxygen functional groups for biaryl construction. Angew Chem Int Ed 55:3124–3128
Gelderman MP, Simakova O, Clogston JD, Patri AK, Siddiqui SF, Vostal AC, Simak J (2008) Adverse effects of fullerenes on endothelial cells: Fullerenol c60(oh)24 induced tissue factor and icam-i membrane expression and apoptosis in vitro. Int J Nanomedicine 3:59–68
Genady AR, Fong D, Slikboer SR, El-Zaria ME, Swann R, Janzen N, Faraday A, Mcnelles SA, Rezvani M, Sadeghi S, Adronov A, Valliant JF (2020) 99mtc-functionalized single-walled carbon nanotubes for bone targeting. ACS Appl Nano Mater 3:11819–11824
Gifani M, Eddins DJ, Kosuge H, Zhang Y, Paluri SLA, Larson T, Leeper N, Herzenberg LA, Gambhir SS, Mcconnell MV, Ghosn EEB, Smith BR (2021) Ultraselective carbon nanotubes for photoacoustic imaging of inflamed atherosclerotic plaques. Adv Funct Mater 31:2101005
Goodarzi S, Da Ros T, Conde J, Sefat F, Mozafari M (2017) Fullerene: biomedical engineers get to revisit an old friend. Mater Today 20:460–480
Gu F, Hu C, Xia Q, Gong C, Gao S, Chen Z (2018) Aptamer-conjugated multi-walled carbon nanotubes as a new targeted ultrasound contrast agent for the diagnosis of prostate cancer. J Nanopart Res 20:303–303
Gulbake AS, Gaur A, Jain A, Shilpi S, Mehra NK, Gulbake A (2021) Multifunctional carbon nanotubes in drug delivery. In: Mehra NK, Gulbake A (eds) Micro- and nanotechnologies-based product development. CRC Press
Gulbake AS, Jain A, Shilpi S, Kumar P, Gulbake A (2019) Mannosylated multiwalled carbon nanotubes assisted artesunate delivery for cerebral malaria. Int J Appl Pharm 11:24–30
Hwang DW, Kim HY, Li F, Park JY, Kim D, Park JH, Han HS, Byun JW, Lee YS, Jeong JM, Char K, Lee DS (2017) In vivo visualization of endogenous mir-21 using hyaluronic acid-coated graphene oxide for targeted cancer therapy. Biomaterials 121:144–154
Iijima S (1991) Helical microtubules of graphitic carbon. Nature 354:56–58

Iijima S, Ajayan PM, Ichihashi T (1992) Growth model for carbon nanotubes. Phys Rev Lett 69: 3100–3103

Jagadeesan AK, Thangavelu K, Dhananjeyan V (2020) Carbon nanotubes: synthesis, properties and applications. In: Pham P, Goel P, Kumar S, Yadav K (eds) 21st century surface science - a handbook. IntechOpen Limited, London

Jain AK, Dubey V, Mehra NK, Lodhi N, Nahar M, Mishra DK, Jain NK (2009) Carbohydrate-conjugated multiwalled carbon nanotubes: development and characterization. Nanomedicine 5: 432–442

Jariwala DH, Patel D, Wairkar S (2020) Surface functionalization of nanodiamonds for biomedical applications. Mater Sci Eng C 113:110996

Jensen AW, Wilson SR, Schuster DI (1996) Biological applications of fullerenes. Bioorg Med Chem 4:767–779

Jeon I-Y, Dong WC, Kumar NA, Baek J-B (2011) Functionalization of carbon nanotubes. In: Yellampalli S (ed) Carbon nanotubes-polymer nanocomposites. IntechOpen Limited, London

Jin H, Chen WQ, Tang XW, Chiang LY, Yang CY, Schloss JV, Wu JY (2000) Polyhydroxylated c (60), fullerenols, as glutamate receptor antagonists and neuroprotective agents. J Neurosci Res 62:600–607

Kafa H, Wang JT, Rubio N, Klippstein R, Costa PM, Hassan HA, Sosabowski JK, Bansal SS, Preston JE, Abbott NJ, Al-Jamal KT (2016) Translocation of lrp1 targeted carbon nanotubes of different diameters across the blood-brain barrier in vitro and in vivo. J Control Release 225: 217–229

Kam NW, Dai H (2005) Carbon nanotubes as intracellular protein transporters: generality and biological functionality. J Am Chem Soc 127:6021–6026

Karki NRA, Tiwari H, Negi P, Sahoo NP (2020) Theranostics application of graphene-based materials in cancer imaging, targeting and treatment. Tumor progression and metastasis. In: Lasfar A, Cohen-Solal K (eds) Tumor progression and metastasis

Kaur S, Mehra NK, Jain K, Jain NK (2017) Development and evaluation of targeting ligand-anchored cnts as prospective targeted drug delivery system. Artif Cells Nanomed Biotechnol 45: 242–250

Khademhosseini V, Dideban D, Ahmadi M, Ismail R, Heidari H (2018) Single electron transistor scheme based on multiple quantum dot islands: carbon nanotube and fullerene. ECS J Solid State Sci Technol 7:M145–M152

Kim HI, Wang M, Lee SK, Kang J, Nam JD, Ci L, Suhr J (2017) Tensile properties of millimeter-long multi-walled carbon nanotubes. Sci Rep 7:9512

Koh B, Park SB, Yoon E, Yoo HM, Lee D, Heo JN, Ahn S (2019) A(v)β(3)-targeted delivery of camptothecin-encapsulated carbon nanotube-cyclic rgd in 2d and 3d cancer cell culture. J Pharm Sci 108:3704–3712

Krätschmer W, Lamb LD, Fostiropoulos K, Huffman DR (1990) Solid c60: a new form of carbon. Nature 347:354–358

Kumar R, Sahoo S, Joanni E, Singh RK, Tan WK, Kar KK, Matsuda A (2019) Recent progress in the synthesis of graphene and derived materials for next generation electrodes of high performance lithium ion batteries. Prog Energy Combust Sci 75:100786

Kuznietsova H, Dziubenko N, Hurmach V, Chereschuk I, Motuziuk O, Ogloblya O, Prylutskyy Y (2020) Water-soluble pristine c(60) fullerenes inhibit liver fibrotic alteration and prevent liver cirrhosis in rats. Oxidative Med Cell Longev 2020:8061246

Lee XJ, Hiew BYZ, Lai KC, Lee LY, Gan S, Thangalazhy-Gopakumar S, Rigby S (2019) Review on graphene and its derivatives: synthesis methods and potential industrial implementation. J Taiwan Inst Chem Eng 98:163–180

Li S, Li Z, Ke B, He Z, Cui Y, Pan Z, Li D, Huang S, Lai C, Su J (2019) Magnetic multi-walled carbon nanotubes modified with polyaluminium chloride for removal of humic acid from aqueous solution. J Mol Liq 279:241–250

Li T, Xiao L, Yang J, Ding M, Zhou Z, Laconte L, Jin L, Dorn HC, Li X (2017) Trimetallic nitride endohedral fullerenes carboxyl-gd(3)n@c(80): a new theranostic agent for combating oxidative stress and resolving inflammation. ACS Appl Mater Interfaces 9:17681–17687

Liu Z, Tabakman SM, Chen Z, Dai H (2009) Preparation of carbon nanotube bioconjugates for biomedical applications. Nat Protoc 4:1372–1382

Lu B, Li T, Zhao H, Li X, Gao C, Zhang S, Xie E (2012) Graphene-based composite materials beneficial to wound healing. Nanoscale 4:2978–2982

Mahmoudi M, Akhavan O, Ghavami M, Rezaee F, Ghiasi SM (2012) Graphene oxide strongly inhibits amyloid beta fibrillation. Nanoscale 4:7322–7325

Maiti D, Tong X, Mou X, Yang K (2019) Carbon-based nanomaterials for biomedical applications: a recent study. Front Pharmacol 9

Meher J, Kesharwani P, Chaurasia M, Singh A & Chourasia M (2018) Carbon nanotubes (cnts): A novel drug delivery tool in brain tumor treatment. *In:* P, K (ed.) *Nanotechnology-based targeted drug delivery systems for brain tumor*

Mignani S, Shi X, Ceña V, Rodrigues J, Tomas H, Majoral JP (2021) Engineered non-invasive functionalized dendrimer/dendron-entrapped/complexed gold nanoparticles as a novel class of theranostic (radio)pharmaceuticals in cancer therapy. J Control Release 332:346–366

Orsu P, Koyyada A (2020) Recent progresses and challenges in graphene based nano materials for advanced therapeutical applications: a comprehensive review. Mater Today Commun 22: 100823

Pandey P, Dahiya M (2016) Carbon nanotubes: types, methods of preparation and applications. Carbon 1:15–21

Partha R, Conyers JL (2009) Biomedical applications of functionalized fullerene-based nanomaterials. Int J Nanomedicine 4:261–275

Patel DK, Kim HB, Dutta SD, Ganguly K, Lim KT (2020) Carbon nanotubes-based nanomaterials and their agricultural and biotechnological applications. Materials (Basel, Switzerland) 13

Peci T, Dennis TJS, Baxendale M (2015) Iron-filled multiwalled carbon nanotubes surface-functionalized with paramagnetic gd (iii): a candidate dual-functioning mri contrast agent and magnetic hyperthermia structure. Carbon 87:226–232

Pinals RL, Ledesma F, Yang D, Navarro N, Jeong S, Pak JE, Kuo L, Chuang Y-C, Cheng Y-W, Sun H-Y, Landry MP (2021) Rapid sars-cov-2 spike protein detection by carbon nanotube-based near-infrared nanosensors. Nano Lett 21:2272–2280

Prabhakar N, Näreoja T, Von Haartman E, Karaman DŞ, Jiang H, Koho S, Dolenko TA, Hänninen PE, Vlasov DI, Ralchenko VG, Hosomi S, Vlasov II, Sahlgren C, Rosenholm JM (2013) Core–shell designs of photoluminescent nanodiamonds with porous silica coatings for bioimaging and drug delivery ii: application. Nanoscale 5:3713–3722

Prajapati SK, Jain A, Shrivastava C, Jain AK (2019) Hyaluronic acid conjugated multi-walled carbon nanotubes for colon cancer targeting. Int J Biol Macromol 123:691–703

Prajapati SK, Malaiya A, Kesharwani P, Soni D, Jain A (2022) Biomedical applications and toxicities of carbon nanotubes. Drug Chem Toxicol 45:435–450

Qin J-X, Yang X-G, Lv C-F, Li Y-Z, Liu K-K, Zang J-H, Yang X, Dong L, Shan C-X (2021) Nanodiamonds: synthesis, properties, and applications in nanomedicine. Mater Des 210:110091

Raval JP, Joshi P, Chejara DR (2018) Carbon nanotube for targeted drug delivery. In: Inamuddin AAM, Mohammad A (eds) Applications of nanocomposite materials in drug delivery. Woodhead Publishing

Rhazouani A, Gamrani H, El Achaby M, Aziz K, Gebrati L, Uddin MS, Aziz F (2021) Synthesis and toxicity of graphene oxide nanoparticles: a literature review of *in vitro* and *in vivo* studies. Biomed Res Int 2021:5518999

Roy S, Jaiswal A (2020) DNA binding and nir triggered DNA release from quaternary ammonium modified poly(allylamine hydrochloride) functionalized and folic acid conjugated reduced graphene oxide nanocomposites. Int J Biol Macromol 153:931–941

Sahu A, Jain A, Gulbake A (2017) The role of carbon nanotubes in nanobiomedicines. Int J Appl Pharm 9:235

Saitoh Y, Miyanishi A, Mizuno H, Kato S, Aoshima H, Kokubo K, Miwa N (2011) Super-highly hydroxylated fullerene derivative protects human keratinocytes from uv-induced cell injuries together with the decreases in intracellular ros generation and DNA damages. J Photochem Photobiol B, Biol 102:69–76

Salah LS, Ouslimani N, Bousba D, Huynen I, Danlée Y, Aksas H (2021) Carbon nanotubes (cnts) from synthesis to functionalized (cnts) using conventional and new chemical approaches. J Nanomater 2021:4972770

Sanginario A, Miccoli B, Demarchi D (2017) Carbon nanotubes as an effective opportunity for cancer diagnosis and treatment. Biosensors 7:9

Sari AH, Khazali A, Parhizgar SS (2018) Synthesis and characterization of long-cnts by electrical arc discharge in deionized water and nacl solution. Int Nano Lett 8:19–23

Sarkar A, Bera S, Chakraborty AK (2020) Coni2s4-reduced graphene oxide nanohybrid: an excellent counter electrode for pt-free dssc. Sol Energy 208:139–149

Schrand AM, Dai L, Schlager JJ, Hussain SM, Osawa E (2007) Differential biocompatibility of carbon nanotubes and nanodiamonds. Diam Relat Mater 16:2118–2123

Shi J, Chen Z, Wang L, Wang B, Xu L, Hou L, Zhang Z (2016) A tumor-specific cleavable nanosystem of peg-modified c60@au hybrid aggregates for radio frequency-controlled release, hyperthermia, photodynamic therapy and x-ray imaging. Acta Biomater 29:282–297

Shi J, Yu X, Wang L, Liu Y, Gao J, Zhang J, Ma R, Liu R, Zhang Z (2013) Pegylated fullerene/iron oxide nanocomposites for photodynamic therapy, targeted drug delivery and mr imaging. Biomaterials 34:9666–9677

Shi Kam NW, Jessop TC, Wender PA, Dai H (2004) Nanotube molecular transporters: internalization of carbon nanotube-protein conjugates into mammalian cells. J Am Chem Soc 126:6850–6851

Simon J, Flahaut E, Golzio M (2019) Overview of carbon nanotubes for biomedical applications. Materials (Basel, Switzerland) 12:624

Smith AT, Lachance AM, Zeng S, Liu B, Sun L (2019) Synthesis, properties, and applications of graphene oxide/reduced graphene oxide and their nanocomposites. Nano Mater Sci 1:31–47

Speranza G (2021) Carbon nanomaterials: synthesis, functionalization and sensing applications. Nanomaterials (Basel, Switzerland) 11:967

Su Y, Liu Y, Xu X, Zhou J, Xu L, Xu X, Wang D, Li M, Chen K, Wang W (2018) On-demand versatile prodrug nanomicelle for tumor-specific bioimaging and photothermal-chemo synergistic cancer therapy. ACS Appl Mater Interfaces 10:38700–38714

Sun D, Yan X, Lang J, Xue Q (2013) High performance supercapacitor electrode based on graphene paper via flame-induced reduction of graphene oxide paper. J Power Sources 222:52–58

Syama S, Mohanan PV (2019) Comprehensive application of graphene: emphasis on biomedical concerns. Nano-Micro Lett 11:6

Tanzi L, Terreni M, Zhang Y (2022) Synthesis and biological application of glyco- and peptide derivatives of fullerene c60. Eur J Med Chem 230:114104

Theek B, Rizzo LY, Ehling J, Kiessling F, Lammers T (2014) The theranostic path to personalized nanomedicine. Clin Transl Imaging 2:66–76

Tinwala H, Wairkar S (2019) Production, surface modification and biomedical applications of nanodiamonds: a sparkling tool for theranostics. Mater Sci Eng C 97:913–931

Usman MS, Hussein MZ, Kura AU, Fakurazi S, Masarudin MJ, Ahmad Saad FF (2018) Graphene oxide as a nanocarrier for a theranostics delivery system of protocatechuic acid and gadolinium/gold nanoparticles. Molecules 23

Vander Wal RL, Berger GM, Ticich TM (2003) Carbon nanotube synthesis in a flame using laser ablation for in situ catalyst generation. Appl Phys A 77:885–889

Wang L, Wang Y, Hao J, Dong S (2017) Magnetic fullerene-DNA/hyaluronic acid nanovehicles with magnetism/reduction dual-responsive triggered release. Biomacromolecules 18:1029–1038

Wang Y, Wang H, Liu D, Song S, Wang X, Zhang H (2013) Graphene oxide covalently grafted upconversion nanoparticles for combined nir mediated imaging and photothermal/photodynamic cancer therapy. Biomaterials 34:7715–7724

Xie L, Wang G, Zhou H, Zhang F, Guo Z, Liu C, Zhang X, Zhu L (2016) Functional long circulating single walled carbon nanotubes for fluorescent/photoacoustic imaging-guided enhanced phototherapy. Biomaterials 103:219–228

Yamawaki H, Iwai N (2006) Cytotoxicity of water-soluble fullerene in vascular endothelial cells. Am J Physiol Cell Physiol 290:C1495-502

Yin JJ, Lao F, Fu PP, Wamer WG, Zhao Y, Wang PC, Qiu Y, Sun B, Xing G, Dong J, Liang XJ, Chen C (2009) The scavenging of reactive oxygen species and the potential for cell protection by functionalized fullerene materials. Biomaterials 30:611–621

Yin JJ, Lao F, Meng J, Fu PP, Zhao Y, Xing G, Gao X, Sun B, Wang PC, Chen C, Liang XJ (2008) Inhibition of tumor growth by endohedral metallofullerenol nanoparticles optimized as reactive oxygen species scavenger. Mol Pharmacol 74:1132–1140

Zare H, Ahmadi S, Ghasemi A, Ghanbari M, Rabiee N, Bagherzadeh M, Karimi M, Webster TJ, Hamblin MR, Mostafavi E (2021) Carbon nanotubes: smart drug/gene delivery carriers. Int J Nanomedicine 16:1681–1706

Zhao Y, Zhao T, Cao Y, Sun J, Zhou Q, Chen H, Guo S, Wang Y, Zhen Y, Liang X-J, Zhang S (2021a) Temperature-sensitive lipid-coated carbon nanotubes for synergistic photothermal therapy and gene therapy. ACS Nano 15:6517–6529

Zhao Y, Shen X, Ma R, Hou Y, Qian Y, Fan C (2021b) Biological and biocompatible characteristics of fullerenols nanomaterials for tissue engineering. Histol Histopathol 36:725–731

Zhu Y, Li J, Li W, Zhang Y, Yang X, Chen N, Sun Y, Zhao Y, Fan C, Huang Q (2012) The biocompatibility of nanodiamonds and their application in drug delivery systems. Theranostics 2:302–312

Quantum Dots: Functionalization and Theranostic Applications

10

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Abstract

In the last decade, nanotechnology has shown incredible growth and established itself as valuable technology to prepare highly specific biomedical appliances. Quantum dots (QDs) are one such example of nanotechnological product, which is a crystalline semiconductor material able to produce bright fluorescent light upon excitation. The size of the QDs ranges from 2 to 10 nm, which are mostly made up of inorganic compounds like cadmium (Cd), selenium (Se), tellurium (Te), zinc (Zn), sulphur (S), etc. or organic materials like citric acid, various amino acid, carbohydrates, etc. Contrasting with commonly used organic dyes, it has unique optical and electrical properties that mainly assist in bioimaging, cellular imaging, biosensing, drug delivery, or gene delivery. Functionalization of QDs with various surface modification strategies and bioconjugation with the bioactive molecule enhance its biomedical applications by controlling the cellular toxicity exerted by the core of QDs. In this chapter, we have reviewed the QDs, starting from its method of preparation to derivatives, functionalization strategies, and detailed discussion on its theranostic applications.

Keywords

Quantum dots · Carbon quantum dots · Cancer imaging · Nanoparticles · Chemotherapy · Theranostic nanocarriers

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Abbreviations

Ag-QDs	Silver quantum dots
BBB	Blood-brain barrier
CD-Asp	New-type carbon dots
CdS	Cadmium sulphide
CDs	Carbon dots
CdSe	Cadmium selenide
CdTe	Cadmium telluride
CNPs	Carbon nanoparticles
CO	Chemical oxidation
CQDs	Carbon quantum dots
DMF	Dimethylformamide
FCDs	Fluorine-doped carbon dots
GO	Graphene oxide
GQD	Graphene quantum dot
hMSCs	Human mesenchymal stem cells
IgG	Immunoglobulin G
N-CQDs	Nitrogen-doped carbon quantum dots
PbS	Lead sulphide
PDT	Photodynamic therapy
PEG	Polyethylene glycol
PEI	Polyethylenimine
QDNB	Quantum dot nanobead
QDs	Quantum dots
TEM	Transmission electron microscopy
ZnCu	Zinc copper
Zn-QDs	Zinc oxide quantum dots
ZnS	Zinc sulphide

10.1 Introduction

Quantum dots (QDs) are nanometric, fluorescent, semiconductor crystals, first explored by Russian physicist Alexey Ekimov in the 1980s (Matea et al. 2017). The first biological imaging application of QDs was later reported in the year 1998 (Piccinno et al. 2012). Alexey Ekimov has synthesized copper chloride and later cadmium chloride into nanocrystal, which forms in a molten glass matrix and published his observations in the 1980s. In 1982, Alexander Efros, another Russian physicist, explained the behaviour of these nano-sized crystals by the confinement of their electrons (History of Quantum Dots – Nexdot 2022). In Asia, Japanese researcher in 1984 worked on copper chloride QDs in solid matrices. In the year 1993, Moungi Bawendi produced homogenous QDs having less than 5% size

variation. This breakthrough research guided researchers in producing uniform-sized QDs and tunable fluorescence. Following that, QDs have been widely explored for various applications in different fields including solar cell production, biological imaging, drug delivery vehicle, and biosensing. QDs have unique property to generate bright fluorescent light upon excitation through a light source such as a laser or ultraviolet radiation (Bajwa et al. 2016a; Kargozar et al. 2020).

QDs are majorly classified in two major types: one is inorganic QDs (made from various metals), and second is organic QDs or carbon quantum dots (CQDs). Inorganic QDs are prepared from the metals belonging to groups (II) to (VI) and (III) to (V) of the periodic table which includes silver (Ag), cadmium (Cd), mercury (Hg), phosphorus (P), lead (Pb), selenium (Se), etc. For instance, cadmium selenide (CdSe) QDs contain Cd from group (II) and Se from group (VI-a) of the periodic table (Iga et al. 2007). QDs' unique optical and electronic properties established QDs as an emerging fluorescent probe and carrier for biomedical applications.

CQDs and graphene quantum dots (GQDs) (also a part of organic QDs) are prepared from various organic materials; thus they are considered as biocompatible which make them potential candidate for in vivo tissue imaging and drug delivery vehicle to treat various life-threatening diseases. For instance, D'souza et al. (2018) developed mitomycin (an anticancer agent)-loaded CQDs for breast cancer treatment, using *Daucus carota* (wild carrot) roots as carbon source. Mitomycin was allowed to stir with CQDs on magnetic stirrer which leads to the formation of hydrogen bonds between drug and CQDs. The cytotoxicity assay of prepared CQDs was carried out on the MCF-7 breast cancer cells and showed that blank CQDs were non-toxic and biocompatible, while drug-loaded CQDs produced higher cytotoxicity in comparison to free drug, supporting the use of CQDs as prospective theranostic carrier (D'souza et al. 2018). The initial success of QDs in biological imaging, sensing, and detection increased the interest of the scientific community to develop this technology for various clinical applications. QDs are primarily used in biosensing of various metals and biomarkers and imaging of diseased tissues like tumour. Due to lack of information regarding in vivo toxicity and degradation profile of QDs, its clinical use is currently limited. Regarding applications of QDs in drug delivery, the focus is on two major areas: using QDs as (i) drug delivery carriers and (ii) imaging or sensing probe (Jha et al. 2018).

10.2 Properties of Quantum Dots

QDs have eye-touching structural, optical, and electronic properties which provide them unique ability to fluoresce upon excitation without photobleaching. Important properties of QDs are mentioned in Table 10.1.

Table 10.1 Properties of QDs

Core structure property	• Inorganic QDs mainly consist of a metalloid crystalline core, made up of materials like cadmium tellurium (CdTe), cadmium sulphide (CdS), indium phosphate, etc. • The core is stabilized by the semiconductor shell to improve its physical as well as optical properties (Jha et al. 2018). • Coating or capping of biocompatible material or polymer layers to the core, for instance, polyethylene glycol (PEG), enhances water solubility and desired provide bioactivity (Gidwani et al. 2021; Rizvi et al. 2012).
Optical properties	• The electrons in the valence band travels into the conduction band when a high-energy photon is absorbed by the semiconductor QDs. This creates a positively charged hole in valence band. During excitation, i.e. electron-hole pair is created when the electron and hole interact due to the coulomb attractive force. When the recombination of the electron and the hole in the valence band occurs, the photons are emitted by the QDs in the form of fluorescent light (Xu 2016). • The band gap between the conduction band and valence band depends on the size of the QDs; the smaller the size, the larger would be the band gap. Thus, the fluorescence can be controlled with changing size during synthesis (Bera et al. 2010).
Biological properties	• The synthesized QDs undergo surface modifications. The surface modification enables the QDs to target a particular tissue. The encapsulation and ligand exchange strategies could produce QDs having the ability to be dispersed in water, with better stability in wide range of pH, and also reduce non-specific binding to cellular components (Rosenthal et al. 2011). • The bioconjugation method of QDs also enables its wide application in drug delivery, imaging, and gene delivery. • The protein conjugated QDs can be used as probes in biological immunoassay and live cell imaging (Vasudevan et al. 2015).

10.3 Methods of Preparation

Since the discovery of QDs by Ekimov in a glass matrix in 1981, QDs have been synthesized using several methods. Mainly, these methods are classified in two main approaches, i.e. top-down approaches and bottom-up approaches. Depending on the manufacturing route, variation in the diameter of QDs from nanometres to a few micrometres occurs (Brichkin and Razumov 2016). The bottom-up approaches, like the organometallic route, fabricates QDs with excellent optical properties. In the organometallic route, it involves steps like nucleation, growth, and termination, which are conducted in organic solvents under a high temperature and inert environment. The derived QDs from this method results in high quantum yield and sharp emission peak, ensuring better bioimaging applications (Mozafari et al. 2013). Different QDs that were synthesized using organometallic routes are CdTe/CdS and lead sulphur (PbS) QDs. The synthesized QDs further undergo various surface modifications before their biological use (Yu et al. 2012).

Aqueous solution-based synthesis is a simple and powerful route used in producing all kinds of nanomaterials including QDs. In comparison to other routes, it

provides the advantage of greater compatibility and flexibility (Wang et al. 2009). Some inorganic QDs and most of the CQDs were prepared by using solution-based techniques like CdS and zinc selenium (ZnSe) QDs (Pawar et al. 2015; Ding et al. 2014). As compared to organometallic QDs, aqueous QDs have a smaller hydrodynamic diameter (approximately 5–10 nm). A major limitation of the aqueous QDs is their poor optical properties.

Apart from these two approaches, another technique is used to stabilize QDs, where biomolecules such as peptides and polysaccharides have been used as a template (Lei et al. 2013). The biocompatibility and stability of QDs can improve on capping with nucleic acid and other biomolecules which is helpful for cell imaging studies. For instance, Kasotakis et al. (2014) proposed the decoration of different sizes of CdSe@ZnS core-shell QDs with two-dimensional peptide fibrils. Then, QDs were capped with trioctyl phosphine oxide molecules. Results showed better stability in aqueous media for peptide-capped QDs (Kasotakis et al. 2014). A new route of synthesizing QDs by using living organisms, which provides ecofriendly and non-toxic QDs. For example, the synthesis of CdS QDs has been carried out in various bacterial strains such as *Escherichia coli* (Depeursinge et al. 2010), *Bacillus megatherium* (Prakash et al. 2010), and *Gluconacetobacter xylinus* (Narayanan and Sakthivel 2010).

Basically, preparation of QDs requires carbonization, which can be achieved by different techniques. These techniques are summarized in two sections: top-down and bottom-up approaches. Some scientists have mentioned CQDs as carbon nanoparticles (CNPs) in their research which is ultimately the same thing. Table 10.2 summarizes the starting material and method of preparation with important characteristics of various QDs prepared by scientists for biomedical applications.

10.3.1 Top-Down Approaches

Top-down methods involve the breakdown of relatively large particles into smaller molecules. Various top-down approaches for the synthesis of QDs are discussed in the following sub-sections.

10.3.1.1 Electrochemical/Chemical Oxidation

Electrochemical oxidation involves the use of strong acid to exfoliate CQDs from carbon fibres, carbon nanotubes, and other carbon sources. It is a commonly used top-down approach for the production of QDs with high purity, high yield, low cost, and easy size optimization (Zhou et al. 2007). For example, Ray et al. (2009) synthesized CNPs via nitric acid oxidation of carbon soot. The size of the produced CNPs was 2–6 nm with 3% of quantum yield, showing green fluorescence on exposing the CNPs under ultraviolet rays. Cellular uptake study showed that CNPs do not require further functionalization to enter inside the cells. The researchers had carried out the trypan blue assay and MTT assay using HepG2 cells (human hepatoblastoma). It was found that the cell survival rate was in between

Table 10.2 Summary of QDs with its source, method of preparation, fluorescent colour, size, and application

Method of preparation	Carbon source	Colour	Size	Application	Reference
Electrochemical oxidation	Urea, sodium citrate	Blue	1–3.5 nm	In the detection of mercury ion (Hg^{2+})	Hou et al. (2015)
Electrochemical oxidation	Carbon nanotubes	Blue	2.8 ± 0.5 nm	Biological labelling and optoelectronics	Zhou et al. (2007)
Hydrothermal treatment	Glucosamine HCL	Green	15–70 nm	Cell labelling and bioimaging	Yang et al. (2011)
Hydrothermal treatment	Citric acid + isoleucine	Violet	6–15 nm	Biosensor for detection of Fe^{3+} ions	Jiang et al. (2015)
Laser ablation	Graphite powders	–	Ultra-small 1 nm	Catalytic and sensing application	Nguyen et al. (2019)
Laser ablation	Graphite in ethylenediamine and polyethylenimine	Violet	6–15 nm	Fluorescent imaging and labelling	Kaczmarek et al. (2021)
Ultrasonic treatment	Glucose, HCL/NaOH	Blue	5–10 nm	As biosensors and in bioimaging	Li et al. (2011)
Arc discharge	TiO_2 nanostructure coupled with carbon dots (CDs)	–	Average size of 27 nm	Photocatalytic applications	Biazar et al. (2018)
Arc discharge	Carbon by-products	–	Average size of 27 nm	Optoelectronic applications	Su et al. (2014)
Microwave-assisted	Glycerol	Indigo	9 ± 1.1 nm	Gene delivery in lung cancer	Wu et al. (2016)
Microwave-assisted	Citric acid, urea, and thiourea	Green	2–6 nm	Photodynamic therapy, bioimaging^{-}	Bourlinos et al. (2015)
Pyrolysis	D-glucose	Yellow	2.28 ± 0.42 nm	Diagnosis of brain cancer cells	Zheng et al. (2015)
Pyrolysis	Sodium alginate	Blue	<10 nm	In the diagnosis of scurvy by detecting ascorbic acid	Fung et al. (2016)

90 and 100% in less than 0.5 mg/mL of CNPs, which proves that the prepared CNPs have low cytotoxicity. Although the cellular death was observed at higher concentration (>1 mg/mL), this concentration is 100–1000 times higher than the concentration required for cell imaging (Ray et al. 2009). Peng and Travas-Sejdic have prepared luminescent CDs using carbohydrate as a carbon precursor. Process was started with the dehydration of carbohydrate with concentrated sulphuric acid (H_2SO_4) to produce carbonaceous materials. These carbonaceous materials were further treated with nitric acid to get individual carbogenic nanoparticle. The luminescent carbogenic dots were obtained after passivating nanoparticles using amine-terminated compounds. The surface passivation is important for improving the photoluminescence intensity of the QDs as well as the quantum yield (Peng and Travas-sejdic 2009).

10.3.1.2 Laser Ablation

Laser ablation is a single-step reaction method for the preparation of CDs, which involves the use of high-energy laser and carbon source for the synthesis of CDs. High-energy laser is useful in generating high temperature and pressure quickly at desired surface. For instance, Sun et al. (2006) have produced fluorescent CDs by using laser ablation technique. The complete process was divided in three parts: baking, annealing, and curing in presence of argon flow by hot pressing the graphite powder and cement. The sample and its aqueous suspension showed no detectable photoluminescence, although the surface passivation of the CDs with organic compounds such as diamine-terminated oligomeric PEG showed bright luminescence (Sun et al. 2006). Moreover, Calabro et al. (2018) developed a one-pot pulse laser ablation process and chemical oxidation (CO) process for the synthesis of GQDs from carbon nano-onions. The photoluminescence result of the LA-GQDs showed a blue-shifted emission in comparison to CO-GQDs, mostly due to the uniform particle size and surface functional groups. Also, the LA-GQDs resulted into decreased thickness and smaller and uniform size as compared to the product produced from CO which proves the efficiency of laser ablation method over CO method (Calabro et al. 2018).

10.3.1.3 Ultrasonic Treatment

This method uses the high-energy ultrasonic waves to produce the QDs by breaking the large carbon materials. Carbon materials are allowed to dissolve in aqueous alkaline or acidic solution for efficient breakdown of precursor materials. Li et al. (2011) prepared fluorescent CNPs from glucose by an acid-assisted ultrasonic treatment. The transmission electron microscopy (TEM) analysis revealed the spherical shape of the prepared CNPs of less than 5 nm. The FTIR spectroscopy of the CNPs showed the C-O stretching and O-H bending vibration confirming the presence of large number of hydroxyl groups. The abundant hydroxyl groups on the surface of CNPs provides high hydrophobicity and stability in the aqueous system. This CNPs also exhibited greater stability (>6 months) and high quantum yield (≈ 7%). The dispersion of prepared CNPs in aqueous medium exhibited an excellent photoluminescence property without any surface functionalization making it a

suitable candidate for drug delivery, bioimaging, and biosensing application (Li et al. 2011). Similarly, Lu and Zhou (2019) carried out the ultrasonic treatment of dopamine in dimethylformamide (DMF) to synthesize nitrogen-doped CQDs (N-CQDs) intended for the detection of ferrous (Fe^{2+}) ion by emitting the blue fluorescent. The emission spectra showed that on addition of Fe^{2+}, the reduction in fluorescent intensity confirmed the fluorescence quenching associated with Fe^{2+} ion. The researchers have carried out the cell imaging and cell cytotoxicity studies using human hepatoblastoma (HepG2) cells as a representative of cancer cells. The confocal laser scanning microscopy analysis showed a bright blue fluorescence at 364 nm confirming the entry of N-CQDs in the HepG2 cancer cells. The cell cytotoxicity assay of N-CQDs showed about 100% HepG2 cell viability at a N-CQD concentration below 100 μg/ml. The cell viability was near to 90% even at 300 μg/ml concentration of N-CQDs showing its low toxicity even at higher concentration (Lu and Zhou 2019).

10.3.1.4 Arc Discharge (ACD)

This method uses the high-energy gas plasma to acquire decomposition of carbon materials. The reaction is carried out in the sealed reactor generating high-energy plasma. The electric current is allowed to pass between anode (filled with gas plasma) and cathode that leads to breakdown of carbon material into smaller compounds (Gidwani et al. 2021). During preparation carbon vapor aggregated on cathode was cooled down, and the resulting powder was collected. The QDs obtained from this method have good aqueous solubility (Arora and Sharma 2014). Biazar et al. (2018) prepared TiO_2 QDs via the arc discharge method in a deionized water. Two titanium rods were used to apply arc discharge to synthesize TiO_2 nanoparticles with the purity of 99.5%. The morphological and size distribution evaluation by field emission scanning electron microscope analysis revealed its spherical shape with mean particle size of 27 nm. Optical properties of QDs showed a maximum absorption near 500 nm (Biazar et al. 2018).

These techniques are mostly used in the preparation of inorganic QDs. However, the use in the preparation of CQDs has produced satisfactory results. QDs obtained by these methods show better fluorescence properties.

10.3.2 Bottom-up Approaches

Bottom-up approach involves formation of smaller carbon structures from the various precursor materials for the synthesis of QDs. Various bottom-up approaches are discussed in following section.

10.3.2.1 Hydrothermal/Solvothermal Synthesis

Hydrothermal synthesis is a cost-effective approach for the preparation of CQDs from various carbon sources like amines, saccharides, and organic acids (Sharma and Das 2019). The method is used to produce water-soluble QDs having a size of around 10 nm. The carbonization occurs at high temperatures; thus Teflon-coated

stainless steel autoclave is exclusively used. The carbon precursor or polymer is dissolved into the solvent and placed in the heating chamber at a high temperature (Guo et al. 2014). Zhang et al. (2010) used hydrothermal method for the synthesis of carbon nanoparticles from L-ascorbic acid. Reaction mixture was prepared by dissolving L-ascorbic acid in water and ethanol. Then, the mixture was heated at 180 °C for 4 h in an autoclave followed by cooling at room temperature. The prepared CNPs exhibited higher photoluminescence efficiencies (6.79%) than the CNPs prepared from other methods. The field emission TEM analysis reveals that the particles were well dispersed and uniform in size as compared to particle synthesized from other methods (Zhang et al. 2010). Likewise, scientists have also prepared CNPs by a one-step hydrothermal synthesis by dissolving 0.5 gm dopamine into 10 mL water (Qu et al. 2013). Li et al. (2015) used ammonium citrate and ethylenediamine for the synthesis of N-CQDs capable to detect mercuric and iodide ions. The heating of the aqueous solution of ammonium citrate and ethylenediamine at 200 °C temperature for 5 h was carried out. The prepared N-CQDs showed an average diameter of 4.8 nm and a quantum yield of 66.8% (Li et al. 2015).

10.3.2.2 Microwave-Assisted Synthesis

Microwave-assisted synthesis uses the high-intensity microwaves, which reduces the processing time significantly (from hours to minutes). This method is the first choice of researchers due to its ability to produce homogenous and low-cost QDs. Commonly used carbon precursor includes carbohydrates like glucose and fructose; amino acids like beta-alanine, arginine, and cysteine; and other carbon-based molecules like citric acid. For example, He et al. (2015) proposed the rapid microwave-assisted synthesis of fluorescent CDs intended for cellular targeting and cellular imaging. The CDs were synthesized with the condensation and carbonization reaction of citric acid and ethylenediamine in microwave under controlled temperature. The researchers carried out the fluorescence imaging of live cells which are stained with CDs. A bright florescence and uniform staining of the live cells were reported. The MTT assay was carried out on the 3 T3 cells (fibroblast cell line), which showed a cell viability of 90% when treated with CDs at concentration up to 0.8 mg/ml, indicating its low cellular toxicity. The researcher also performed the in vivo fluorescence imaging on mice having HeLa tumour. The CDs were injected intravenously showing the incremental accumulation of QDs at tumour site with time, upon excitation at 405 nm (He et al. 2015).

Wang et al. (2014) synthesized water-soluble CDs using citric acid as carbon source and tryptophan as a passivating agent as well as a nitrogen-doping agent. The prepared CDs were subjected for the complex formation with polyethylenimine (PEI) through electrostatic interaction between negatively charged CDs and positively charged PEI. The synthesized CDs were used for the efficient delivery of the siRNA into human gastric cancer cell line MGC-803. The CDs-PEI complexes having a positive charge were found efficient in enhancing the delivery and cellular uptake of the negatively charge siRNA. The confocal laser scanning microscopic images confirm the internalization of siRNA assisted by the CDs-PEI complex. The scientist also performed real-time polymerase chain reaction and Western blot assay

to analyse the gene-silencing efficiency of the CDs-PEI complexes. The real-time polymerase chain reaction and Western blot assay results showed that the CDs-PEI complexes strongly inhibit the expression of survivin protein; thus normal function of survivin like inhibiting apoptosis and regulating mitosis will be disturbed which leads to tumour cell death (Wang et al. 2014). Due to its ability to form monodisperse QDs within a few minutes, it is widely used by the scientific community.

10.3.2.3 Thermal Decomposition

In general, thermal decomposition involves the chemical decomposition of compounds by heat. Thermal decomposition method is either reversible or irreversible depending on starting materials, for example, decomposition of starch, proteins, etc. is reversible, while the decomposition of ammonium chloride, limestone, etc. is irreversible. The carbonization carried out during thermal decomposition usually performed at a very high temperature. Easy scale-up ability and operational functioning are the two main eye-touching features of this method. For example, Ludmerczki et al. (2019) carried out the thermal decomposition of the citric acid (carbon source) to produce luminescent CDs. The citric acid CD stability in aqueous medium is low, and hence the researcher used 3-aminopropyltriethoxysilane (APTES) for its surface functionalization, which increased the fluorescence intensity and inhibited the fluorescence quenching in aqueous medium. The TEM analysis revealed the maximum particle size distribution between 5 and 15 nm (Ludmerczki et al. 2019). Wan et al. (2016) have proposed a strategy for the preparation of CDs and graphene-like carbon sheets by thermal decomposition of L-cysteine (Wan et al. 2016).

10.3.2.4 Pyrolysis

In this method, the organic material undergoes heating in the absence of oxygen or an inert atmosphere. The chemical and the physical changes of the carbon source under inert atmosphere resulted in the formation of carbon-containing solid residues. It required high temperature and controlled pressure. L-aspartic acid, D-glucose, and citric acid are some of the commonly used carbon sources for the preparation of CDs through this method (Khayal et al. 2021). For instance, Zheng et al. (2015) synthesized self-targeting CDs from equimolar mixture of L-aspartic acid and d-glucose as precursor for the diagnosis of brain cancer cells. The MTT assay was performed for the cytotoxicity study of prepared CD-Asp (new-type CDs) using C6 glioma and noncancerous L929 mouse fibroblast cells. A survival rate of $>75\%$ was observed in 500 μg/mL concentration after incubating the CDs for 48 h at six varying concentrations (i.e. 10–500 μg/mL). To study the selectivity of the prepared CDs towards the two cells, i.e. C6 cells and L929 cells, the researcher conducted the confocal laser scanning microscopic (CLSM) imaging of respective cells treated with CD-Asp at optimum concentration (2 mg/mL). It was found that the fluorescence intensity in case of C6 cells was much stronger than L929 cells, confirming the greater selectivity towards the C6 cells as compared to the L929 cells. To study the glioma targeting efficiency of the CD synthesized from aspartic acid, the researcher compared the in vivo imaging of the mice injected with CD synthesized from

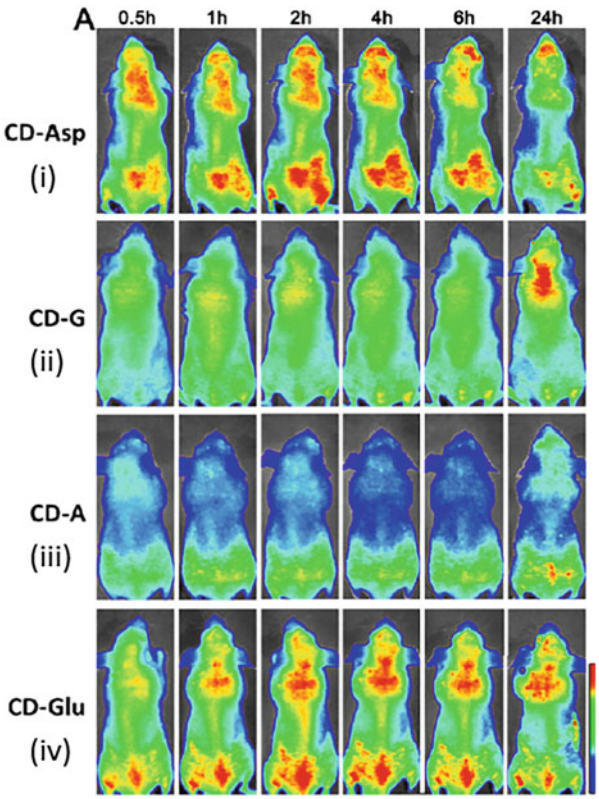

Fig. 10.1 In vivo imaging of glioma-bearing mice injected with CD-Asp (i), CD-glutamic acid (ii), CD-aspartic acid (iii), and CD-glucose (iv) (Zheng et al. 2015)

L-aspartic acid, D-glucose, and glutamic acid. It was found that the CD-Asp has higher intensity as compared to the other three, with better target efficiency towards glioma cells. The results suggested that these CD-Asp could act as a fluorescent imaging agent for glioma diagnosis as shown in Fig. 10.1 (Zheng et al. 2015).

Overall, bottom-up approaches like pyrolysis and hydrothermal method are the simple and scalable method for the preparation of QDs. However, more sophisticated instrument is required for better reproducibility of same-sized QDs at larger scale.

10.4 Functionalization of Quantum Dots

QDs are immensely smaller crystalline and made up of metalloid inorganic core. The accumulation of these metals in the body resulted in cell toxicity. Thus, for biological compatibility and its usage in various biomedical applications, the QDs should be hydrophilic and non-toxic. Surface functionalization of various QDs is carried out to modify their properties to enhance their application unmodified. QDs upon long-term exposure to ionic media or cellular media lead to metal ion toxicity

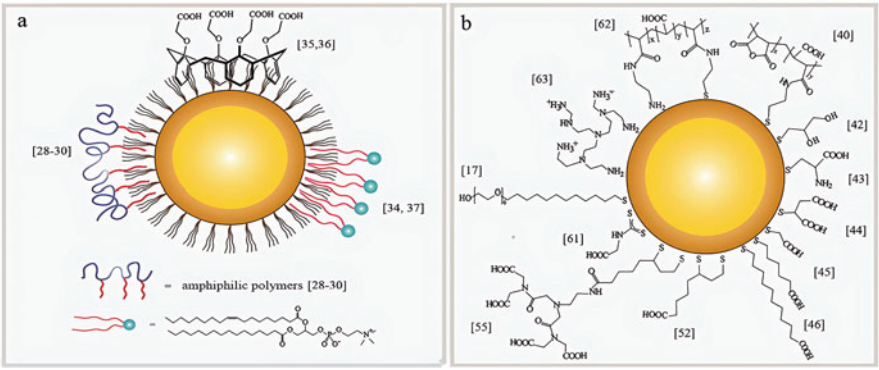

Fig. 10.2 Surface functionalization of QDs. (**a**) Coating with amphiphilic molecules and (**b**) surface modification through ligand exchange (Bilan et al. 2015)

and undergo oxidation, photochemical degradation, and metal ion leaching, which are toxic for biological systems. So, surface functionalization helps in improving the QDs' biological performance which is generally performed by two methods as described in the following section. Further various functionalization and conjugation strategies used with QDs are shown graphically in Fig. 10.2a, b**.**

10.4.1 Non-covalent Binding

The binding of molecules through non-covalent binding is due to the electrostatic interaction or hydrophobic interaction. In comparison to the covalent bonding, this interaction is much weaker and affects the stability of bioconjugation (Zhan et al. 2012). Non-covalent bonding is significantly impacted by the charge on the surface of QDs and not of the chemical groups present on QDs. The involvement of opposite charges resulted in the formation of the conjugated complex of the QDs and the biomolecules (Gonçalves et al. 2010).

10.4.2 Covalent Binding

In covalent bonding, functional groups of QDs bind with the biomolecule's functional groups using various types of chemical conjugation reactions as discussed below.

10.4.2.1 Conjugation with Carboxyl-Containing QDs

Carboxyl group-containing QDs could be conjugated with amine groups present on the amino acids of proteins, peptides, and antibodies. The formation of amine bonds improve the water solubility and storage of proteins and peptides (Cayuela et al.

2016). The various crosslinkers are used to bind the biomolecule to QDs, e.g. EDC {1-ethyl-3-(dimethyl aminopropyl) carbodiimide hydrochloride} which leads to the formation of an amide bond between the carboxyl group of QDs and amine group of biomolecules. ZnS QDs conjugated with dopamine are a prime example of carboxyl group-containing QDs conjugated with a biomolecule through amine bond (Matea et al. 2017; Martynenko et al. 2017).

10.4.2.2 Conjugation with Amine-Containing QDs

It is the most widely used conjugation, where groups like thiol and carboxylic acid of biomolecules like peptides, protein, and antibodies are conjugated with the QDs containing amine groups. For example, QDs conjugated with the anti-*Escherichia coli* antibodies were used as an immunosensor in the detection of *E. coli* (Martynenko et al. 2017).

10.4.2.3 Conjugation with Hydroxyl- or Aldehyde-Containing QDs

Like amine-containing QDs, hydroxyl- and aldehyde-containing QDs are preferred due to its water solubility and capacity to adsorb protein or peptides on its surface. Generally, hydroxyl- or aldehyde-containing QDs react with amine groups of proteins or peptide and form Schiff's base which subsequently reduced to secondary amine group. For instance, Martynenko et al. (2017) conjugated CdSe/ZnS QDs with IgG antibody by using poly[methylene(phenylene isocyanate)] coupling agent for preparing immune histochemical diagnostic probe (Gidwani et al. 2021).

10.4.2.4 Conjugation with Thiol-Containing QDs

Generally, thiol-containing QDs bind with the sulphur-containing biomolecules like cysteine and methionine. The main reason behind the formation of disulphide bond is to improve the water solubility of poorly soluble QDs. For example, Brunetti et al. (2020) have conjugated cysteine residue of tetra-branched peptide NT4 with QDs for active targeting in cancer cells. Results of cell cytotoxicity study on HT-29 cancer cells revealed better cytotoxicity in NT4-labelled QDs due to enhanced internalization by NT4 peptide (Brunetti et al. 2020).

PEG is one of the most commonly used reagents for surface functionalization. It is biocompatible and resistant to non-specific binding (Bentzen et al. 2005). Shen et al. (2012) proposed a one-pot hydrothermal reaction for the synthesis of GQDs using small graphene oxide (GO) sheets and PEG as a precursor. Scientists have observed two times higher photoluminescence compared to GO sheets and 28% quantum yield at 360 nm (Shen et al. 2012).

At last, functionalization of QDs will improve the biocompatibility and in vivo performance. Chemical conjugation by using various coupling reagent is the choice of functionalization method due to its stability compared to non-covalent attachments.

10.5 Role of Quantum Dots in Drug Delivery

QDs especially CQDs are used as a vehicle for drug delivery or gene delivery because of their low production cost, easy availability of raw materials, biocompatibility, and facile surface functionalization (Sharma and Das 2019). The versatile nature of QDs provides the ability to use it as drug delivery carrier as well as imaging probe alone or simultaneously. The QDs find a vital role in the delivery of drugs in various life-threatening diseases through encapsulation or other approaches. QDs provide wide theranostic platforms, acting as the main nanocarrier, biosensor, and imaging agent.

10.5.1 Cancer

QDs are being widely explored as imaging agent in the diagnosis of cancerous tumours. Each type of cancer overexpresses some specific biomarkers, and accordingly functionalization of QDs with suitable ligand has been explored for active targeting as well as diagnosis of tumours. Metastatic cancers require an efficient detection system. For this purpose, QDs conjugated with the antibody or other targeting ligand are helpful to acquire real-time imaging of tumour (Bajwa et al. 2016b; Wu et al. 2003). Olerile et al. (2017) reported the study of paclitaxel co-loaded with CdTe/CdS/ZnS QDs in nanostructured lipid carriers (NLCs), prepared by emulsion evaporation and low-temperature solidification method. To examine the tumour size and location, co-loaded NLCs were injected in mice. During animal imaging study, researcher observed that, with the increase in time, the fluorescent intensity is decreasing (Fig. 10.3). During the initial hours, the tumour showed high uptake, while the liver accounted barely any fluorescence, but as the time passes, the uptake by the tumour significantly decreases, while level of nanoparticles increases in the liver. This indicates the ability of the co-loaded NLCs in targeting and detection of H22 tumour (Olerile et al. 2017). Similarly, inorganic QDs were explored for the same application by Cai and co-workers (2016). They have carried out the loading of the drug doxorubicin onto zinc oxide QDs (Zn-QDs). Zn-QDs have an average size of 3 nm. Further, PEG and hyaluronic acid were used to functionalize Zn-QDs to target overexpressed glycoprotein CD44 receptors in cancer cells via hyaluronic acid as a ligand for CD44 receptors. The results of cytotoxicity assay with A549 cells revealed that the ZnO QDs dissociate in an aqueous media and produced Zn^{2+} ions inside the cells causing cellular death. The assay also reported that the hyaluronic acid-containing conjugate has higher antitumour activity than the PEG-ZnO QDs mainly due to the endocytosis caused by the hyaluronic acid and its rapid dissolution under acidic conditions (Cai et al. 2016). Outcomes of various research suggested that theranostic nanocarrier could be useful in determining the dosage which significantly varies from patient to patient in case of cancer.

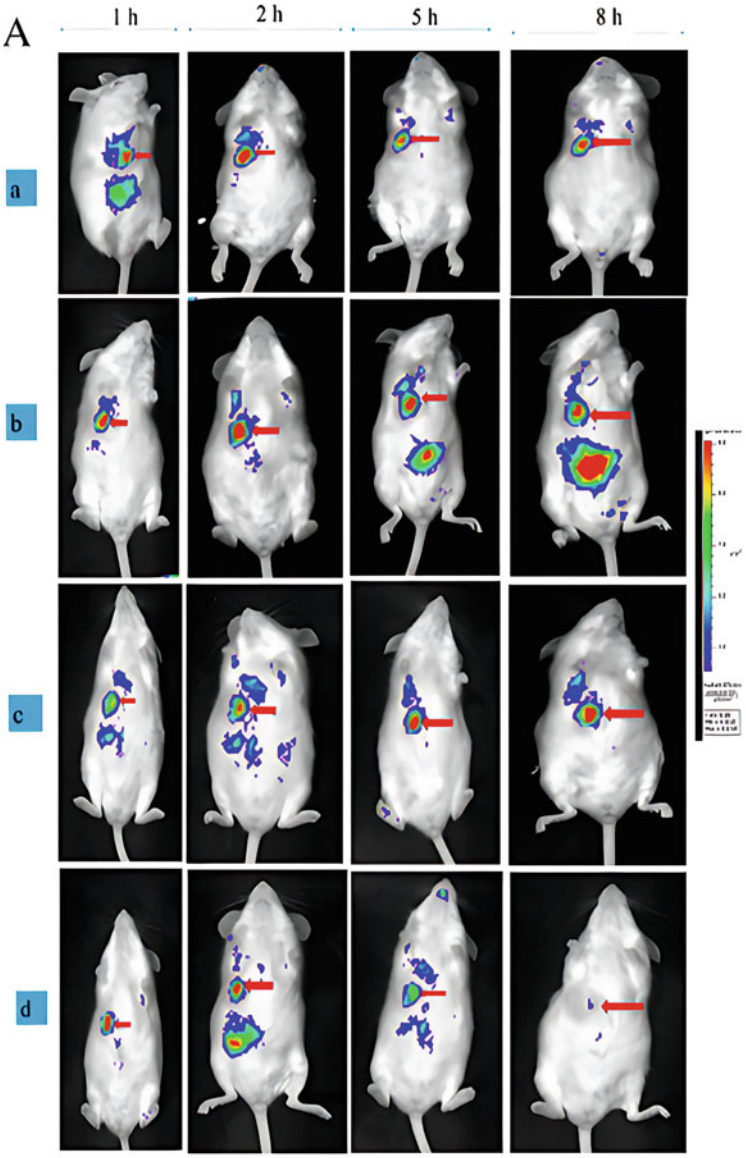

Fig. 10.3 NIR-fluorescence imaging of tumour-bearing Kunming mice showing in vivo biodistribution of NLCs co-loaded with paclitaxel and QDs (Olerile et al. 2017)

10.5.2 Neurodegenerative Disorders

The brain is an important part of the human body and the control centre, which regulates the various biochemical functions. Neurodegenerative diseases are primarily characterized by progressive loss of selective neurons, which are important to perform various biochemical activity. The classification of neurodegenerative diseases is based on anatomical distribution like pyramidal and extrapyramidal disorders or depends on clinical features like dementia, Parkinson's disease, etc. The treatment for these disorders is available only to relieve symptoms and does not reduce or stop the progression of underlying pathology (Begley and Brightman 2003). The major concern for delivering the drug to the brain is the blood-brain barrier (BBB) which consists of tight junctions of cerebral endothelial cells, choroid plexus epithelial cells, and cells of the arachnoid epithelium. The extreme tight junction of the BBB notably reduces the penetration of polar solutes between systemic circulation to the brain (Kooij et al. 2005).

10.5.2.1 Alzheimer's Disease (AD)

It is a progressive neurodegenerative disease with dementia as the main symptom amongst older age individuals. AD gradually reduces one's ability to learn and understand. Due to the complexity of the brain, pathophysiology of AD is fully not known, although common histopathologic features include the aggregation of tau protein in intracellular neurofibrillary tangles and extracellular beta-amyloid plaques are considered as indicative markers (Winslow et al. 2011). With the complexity and multifactorial nature of AD, new therapeutic approaches such as QDs are widely exploited to target specific receptors. For example, Liu et al. (2015) developed GQD from the oxidation of graphene sheets in concentrated sulphuric acid which can prevent the accumulation of Aβ peptides. The GQDs synthesized were characterized by TEM and atomic force microscopy which showed average diameter of 8 nm. The researcher carried out the thioflavin T (ThT) fluorescence assay to investigate the $A\beta_{1-42}$ aggregate inhibition by the synthesized GQDs. The ThT which is a fluorescent dye undergoes combination with the amyloid fibrils resulting into the increase in fluorescent intensity with increasing conjugation. The aggregation of amyloid fibrils was observed at the end of lag phase with rapid increase in fluorescent intensity. The presence of GQDs exhibited decrease in ThT fluorescent value indicating the suppression of amyloid fibrils by the prepared GQDs. The investigation revealed that the GQDs suppressed the formation of amyloid fibrils and also indicates that the peptide (glycine-proline-glutamate) is a promising candidate in functionalization of QDs (Liu et al. 2015). Similarly, Xiao and co-workers have designed GQDs and conjugated with peptide glycine-proline-glutamate for inhibition of the $A\beta_{1-42}$ aggregation. Results of ThT assay revealed better inhibitory effect towards Aβ fibrils in comparison to the resveratrol used as a reference compound (Xiao et al. 2016).

10.5.2.2 Parkinson's Disease

It is the second most prevailing neuro-disorder following AD. The loss of dopaminergic neurons in substantive nigra is a common feature of Parkinson. Mitochondrial dysfunction, apoptosis, oxidative stress, etc. are the other major pathogenesis of Parkinson's disease (Sarkar et al. 2016). In recent years, GQDs are proved to be effective in preventing α-synuclein accumulation and fibrillation. GQDs dissolve the adult fibres by direct reaction and reduce mitochondrial disorders. For instance, Kim et al. (2018) investigated the GQDs role in causing disaggregation of fibrils through direct interaction and inhibit fibrilization of α-synuclein fibrils. The TEM images revealed that the GQD-treated α-synuclein fibrils showed an increase in the number of short fibril fragments during the initial 24-h incubation due to the dissociation, while the fibrils' number started to decrease from third day. At the end of the seventh day, the fibril fragments were not detectable due to the rapid reduction, which indicated the fibril dissociation by the GQDs (Kim et al. 2018). The major problem with the traditional drug delivery system is its poor penetration capacity through BBB, but the nanocarriers such as GQD could improve the BBB permeability due to its unique physical and chemical properties and nanometric size. One such research was carried out with GQD-biotin conjugate. The results of immunohistochemical analysis of the brain confirmed the brain penetration and accessibility of GQD-biotin conjugate (Kim et al. 2018). Researches published in the past few years revealed that theranostic nanocarriers are found to be beneficial in diagnosis of various neurodegenerative disorders.

10.5.3 Infectious Diseases

Infectious diseases are the second leading cause of death worldwide. Acute lower respiratory tract infections, HIV/AIDS, diarrheal diseases, tuberculosis, and malaria are the infectious diseases that cause most of the deaths globally (Bagre et al. 2022; Fauci 2001). QDs have superior luminescent properties and easy surface functionalization capacity, which favours its use for bioimaging. Mostly inorganic QDs like CdSe, CdTe, CuO, and ZnO are the best choices for bioimaging as they possess excellent luminescent properties. The mechanism of QDs as an antimicrobial is due its tendency to (i) generate reactive oxygen species in biological system, (ii) induce cell membrane degradation, and (iii) inhibit cell proliferation. Martinez et al. (2018), developed clinical imaging tools by using luminescent ZnO QDs, which have a high surface to volume ratio and strong surface chemistry, fulfilling all the requirements to adequately treat bacterial infections (Martínez-Carmona et al. 2018). For example, Łoczechin et al. (2019) investigated the role of seven different CQDs which were functionalized with boronic acid for antiviral activity to treat human coronavirus (HCoV-229E) infections. The scientist synthesized two generations of CQDs to investigate the inhibition of host cell infections by HCoV-229E coronavirus. The first-generation boronic acid-modified CQDs (i.e. CQDs-1, CQDs-2, CQDs-3, and CQDs-4) were synthesized by using citric acid and ethylenediamine as starting material. The second-generation CQDs (i.e. CQDs-5,

CQDs-6, and CQDs-7) were obtained by the hydrothermal carbonization of phenylboronic acid and 4-aminophenylboronic acid. The cytotoxicity assay was caried out by using Huh-7 cell lines (an immortal cell line composed of epithelial-like, tumourigenic cells). The results showed that these different CQDs were biocompatible and have low cellular toxicity even at higher concentration (100 $\mu g/mL^{-1}$). Further, the CQDs were also found to be responsible for the decrease in replication rate of virus due to some receptor-based interaction with viral surfaces (Łoczechin et al. 2019). QD-based nanotheranostic carrier was found effective in diagnosis as well as treating various infectious diseases.

10.5.4 Gene Delivery

It is a newer strategy used in the treatment of various hereditary and genetic disorders. The combination of QDs as bioimaging tool with drug or gene delivery forms a nanohybrid which improves the efficiency in delivery of drugs and offers a breakthrough in the diagnosis strategy. Gene delivery offers much better clinical outcomes for inherited diseases compared to other treatment options. Yang et al. (2014) studied the effect of PEI-coated QDs and its efficiency for gene delivery into human mesenchymal stem cells (hMSCs). The hMSCs were treated with various types of QD-bundled nanoparticles (named as Qdot 525, Qdot 565, Qdot 605, and Qdot 655 which have incrementally higher fluorescent intensity) and then transplanted into Balb/c mice for the in vivo investigation of gene delivery and cell-tracking behaviour by QDs. Using the bioimaging techniques like confocal laser scanning microscopy, the gene complexed QD-bundled NPs were successfully detected in the hMSCs as shown in Fig. 10.4 (Yang et al. 2014).

Zuo et al. (2018) proposed the design of fluorine-doped CDs (FCDs) for gene delivery using tetrafluoroterephthalic acid as fluorine source while branched polyethylenimine (b-PEI) is used to create positive charge site. The positive charges of b-PEI facilitated binding with DNA having negative charge. The researcher observed that the FCDs showed high gene delivery efficiency in stem cells, primary cells, and other common cell lines. This is mainly due to the doping of CDs by fluorine which enhanced the affinity of encapsulated DNA to the cytomembrane. It

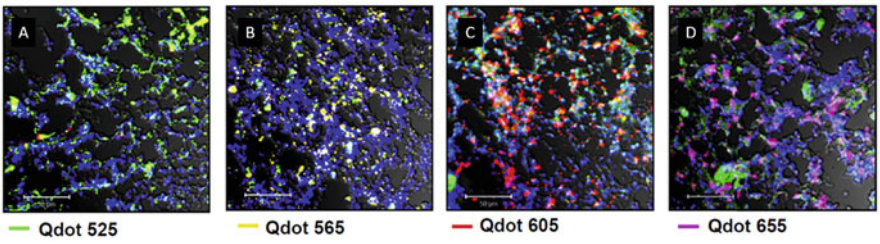

Fig. 10.4 Confocal fluorescence image of transplanted region with multiple QD-bundled NP-transfected hMSCs (Yang et al. 2014)

was also concluded that the FCDs exhibited high gene delivery efficiency with low DNA dose as well as at high concentration of serum. The evaluation of the imaging property of the FCDs was carried out using the HEK 293 T cells and A549 cells. A bright fluorescence was observed due to the staining of the cytoplast indicating that FCDs crossed through the cell membrane and entered into the cell (Zuo et al. 2018).

Overall, QDs were found advantageous in diagnosis and imaging of various diseases. Moreover, functionalization of QDs or incorporation of QDs in various nanocarriers also decrease its toxicity and improve the biocompatibility. Specifically, for cancer treatment, QD-based theranostic nanocarriers are found beneficial in tracking the tumour suppression at regular intervals.

10.6 Safety and Toxicity Perspectives of Quantum Dots

QDs emerged as a possible replacement for organic dyes and find major applications in biological imaging, drug delivery, and biosensing application due to its imperative benefits like excellent fluorescent properties, good chemical stability, and broad excitation range. Besides all these advantages and applications, the toxicity of QDs is a major concern, which restricts its usage in day-to-day life. The physicochemical properties of individual QDs determine their potential toxicity that mostly depends on parameters like size, starting material, dose, route of administration, surface charge, etc. In general, it was observed that inorganic QDs were more toxic towards the biological system compared to the CQDs (Reshma and Mohanan 2019).

The extensive use of QDs for in vivo studies has been minimal due to the toxicity induced by the leakage of heavy metal ions into biological systems from As-, Cd-, and Pb-containing QDs (II–IV, IV–VI, and III–V groups of periodic tables). The toxicity of QDs is also governed by the surface of the QDs, covered by the shells or protected by ligands. The toxicity of QDs causes different DNA damages and affects cell growth, proliferation, and cell viability. The mechanisms explaining the toxicity of QDs are charge-induced disruption of cell membrane, formation of free radicals, and interaction of QDs with cellular components. The inner metal core is made up of toxic metals like As, Cd, Se, Te, and Pb shielded by the outer surface layers mostly ZnS shell which enhance hydrophilicity as well as quantum yield resulting into increased solubility as well as macromolecule interactions. The addition of a shell (ZnS or silica) to the core reduces oxidation and degradation. Photolytic reactions, oxidations, and low-pH conditions caused degradation of the outermost layer leading to the release of toxic substances in biological system. To minimize the toxicity of the QDs, scientists have explored surface modification strategies like functionalization and polymerization. Nagy et al. (2012) used CdSe QDs and tested them on primary human cells to find the contribution of surface charge, size, and functionalization. Scientists have observed that toxicity induced by the QDs was independent to generation of reactive oxygen species. Additionally, toxicity induced by negatively charged QDs was due to increased gene expression of pro-inflammatory cytokines, while more toxicity was observed by positively charged QDs due to its ability to change genes associated with mitochondrial

function. Also, the researchers have concluded that surface charge has the highest while particle size and functionalization have comparatively lower impact on biocompatibility of QDs (Nagy et al. 2012).

Derfus et al. (2004) reported the in vivo toxicity of CdSe-core QDs using primary hepatocytes as a liver model. More than 25% of Cd accumulated in the rat liver. The hepatic injury is due to the Cd binding to mitochondrial proteins specifically the sulphhydryl groups (Derfus et al. 2004). Chong et al. (2014) performed multiple-dosing toxicity study to evaluate the biosafety of GQD-PEG via in vitro and in vivo assessment. The in vitro study showed that GQDs have very low cellular toxicity due to its high oxygen content and small size (Chong et al. 2014).

Overall, multiple research articles have discussed the toxicity issue related to inorganic and highly charged QDs. Scientific literature also revealed that proper surface functionalization as well as amphiphilic QDs can be explored to enhance the biocompatibility.

10.7 Clinical Status of Quantum Dots

QDs have been used as a diagnostic tool, in drug delivery, biological imaging, and biosensor. The toxicity and the clinical aspect of QDs become crucial for their use in the human body. Numerous researches are available suggesting the positive outcomes of QDs especially CQDs which exhibit relatively low toxicity (Nair et al. 2020). Specifically, in vivo studies with QDs showed satisfactory results in finding tumour location and its size in cancer diagnosis.

Several clinical trials involving QDs have been reported in recent times showing its clinical implementation in various disease treatments. Table 10.3 summarized the QDs currently in clinical trials.

10.8 Current Challenges and Future Aspects

The major drawback of QDs is their hazardous cytotoxic and unwanted immune reactions caused by the capping materials and heavy metals. Another major concern is the smaller-sized QD complexes which prevent renal excretion and get concentrated in the liver. Raw QDs have been observed to elicit cytotoxicity in in vitro and in vivo experiments in various research. Although these challenges restrict the application of QDs in biomedical field, however plethora of researches on surface passivation of QDs have shown improvement in biocompatibility of QDs with significant reduction in its cytotoxicity (Jha et al. 2018). QDs decorated with target-specific ligands could be explored successfully in diagnosis and management of cancer and other fatal disease in near future. Further, luminescent hydrophilic QDs could also be another promising candidate for cell and biomolecule labelling (Ahmad et al. 2022; Iga et al. 2007; Patel et al. 2023).

Table 10.3 List of clinical trials on QDs

Study details	Conditions	Interventions	Probable outcomes
Topical fluorescent nanoparticle-conjugated somatostatin analogue for suppression and bioimaging of breast cancer [recruiting] Phase – I	• Breast cancer • Skin cancer • Skin disease	**Drug:** QDs coated with veldoreotide **Drug:** Topical approved placebo	Stable topical QDs coated with veldoreotide Growth inhibition was measured using the sulforhodamine B-based assay Amount of QDs-veldoreotide in the breast periphery was measured using fluorescence-based flow cytometry Growth inhibition was measured by visual determination of breast cancer cells.
Clinical trials of photoelectrochemical immunosensor for early diagnosis of acute myocardial infarction [not yet recruiting]	Acute myocardial infarction	**Device:** Graphene QDs combined with Si nanowire photoelectrochemical immunosensor	Effectiveness of photoelectrochemical immunosensor

10.9 Conclusion

In current time frame, QDs are considered as superior fluorescent agent compared to the traditional dyes, which are costly and toxic. The easy production methods and superordinate fluorescence-producing capacity without photobleaching made QDs assessable for treatment and diagnosis of various diseases. Despite all these benefits, there is still lack of information available regarding degradation behaviour, pharmacokinetic parameters, toxicological evaluation, and regulatory status of QDs. Therefore, more experiments should be done, and much more data should be available, to be sure to do actual clinical applications on humans.

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References

Ahmad J, Rizwanullah M, Suthar T, Albarqi HA, Ahmad MZ, Vuddanda PR et al (2022) Receptor-targeted surface-engineered nanomaterials for breast cancer imaging and theranostic applications. Crit Rev Ther Drug Carrier Syst 39(6):1–44

Arora N, Sharma NN (2014) Arc discharge synthesis of carbon nanotubes: comprehensive review. Diam Relat Mater 50:135–150. https://doi.org/10.1016/j.diamond.2014.10.001

Begley DJ, Brightman MW (2003) Structural and functional aspects of the blood-brain barrier. Prog Drug Res 61:39–78

Bagre A, Patel PR, Naqvi S, Jain K (2022) Emerging concerns of infectious diseases and drug delivery challenges. In: Nanotheranostics for treatment and diagnosis of infectious diseases. Elsevier, pp 1–23. ISBN: 9780323912013

Bajwa N, Mehra NK, Jain K, Jain NK (2016a) Pharmaceutical and biomedical applications of quantum dots. Artif Cells Nanomed Biotechnol 44(3):758–768

Bajwa N, Kumar Mehra N, Jain K, Kumar Jain N (2016b) Targeted anticancer drug delivery through anthracycline antibiotic bearing functionalized quantum dots. Artif Cells Nanomed Biotechnol 44(7):1774–1782

Bentzen EL, Tomlinson ID, Mason J, Gresch P, Warnement MR, Wright D et al (2005) Surface modification to reduce nonspecific binding of quantum dots in live cell assays. Bioconjug Chem 16(6):1488–1494

Bera D, Qian L, Tseng TK, Holloway PH (2010) Quantum dots and their multimodal applications: a review. Materials 3(4):2260–2345

Biazar N, Poursalehi R, Delavari H (2018) Optical and structural properties of carbon dots/TiO2 nanostructures prepared via DC arc discharge in liquid. AIP Conf Proc 1920:1–5

Bilan R, Fleury F, Nabiev I, Sukhanova A (2015) Quantum dot surface chemistry and functionalization for cell targeting and imaging. Bioconjug Chem 26(4):609–624

Bourlinos AB, Trivizas G, Karakassides MA, Baikousi M, Kouloumpis A, Gournis D et al (2015) Green and simple route toward boron doped carbon dots with significantly enhanced non-linear optical properties. Carbon N Y 83:173–179. https://doi.org/10.1016/j.carbon.2014.11.032

Brichkin SB, Razumov VF (2016) Colloidal quantum dots: synthesis, properties and applications. Russ Chem Rev 85(12):1297–1312

Brunetti J, Piantini S, Fragai M, Scali S, Cipriani G, Depau L, Pini A, Falciani C, Menichetti S, Bracci L (2020) A new NT4 peptide-based drug delivery system for cancer treatment. Molecules 25(5):1088

Cai X, Luo Y, Zhang W, Du D, Lin Y (2016) PH-sensitive ZnO quantum dots-doxorubicin nanoparticles for lung cancer targeted drug delivery. ACS Appl Mater Interfaces 8(34): 22442–22450

Calabro RL, Yang DS, Kim DY (2018) Liquid-phase laser ablation synthesis of graphene quantum dots from carbon nano-onions: comparison with chemical oxidation. J Colloid Interface Sci 527: 132–140. https://doi.org/10.1016/j.jcis.2018.04.113

Cayuela A, Soriano ML, Carrillo-Carrión C, Valcárcel M (2016) Semiconductor and carbon-based fluorescent nanodots: the need for consistency. Chem Commun 52(7):1311–1326

Chong Y, Ma Y, Shen H, Tu X, Zhou X, Xu J et al (2014) The in vitro and in vivo toxicity of graphene quantum dots. Biomaterials 35(19):5041–5048

D'souza SL, Chettiar SS, Koduru JR, Kailasa SK (2018) Synthesis of fluorescent carbon dots using Daucus carota subsp. sativus roots for mitomycin drug delivery. Optik (Stuttg) 158:893–900. https://doi.org/10.1016/j.ijleo.2017.12.200

Depeursinge A, Racoceanu D, Iavindrasana J, Cohen G, Platon A, Poletti P-A et al (2010) Fusing visual and clinical information for lung tissue classification in HRCT data. Artif Intell Med 11: ARTMED1118

Derfus AM, Chan WCW, Bhatia SN (2004) Probing the cytotoxicity of semiconductor quantum dots. Nano Lett 4(1):11–18

Ding Y, Shen SZ, Sun H, Sun K, Liu F (2014) Synthesis of l-glutathione-capped-ZnSe quantum dots for the sensitive and selective determination of copper ion in aqueous solutions. Sensors Actuators B Chem 203:35–43. https://doi.org/10.1016/j.snb.2014.06.054

Fauci AS (2001) Infectious diseases: considerations for the 21st century. Clin Infect Dis 32(5):675–685

Fung J, Fong Y, Chin SF, Ng SM (2016) Author ' s accepted manuscript carbon dots as sensing probe. Biosens Bioelectron. https://doi.org/10.1016/j.bios.2016.05.087

Gidwani B, Sahu V, Shankar S, Pandey R, Joshi V (2021) Quantum dots : prospectives , toxicity , advances and applications. J Drug Deliv Sci Technol 61(January):102308. https://doi.org/10.1016/j.jddst.2020.102308

Gonçalves H, Jorge PAS, Fernandes JRA, Esteves da Silva JCG (2010) Hg(II) sensing based on functionalized carbon dots obtained by direct laser ablation. Sensors Actuators B Chem 145(2):702–707

Guo CX, Zhao D, Zhao Q, Wang P, Lu X (2014) Na+−functionalized carbon quantum dots: a new draw solute in forward osmosis for seawater desalination. Chem Commun 50(55):7318–7321

He H, Wang X, Feng Z, Cheng T, Sun X, Sun Y et al (2015) Rapid microwave-assisted synthesis of ultra-bright fluorescent carbon dots for live cell staining, cell-specific targeting and in vivo imaging. J Mater Chem B 3(24):4786–4789

History of Quantum Dots – Nexdot. [cited 2022 Jun 24]. Available from: https://nexdot.fr/en/history-of-quantum-dots/

Hou Y, Lu Q, Deng J, Li H, Zhang Y (2015) Analytica Chimica acta one-pot electrochemical synthesis of functionalized fl uorescent carbon dots and their selective sensing for mercury ion. Anal Chim Acta:1–6. https://doi.org/10.1016/j.aca.2015.01.039

Iga AM, Robertson JHP, Winslet MC, Seifalian AM (2007) Clinical potential of quantum dots. J Biomed Biotechnol 2007

Jiang Y, Han Q, Jin C, Zhang J, Wang B (2015) A fluorescence turn-off chemosensor based on N-doped carbon quantum dots for detection of Fe3+ in aqueous solution. Mater Lett 141:366–368. https://doi.org/10.1016/j.matlet.2014.10.168

Jha S, Mathur P, Ramteke S, Jain NK (2018) Pharmaceutical potential of quantum dots. Artif Cells Nanomed Biotechnol 46(sup1):57–65. https://doi.org/10.1080/21691401.2017.1411932

Kaczmarek A, Hoffman J, Morgiel J, Mościcki T, Stobiński L, Szymański Z et al (2021) Luminescent carbon dots synthesized by the laser ablation of graphite in polyethylenimine and ethylenediamine. Materials (Basel) 14(4):1–13

Kargozar S, Hoseini SJ, Milan PB, Hooshmand S, Kim HW, Mozafari M (2020) Quantum dots: a review from concept to clinic. Biotechnol J 15(12):2000117

Kasotakis E, Kostopoulou A, Spuch-Calvar M, Androulidaki M, Pelekanos N, Kanaras AG et al (2014) Assembly of quantum dots on peptide nanostructures and their spectroscopic properties. Appl Phys A Mater Sci Process 116(3):977–985

Khayal A, Dawane V, Yadav VK, Yadav KK, Khan SH (2021) State of art Technology : synthesis of carbon dots and their potential applications in biomedical. Res Environ Remediation. (August)

Kim D, Yoo JM, Hwang H, Lee J, Lee SH, Yun SP et al (2018) Graphene quantum dots prevent α-synucleinopathy in Parkinson's disease. Nat Nanotechnol 13(9):812–818. https://doi.org/10.1038/s41565-018-0179-y

Kooij G, Van Horssen J, De Vries E (2005) Tight junctions of the blood-brain barrier. Blood-Brain Barrier Microenviron Basic Physiol Neurol Dis 38:47–69

Lei KW, West T, Zhu XY (2013) Template-assembly of quantum dot molecules. J Phys Chem B 117(16):4582–4586

Li H, He X, Liu Y, Huang H, Lian S, Lee ST et al (2011) One-step ultrasonic synthesis of water-soluble carbon nanoparticles with excellent photoluminescent properties. Carbon N Y 49(2):605–609. https://doi.org/10.1016/j.carbon.2010.10.004

Li Z, Yu H, Bian T, Zhao Y, Zhou C, Shang L et al (2015) Highly luminescent nitrogen-doped carbon quantum dots as effective fluorescent probes for mercuric and iodide ions. J Mater Chem C 3(9):1922–1928

Liu Y, Xu LP, Dai W, Dong H, Wen Y, Zhang X (2015) Graphene quantum dots for the inhibition of β amyloid aggregation. Nanoscale 7(45):19060–19065

Łoczechin A, Séron K, Barras A, Giovanelli E, Belouzard S, Chen YT et al (2019) Functional carbon quantum dots as medical countermeasures to human coronavirus. ACS Appl Mater Interfaces 11(46):42964–42974

Lu M, Zhou L (2019) Materials Science & Engineering C one-step sonochemical synthesis of versatile nitrogen-doped carbon quantum dots for sensitive detection of Fe 2 + ions and temperature in vitro. Mater Sci Eng C 101(March):352–359. https://doi.org/10.1016/j.msec.2019.03.109

Ludmerczki R, Mura S, Carbonaro CM, Mandity IM, Carraro M, Senes N et al (2019) Carbon dots from citric acid and its intermediates formed by thermal decomposition. Chem A Eur J 25(51):11963–11974

Martínez-Carmona M, Gun'Ko Y, Vallet-Regí M (2018) Zno nanostructures for drug delivery and theranostic applications. Nano 8(4):1–27

Martynenko IV, Litvin AP, Purcell-Milton F, Baranov AV, Fedorov AV, Gun'Ko YK (2017) Application of semiconductor quantum dots in bioimaging and biosensing. J Mater Chem B 5(33):6701–6727

Matea CT, Mocan T, Tabaran F, Pop T, Mosteanu O, Puia C et al (2017) Quantum dots in imaging, drug delivery and sensor applications. Int J Nanomedicine 12:5421–5431

Mozafari M, Moztarzadeh F, Seifalian AM, Tayebi L (2013) Self-assembly of PbS hollow sphere quantum dots via gas-bubble technique for early cancer diagnosis. J Lumin 133:188–193. https://doi.org/10.1016/j.jlumin.2011.12.054

Nagy A, Steinbrück A, Gao J, Doggett N, Hollingsworth JA, Iyer R (2012) Comprehensive analysis of the effects of CdSe quantum dot size, surface charge, and functionalization on primary human lung cells. ACS Nano 6(6):4748–4762

Nair A, Haponiuk JT, Thomas S, Gopi S (2020) Natural carbon-based quantum dots and their applications in drug delivery: a review. Biomed Pharmacother [Internet] 132:110834. https://doi.org/10.1016/j.biopha.2020.110834

Narayanan KB, Sakthivel N (2010) Biological synthesis of metal nanoparticles by microbes. Adv Colloid Interf Sci 156(1–2):1–13. https://doi.org/10.1016/j.cis.2010.02.001

Nguyen V, Zhao N, Yan L, Zhong P, Nguyen VC, Le PH (2019) Double-pulse femtosecond laser ablation for synthesis of ultrasmall carbon nanodots. Mater Res Express 7:1

Olerile LD, Liu Y, Zhang B, Wang T, Mu S, Zhang J et al (2017) Near-infrared mediated quantum dots and paclitaxel co-loaded nanostructured lipid carriers for cancer theragnostic. Colloids Surf B Biointerfaces 150:121–130. https://doi.org/10.1016/j.colsurfb.2016.11.032

Patel P, Kumar K, Jain VK, Popli H, Yadav AK, Jain K (2023) Nanotheranostics for diagnosis and treatment of breast cancer. Curr Pharm Des 29(10):732–747

Pawar SP, Gore AH, Walekar LS, Anbhule PV, Patil SR, Kolekar GB (2015) Turn-on fluorescence probe for selective and sensitive detection of d-penicillamine by CdS quantum dots in aqueous media: application to pharmaceutical formulation. Sensors Actuators B Chem 209:911–918. https://doi.org/10.1016/j.snb.2014.12.064

Peng H, Travas-Sejdic J (2009) Simple aqueous solution route to luminescent carbogenic dots from carbohydrates. Chem Mater 21(23):5563–5565

Piccinno F, Gottschalk F, Seeger S, Nowack B (2012) Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world. J Nanopart Res 14:9

Prakash A, Sharma S, Ahmad N, Ghosh A, Sinha P (2010) Bacteria mediated extracellular synthesis of metallic nanoparticles. Int Res J Biotechnol 1(5):71–79

Qu K, Wang J, Ren J, Qu X (2013) Carbon dots prepared by hydrothermal treatment of dopamine as an effective fluorescent sensing platform for the label-free detection of iron(III) ions and dopamine. Chem A Eur J 19(22):7243–7249

Ray SC, Saha A, Jana NR, Sarkar R (2009) Fluorescent carbon nanoparticles: synthesis, characterization, and bioimaging application. J Phys Chem C 113(43):18546–18551

Reshma VG, Mohanan PV (2019) Quantum dots: applications and safety consequences. J Lumin 205:287–298

Rizvi SB, Yildirimer L, Ghaderi S, Ramesh B, Seifalian AM, Keshtgar M (2012) A novel POSS-coated quantum dot for biological application. Int J Nanomedicine 7:3915–3927

Rosenthal SJ, Chang JC, Kovtun O, Mcbride JR, Tomlinson ID (2011) Review biocompatible quantum dots for biological applications. Chem Biol 18(1):10–24. https://doi.org/10.1016/j.chembiol.2010.11.013

Sarkar S, Raymick J, Imam S (2016) Neuroprotective and therapeutic strategies against Parkinson's disease: recent perspectives. Int J Mol Sci 17:6

Sharma A, Das J (2019) Small molecules derived carbon dots: synthesis and applications in sensing, catalysis, imaging, and biomedicine. J Nanobiotechnol 17(1):1–24. https://doi.org/10.1186/s12951-019-0525-8

Shen J, Zhu Y, Yang X, Zong J, Zhang J, Li C (2012) One-pot hydrothermal synthesis of graphene quantum dots surface-passivated by polyethylene glycol and their photoelectric conversion under near-infrared light. New J Chem 36(1):97–101

Su Y, Xie M, Lu X, Wei H, Geng H, Yang Z et al (2014) Facile synthesis and photoelectric properties of carbon dots with upconversion fluorescence using arc-synthesized carbon by-products. RSC Adv 4(10):4839–4842

Sun YP, Zhou B, Lin Y, Wang W, Fernando KAS, Pathak P et al (2006) Quantum-sized carbon dots for bright and colorful photoluminescence. J Am Chem Soc 128(24):7756–7757

Vasudevan D, Gaddam RR, Trinchi A, Cole I (2015) Core-shell quantum dots: properties and applications. J Alloys Compd 636(February):395–404. https://doi.org/10.1016/j.jallcom.2015.02.102

Wan JY, Yang Z, Liu ZG, Wang HX (2016) Ionic liquid-assisted thermal decomposition synthesis of carbon dots and graphene-like carbon sheets for optoelectronic application. RSC Adv 6(66):61292–61300

Wang D, Xie T, Li Y (2009) Nanocrystals: solution-based synthesis and applications as nanocatalysts. Nano Res 2(1):30–46

Wang Q, Zhang C, Shen G, Liu H, Fu H, Cui D (2014) Fluorescent carbon dots as an efficient siRNA nanocarrier for its interference therapy in gastric cancer cells. J Nanobiotechnol 12(1):1–12

Winslow BT, Onysko M, Stob CM, Hazlewood KA (2011) Treatment of Alzheimer disease. Am Fam Physician 83(12):1403–1412

Wu X, Liu H, Liu J, Haley KN, Treadway JA, Larson JP et al (2003) Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. Nat Biotechnol 21(1):41–46

Wu YF, Wu HC, Kuan CH, Lin CJ, Wang LW, Chang CW et al (2016) Multi-functionalized carbon dots as theranostic nanoagent for gene delivery in lung cancer therapy. Sci Rep 6(October 2015):1–12. https://doi.org/10.1038/srep21170

Xiao S, Zhou D, Luan P, Gu B, Feng L, Fan S et al (2016) Graphene quantum dots conjugated neuroprotective peptide improve learning and memory capability. Biomaterials 106:98–110. https://doi.org/10.1016/j.biomaterials.2016.08.021

Xu H (2016) Fluorescence properties of quantum dots and their utilization in bioimaging. 1–2 p. https://www.diva-portal.org/smash/get/diva2:957626/FULLTEXT02.pdf

Yang HN, Park JS, Jeon SY, Park W, Na K, Park KH (2014) The effect of quantum dot size and poly(ethylenimine) coating on the efficiency of gene delivery into human mesenchymal stem cells. Biomaterials 35(29):8439–8449. https://doi.org/10.1016/j.biomaterials.2014.06.024

Yang ZC, Li X, Wang J (2011) Intrinsically fluorescent nitrogen-containing carbon nanoparticles synthesized by a hydrothermal process. Carbon 49(15):5207–5212

Yu Y, Zhang K, Sun S (2012) One-pot aqueous synthesis of near infrared emitting PbS quantum dots. Appl Surf Sci 258(18):7181–7187. https://doi.org/10.1016/j.apsusc.2012.04.031

Zhan HJ, Zhou PJ, He ZY, Tian Y (2012) Microwave-assisted aqueous synthesis of small-sized, highly luminescent CdSeS/ZnS core/shell quantum dots for live cell imaging. Eur J Inorg Chem 15:2487–2493

Zhang B, Liu CY, Liu Y (2010) A novel one-step approach to synthesize fluorescent carbon nanoparticles. Eur J Inorg Chem 28:4411–4414

Zheng M, Ruan S, Liu S, Sun T, Qu D, Zhao H, Xie Z, Gao H, Jing X, Sun Z (2015) Self-targeting fluorescent carbon dots for diagnosis of brain cancer cells. ACS Nano 9(11):11455–11461

Zhou J, Booker C, Li R, Zhou X, Sham TK, Sun X, Ding Z (2007) An electrochemical avenue to blue luminescent nanocrystals from multiwalled carbon nanotubes (MWCNTs). J Am Chem Soc 129(4):744–745

Zuo G, Xie A, Pan X, Su T, Li J, Dong W (2018) Fluorine-doped cationic carbon dots for efficient gene delivery. ACS Appl Nano Mater 1(5):2376–2385

Functional Nanogels and Hydrogels: A Multipronged Nanotherapy in Drug Delivery and Imaging

11

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Abstract

Materials like nanogel and hydrogel have numerous uses, especially in the biomedical and pharmaceutical sectors. Their abilities to load pharmaceuticals of all kinds, including hydrophobic ones and biomolecules, while maintaining form and mechanical qualities, as well as their capacity to absorb large amounts of aqueous solutions, offer a notion of their diversity and growing demand. Numerous techniques of synthesis have been identified, particularly for chemical/permanent hydrogels, as they have been studied extensively over a long period of time. Like this, stimuli-responsive hydrogels, commonly referred to as intelligent materials, have been investigated in order to improve the regulation of qualities like targeting and drug release. The uses for the so-called twenty-first-century materials have been expanded even further by studying hydrogel on the micro- and nanoscales and manipulating the particle size. Our goal in writing this article was to provide a summary of recent research on the synthesis processes, biological uses, and pharmaceutical applications of macro-, micro-, and nanogels.

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Keywords

Gels · Nanogels · Hydrogels · Stimuli responsive · Biomedical · Pharmaceutical applications

11.1 Introduction

Gels are characterized as semi-unbending frameworks in which the development of the scattering medium is confined by joining three-dimensional organization of particles or solvated macromolecules of the scattered stage. The word "gel" is taken from "gelatin" and both "gel" and "jam," which mean "freeze" or "coagulate" (Anitha et al. 2012; Anitha et al. 2011). This introduction shows the fundamental thought of a fluid setting to a solid-like material that doesn't stream, however is versatile and holds some fluid attributes. Utilization of the term "gel" as a grouping began during the last part of the 1800s as scientific experts endeavored to arrange semisolid substances as per their phenomenological attributes instead of their atomic syntheses. Around then, insightful strategies expected to decide compound constructions were deficient. The research fraternity has characterized gels (in some cases called jams) as semisolid frameworks containing either suspensions comprised of little inorganic particles or huge natural atoms interpenetrated by a fluid (Ali et al. 2011).

In drug applications, water and hydroalcoholic arrangements are generally normal. Numerous polymer gels show reversibility between the gel state and sol, which is the liquid stage containing the scattered or disintegrated macromolecule. Be that as it may, the arrangement of some polymer gels is irreversible because their chains are covalently fortified. The three-dimensional organizations framed in two-stage gels and jams are shaped by a few inorganic colloidal muds. The development of these inorganic gels is reversible. Gels are for the most part viewed as more unbending than jams since gels contain more covalent cross-links, a higher thickness of actual bonds, or just less fluid.

A hydrogel 3D structure is composed of hydrophilic polymeric or chemical compounds that may expand in aqueous solvent and hold large amounts of water, while preserving their appearance due to synthetic or actual cross-linking of the polymer chains. Water ought to establish basically small volume of the individual contents for the constituents of hydrogel. Because of their high water content, hydrogels also have a level of ability that is almost identical to that of regular tissue. Because of the presence of hydrophilic groups like amide, acids, acetamides, and bisulfates, the organization is hydrophilic. Hydrogels go through a large volume stage or sol-gel transition in response to physical aggitation like shaking the container (Bae et al. 2008). The change in temperature, electrical fields, pressure pH, particle size etc causes the conformational changes in the architecture of gel and results in sol-gel transition or vice versa. Such conformational changes are reversible and once the trigger like change in pressure etc are removed, the hydrogels regain their gel state (Bailon and Berthold 1998). The main determinants of, how hydrogels

respond to external modifications, are the constituent monomer of gel forming polymer, the surface charge, the pendant chains, and the degree of cross-linking. Numerous original works, surveys, and monographs are published, which focused on the composition, characteristics, and applications of hydrogels (Bazile et al. 1992; Bhaskaran and Lakshmi 2009).

Nanogel is composed of nanosize range of gel structure composed of various polymers (synthetic and natural). Nanotechnology have enabled the researchers to design various nanogel frameworks for sustained and controlled including targeted delivery of therapeutic agents. By applying fundamentals of polymer science, scientists have designed various nanogels, which has gained the remarkable attention toward nano-drug delivery system and tissue engineering stream with promising outcomes in the future (Baviskar et al. 2011; Bombardelli 1991). Briefly, the nanogels could be defined as the nanosized three-dimensional structures, which are composed of monomer units and having ability to swelled and spread with ability to deliver the drug molecules in controlled manner for therapeutic applications.

11.2 Hydrogels

Hydrogel comprises 3D network of hydrophilic polymers that can swell in water and store a considerable amount of water while preserving structural integrity. This is accomplished via the chemical or physical cross-linking of individual polymer chains (Fig. 11.1). A material must contain at least 10% water by weight (or volume) to be considered a hydrogel. Hydrogels are comparable to natural tissue in terms of flexibility due to their high water content (Chakravarthi et al. 2010; Chang et al. 2010). Hydrogels can absorb water due to the hydrophilic functional groups attached to the polymeric backbone, and they are resistant to disintegrating due to cross-links between network chains (Cheng et al. 2007). Hydrogels undergo a considerable volume-phase shift, also referred to as a solgel-phase changing, which is influenced by the specific chemical and physical factors.

In contrast to pH, ions, and particular chemical compositions, which are examples of chemical or biochemical stimuli, temperature, electric and magnetic fields, solvent composition, light intensity, and pressure are examples of physical stimuli. However, because these conformational changes are frequently reversible, the hydrogels can usually revert to their initial configuration after a reaction as soon as the trigger is taken away. The nature of hydrogels reaction to outside stimuli depends on the type of monomer, charge density, pendant chains, and degree of cross-linkage. According to the given external stimulus, the size of the response is similarly proportionate (Garcion et al. 2006; Wang et al. 2015; Fromen et al. 2016).

Over the past two decades, synthetic hydrogels, which have a longer service life, a larger water absorption capacity, and a higher gel strength, have steadily replaced natural hydrogels. On the other hand, synthetic polymers often have clearly defined structures that can be changed to achieve tailored degradability and utility. Completely synthetic materials can be used to create hydrogels. Additionally, it

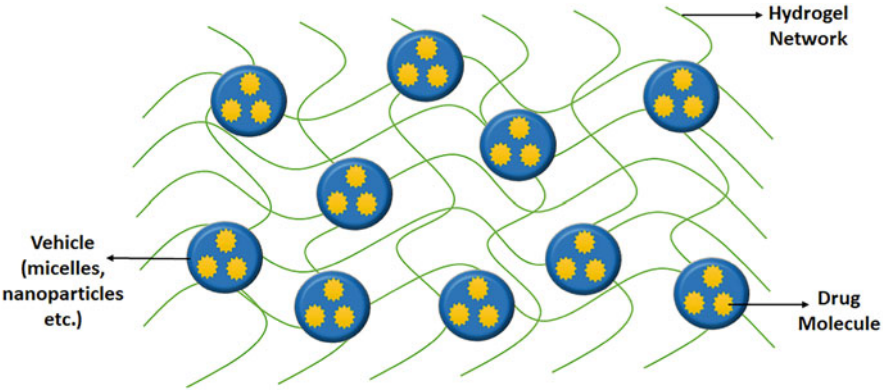

Fig. 11.1 Illustration of a typical hydrogel structure

maintains its stability in the presence of both considerable and abrupt temperature changes (Sorger et al. 2020).

The two- or multi-component hydrogels are composed of a three-dimensional network of polymer chains and water that connects macromolecules. Such structures in equilibrium can contain varying amounts of water depending on the properties of the polymer(s) used as well as the type and density of the network joints; typically, in the swollen state, the mass fraction of water in a hydrogel is much higher than the mass fraction of polymer. Water-soluble synthetic polymers that are cross-linked are often used to induce significant swelling (Sorger et al. 2020; David et al. 1997).

In addition to multistep processes like the synthesis of reactive polymer molecules and their subsequent cross-linking, which may involve reacting polymers with suitable cross-linking agents, one-step processes like polymerization and parallel cross-linking of multifunctional monomers can also be used to make hydrogels. The polymer engineer may create polymer networks with characteristics like biodegradation, mechanical strength, and chemical and biological reaction to stimuli. They can also manipulate the structure at the molecular level by changing the cross-linking density (Deshpandey et al. 2008).

11.2.1 Hydrogel Classification

The following criteria are used to categorize hydrogel products (Fig. 11.2).

11.2.1.1 Source

The hydrogels are categorized in two categories, i.e., natural polymeric hydrogel and synthetic polymer hydrogel (Dhar et al. 2008).

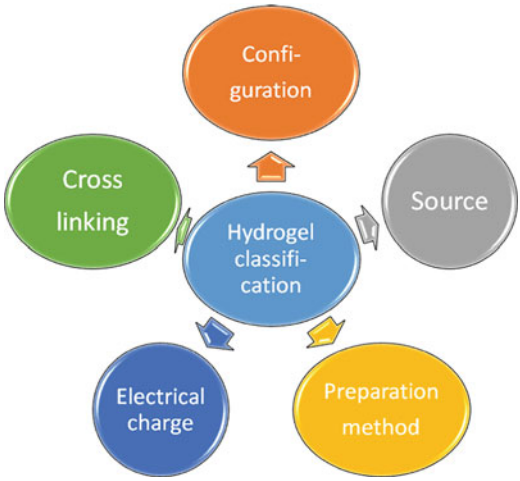

Fig. 11.2 Classification of hydrogels

11.2.1.2 Configuration

The structures of hydrogels can also be categorized as amorphous, semicrystalline, crystalline, and hydrocolloid aggregates.

11.2.1.3 Depending on the Cross-Linking Type

There are two categories for the chemical and physical nature of the cross-linked connection on the hydrogels. The chemically cross-linked hydrogels are stable complexed compared to the physical complexed network hydrogels, which contain momentary connection brought by the polymeric structure or by physical interaction like ionic bond, hydrogen bonds, or lipophilic bonds.

11.2.1.4 Based on the Electrical Charge

Based on the absence and presence of electrical charge on the cross-linked structure, the hydrogel is categorized in five groups:

- Nonionic (neutral).
- Ionic (including an ionic and cationic).
- Amphoteric electrolytes containing both acidic and basic groups.
- Zwitterionic (polybetaines) containing both an ionic and cationic groups in each structural repeating unit (Dreis et al. 2007; Esmaeili et al. 2008).

11.2.1.5 Based on the Method of Preparations

Some important kinds of hydrogels emerge because of the preparation procedure.

- **Homopolymeric:** Polymer network created from a single species of monomer, which is a basic structural unit constituting any polymer network, is referred to as homopolymeric hydrogels. Depending on the monomer and polymerization process, homopolymers may have a cross-linked skeletal structure (Garcia-Closas et al. 2007).

- **Copolymeric:** Copolymeric hydrogels are made up of two or more monomer species, each with at least one hydrophilic component, that are organized in a random, block, or alternating pattern along the polymer network's chain (Lee and Yoo 2008).
- **Multi-polymeric:** An important family of hydrogels is the interpenetrating polymeric hydrogel (IPN), which is made up of two cross-linked synthetic and/or natural polymer components held together in a network structure. One component is a cross-linked polymer, while the other is a non-cross-linked polymer in a semi-IPN hydrogel (Lee et al. 2009).

11.2.2 Technologies Adopted in Hydrogel Synthesis

Hydrogels are polymer networks having hydrophilic properties. While hydrophilic monomers are used to make most hydrogels, hydrophobic monomers are occasionally used to alter the features of hydrogel for specific events. Additionally, hydrogel can be synthesized by employing both natural and artificial polymers (Gill et al. 2011). The artificial polymers are chemically more powerful compared to the natural polymers. Their mechanical strength delays the pace of degradation, but it also contributes to durability. These two opposing features should be harmonized through ideal design. If the natural polymers have the right functional groups or have been functionalized with radically polymerizable groups, it can also be employed to create hydrogels from them (Pushpavanam et al. 2020). Some of the most common methods of hydrogel synthesis utilizing various polymer system, copolymer, and monomer are elaborated in Table 11.1.

The hydrogel comprises water-soluble structure that has undergone various sort of cross-linking to create an elastic structure. As a result, a hydrogel can be created using any method for creating a cross-linked polymer. Hydrogels are frequently created via copolymerization/cross-linking free radical polymerizations, which combine hydrophilic monomers with multifunctional cross-linkers. Hydrogels are produced via a variety of cross-linking techniques using hydrophilic straight polymer chain of both synthetic and natural polymer (Baldock et al. 2020):

- Linking polymer chains via chemical reaction.
- Using ionizing radiation to generate main-chain free radicals, which can recombine as cross-link junctions.
- Physical interactions such as entanglements, electrostatics, and crystallite formation (Rabaeh et al. 2021).

Gels can be produced using a variety of polymerization processes, including bulk, solution, and suspension polymerization. Monomer, initiator, and cross-linker are the three substances needed to make hydrogels. Diluents like water or other aqueous solutions can be employed to regulate the heat of polymerization and the characteristics of the resulting hydrogel (Gupta and Sharma 2011). The hydrogel mass must subsequently be cleaned to remove any impurities left over from the preparation process.

Table 11.1 Elaboration of various methods of hydrogel synthesis and their applications (Gill et al. 2011; Brubaker et al. 2016)

S. no.	Hydrogel	Monomer type	Cross-linker	Application
1	Self-assembled peptide hydrogel	Acrylate-modulated PEG hyaluronic acid	None	Tissue regeneration
2	Semi-interpenetrating system	Acrylic acid copolymer	N,N′-methylenebisacrylamide	Drug delivery
3	Copolymer hydrogel	Methacrylic acid, carboxy methyl cellulose	Tetraethylene glycol dimethacrylate	Drug delivery, dressing material
4	Interpenetrating system	Poly(N-isopropylacrylamide) chitosan	N,N′-methylenebisacrylamide	Drug delivery
5	Homopolymer hydrogel	Poly(2-hydroxyethyl methacrylate) polyethylene glycol (PEG)	PEG, tetraethylene glycol dimethacrylate	Drug delivery, contact lens material

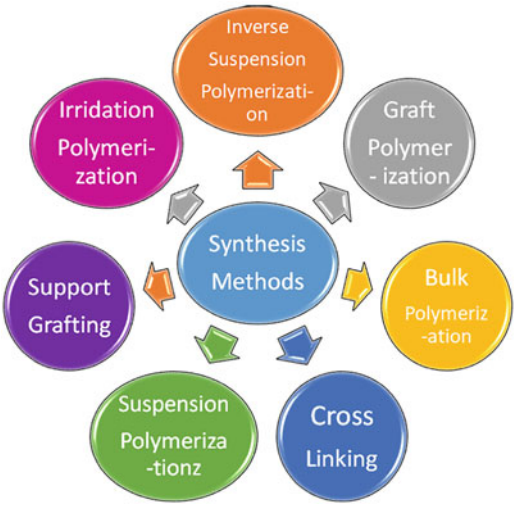

Fig. 11.3 Elaboration of synthesis methods of hydrogel

Unreacted monomers, initiators, cross-linkers, and unwanted compounds created by side reactions are just a few examples of these contaminants (Hana et al. 2009). The following describes the various hydrogel applications (Fig. 11.3).

11.2.2.1 Inverse-Suspension Polymerization

Based on acrylamide, acrylic acid, and its salts, hydrogels have been produced using diluted solution and inverse-suspension polymerization. Less research has been done on highly concentrated solution polymerization of patented acrylic monomers. In a concentrated (43.6 wt%) solution polymerization, the research fraternity has produced an acrylic acid-sodium acrylate superabsorbent using potassium persulfate as a thermal initiator. Typically, polar monomers are utilized to produce hydrogels. They can be classified into natural polymer hydrogels, synthetic polymer hydrogels, and combinations of the two classes based on their starting ingredients (Kohli et al. 2007; Houchin and Topp 2009).

11.2.2.2 Graft Polymerization

The graft polymerization-based nanogel can be produced using a variety of techniques, including graft polymerization, cross-linking polymerization, network growth of water-soluble polymers, and radiation cross-linking. There are many kinds of hydrogels, but the ones that are most frequently employed are weakly cross-linked acrylate and acrylic acid copolymers, grafted starch-acrylic acid polymers generated by inverse suspension, emulsion polymerization, and solution polymerization. The polymerization procedures are explained in the following sections (Hureaux et al. 2010).

11.2.2.3 Bulk Polymerization

Many vinyl monomers have the potential to be used in hydrogel production. One or more types of monomers can be used to make bulk hydrogels. Because of the large range of monomers available, it is possible to make a hydrogel with the appropriate

physical qualities for a specific application (Jain et al. 2010). In most hydrogel formulations, a modest amount of cross-linking agent is used. Radiation, UV light, or chemical catalysts are often employed to start the polymerization reaction. The types of solvents and monomers utilized have an impact on the suitable initiator selection. The polymerized hydrogel can be created in a variety of forms, including membranes, rods, particles, and emulsions. Bulk polymerization is the most basic method, simply requiring monomer and monomer-soluble initiators. The high concentration of monomer causes a high rate of polymerization and degree of polymerization (Jaiswal et al. 2010). The viscosity of the reaction, on the other hand, increases dramatically with the conversion that creates heat during polymerization.

These problems can be avoided by controlling the reaction at low conversions. A homogeneous hydrogel created by bulk polymerizing monomers yields a glassy, translucent, and very rigid polymer matrix. The glassy matrix expands and becomes soft and flexible when submerged in water (Jayakumar et al. 2011).

11.2.2.4 Solution Polymerization or Cross-Linking

The non-charge or ionic monomers are composite with the diverse cross-linking mediators in solution copolymerization or cross-linking reactions**.** UV irradiation or a redox initiator system is used to start the polymerization process. The existence of a heat sink in the form of a solvent is the primary advantage of solution polymerization versus bulk polymerization (Jeong et al. 2000). To eliminate the monomers, oligomers, cross-linking agent, initiator, soluble and extractable polymer, and other impurities, the produced hydrogel must be cleared with sterile water. When the water contents surpass the amount of water employed during polymerization, phase separation takes place with heterogeneous hydrogel formation. Water, ethanol, water-ethanol mixes, and benzoyl alcohol are common solvents for solution polymerization of hydrogels. After the gel has been formed, the synthesis solvent can be eliminated by expanding the hydrogel in water media (Joshi Jr and Phansopkar 2022).

11.2.2.5 Suspension Polymerization or Inverse-Suspension Polymerization

Dispersion polymerization is a beneficial method since no grinding is necessary because the products are obtained as powder or microspheres (beads). Because the water-in-oil (*W/O*) procedure is utilized instead of the more common oil-in-water (*O/W*) method, the polymerization is known as "inverse suspension" (Bergueiro et al. 2022).

This approach disperses the monomers and initiator as a homogenous mixture in the hydrocarbon phase. The monomer solution viscosity, agitation rate, rotor configuration, and dispersant type mostly determine the resin particle size and form. Hetero-phase polymerizations have already been the subject of various in-depth discussions. The dispersion requires continuous agitation as well as the addition of a low hydrophilic-lipophilic-balance (HLB) suspending agent because it is thermodynamically unstable (Kabanov and Vinogradov 2009).

11.2.2.6 Grafting to a Support

Hydrogels made by bulk polymerization have a fragile structure by nature. The hydrogel can also be spliced on the surface coated onto the powerful base to improve its mechanical qualities. This process includes generating free radicals on a stronger support surface and then polymerizing monomers directly onto it, resulting in a covalently bound chain of monomers. Hydrogels have been synthesized from a variety of polymeric substrates using grafting techniques (Zhenghong et al. 2009).

11.2.2.7 Polymerization by Irradiation

To prepare unsaturated compound hydrogels, high-energy ionizing radiation are employed as provoker. Production of radicals on the polymer chains occurs when an aqueous polymer solution is irradiated.

In addition, radiolysis of water molecules produces hydroxyl radicals, which attack polymer chains and result in the formation of macro-radicals (Zweers et al. 2004). The production of covalent bonds arises from the recombination of macro-radicals on distinct chains, resulting in the formation of a cross-linked structure. Polyvinyl alcohol, polyethylene glycol, and polypropylene glycol are examples of polymers cross-linked by the radiation technique (acrylic acid). The creation of reasonably pure and initiator-free hydrogels is a chief feature of radiation initiation over chemical initiator (Kim et al. 2008).

11.2.3 Properties of Hydrogel

The multifunctional features of ideal hydrogel materials are as follows:

- The highest absorption capacity (maximum equilibrium swelling) in saline.
- Desired rate of absorption (preferred particle size and porosity) depending on the application requirement.
- The highest absorbency under load (AUL).
- The lowest soluble content and residual monomer.
- The lowest price.
- The highest durability and stability in the swelling environment and during the storage.
- The highest biodegradability without formation of toxic species following the degradation.
- pH neutrality after swelling in water.
- Colorless, odorless, and nontoxic.
- Photo stability.
- Rewetting capability (if required): the hydrogel has to be able to give back the imbibed solution or to maintain it, depending on the application requirement (e.g., in agricultural or hygienic applications) (Kumar et al. 2015; Nambara et al. 2016; Brubaker et al. 2016; Kim et al. 2012).

A hydrogel sample cannot, of course, simultaneously satisfy all of the aforementioned criteria. The synthetic materials required to get the utmost level of some of these attributes will render the remaining ones ineffective. As a result, to achieve a suitable balance between the qualities, the production reaction variables must be tweaked through practice. For instance, drug delivery hydrogels must be porous and responsive to pH or temperature, whereas hygienic hydrogel products must have the maximum absorption capacity, and the minimal enduring monomer (Lee et al. 2011).

11.2.4 Theranostic Applications of Hydrogels

The hydrogels possess numerous applications in various sectors (Fig. 11.4) such as pharmaceutical industries, biomedical industries, agro-medical industries, etc.; some of the most important application are as follows.

11.2.4.1 Active and Passive Drug Delivery

By dispensing pharmaceutical at specific volume for predetermined time interval, controlled drug delivery systems (DDS) have been used to alleviate the limitations of conventional drug formulations. Due to their remarkable qualities, hydrogels are an excellent substitute for traditional pharmaceutical administration methods. Greater porosity, controlling the degree of matrix cross-linking and the hydrogel's affinity for the aqueous environment where swelling occurs, will enable the creation of hydrogel structures (Li et al. 2011). This enables the hydrogel for the maximum permeation capacity to various types of pharmaceuticals, enabling the loading and

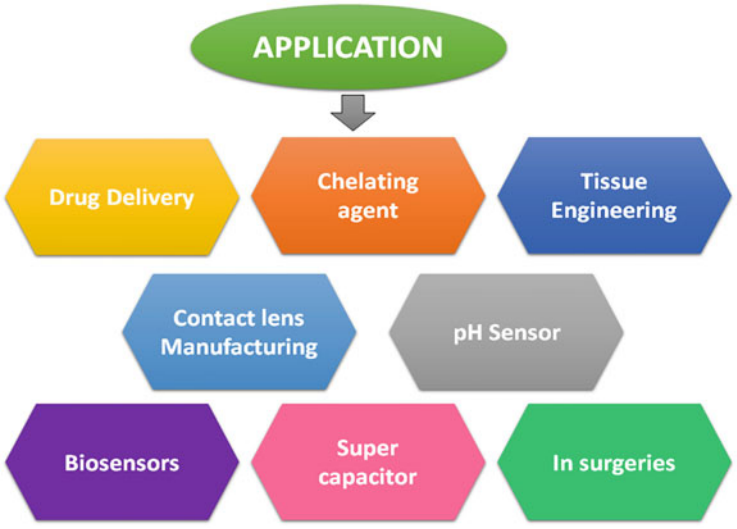

Fig. 11.4 Elaboration of hydrogel application and uses

release of drugs under the desired conditions. The main advantage of hydrogels in drug delivery studies is their capacity for sustained release, which enables the administration of high concentrations of active pharmaceutical ingredients to a given area over extended time duration (Liu et al. 2012).

To increase the binding of a loaded drug to the hydrogel matrix and extend the time of drug release, physical (electrostatic interactions) and chemical (covalent bonding) techniques can be used. A number of drugs can be stored in hydrogels where they are shielded from potentially dangerous environments and released gradually over time. Drug release on demand can be initiated by regional variation in temperature, enzyme pH, and other physical factors (Lo et al. 2009).

11.2.4.2 Elimination of Dyes and Heavy Metal Ions (Chelating Agent)

Hydrogels made of synthetic cross-linked polyacrylate have been utilized to lessen the toxicity of heavy metals in aqueous media. However, due to their high price, these synthetic materials might not be a viable solution on a broad scale. Utilizing well-known adsorption techniques, which can be versatile in design and operation and also have the added benefit of allowing for the reuse of treated effluent (Feinberg et al. 2013), heavy metal ion pollution can be cleaned. It is typically possible to replenish the adsorbent to make the process more economical because the adsorption process is typically reversible. To remove metal ions from aquatic environments, biopolymer-based hydrogels made of chitosan, alginate, starch, and cellulose derivatives have been used. It has been demonstrated that the hydrogel's functional groups can change how heavy metal ions bind to surfaces and how much they can bind. This is because more complex mechanisms than just sorption are involved in the elimination of metal ions, such as chelating and ion exchange (Azegami et al. 2018).

11.2.4.3 Scaffolds in Tissue Engineering

Because of their comparable topologies to the extracellular matrix found in many tissues, the ease with which they can be produced, and the low invasiveness with which they can be administered, hydrogels are fascinating scaffold materials. The intended scaffold application and the environment in which the scaffold will be placed dictate the physical attributes, mass transport properties, and biological features of optimal framework strategy and constituent's assortment for every special advantage (Kong et al. 2013). Hydrogels for tissue engineering scaffolds can be made from both synthetic and naturally occurring materials. As all-purpose bulking agents, alginate, chitosan, and collagen hydrogel scaffolds have demonstrated potential. Synthetic hydrogels are frequently employed as anti-adhesive materials since cells lack adhesion receptors for them and, if appropriately made, proteins do not readily absorb on them. Polyethylene glycol (PEG) is a chemical that has been used to prevent postoperative adhesions (Muraoka et al. 2019).

11.2.4.4 Contact Lenses

One class of materials used to make contact lenses is silicon hydrogels. These hydrogels have strong swelling characteristics and a high oxygen permeability,

which makes them perfect for contact lens constructions. They are an evolution of basic hydrogels.

Beneficial characteristics are brought about by their structure, which combines hydrophobic silicones with hydrophilic chains to produce a composite that is acceptable in terms of both physical properties and optical properties (Mohamed and van der Walle 2008). When used frequently, an interconnected network made of linear or branched hydrophilic polymer chains can be incorporated into the polymer's structure to prevent lens dryness. This suggests that the "wetting chains" and the network of patterned hydrogels are only physically connected, without any covalent connections. The bulk of the requirements for use in a range of physiological situations can be met by the hydrogels employed in the production of contact lenses (Naik et al. 2012). To make a hydrogel material contact lens comfortable to wear, there are several needs including water content, strong mechanical characteristics, oxygen permeability, surface wettability, ideal optical capabilities, consistency against chemical reaction, inert nature, and sufficient microbiological resistance for live cells (Oh et al. 2009).

11.2.4.5 Sensors for pH

Hydrogels or polymers that respond to stimuli can substantially change their volume in response to even minor changes in each environment. Due to ionization, cationic polyelectrolytes dissolve (swell) more easily than anionic polyelectrolytes at low pH. The ability of hydrogels to mechanically deform or strain a transduction element, resulting in a change in that element's specific property or a change in a measurable distance, forms the basis of hydrogel-based transducer function. There are two types of transducers: mechanical (such as microcantilevers and bending plate transducers) and optical (such as reflecting diaphragms and fiber Bragg grating sensors) (Oh et al. 2010; Yassin et al. 2010).

11.2.4.6 Biosensors

Hydrogels are naturally biocompatible because of their high water content and hydrophilic characteristics, which are like the void-filling element of the extracellular matrix. As a result, covering and coating the sensor parts of biosensors with hydrogels makes sense to avoid unintended interactions with biological molecules or cells (Blander 2016). Enzymes and other biomolecules may maintain their functional and active structure in hydrogels, which can be employed as immobilization matrices for biosensing elements. To sustain living biological components, such as cells, in biosensors, hydrogels can be used as a 3D matrix or support or coated to the surface of sensing devices, such as electrodes. Cell preservation in a hydrogel matrix for a set amount of time and pathogen detection find further applications in this field (Clausen and Stoitzner 2015).

11.2.4.7 Spinal Cord Damage Treated with Injectable Hydrogel

The microscopic gaps from spinal cord tissue and divided portions produced following spinal cord injury can be filled with liquid hydrogel that has been converted in vivo to gel phase.

By promoting cellular penetration and matrix synthesis, the gel, which is now acting as a scaffold, will minimize empty areas and act as a guide for the regeneration of injured cord tissue. Prefabricated scaffolds won't need to be made specifically for each patient, and it won't be essential to detach healthy tissue from the injury location in order to insert the assembled framework leading to extensive adverse effects and toxicity (Kayal and Ramanujan 2010). Patients like the comfort and painlessness of injectable hydrogel systems. In situ polymer solutions can easily be combined with cells or bioactive substances, and these mixtures can swiftly create 3D microenvironments with any desired defect shape.

11.2.4.8 Supercapacitor Hydrogels

Considering their outstanding solid-liquid interface, potent electric characteristics, and mechanical flexibility, electrically conducting polymer hydrogels show tremendous potential for the anticipated integration (Kievit et al. 2011). They also show the effective constituent's junction for creating springy energy storage systems. Supercapacitor hydrogels are gaining a lot of attention as potential new power sources because conducting polymers like polypyrrole derivatives offer the specific electrical features of metal with appealing characteristics connected with primitive polymers, like flexible construction and processing flexibility. Flexible solid-state supercapacitors can store a lot of energy and have a high-power density (Yuba et al. 2017). They also have a long cycle life.

11.3 Nanogels

Nanogels are cross-linked swellable polymer networks that do not dissolve in aqueous media and are three-dimensional hydrogel materials with a high water-holding capacity (Fig. 11.5). Numerous organic or synthetic polymers, or a combination of the two, can be used to create nanogels. Nanogels' chemical makeup can be adjusted to better control their size, charge, porosity, amphiphilicity, softness, and degradability (Park et al. 2009). Although more recent developments in synthetic processes have made it feasible to create nanogels in a variety of shapes, they are typically spherical particles. To maintain structural integrity, they can also feature a

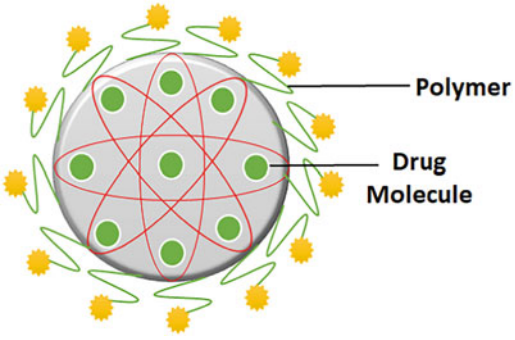

Fig. 11.5 Elaboration of a typical nanogel structure

core-shell or core-shell-corona structure with at least one cross-linked layer. Due to their hydrophilic nature and great biocompatibility, as well as their distinctive physical properties, nanogels have a particular advantage over various nanomaterials blends for the biomedical application usage (Prakash and Thiagarajan 2011).

In addition to shielding the cargo from deterioration and elimination, nanogels' unique characteristics including stimuli-responsive behavior, softness, and swelling also aid in inducing a regulated, triggered response at the target area. By correctly changing the materials used in their creation, they can host a range of guest molecules, from inorganic nanoparticles to bio-macromolecules like proteins and DNA, without damaging their gel-like behavior. Whereas, polymer based nanoparticulate systems are rigid and lacks the ability to encapsulate drug molecules and other bioactives with different physicochemical characteristics (Su et al. 2011). Despite having special material properties like optical activity, electrical conductivity, and magnetic properties that make them ideal for in vivo diagnostic and imaging applications, inorganic nanomaterials are limited by poor colloidal stability, low aqueous solubility, and rapid elimination by the mononuclear phagocytic system (MPS) (Sun et al. 2008). Imaging probes are further employed as transporter in polymeric nanogels, increasing their stability and usefulness. As a result, a new class of drugs called as "nanohybrids," which are nanogels with inorganic components, was created. These nanohybrids might contain a variety of substances for imaging and diagnosing different disorders. The macromolecular architecture of nanogels allow to incorporate small molecules and due to their nanoscale size is that they can be passively targeted (Fig. 11.6) or may be conjugated with a targeting ligand to target a specific area of interest. Despite having a wide

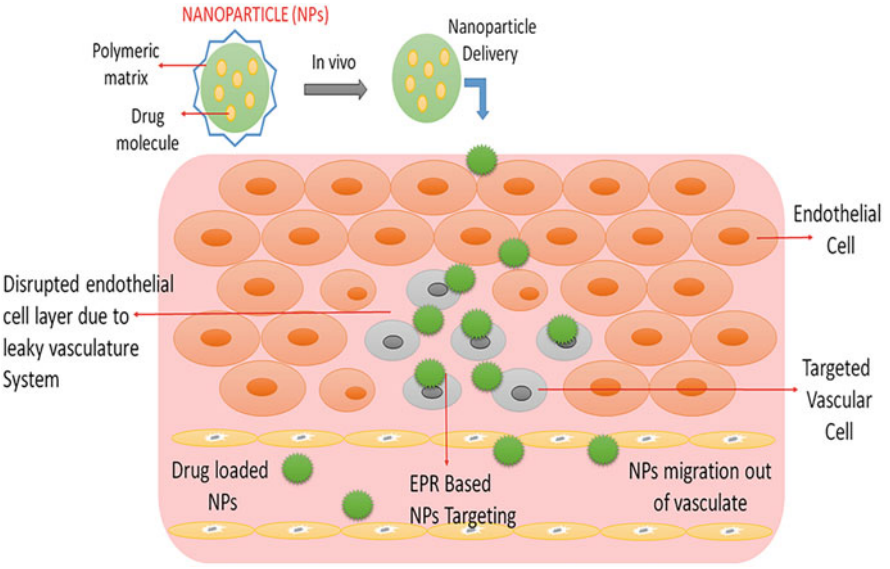

Fig. 11.6 Diagrammatic illustration of passive targeting mechanism by nanogel particles

variety of applications, nanogels have not yet been used in therapeutic settings. Numerous thorough and increasingly specialized review papers on the creation and use of nanogels have lately been published (Prasanth et al. 2011).

11.3.1 Nanogel Synthesis Methodologies

Numerous techniques can be used to create nanogels. Depending on the kind of cross-linking, nanogels have historically been categorized as both physically and chemically complexed (Rejinold et al. 2011a). When low molecular weight monomers are polymerized or when polymer precursors are cross-linked, covalent bonds are formed between the polymer chains. Heterogeneous polymerization processes with bi- or multifunctional cross-linkers are the most popular methods for creating chemically cross-linked nanogels. Nanogels with various compositions, sizes, and topologies, such as core-shell and hollow nanogel particles, can be made using both traditional and controlled/living radical polymerization processes. The incorporation of functionalities in the interior or on the surface of nanogels is also made possible using functional initiators and macro-initiators, which provide multivalent bioconjugation (Rejinold et al. 2011b). To produce nanogels from polymer precursors, various cross-linking techniques have been devised, including amide cross-linking, photo-induced cross-linking, enzyme-mediated cross-linking, Schiff base reactions, thiol-disulfide exchange, and click chemistry. The cross-linking of preexisting core-shell self-assemblies, such as polymer micelles, allows the nanogels to exhibit a high degree of spatial organization. Recent developments in nanoscale manufacturing methods have enhanced the potential for high-throughput synthesis of well-defined nanogels with precise control over size, shape, deformability, and surface chemistry (Sahu and Ahmad 2010). Even when produced under benign conditions, systems that are physically cross-linked are more brittle than those that are covalently cross-linked because of feeble bonds such as weak ionic complex, hydrogen bonds, or lipophilic low-resistance bonds. The research community has investigated how hydrophobically modified polymers and other related polymers can be used to create useful nanogels. Controlling the particle size, which requires fine-tuning of polymer concentrations or ambient conditions like temperature, pH, and ionic strength, is one of the difficulties in the manufacture of nanogels by such polymers (Salaun and Vroman 2009). The composition, architecture, and activity of cross-linked nanogels now exhibit unparalleled diversity and control because of advancements in polymer chemistry, giving scientists more freedom to modify their properties to suit particular biological requirements.

11.3.2 Nanogels' Properties

The versatility of nanogel makes it an ideal nanocarrier for therapeutic transport, internalization of APIs, controlled release, etc. (Siegel et al. 2006). The following are the fundamental characteristics of nanogels (Fig. 11.7).

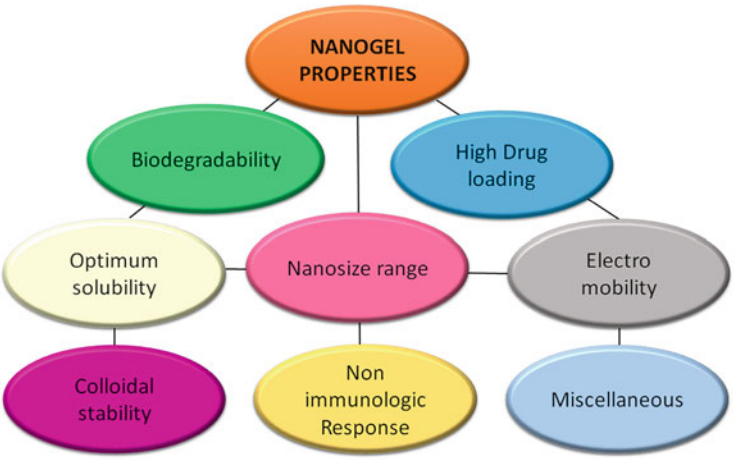

Fig. 11.7 Illustration of nanogel properties

11.3.2.1 Biocompatibility and Degradability

Nanogel-based drug delivery systems are highly biocompatible and biodegradable; as a result, this field is now very promising. The fastest swelling and de-swelling properties of nanogels are their most favorable feature (Shah et al. 2010).

11.3.2.2 Elevated Drug Encapsulation Potential

The feasible molecules contained in the polymeric structure are primarily responsible for the elevated drug encapsulation of nanogel system. Advantages like drug transport, logistic, and release are greatly influenced by functional groups, and some functional groups show excellent promise for conjugation with medicines, bioactives, and peptides in targeted applications. These functional groups are crucial for the development of hydrogen bonds and van der Waals forces in nanogel networks leading to the targeted drug release, drug transport, and so on. Due to the presence of these pendent functional groups, drug loading characteristics at the drug/protein interface are also higher (Sheihet et al. 2012).

11.3.2.3 Particle Size

The diameter of nanogels typically range from 20 to 200 nm. This appropriate size range is essential for preventing rapid renal segregation, while remaining small enough to avoid reticuloendothelial system (RES) uptake. The blood brain barrier (BBB) is easily crossed by nanosized substances, indicating possible penetration capability (Shigemasa et al. 1994).

11.3.2.4 Solubility

In their gel system, nanogels may effectively solubilize hydrophobic medications and biomolecules. Additionally, they can effectively solubilize diagnostic chemicals within their gel network (Shimizu et al. 2008).

11.3.2.5 Electromobility

The main benefit of nanogel systems is their ease of synthesis and preparation, which use little energy and do not require the use of sophisticated mechanical systems and equipment. Simple sonication and homogenization are adequate for the efficient entrapment and encapsulation of medicines, biomolecules, proteins, and peptides in nanogel networks (Embgenbroich and Burgdorf 2018).

11.3.2.6 Colloidal Consistency

In comparison to surfactant micelles, polymeric micellar nanogel systems are more stable and have low critical micellar concentrations, a moderate rate of dissociation, and extended storage of loaded drugs or bioactives (Gros and Amigorena 2019).

11.3.2.7 Non-immunologic Response

Drug delivery methods based on nanogels typically don't show any immunological effects or reactions.

11.3.2.8 Others

The efficiency with which nanogel can encapsulate and deliver both hydrophilic and hydrophobic drugs depends on several variables, including pH, temperature, functional groups (hydrophilic/hydrophobic groups) in the polymeric network, cross-linking density of the gels, surfactant concentration, and the type of cross-linker present in the nanogel system (Yi et al. 2017).

11.3.3 Categories of Nanogel

There are chiefly two types of categories in nanogel system (Xu et al. 2007) (Fig. 11.8):

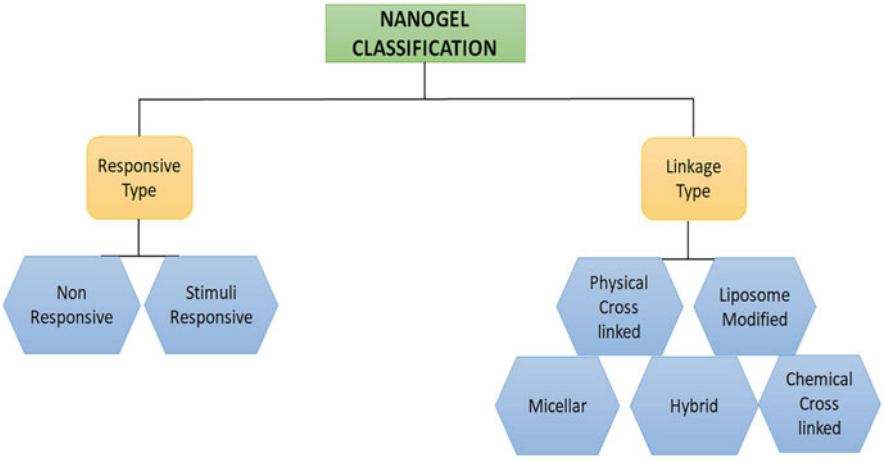

Fig. 11.8 Illustration of nanogel classification

11.3.3.1 On the Basis of Response

It can also be subclassified into the following subcategories.

Nonresponsive Nanogels

They merely swell in a suitable solvent, such as water, as a result of a straightforward absorption process (Sorina et al. 2011). They swell or de-swell in response to changes in environmental factors as pH, temperature, humidity, magnetic field, ionic strength, etc.

Stimuli-Responsive Nanogel

Those who respond to many environmental stimuli are referred to as multi-responsive nanogels (Uhrich et al. 1999).

11.3.3.2 On the Basis of Linkage

The second category of nanogel is based on the nature of linkage in nanogel structure. The following subcategories were added to this classification.

Physically Cross-Linked Nanogel

Physically cross-linked gels, also known as pseudogels, are produced by weak linkage forces such as van der Waals forces, hydrophobic contacts, electrostatic interactions, or hydrogen binding, among others. Physically cross-linked gels can be produced using many simple methods. These systems are quite sensitive, and their responsiveness is greatly influenced by elements including temperature, medium ionic strength, cross-linkers, and polymer content. By complexing polymeric chains with opposing charges and joining amphiphilic block copolymers, micro- and nanogels can be made in a matter of minutes. In addition, polymeric chains can aggregate or self-assemble to create physical gels (Tamura et al. 2006; Tan et al. 2008).

Liposome-Modified Nanogels

In a wide range of illness therapies, liposome-modified nanogels are proving to be excellent drug carriers. Liposomes are present in the network matrix of these nanogels, and their release is regulated by a variety of variables, including pH, temperature, and ionic charge. In contrast, at pH 5.5, succinylated polyglycerol liposomes considerably transport calcium to the cytoplasm. Additionally, thermo-responsive triggered manner of drug release is demonstrated by transdermal administration of liposome-customized nanogel made of poly(N-isopropylacrylamide) polymeric molecules (Teskac and Kristi 2010).

Micellar Nanogels

In aqueous conditions, amphiphilic blocks or graft copolymers are assembled supramolecularly to form these delicate nanogels. Micellar nanogels have the shape of a central core casing. The hydrophobic block piece that makes up the core is encased in a hydrophilic polymer segment. The hydrophilic portion is essential for the creation of hydrogen bonds, which provide the micelles' stable core-shell shape. These core-shell micellar nanogels are extremely stable and stimuli

responsive in nature. The enormous surface area of the core-shell nanogel allows for precise lodging of pharmaceuticals or bioactives produced via a physical entrapment technique (Tobio et al. 1998). Therefore, the hydrophobic core's drug component or bioactive is shielded from hydrolysis and enzymatic degradation. The creation of Y-shaped micelles constructed of biodegradable, nontoxic, and pH-responsive polymers, such as PLGA, chitosan, and poly(oleic acid-N-isopropylacrylamide), among others, is being studied by the scientific community for efficient drug administration (Urbinati et al. 2010).

Hybrid Nanogel

Nanoparticle gels of varied size are disseminated in natural or synthetic medium composing the hybrid nanogel. These hybrid nanogels are extensively delicate because of how they are built structurally. Researchers found that the hybrid nanogel, which is made in an aqueous medium by the self-assembly of polymers, is particularly effective at releasing drugs quickly to the desired spot. These nanogels are made using a variety of polymers, including pullulan-PNIPAM, polysaccharides, etc. (Vinogradov et al. 2005). The cholesterol-pullulan hybrid nanogel has undergone extensive research and has been found to be a promising drug delivery system for DNA, RNA, proteins, peptides, oligosaccharides, and other bioactive substances. Insulin and antineoplastic drugs can be delivered on-site and as needed using hybrid nanogels. These hybrid nanocomposites consist of extensively precise features and are sensitive to external spurs such as delivering cholesterol pullulan via pullulan consisting of cholesterol nanogel that was created using a self-assembling or aggregated technique and is highly effective at doing so because of its pH and temperature-sensitive trigger mechanism (Wang et al. 2011).

Chemically Cross-Linked Nanogel

Covalent bonding is a method for creating chemically cross-linked nanogel. It shows an intense effect in the nanogel structure due to the presence of specific functional moieties and chemical bond leading to the varied properties and nature. This method has been successful in producing a variety of nanogels (Wang et al. 2008). In the presence of multifunctional cross-linkers, vinyl monomers are employed to speed up the polymerization of hydrophilic polymers and hydrophilic-hydrophobic copolymers. These cross-linkers have a big impact on the physiochemical properties of nanogel and the stability of nano-formulations. The size, form, and morphology of nanogels are significantly influenced by the type of cross-linkers used. For instance, when pendant thiol groups created by the disulfide cross-linking method are present, biodegradable PLGA-PEG diblock nanogel displays a size range between 20 and 200 nm. The application of "green chemistry" in the synthesis process enabled the completion of the whole polymerization process (Wu and Wang 2001).

11.3.4 Nanogel Uses in Theranostics

The nanogel has several applications and uses in various fields. Table 11.2 elaborates on the key **theranostic applications of nanogels.**

Table 11.2 Theranostic application of nanogel-based delivery system

S. no.	Polymer system	Nanogel characteristics	Inference
1.	Acetylated chondroitin sulfate	Self-assembled	Doxorubicin encapsulation
2.	Heparin pluronic gel	Self-assembled	Delivery of RNA-based enzyme to targeted cell
3.	Chitosan glycol complexed with 3-diethylaminopropyl molecule	pH stimuli	Elevated doxorubicin internalization
4.	PEG and polyethylenimine complex	Polyplex nanogel system	Low toxicity with elevated therapeutic effect
5.	Polyethylenimine nanogel system	Size dependent nanogel	Elevated gene hTERT-CD-TK lung cancer targeting
6.	Cross-linked PEG-pluronic-polyethylenimine	Biocompatible nanogel	5-triphosphorylated ribavirin reduced delivery
7.	Folate pullulan pheophorbide	Self-oxidized polysaccharide nanogel	Reduced pheophorbide toxicity
8.	Cholesterol-bearing pullulan nanogel	Sustained release nanogel	Recombinant marine interleukin-12 tumor immunotherapy

11.3.4.1 Nanogel as Therapeutic Drug Transporter

Nanogels can store up to 30% of the weight of biological molecules and pharmaceuticals due to swelling caused by electrostatic, van der Waals, and/or hydrophobic interactions, covalent bonding with the polymer chains, and other interactions.

In comparison to liposomes and polymeric micelles, these loading capacities are noticeably higher. Drug loading causes the nanogels to collapse, resulting in stable nanoparticles that contain the biological agent. By spreading hydrophilic polymers (like PEG) in a nanogel structure, aggregate formation can be prevented. Hydrophilic polymer chains are exposed at the surface and form a barrier surrounding the nanogel when the drug-nanogel combination fails (Allison 2008). Both the creation of a wide variety of drug formulations and the incorporation of numerous therapeutic cargos into a single nanogel carrier are made possible by the flexibility and control of polymer chemistry. Temperature- or pH-induced volume collapse-induced drug release may be of interest to drug delivery applications. The surface of the nanogel can be functionalized to aid in their selective accumulation in the target tissue or cells. Nanogels are being created to carry, protect, target, and release therapeutic compounds in a geographically and chronologically regulated manner due to their rational design, which enables a variety of applications (Alsarra and Alarifi 2004).

- For small therapeutic molecule delivery.
- For oligonucleotide delivery.
- For delivery of protein therapeutics.
- For combination drug delivery.

11.3.4.2 Nanogel as Imaging and Diagnosing Tool

Because of their fluid-like transport characteristics, high water content, structural plasticity, and biocompatibility, nanogels make excellent carriers for range visualization measurement and disproportion candidates. The addition of many functional moieties to the outer or inner surface of nanogels makes it possible to incorporate/conjugate a variety of inorganic nanoparticle, coloring agents, and detection moieties (Anderson and Shive 1997). When given as nonencapsulated entities, magnetic nanoparticles, such as iron oxide, have been shown to exhibit inferior colloidal stability and sensitivity compared to when they are encased in cross-linked nanogels. Nanogels enable a significant cargo of magnetic nanoparticles to be confined due to the cluster effect, leading to noticeably greater local magnetic fields. The hydrogel coating increases relaxivities even more by lowering the water diffusion coefficient close to the particles and increasing the interaction between water protons and the strong magnetic fields at the particle's surface (Yoo and Park 2004). The thickness of the gel layer covering the magnetic particle affects how much water molecule diffusivity is lowered. By adjusting the swelling/de-swelling transition of the nanogel matrix, this would provide partial control of relaxation times. Without the aid of any inorganic paramagnetic material, one of the studies has found that PMA nanogels can function as pH sensors for magnetic resonance imaging (MRI). Since connected water molecules' mobility is severely restricted, shrinking pH-sensitive nanogels at an acidic pH produces stiffer structures and slower rotational motions of the polymer chains than in the inflated state, which reduces the transverse relaxation time (Yoshizawa et al. 2011). The freedom of tumbling motion is another element that influences the length of time that contrast agents like Gd chelates remain relaxed. Conjugating the contrast agent with a macromolecular system is one of the most effective methods for preventing tumbling. For entrapping such compounds, nanogels are the ideal hydrophilic substrate with variable loading capacity, and it has been demonstrated that this increases relaxivity. Gd^{3+} is a dangerous ion; hence it is administered to patients in the phase of chelates. Additionally including chelates, the trans-metalation activities, which include replacing the chelated metal ion with a rival ion, might result in toxicity (Hao et al. 2005). It has been demonstrated that nanogels based on DTPA (diethylenetriaminepentaacetic acid) are extensively resistant to the trans-metalation reaction compared to bare chelates. Additionally, the toxicity of inorganic nanoparticles can be decreased by encasing them in nanogels. Furthermore, it has been established that the size and shape of gold and silver nanoparticles have a major impact on their electrical and optical properties, necessitating their encapsulation in nanogel for colloidal stability and imaging uniformity (Hasegawa et al. 2009). The interparticle distance between gold nanoparticles can be reversibly altered by the phenomenon of de-swelling and swelling of the neighboring nanogel structure I response to the alteration of the microenvironment, changing their optical properties similarly to contrast agents. This may provide some tissue selectivity for the imaging function:

- As MR contrast agents.
- For PET imaging.

- For optical imaging.
- For multimodal imaging agents (Hogg 2007).

In in vivo and clinical studies, particularly for the treatment of cancer, nanogels have already been used as DDS. Recombinant murine interleukin-12 (IL-12), when administered subcutaneously to mice with subcutaneous fibrosarcoma and then incubated at room temperature, causes a sustained increase in IL-12 levels in the sera and significantly slows the growth of the tumor. In a clinical investigation (Changcheng et al. 2011), cholesterol pullulan (CHP) nanogels demonstrated great potential for peptide delivery. Nine patients got 300 g doses of the CHP-HER-2 vaccine given every 2 weeks along with booster shots. With only a slight amount of skin pain at the injection site, the immunization was well accepted. A $CD4^+$ and $CD8^+$ T-cell response was seen in all the patients, proving that the treatment was effective.

By decreasing cytotoxicity in nervous system cells and boosting binding ability to an oligomer in the treatment of Alzheimer's disease, the CHP nanogels have also shown their potential for use in clinical trials (Changediya et al. 2011). The PEO-b-PMA diblock copolymers were used to produce nanogels having free OH groups at the PEO termini. Nanogels were then infused with cisplatin or doxorubicin, and they were joined to activated folic acid with terminal amino groups. Targeted nanogels can find their target when folate receptor-a is overexpressed in human ovarian carcinomas A2780. In a subcutaneous injection of the A2780 model into mice given a diet low in folate, intravenously, compared to the free medication, treatment of the targeted nanogels enhanced the antitumor effect of cisplatin, while reducing kidney toxicity (Zhang et al. 2005). An optically sensitive insulin-loaded silver nanoparticle nanogel composed of poly(4-vinylphenylboronic acid-co-2-(dimethylamino) ethyl acrylate) has revolutionized clinical studies. The development of antibiotic-conjugated nanogels and their in vivo application have resulted in a promising initial clinical trial strategy. Nanogels appear to be promising candidates for drug delivery systems, while more single-cell research is necessary. Which nanogels prefer cytosolic over endosomal or nuclear destinations, for example, will be revealed through research into the mechanisms of uptake at the level of neurons and/or glial cells in the central nervous system as well as at the blood-brain barrier. Such research is required if nanogels are to be marketed as precise medication delivery devices for subcellular targeting.

11.4 Conclusion

Gels, microgels, hydrogels, and nanogels have been developed over time to become capable of acting as therapeutic transporter system for numerous therapeutic and diagnostic particles. The swelling behavior and softness in the gel structure are outcomes of the improvement in formulation technique and a better comprehension of material characteristics. With this knowledge, we can investigate how they might be used in various biomedical fields and explore the possibility of modifying these

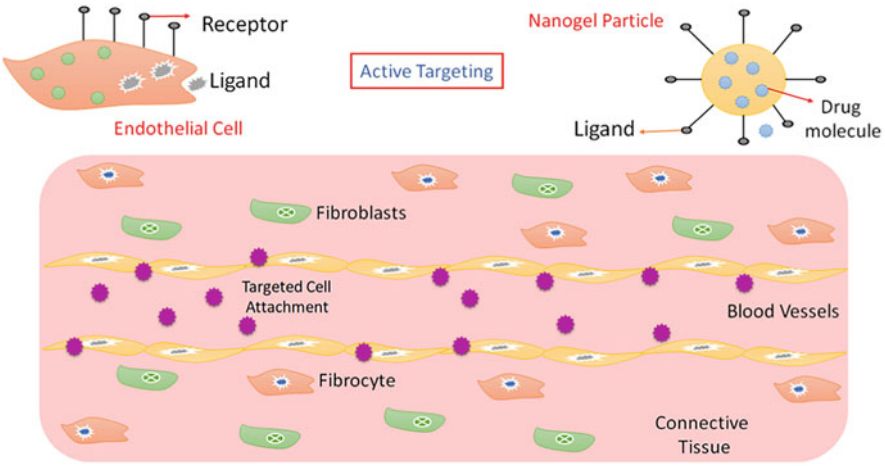

Fig. 11.9 Elaboration of ligand-receptor-mediated nanogel particle active targeting mechanism via selective cell surface binding

qualities to our advantage. Our knowledge of their behavior in vivo has improved because of advancements in analytical techniques, which can assist direct efforts to augment the dilapidation and pharmacokinetic contours in order to provoke future nanogels. Proteins, peptides, nucleic acids, and inorganic nanoparticles made of gold, silver, or iron oxide can all be synthesized as small molecules to interact with gels, including hydrogel and nanogel. They have evolved in recent years into multidrug carriers and multimodal imaging agents. They can also combine two or more agents, depending on the purpose. To deliver targeting ligands to a particular receptor and point them in the right direction, they can be surface functionalized (Fig. 11.9). Their cross-linked network expands because of easy volume-phase changes so that they may be sensitive to environmental or peripheral spurs. This makes it possible to control the release of drugs geographically and temporally and/or the activation of reporter molecules, which can then produce signals for imaging and diagnostics. Due to their qualities, they perform better than traditional nanoparticulate materials in terms of application. Only a few nanogels have been investigated in clinical trials, despite the advancements made in gel design to date. Because of the system's complexity and advanced structural elements, the intended result requires meticulous nanogel engineering. Another challenge is overcoming repeatability from batch to batch and scalable production. Both getting the payload where it must go and getting the nanogels out of there quickly after they've served their purpose in vivo are difficult tasks. Several studies have looked at the effectiveness and safety of gel formulations, but there are few reports on their long-term accumulation and breakdown properties. The eventual shift of the nanogels from bench to bedside will be aided by advancements in design as well as thorough investigations into their in vivo behavior.

References

Ali I, Rahis-ud-din SK, Aboul-Enein HY, Rather MA (2011) Social aspects of cancer genesis. Cancer Ther 8:6–14

Allison SD (2008) Effect of structural relaxation on the preparation and drug release behavior of poly (lactic-co-glycolic) acid microparticles drug delivery systems. J Pharm Sci 97:2022–2035

Alsarra IA, Alarifi MN (2004) Validated liquid chromatographic determination of 5- fluorouracil in human plasma. J Chromatogr B 804:435–439

Anderson JM, Shive MS (1997) Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv Drug Deliv Rev 28:5–24

Anitha A, Chennazhi KP, Nair SV, Jayakumar R (2012) 5-Flourouracil loaded N, O-carboxymethyl chitosan nanoparticles as an anticancer nanomedicine for breast cancer. J Biomed Nanotechnol 8:1–14

Anitha A, Deepagan VG, Divya VVM, Deepthy M, Nair SV, Jayakumar R (2011) Preparation, characterization, in vitro drug release and biological studies of curcumin loaded dextran sulphate–chitosan nanoparticles. CarbohydrPolym 84:1158–1164

Azegami T, Yuki Y, Hayashi K, Hishikawa A, Sawada S, Ishige K, Akiyoshi K, Kiyono H, Itoh H (2018) Intranasal vaccination against angiotensin II type 1 receptor and pneumococcal surface protein a attenuates hypertension and pneumococcal infection in rodents. J Hypertens 36:387–394

Bae KH, Mok H, Park TG (2008) Synthesis, characterization and intracellular delivery of reducible heparin nanogels for apoptotic cell death. Biomaterials 29:3376–3383

Bailon P, Berthold W (1998) Polyethylene glycol-conjugated pharmaceutical proteins. Pharm Sci Technol Today 1:352–356

Baldock C, Karger CP, Zaidi H (2020) Gel dosimetry provides the optimal end-to-end quality assurance dosimetry for MR-linacs. Med Phys 47:3259–3262

Baviskar DT, Chaudhari RD, Kale MT, Jain DK (2011) Recent advances on tumor targeted drug delivery system, an overview. J Biomed Pharma Sci 1:32–42

Bazile DV, Ropert C, Huve P, Verrecchia T, Marlard M, Frydman A, Veillard M, Spenlehauer G (1992) Body distribution of fully biodegradable [14C]-poly (lactic acid) nanoparticles coated with albumin after parenteral administration to rats. Biomaterials 13:1093–1102

Bergueiro J, Glitscher EA, Calderón M (2022) A hybrid thermoresponsive plasmonic nanogel designed for NIR-mediated chemotherapy. Biomat Adv 137(2):12842

Bhaskaran S, Lakshmi PK (2009) Comparative evaluation of niosome formulations prepared by different techniques. Acta Pharm Sci 51:27–32

Blander JM (2016) The comings and goings of MHC class I molecules herald a new Dawn in cross-presentation. Immunol Rev 272:65–79

Bombardelli E (1991) Phytosome: new cosmetic delivery system. Boll Chim Farm 130:431–438

Brubaker CE, Panagiotou V, Demurtas D, Bonner DK, Swartz MA, Hubbell JA (2016) A cationic micelle complex improves CD8+ T cell responses in vaccination against unmodified protein antigen. ACS Biomater Sci Eng 2:231–240

Chakravarthi SS, De S, Miller DW, Robbinson DH (2010) Comparison of anti-tumor efficacy of paclitaxel delivered in nano- and microparticles. Int J Pharm 383:37–44

Chang RS, Kim J, Lee HY, Han S-E, Na J, Kim K, Kwon IC, Kim YB, Oh Y-K (2010) Reduced dose-limiting toxicity of intraperitoneal mitoxantrone chemotherapy using cardiolipin-based anionic liposomes. Nanomed Nanotechnol 6(6):769–776

Changcheng H, Kexin J, Xu Z, Mei X, Zhiyong L, Huiliang W (2011) Nanoparticles, microgels and bulk hydrogels with very high mechanical strength starting from micelles. Soft Matter 7:2943–2952

Changediya V, Khadke M, Devdh ES (2011) Phytosomes, new approach for delivering herbal drug with improved bioavailability. Res J Pharm, Biol Chem Sci 2:57–68

Cheng J, Teply BA, Sherifi I, Sung J, Luther G, Gu FX, Levy-Nissenbaum E, Radovic-Moreno AF, Langer R, Farokhzad OC (2007) Formulation of functionalized PLGA-PEG nanoparticles for in vivo targeted drug delivery. Biomaterials 28:869–876

Clausen BE, Stoitzner P (2015) Functional specialization of skin dendritic cell subsets in regulating T cell responses. Front Immunol 6:1–19

David L, Nahrwold DL, Dawes LG (1997) Biliary neoplasms in surgery. In: Greenfield LJ (ed) Scientific principles and practice, 2nd edn. Raven, Philadelphia, pp 1056–1066

Deshpandey DD, Shankhe K, Amiji M (2008) Novel multimodal omega-3-fatty acid oil containing nanoemulsion formulation for the treatment of inflammation and endothelial dysfunction in atherosclerosis. J Am Coll Cardiol 2:25–30

Dhar S, Gu FX, Langer R, Farokhzad OC, Lippard SJ (2008) Targeted delivery of cisplatin to prostate cancer cells by aptamer functionalized Pt (IV) prodrug-PLGA–PEG nanoparticles. Proc Natl Acad Sci U S A 105:17356–17361

Dreis S, Rothweiler F, Michaelis M, Cinatl JJ, Kreuter J, Langer K (2007) Preparation, characterization and maintenance of drug efficacy of doxorubicin-loaded human serum albumin (HSA) nanoparticles. Int J Pharm 341:207–214

Embgenbroich M, Burgdorf S (2018) Current concepts of antigen cross-presentation. Front Immunol 9:1643

Esmaeili F, Ghahreman IMH, Esmaeili B, Khoshayand MR, Atyabi F, Dinarvand R (2008) PLGA nanoparticles of different surface properties: preparation and evaluation of their body distribution. Int J Pharm 349:249–255

Feinberg H, Rowntree TJW, Tan SLW, Drickamer K, Weis WI, Taylor ME (2013) Common polymorphisms in human langerin change specificity for glycan ligands. J Biol Chem 288: 36762–36771

Fromen CA, Rahhal TB, Robbins GR, Kai MP, Shen TW, Luft JC, DeSimone JM (2016) Nanoparticle surface charge impacts distribution, uptake and lymph node trafficking by pulmonary antigen-presenting cells. Nanomedicine 12:677–687

Garcia-Closas R, Garcia-Closas M, Kogevinas M, Malats N, Silverman D, Serra C, Tardon A, Carrato A, Castano- Vinyals G, Dosemeci M, Moore L, Rothman N, Sinha R (2007) Food, nutrient and heterocyclic amine intake and the risk of bladder cancer. Eur J Cancer 43(11): 1731–1740

Garcion E, Lamprecht A, Heurtault B, Paillard A, Aubert-Pouessel A, Denizot B, Menei P, Benoıt JP (2006) A new generation of anticancer, drug-loaded, colloidal vectors reverses multidrug resistance in glioma and reduces tumor progression in rats. Mol Cancer Ther 5(7):1710–1722

Gill KK, Nazzal S, Kaddoumi A (2011) Paclitaxel loaded PEG (5000)-DSPE micelles as pulmonary delivery platform, formulation characterization, tissue distribution, plasma pharmacokinetics, and toxicological evaluation. Eur J Pharm Biopharm 79:276–284

Gros M, Amigorena S (2019) Regulation of antigen export to the cytosol during cross-presentation. Front Immunol:41

Gupta M, Sharma V (2011) Targeted drug delivery system, a review. Res J Chem Sci 1:135–138

Hana X, Liua J, Liua M, Xiea C, Zhana C, Gua B, Liua Y, Fenga L, Lua W (2009) NC-loaded folate-conjugated polymer micelles as tumor targeted drug delivery system, preparation and evaluation in vitro. Int J Pharm 372:125–131

Hao YL, Deng YJ, Chen Y, Wang XM, Jun H, Zhong SXB (2005) In vitro and in vivo studies of different liposomes containing topotecan. Arch Pharm Res 28:626–635

Hasegawa U, Sawada SI, Shimizu T, Kishida T, Otsuji E, Mazda O, Akiyoshi K (2009) Raspberry-like assembly of crosslinking nanogels for protein delivery. J Control Release 140:312–317

Hogg N (2007) Red meat and colon cancer, Heme proteins and nitrite in the gut. A commentary on diet-induced endogenous formation of nitroso compounds in the GI tract. Free Radic Biol Med 43:1037–1039

Houchin ML, Topp EM (2009) Physical properties of PLGA films during polymer degradation. J Appl Polym Sci 114:2848–2854

Hureaux J, Lagarce F, Gagnadoux F, Marie-Christine R, Moal V, Urban T, Benoit JP (2010) Toxicological study and efficacy of blank and paclitaxel-loaded lipid nanocapsules after i.v. administration in mice. Pharm Res 27:421–430

Jain A, Agarwal A, Majumder S, Lariya N, Kharya A, Himanshu A, Majumdar S, Agrawal GP (2010) Mannosylated solid lipid nanoparticles as vectors for site-specific delivery of an anti-cancer drug. J Control Release 148:359–367

Jaiswal MK, Banerjee R, Pradhan P, Bahadur D (2010) Thermal behavior of magnetically modalized poly (N-isopropylacrylamide)-chitosan based nanohydrogel. Colloids Surf B Biointerfaces 81(1):185–194

Jayakumar R, Chennazhi KP, Nair SV, Rejinold N (2011) The art, method, manner, process and system of preparation of alpha chitin nanogels for drug delivery and imaging applications, Indian Patent No. 357/CHE/2011 A

Jeong B, Bae YH, Kim SW (2000) In situ gelation of PEG-PLGA-PEG triblock copolymer aqueous solutions and degradation thereof. J Biomed Mater Res 50:171–177

Joshi MV Jr, Phansopkar P (2022) Superior replacement of medicinal gel with ayurvedic nanogel as a coupling medium for electrotherapeutic treatment of osteoarthritis: a review article. Cureus 1(14):e28658

Kabanov AV, Vinogradov S (2009) Nanogels as pharmaceutical carriers, finite networks of infinite capabilities. Angew Chem Int Ed 48:5418–5429

Kayal S, Ramanujan RV (2010) Doxorubicin loaded PVA coated iron oxide nanoparticles for targeted drug delivery. Mater Sci Eng C 30:484–490

Kievit FM, Wang FY, Fang C, Mok H, Wang K, Silber JR, Ellenbogen RG, Zhang M (2011) Doxorubicin loaded iron oxide nanoparticles overcome multidrug resistance in cancer in vitro. J Control Release 152:76–83

Kim JH, Bae SM, Na MH, Shin H, Yang YJ, Min KH, Choi KY, Kim K, Park RW, Kwon IC, Lee BH, Hoffman AS, Kim IS (2012) Facilitated intracellular delivery of peptide-guided nanoparticles in tumor tissues. J Control Release 157(3):493–499

Kim S, Park KM, Ko JY, Kwon IC, Cho HG, Kang D, Yu IT, Kim K, Na K (2008) Minimalism in fabrication of self-organized nanogels holding both anti-cancer drug and targeting moiety. Colloids Surf B Biointerfaces 63:55–63

Kohli E, Han HY, Zeman AD, Vinogradov SV (2007) Formulation of biodegradable nanogel carriers with 5′-triphosphates of nucleoside analogs that display a reduced cytotoxicity and enhanced drug activity. J Control Release 121:19–27

Kong IG, Sato A, Yuki Y, Nochi T, Takahashi H, Sawada S, Mejima M et al (2013) Nanogel-based PspA intranasal vaccine prevents invasive disease and nasal colonization by Streptococcus pneumoniae. Infect Immun 81:1625–1634

Kumar S, Anselmo AC, Banerjee A, Zakrewsky M, Mitragotri S (2015) Shape and size-dependent immune response to antigen-carrying nanoparticles. J Control Release 220:141–148

Lee J, Yoo HS (2008) Pluronic decorated nanogels with temperature responsive volume transitions, cytotoxicities and transfection efficiencies. Eur J Pharm Biopharm 70:506–513

Lee S, Yun MH, Jeong SW, Hoon C, Kim JY, Seo MH, Pai CM, Kim SO (2011) Development of docetaxel-loaded intravenous formulation, nanogel-PM using polymer-based delivery system. J Control Release 155(2):262–271

Lee Y, Park SY, Kim C, Park TG (2009) Thermally triggered intracellular explosion of volume transition nanogels for necrotic cell death. J Control Release 135:89–95

Li N, Wang J, Yang X, Li L (2011) Novel nanogels as drug delivery systems for poorly soluble anticancer drugs. Colloids Surf B Biointerfaces 83:237–244

Liu X, Sun J, Chen X, Wang S, Scot H, Zhan X, Zhan Q (2012) Pharmacokinetics, tissue distribution and anti-tumour efficacy of paclitaxel delivered by polyvinylpyrrolidone solid dispersion. J Pharm Pharmacol:775–782

Lo JT, Chen BH, Lee TM, Han J, Li JL (2009) Self emulsifying o/w formulations of paclitaxel prepared from mixed nonionic surfactants. J Pharm Sci 99:2320–2332

Mohamed F, van der Walle CF (2008) Engineering biodegradable polyester particles with specific drug targeting and drug release properties. J Pharm Sci 97:71–87

Muraoka D, Seo N, Hayashi T, Tahara Y, Fujii K, Tawara I, Miyahara Y, Okamori K, Yagita H, Imoto S, Yamaguchi R, Komura M, Miyano S, Goto M, Sawada S, Asai A, Ikeda H, Akiyoshi K, Harada N, Shiku H (2019) Antigen delivery targeted to tumor-associated macrophages overcomes tumor immune resistance. J Clin Invest 129:1278–1294

Naik S, Patel D, Chuttani K, Mishra AK, Misra A (2012) In vitro mechanistic study of cell death and in vivo performance evaluation of RGD grafted PEGgylated docetaxel liposomes in breast cancer. Nanomed Nanotechnol 8:951–962

Nambara K, Niikura K, Mitomo H, Ninomiya T, Takeuchi C, Wei J, Matsuo Y, Ijiro K (2016) Reverse size dependences of the cellular uptake of triangular and spherical gold nanoparticles. Langmuir 32:12559–12567

Oh JK, Bencherif SA, Matyjaszewski K (2009) Atom transfer radical polymerization in inverse miniemulsion; a versatile route towards preparation and functionalization of microgel/nanogels for targeted drug delivery applications. Polymers 50:4407–4423

Oh NM, Oh KT, Baik HJ, Lee BR, Lee AH (2010) A self organized 3-diethylaminopropyl bearing glycol chitosan nanogel for tumor acidic pH targeting, in-vitro evaluation. Colloids Surf B Biointerfaces 78:120–126

Park JH, Chi SC, Lee WS, Lee WM, Koo YB, Yong CS, Choi HG, Woo JS (2009) Toxicity studies of Cremophor-free paclitaxel solid dispersion formulated by a supercritical anti solvent process. Arch Pharm Res 32:139–148

Prakash R, Thiagarajan P (2011) Nanoemulsions for drug delivery through different routes. Res Biotech 2:9–13

Prasanth VV, Chakraborthy A, Mathew ST, Mathapan R (2011) Microspheres - an overview. Int J Res Pharm Biomed Sci 2:232–238

Pushpavanam K, Inamdar S, Dutta S, Bista T, Sokolowski T, Sapareto S, Rege K (2020) Plasmonic gel nanocomposites for detection of high energy electrons. J Mater Chem B 8:4930–4939

Rabaeh KA, Eyadeh MM, Hailat TF, Madas BG, Aldweri FM, Almomani AM, Awad SI (2021) Improvement on the performance of chemically cross-linked fricke methylthymol-blue radiochromic gel dosimeter by addition of dimethyl sulfoxide. Radiat Meas 141:106540

Rejinold NS, Muthunarayanan M, Chennazhi KP, Jayakumar R (2011b) Curcumin loaded fibrinogen nanoparticles for cancer drug delivery. J Biomed Nanotechnol 7:521–534

Rejinold NS, Sreerekha PR, Chennazhi KP, Nair SV, Jayakumar R (2011a) Biocompatible, biodegradable and thermo-sensitive chitosan-g-poly (N- isopropylacrylamide) nanocarrier for curcumin drug delivery. Int J Biol Macromol 49:161–172

Sahu MK, Ahmad D (2010) Development and optimization of fixed dose antihypertensive combination drugs using double layer sustained release microsphere technology. Int J Pharm Biomed Res 1:114–123

Salaun F, Vroman I (2009) Curcumin loaded nanocapsules, formulation and influence of the nanoencapsulation processes variables on the physico-chemical characteristics of the particles. Int J Chem React Eng 7:A55–A61

Shah P, Bhalodia D, Shelat P (2010) Nanoemulsion: a pharmaceutical review. Syst Rev Pharm 3: 24–32

Sheihet L, Garbuzenko OB, Bushman J, Gounder MK, Minko T, Kohn J (2012) Paclitaxel in tyrosine-derived nanospheres as a potential anti-cancer agent, in vivo evaluation of toxicity and efficacy in comparison with paclitaxel in Cremophor. Eur J Pharm Sci 45:320–329

Shigemasa Y, Saito K, Sashiwa H, Saimoto H (1994) Enzymatic degradation of chitins and partially deacetylated chitins. Int J Biol Macromol 16:43–49

Shimizu T, Kishida T, Hasegawa U, Ueda Y, Imanishi J, Yamagishi H, Akiyoshi K, Otsuji E, Mazda O (2008) Nanogel DDS enables sustained release of IL-12 for tumor immunotherapy. Biochem Biophys Res Commun 367:330–335

Siegel SJ, Kahn JB, Metzger K, Winey KI, Werner K, Dan N (2006) Effect of drug type on the degradation rate of PLGA matrices. Eur J Pharm Biopharm 64:287–293

Sorger D, Stadtmann H, Sprengel W (2020) Fading study and readout optimization for routinely use of LiF:Mg,Ti thermoluminescent detectors for personal dosimetry. Radiat Meas 135:106342

Sorina AG, Adi G, Corina R (2011) Systems based on dendrimers and antitumoral drug synthesized by non-covalent method: the influence of dendrimers generation. MaterialePlastice 48:17–22

Su M, Zhao M, Luo Y, Lin X, Xu L, He H, Xu H, Tang X (2011) Evaluation of the efficacy, toxicity and safety of vinorelbine incorporated in a lipid emulsion. Int J Pharm 411:188–196

Sun B, Ranganathan B, Feng SS (2008) Multifunctional poly (D,L- lactide-co-glycolide)/montmorillonite PLGA/MMT nanoparticles decorated by trastuzumab for targeted chemotherapy of breast cancer. Biomaterials 29:475–486

Tamura H, Nagahama H, Tokura S (2006) Preparation of hydrogel under mild conditions. Cellulose 13:357

Tan JPK, Zeng AQF, Chang CC, Tam KC (2008) Release kinetics of procaine hydrochloride from pH responsive nanogel, theory and experiments. Int J Pharm 357:305–313

Teskac K, Kristi J (2010) The evidence for solid lipid nanoparticles mediated cell uptake of resveratrol. Int J Pharm 390(1):61–69

Tobio M, Gref R, Sanchez A, Langer R, Alonso MJ (1998) Stealth PLA-PEG nanoparticles as protein carriers for nasal administration. Pharm Res 15:270–275

Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM (1999) Polymeric systems for controlled drug release. Chem Rev 99:3181–3198

Urbinati G, Marsaud V, Plassat V, Fattal E, Lesieur S, Michel J (2010) Renoir liposomes loaded with histone deacetylase inhibitors for breast cancer therapy. Int J Pharm 397:184–193

Vinogradov SV, Zeman AD, Batrakova EV, Kabanov AV (2005) Polyplex nanogel formulation for drug delivery of cytotoxic nucleoside analogs. J Control Release 107:143–157

Wang C, Wang YJ, Fan M, Luo F, Qian Z (2011) Characterization, pharmacokinetic and disposition of novel nanoscale preparations of paclitaxel. Int J Pharm 414:251–259

Wang D, Sun B, Feng M, Feng H, Gong W, Liu Q, Ge S (2015) Role of scavenger receptors in dendritic cell function. Hum Immunol 76:442–446

Wang Q, Xu H, Yang X, Yang Y (2008) Drug release behavior from in situ gelatinized thermosensitive nanogel aqueous dispersions. Int J Pharm 361:189–193

Wu XS, Wang N (2001) Synthesis, characterization, biodegradation, and drug delivery application of biodegradable lactic/glycolic acid polymers. Part II: biodegradation. J Biomater Sci Polym Ed 12:21–34

Xu D, Yao S, Liu Y, Sheng K, Hong J, Gong P, Dong L (2007) Size dependent properties of M-PEIs nanogels for gene delivery in cancer cells. Int J Pharm 338:291–296

Yassin AEB, Anwer MK, Mowafy HA, El-Bagory IM, Bayomi MA, Alsarra IA (2010) Optimization of 5-fluorouracil solid-lipid nanoparticles: a preliminary study to treat colon cancer. J Med Sci 7:398–408

Yi BG, Park OK, Jeong MS, Kwon SH, Jung JI, Lee S, Ryoo S, Kim SE, Kim JW, Moon WJ, Park K (2017) In vitro photodynamic effects of scavenger receptor targeted-photoactivatable nanoagents on activated macrophages. Int J Biol Macromol 97:181–189

Yoo HS, Park TG (2004) Folate receptor targeted biodegradable polymeric doxorubicin micelles. J Control Release 96:273–283

Yoshizawa Y, Kono Y, Ogawara KI, Kimura T, Higaki K (2011) PEG liposomalization of paclitaxel improved its in vivo disposition and anti-tumor efficacy. Int J Pharm 412:132–141

Yuba E, Yamaguchi A, Yoshizaki Y, Harada A, Kono K (2017) Bioactive polysaccharide-based PH-sensitive polymers for cytoplasmic delivery of antigen and activation of antigen-specific immunity. Biomaterials 120:32–45

Zhang JQ, Zhang ZR, Yang H, Tan QY, Qin SR, Qiu XL (2005) Lyophilized paclitaxel magnetoliposomes as a potential drug delivery system for breast carcinoma via parenteral administration, in vitro and in vivo studies. Pharm Res 22:573–583

Zhenghong X, Lingli C, Wangwen G, Gao U, Liping L, Zhang Z, Li Y (2009) The performance of docetaxel-loaded solid lipid nanoparticles targeted to hepatocellular carcinoma. Biomaterials 30:226–232

Zweers ML, Engbers GH, Grijpma DW, Feijen J (2004) In vitro degradation of nanoparticles prepared from polymers based on DL-lactide, glycolide and poly (ethylene oxide). J Control Release 100:347–351

Theranostic Applications of Functionalized Exosomes

12

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Abstract

Exosomes are cell membrane-derived vesicles which play an important role in cellular communication and act as transporters for proteins and genetic material. Due to their natural origin and low immunogenicity and toxicity, they are exploited as drug delivery for therapeutics as well as for diagnostics. The function of exosomes depends on the source from where they are derived. These vesicles have a diameter below 100 nm and can be characterized by nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), and dynamic light scattering (DLS). Various techniques are explored for surface functionalization for targeted delivery of therapeutics. Most commonly used techniques include click chemistry and genetic engineering. Exosomes prove to have great potential when it comes to diagnosis and targeted therapy. Developing exosome-based diagnostic techniques to detect disease precisely and early as well as treat disease marks a new era of personalized radiology and nuclear medicine. As circulating drug delivery vehicles for novel therapeutic modalities, exosomes offer a new platform for diagnostics and therapeutics.

Keywords

Extracellular vesicles · Nanocarriers · Drug delivery · Functionalization · Theranostics

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12.1 Introduction

The present world of nanodrug delivery systems is dominated by liposomes and polymeric nanoparticles. These drug delivery carriers have been used to deliver a plethora of actives, although issues related to stability, toxicity, and circulating capability need to be solved. These shortcomings are overcome by exosomes which are considered a superior choice considering the longer half-life, better tumor and tissue targeting, and biocompatibility (Patil et al. 2020). Exosomes are extracellular vesicles secreted by different types of cells. The extracellular vesicles are classified based on their origin and size into exosomes, microvesicles, and apoptotic bodies. The diameter of these vesicles ranges from 30 nm to 150 nm, 50 nm to 1 μm, and 50 nm to 5 μm, respectively. These exosomes are further classified as large exosomes (90–120 nm), small exosomes (60–80 nm), and exomeres (~35 nm) (Bunggulawa et al. 2018). A flowchart of the classification is shown in Fig. 12.1.

Exosomes are very first described in the 1980s as membrane-derived extracellular vesicles secreted from different types of cells (Orr et al. 1987). These nanosized vesicles range from 30 to 200 nm after getting released into the extracellular environment and are involved in a plethora of biological responses. These nanovesicles are formed from the membrane of late endosomes through the process of invagination and budding (Théry et al. 2002). The formation process of exosomes involves invagination of plasma membrane forming multivesicular bodies (MVBs). These MVBs contain intraluminal vesicles which are further secreted as exosomes as shown in Fig. 12.2.

Intercellular communication is important for cells in order to acclimatize with the events occurring in inter- and intracellular matrices. The fact that exosomes are responsible for intercellular communication and have the potential to transfer the actives to recipient cells has gained an enormous attention in the past decade (Milane et al. 2015). Exosomes play an important role in physiological and pathological process as well. The function of the exosomes depends on the source from where they are isolated or the nature of cells from which they are produced. Exosomes contain different types of mRNAs, lipids, and proteins. Research showed that exosomes contain different proteins that are involved in various cell-specific

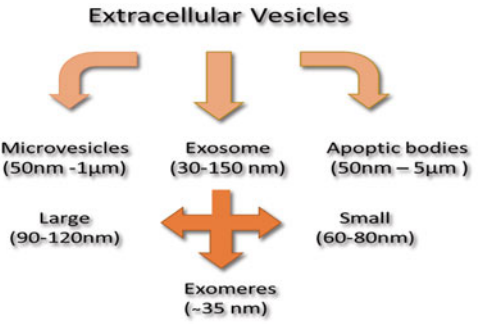

Fig. 12.1 Classification of extracellular vesicles

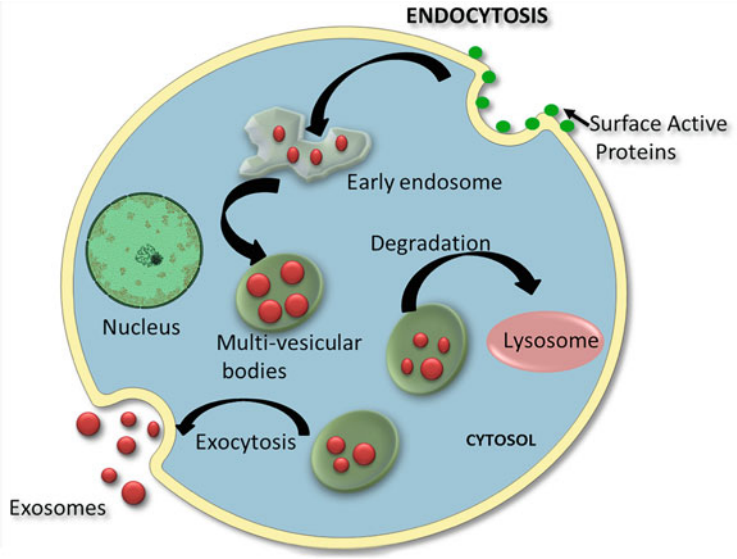

Fig. 12.2 Biogenesis of exosomes

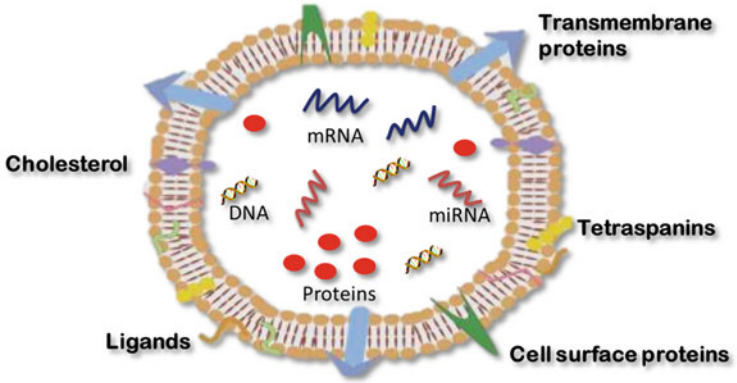

Fig. 12.3 Exosome structure and its contents

functions, lipids, cytokines, and numerous bioactive substances (Jeppesen et al. 2019). Exosomal proteins are classified into two categories. The first category of proteins facilitates vesicle formation and secretion. These include heat shock proteins (HSP-70 and HSP-90), endosomal sorting complex required for transport (ESCRT) complex-related proteins (Alix and TSG101), and membrane transport fusion proteins. The second category of proteins is related to their progenitor cells and possesses specific components. The basic representation and structure of exosomes and its contents are shown in Fig. 12.3. In this chapter we have discussed the origin of exosomes derived from different cells, techniques associated with

isolation of exosomes, and characterization of these extracellular bodies. The chapter also includes functionalization of exosomes for targeted therapy and their applications in therapeutics and diagnostics in brain, cancer, and skin conditions.

12.2 Origin of Exosomes

Exosomes are of endosomal origin**.** Exosomes derived from different cells demonstrate different roles in the body. Some of the sources from where exosomes are secreted are given below.

12.2.1 Macrophage Derived

Macrophages are type of white blood cells that kill infection-causing microorganisms. There are three types of macrophages depending on the function, namely, classically activated (M1) macrophages, wound-healing macrophages (also known as alternatively activated (M2) macrophages), and regulatory macrophages (Mregs). Macrophages are heterogeneous in nature. Therefore, exosomes isolated from the macrophage of a particular organ have a specific function (Li et al. 2020). Studies have been focused on various roles of exosomes isolated from macrophages including inflammation, injury, and repair. Li et al. demonstrated that exosomes derived from macrophage RAW 264.7 cell line prevented inflammation and eventually aided in diabetic wound healing. The derived exosomes enhanced the pro-inflammatory cytokines to improve angiogenesis and reepithelialization in diabetic wounds. To further explore the anti-inflammatory effects of exosomes, lipopolysaccharide (LPS) was used along with high concentration of exosomes for a strong inflammatory response. Results showed that angiogenesis-promoting and anti-inflammatory effects were reversed in LPS and high-concentration exosome group in comparison with the exosome treatment group alone (Li et al. 2019). Another study by Bouchareychas et al. isolating exosomes from bone marrow-derived macrophages (BMDM-exo) experimented that these exosomes contain anti-inflammatory microRNA-99a/146b/378a. These BMDM-exo when polarized with interleukin-4 (IL-4) cytokine (BMDM-IL-4-exo) further enhanced the mRNA which in turn suppresses inflammation via NF-κB pathway and TNF-α signaling. In vivo studies using $Apoe^{-/-}$ mice with repeated infusions of BMDM-IL-4-exo showed diminished hematopoiesis in bone marrow, myeloid cell count, and macrophages in aortic root lesions. Reduction in necrotic lesion area was also observed which eventually stabilizes atheroma indicating a useful approach for inflammatory disorders using macrophage-derived exosomes (Bouchareychas et al. 2020). Ye et al. studied the effects alveolar macrophage-derived exosome has on severe acute lung injury (ALI). ALI was induced with lipopolysaccharide, and exosomes were isolated from bronchoalveolar lavage fluid (BALF) at different time intervals. Release patterns of anti-inflammatory cytokines (IL-4, IL-13, and

IL-10) and fibrotic cytokines (TGFβ and FGF) were examined. Results revealed that exosomes secreted the early pro-inflammatory cytokines which eventually activated neutrophils responsible for polarizing macrophages to M2c that causes post-ALI fibrosis (Ye et al. 2020). Wang et al. observed the role of miRNA (miR) in inflammation regulation and cardiac injury. After myocardial infarction (MI), it was observed that miR-155 expression was upregulated in mice heart. MiR-155-containing exosomes isolated from macrophages inhibited cardiac fibroblast proliferation by downregulating Son of Sevenless 1 expression and promoted inflammation by decreasing suppressor of cytokine signaling 1 expression. The effect of miR-155 on MI was also observed in vivo. A significant higher survival rate was observed in miR-155$^{-/-}$ mice after MI. Moreover, a higher smooth muscle and collagen deposition was seen. Overall results showed that exosomes are identified as paracrine regulator for fibroblast proliferation and inflammation during cardiac injury and miR-155 inhibitor may have the potential for reducing MI-related adverse effects (Wang et al. 2017a). Wu et al. investigated that M2 macrophage-derived exosomes have the caliber to boost the movement of hepatocellular carcinoma (HCC) which may provide the mechanism of tumor metastasis. Tumor-associated macrophages (TAMs) are the main culprit in tumor aggressiveness. The study examined the effect of TAM on HCC. Outcomes revealed that M2-derived exosomes encouraged TAM-mediated pro-migratory activity with integrin and αMβ2 (CD11b/CD18) specificity. Study demonstrated that CD11b and CD18 are key biomarkers in activated TAMs for HCC diagnosis via matrix metalloproteinase-9 signaling pathway (Wu et al. 2021). Researchers also investigated the role of derived exosomes for the prevention and treatment of myocardial infarction (MI). Liu et al. examined the role of M1-like macrophage-derived exosome in a post-MI environment. It was discovered that pro-inflammatory exosomes exerted an anti-angiogenic effect and expressed pro-inflammatory mRNA (miR-155). MiR-155 when transferred to endothelial cells (ECs) downregulated novel target genes, including Rac family small GTPase 1 (RAC1), p21 (RAC1)-activated kinase 2 (PAK2), sirtuin 1 (Sirt1), and protein kinase AMP-activated catalytic subunit alpha 2 (AMPKα2) and eventually inhibited cardiac dysfunction (Liu et al. 2020a). Experiments have shown that exosomes promote cell migration and invasion in colon cancer. MiRNA-containing exosomes were derived from M2 macrophages (MDE) by Lan et al. which reported that these MDE exhibit a functional role in regulating migration and invasion of colorectal cancer (CRC) cells. MDE expressed miR-21-5p and miR-155-5p which bind to BRG1 coding sequence and downregulate its expression although BRG1 is the key player in promoting metastasis. This reciprocal cross talk between colorectal cancer cells and M2 macrophages provides a new opportunity for the treatment of metastatic colorectal cancer (Lan et al. 2019). Some studies have also shown that exosomes promote cancer progression through a signaling pathway that can serve as a potential molecular target for cancer treatment (Yan et al. 2020).

12.2.2 Tumor Derived

Exosomes released for the tumor cells also known as tumor-derived exosomes (TDEs) contain a variety of materials including proteins, nucleic acids, mRNA, and DNA fragments. Many studies demonstrated that TDEs are active in tumor growth, angiogenesis, invasion, and metastasis. TDEs also serve as biomarkers in cancer theranostics (Zhang and Zhou 2019). Jabbari et al. reviewed breast cancer-derived exosomes and their function in tumorigenesis. The roles of these exosomes include radioresistance, chemoresistance, immunosuppression, and promotion of metastasis, proliferation, and angiogenesis (Jabbari et al. 2020). TDEs serve the dual purpose of biomarkers as well as target for treatment. Mausavi et al. focused on TDEs with a special focus on colorectal cancer. Some of the biomarkers (carcinoembryonic antigen (CEA), epithelial cell adhesion molecule (EpCAM)) are not detected in early stages of colorectal cancer, but miRNAs covered with exosomal membranes are secreted in higher amount by tumor cells when compared with normal cells. CEA and EpCAM both are expressed in abundance on CRC-derived exosomes (Mousavi et al. 2019). Studies have been conducted to investigate the role of TDEs in hematological malignancies as well. In hematological malignancies TDEs are found to suppress antileukemia immunity and reprogram bone marrow environment (Boyiadzis and Whiteside 2017). Due to the poor prognosis of ovarian cancer especially its detection in last stages, TDEs can be used as biomarkers and therapeutic delivery tools which will be more effective in early detection and real-time treatment response monitoring. TDEs provide a detailed vision of intercellular tumor environment with a minimal invasive "liquid biopsy" method which will allow early detection before the metastasis commence (Sharma et al. 2017). The multifaceted nature of TDEs also unveils its potential in the diagnosis and targeted therapy of non-small cell lung cancer (NSCLC) by controlling various pathways. TDEs also serve as noninvasive prognostic markers in NSCLC (Zheng et al. 2018).

12.2.3 Mesenchymal Stem Cell (MSC) Derived

MSCs are pluripotent stem cell present in multiple tissues. They possess distinct features of self-renewal and colony formation (Xunian and Kalluri 2020). They also exhibit anti-inflammatory and immunosuppressive properties. MSC-derived exosomes express markers like CD73, CD44, and CD90, which are characteristic of MSC (Ramos et al. 2016). There are numerous studies depicting the application of MSC in cardioprotection (Zhu et al. 2018), hepatoprotection (Tan et al. 2014) wound healing, tissue repair (Ha et al. 2020; Ma et al. 2019), and neuroprotection (Xian et al. 2019; Riazifar et al. 2019).

12.3 Isolation of Exosomes

Due to the heterogenous nature of exosomes, isolation is a difficult task to perform. Due to the similarity in size with other extracellular vesicles, the isolation process development has greatly impeded. In recent times, some of the isolation techniques are developed providing satisfiable quantity and purity of exosomes. Some of the techniques include centrifugation, size-based, polymer precipitation, capture-based, dielectrophoretic (DEP) separation, and deterministic lateral displacement (DLD) separation. A detailed description of these techniques is given as follows.

12.3.1 Ultracentrifugation

It is the gold standard method used for pelleting exosomes. Out of all the reported exosome isolation methods, ultracentrifugation accounts for an estimated 56% of the isolation techniques. Since this method is time-consuming and laborious, exosome separation from clinical sample is not suitable (Witwer et al. 2013). During ultracentrifugation cellular debris and apoptotic bodies are removed, and exosomes are isolated based on density, size, and shape. Isolation is also based on the amount of centrifugal force applied for separation. The centrifugal force applied can go as high as 1,000,000 × g. Ultracentrifugation is divided into preparative and analytical ultracentrifugation. The former one is used for investigating physicochemical properties and molecular interactions, whereas the later one is used for exosomes (Li et al. 2017). Ultracentrifugation is also used in combination with sucrose cushions to isolate the low-density exosomes. Sequential configuration when combined with sucrose gradient centrifugation provides exosomes of high purity (Zeringer et al. 2015). The widely used method is differential centrifugation for isolation of exosomes in which successive rounds of centrifugation are performed with increasing centrifugation forces in order to pellet out exosomes (Livshits et al. 2015). Many researchers have also reported that ultracentrifugation can produce damaged exosomes due to the high shear forces employed in exosomes (Konoshenko et al. 2018).

12.3.2 Size-Based Filtration

This technique includes ultrafiltration, size-exclusion chromatography (SEC), and sequential filtration. In this technique, the isolation is based on size and molecular weight of the sample. The ultrafiltration process uses membrane filters of various molecular weights. It is a cost-effective method and faster than ultracentrifugation. The repeated washing of the membrane is the downside on this method. Sometimes, pore clogging of the membrane can drastically reduce the process time (Cheruvanky et al. 2007).

SEC utilizes a porous stationary gel phase which sorts out the macromolecules and particulate matter based on their size. Since exosomes cannot enter the gel pores,

they pass with the mobile phase resulting in early elution. SEC provides quick, easy, and low-cost isolation (Sidhom et al. 2020). SEC is used to separate exosomes from bio-fluids using Sepharose 2B and CL-4B packed columns (Sabapatha et al. 2006). SEC has also been used in combination with ultrafiltration to produce a significant higher yield of exosomes and also preserve intact the biophysical and functional properties (Nordin et al. 2015). A comparative study between ultracentrifugation and SEC for isolation of urinary exosomes was conducted by Guan et al. Results revealed superior and more purified yield of exosomes in SEC. Rupturing and insufficient precipitation was seen when isolation was done through ultracentrifugation (Guan et al. 2020).

On the other hand, sequential filtration is a step-by-step process. The first step is a dead-end filtration or normal filtration which separates the cell debris and large apoptotic bodies. The second step further removes the non-exosomal bodies from the exosomes through tangential flow filtration (TFF). Finally, filtration takes place from a track-etched membrane with a uniform pore size to enable size-specific isolation of exosomes (Heinemann and Vykoukal 2017). A kit called ExoMir™ has also been developed by BioScientific Inc. on the principle of sequential filtration (Yang et al. 2020).

12.3.3 Polymer Precipitation

This method is based on the use of water-excluding polymers such as polyethylene glycol (PEG). The polymer reduces the hydration of extracellular vesicles and precipitates them which are further processed for purification. Commercial kits are also developed based on this mechanism such as s ExoQuick, Exo-spin, and PureExo. The isolated exosomes are of high purity, i.e., 10^7–10^9 particles per microgram (Hou et al. 2019). Exo-spin is ideally based on precipitation and size-exclusion chromatography. Ryu et al. combined ultracentrifugation with polymer-based precipitation kit ExoQuick for isolating small extracellular vesicles from human serum. The combined method yielded $59.3 \pm 20.6 \times 10^9$ per ml of serum, whereas with four cycles of ultracentrifugation yielded $29.2 \pm 9.9 \times 10^9$ per ml of serum of extracellular vesicles indicating a feasible method for isolating exosomes (Ryu et al. 2020). Chung et al. also demonstrated that polymer-based precipitation gives higher EV-miRNA recovery from serum to enhance miRNA biomarker performance of gastric cancer (Chung et al. 2020).

12.3.4 Immunoaffinity Capture-Based Isolation

This isolation technique is based on antigen-antibody reaction to capture exosomes. The exosome membrane expresses several proteins on their surface such as CD9, CD63, CD81, EPCAM, and annexin, but they lack their soluble counterparts. Immunoaffinity methods are rapid and easy and yield high-purity exosomes (Oksvold et al. 2015).

Table 12.1 Commercial kits available for exosome isolation

Method	Commercial kit	Manufacturer
Precipitation	Total exosome isolation (TEI)	Invitrogen, MA, USA
	ExoQuick™ exosome precipitation solution (EXQ)	System biosciences, CA, USA
	Ribo™ exosome isolation reagent	RIBO, Guangzhou, China
	EXO-prep	HasnaBioMed life sciences, Estonia
	Invitrogen	Thermo fisher scientific
	PureExo® exosome isolation kit	101 bio, CA, USA
Chromatography	Exosome purification kit	Norgen Biotek Corp
	Exosome isolation kit	Cusabio technology LLC
	qEV isolation column	IZON science ltd
	Exo-spin™	Cell guidance systems, USA
Immunoaffinity	MagCapture™ exosome isolation kit	FUJIFILM Wako pure chemical corporation, Japan
	Exosome isolation and analysis kit	Abcam, USA

12.3.5 Microfluidic-Based Separation

Microfluid systems are the newer techniques which check all the boxes required for separation of exosomes. Microfluidic-based techniques outpass all the conventional techniques which consume time and cost and produce a low yield and purity of exosomes. Microfluidic-based isolation methods are being used in diagnosis, treatment, and other biomedical areas (Guo et al. 2021). Apart from the basic separation factors like size and density, this technique also uses some innovative active methods such as inertial lift force (Dudani et al. 2015), acoustic waves (Wu et al. 2017), deterministic lateral displacement (Wunsch et al. 2016), dielectrophoresis (Wang et al. 2013), and nano-trap wires (Liga et al. 2015).

12.3.6 Isolation Using Commercial Kits

Some readily available kits are also available in the market for isolation based on the abovementioned methods. Table 12.1 lists some of the commercially available kits and method of isolation.

12.4 Characterization of Exosomes

12.4.1 Transmission Electron Microscopy (TEM)

TEM is used to analyze the morphology and size of exosomes. TEM works on the principle of electron beams instead of light. These electron beams passed through the specimen and enable the observer to see the morphology or structure of the

specimen. Prior to the imaging, the specimen is fixed in glutaraldehyde and paraformaldehyde at room temperature. Sometimes, the morphology of exosomes can be affected as the electron beam tends to damage the sample. An upgrade of TEM is cryo-TEM which follows a different protocol for sample preparation. Samples are viewed under liquid nitrogen which prevents any possible damage to the samples (Sokolova et al. 2011).

12.4.2 Nanoparticle Tracking Analysis (NTA)

Application of NTA to exosomes characterization was first done in 2011. NTA is used to calculate the particle size of the sample. It involves light scattering as well as Brownian motion of particles. NTA can measure particle size range of 10–1000 nm, and the diameter of particles is calculated using Stokes-Einstein relationship. NTA can identify particles of different sizes simultaneously in the same solution. Moreover, it can also analyze fluorophores attached to antibodies to detect the antigens on exosomes. NTA has now become the gold standard for characterization of exosomes (Sokolova et al. 2011; Gercel et al. 2012). The important parameter for a successful NTA analysis is the diluent used, which can cause contamination during sample preparation.

12.4.3 Atomic Force Microscopy (AFM)

AFM is used to examine the topography and mechanical properties of exosomes and is a surface-scanning technique. Sample is immobilized on a surface for imaging. AFM scans the surface using a cantilever tip (Parisse et al. 2017). When the nanoscale tip comes in contact with the sample surface, the cantilever bends, and the bending is detected by a laser diode and a split photodetector. Hardij et al. compared the air-tapping and liquid peak force mode of AFM for characterization of extracellular vesicles. The concentration of sample for analysis in air mode is 10 μg/ml and in liquid mode is 5 μg/ml. Liquid mode provides the native size of vesicles with a difference of about six times with the air mode. Liquid mode helps in the preservation of the physiological state of sample and provides highly precise size distribution (Hardij et al. 2013).

12.4.4 Dynamic Light Scattering (DLS)

DLS also known as photon correlation spectroscopy is a physics technique and is used to determine the size distribution of particles in a suspension. The working principle of DLS is based on the Brownian motion of particles. The sample is irradiated with a laser beam and the fluctuations in scattered light are detected by

fast photon detector. This technique is suitable when the sample is monodispersed. The sample with different particle sizes can cause interference in detection. DLS has been effectively used in characterizing the ovarian cancer cell-derived exosomes (Gercel et al. 2012).

12.4.5 Resistive Pulse Sensing (RPS)

RPS is a technique which has a measurement range from 100 nm to 100 μm. It is used to determine the concentration and size of particles in a sample suspension. Tunable RPS is an advanced technique of RPS which is based on the coulter counter method for particle counting. Advantages of TRPS include low operational cost, quick analysis, and wide range of analytes (Maas et al. 2017).

12.4.6 Flow Cytometry

It is a high-throughput and most frequently used technology to characterize exosomal surface proteins. Particles to be measured are labeled with fluorescent dyes. Flow cytometry is based on the principle of light scattering and fluorescence emission when beam of laser of specific wavelength hits the sample in a fluid stream. Early flow cytometry equipment can detect particles from 300 nm to 500 nm, but the latest technique has the lower limit of 100 nm, high resolution, and fluorescent amplification (van der Pol et al. 2010).

In addition to the abovementioned techniques, some other techniques are also being used to characterize exosomes. These include surface plasmon resonance (Thakur et al. 2017), nuclear magnetic resonance, and colorimetric detection (Patil et al. 2020).

12.5 Functionalization

Various approaches have been considered in order to functionalize exosomes for better and targeted therapeutic efficacy. Some of the functionalization methods are listed below.

12.5.1 Click Chemistry/Covalent Modification

This method involves the conjugation of ligands with the surface of exosome with a copper-catalyzed azide alkyne cycloaddition which forms a triazole link. Click chemistry is an easy process due the availability of reagents and mild reaction conditions (Hein et al. 2008). The conjugation does not affect the diameter of exosomes nor their uptake in the cells (Smyth et al. 2014). Jia et al. used click

chemistry method for the functionalization of exosomes. Initially, exosomes were loaded with superparamagnetic iron oxide nanoparticles (SPIONs) and curcumin and then conjugated with neuropilin-1-targeted peptide (RGE peptide) by cycloaddition reaction of sulfonyl azide for glioma theranostics. The resulting exosomes showed better performance than the others in treatment groups (Jia et al. 2018). An et al. developed an ultrasensitive aptasensor for detection of tumor exosomes. The aptasensor was based on click chemistry and DNA hybridization chain reaction. The functionalized lipid electrophiles were conjugated to exosomes through amino and aldehyde group reaction. The concentration of exosomes was quantified by analyzing the electrochemical reduction current of 2,3-diaminophenazine. This method resulted in a sensitive detection of exosomes with a limit of 96 particles/μl (An et al. 2019). Another study conducted by Lee et al. labeled azido-containing exosomes with aza-dibenzylcyclooctyne (ADIBO) fluorescent dyes by bioorthogonal click chemistry reaction. The resulting exosomes were used for in vivo tracking in tumor-bearing mice (Lee et al. 2018).

12.5.2 Genetic Engineering

Exosomal membrane is expressed with a variety of proteins which can be ligated with different moieties for facilitating site-specific delivery. Kim et al. modified exosomes isolated from HEK293 cells. Vectors encoding cardiac-targeting peptide (CTP)-Lamp2b was introduced in the in the HEK239 cells and attached using glycosylation sequences. These genetically modified exosomes were further evaluated for their delivery to heart cells and tissues (Kim et al. 2018). Lydia et al. engineered the dendritic cells to express Lamp2b, an exosomal membrane protein, fused to the neuron-specific rabies virus glycoprotein (RVG) peptide in order to deliver siRNA to the brain (Betzer et al. 2017). Tian et al. also genetically engineered dendritic cells to express Lamp2b fused to av. integrin-specific iRGD peptide (CRGDKGPDC) for breast cancer targeting. pEGFP-C1-RVG-Lamp2b-expressing vector was reengineered by replacing the RVG segment with iRGD. Dendritic cells were transfected with iRGD-Lamp2b fusion proteins using Lipofectamine 2000 reagent. Engineered exosomes were then isolated for further studies (Tian et al. 2014). Wang et al. modified exosome membrane for synergistic approach for angiogenesis. Modification of Arg-Gly-Asp (RBD) peptide to the exosome membrane was done in order to bind with $\alpha v\beta 3$ integrin on the surface of blood vessel (Wang et al. 2017b).

Click chemistry and genetic modification are most widely used approaches for surface functionalization of exosomes. Apart from these methods, some other methods are also employed for surface modification. These include electrostatic interaction and ligand receptor interaction.

12.6 Applications

12.6.1 Theranostic in Brain Disorders

There is a plethora of examples that demonstrate that labeled exosomes can help in in vivo imaging and treatment of various brain disorders (Chang et al. 2018; Saeedi et al. 2019). A novel method was developed by Perets et al. for longitudinal and quantitative in vivo neuroimaging based on classical X-ray computed tomography (CT). They used gold nanoparticles as labeling agents on MSC-derived exosomes. These labeled exosomes were administered intranasally, and X-ray CT was used to track the exosomes' migration and their patterns in various brain pathologies like AD, Parkinson, stroke, and autism. Results revealed that post-96-hr administration labeled exosomes were accumulated in the pathologically related murine brain models. The exosomes were selectively taken up by neuronal cells in the pathological regions suggesting a strong application of exosomes in theranostics (Perets et al. 2019). Khongkow et al. modified gold nanoparticles with neuron-targeted exosome for the delivery to brain cells for theranostic applications. Exosomes were isolated from human embryonic kidney (HEK293T) cells after transfection by Lipofectamine with pcDNA GNSTM-3-RVG-10-Lamp2b-HA vector in order to express Lamp2b protein. Gold nanoparticles coated with modified exosomes had a particle size of 105 ± 10.1 nm and polydispersity index of 0.430 ± 0.06. It was observed that exosome-coated nanoparticles showed higher targeting to brain cells in comparison with non-coated nanoparticles. The accumulation of gold nanoparticles after intravenous injection in mice brain was confirmed by in vivo bioluminescence imaging. Gold nanoparticles coated with surface-modified exosomes were superior to those of unmodified ones in terms of specificity and transcytosis of brain cells (Khongkow et al. 2019). A study conducted by Jia et al. revealed that exosomal synaptic proteins such as growth-associated protein 43 (GAP43), neurogranin, synaptosome-associated protein 25 (SNAP25), and synaptotagmin 1 can act as biomarkers to predict Alzheimer's disease (AD) before cognitive impairment or at an asymptomatic stage. A clinical trial with a total of 320 subjects was conducted, out of which 160 subjects were preclinical AD. Results of trial revealed that these proteins reflect the pathological alterations in AD brain and have the potential to differentiate between AD and amnestic mild cognitive impairment (Jia et al. 2021). Cui et al. reported that MSC-derived exosomes preconditioned with hypoxia could prevent the memory deficits in AD. Exosomes were systemically administered to transgenic APP/PS1 mice. Significant increase in the expression of miR-21 was observed after hypoxic treatment. In preconditioned hypoxia MSC exosome group, improved memory and learning caliber was seen along with lowered plaque deposition and lower Aβ levels. Sharp decrease in the levels of glial fibrillary acidic protein, ionized calcium-binding adaptor molecule 1, TNF-α, IL-1β, and activation of STAT3 and NF-κB was also observed confirming the improvement in cognitive functions by restoration of synaptic dysfunction and regulation of inflammatory responses (Cui

et al. 2018). Alvarez-Erviti et al. loaded dendritic cell-derived exosomes with exogenous siRNA. The exosomes before loading siRNA were functionalized with a neuron-specific RVG peptide and administered intravenously. Results revealed that RVG-targeted exosomes effectively delivered siRNA resulting in the knockdown of BACE1 gene which is a therapeutic target in wild-type mice for AD (Alvarez-Erviti et al. 2011). Qu et al. developed dopamine-loaded blood exosomes for therapeutic intervention of Parkinson's disease (PD). Exosomes subjected to particle size and morphological analysis showed size range of 40 nm–200 nm and spherical morphology. A 15-fold increase was observed in the delivery of dopamine using exosomes to the brain including the substantia nigra and striatum region. Better therapeutic efficacy was also seen in vivo using a PD murine model along with lower systemic toxicity when compared with free dopamine through intravenous administration (Qu et al. 2018). The α-synuclein aggregates are important from pathological standpoint. Liu et al. engineered a hybrid system for clearing the α-synuclein aggregates. The hybrid system acts as a nanoscavenger and immune activation of PD. The system consisted of functionalized exosome coating genechem nanocomplex named rabies virus glycoprotein (RVG) peptide-modified exosome (EXO) curcumin/phenylboronic acid-poly(2-(dimethylamino)ethyl acrylate) nanoparticle/small interfering RNA targeting SNCA (REXO-C/ANP/S). Improved motor behavior was observed in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mice model of PD and reduced the α-synuclein aggregates in diseased neurons (Liu et al. 2020b). Luo et al. loaded epicatechin gallate (ECG) into bovine milk-derived exosomes to observe its therapeutic efficacy as a neuroprotective agent on a rotenone-induced PD model in vitro on SHSY5Y cells. Exosomes entered the cells through caveolae-mediated endocytosis-inhibited autophagy and cell apoptosis induced by rotenone. Enhanced antioxidant and neuroprotective effects were observed as well (Luo et al. 2021). Tian et al. conjugated a peptide (cRGDyK) onto the exosome's surface for cerebral ischemia therapy. These conjugated exosomes were loaded with curcumin and administered in a transient middle cerebral artery occlusion (MCAO) mice model. Results showed that conjugated exosomes targeted the lesion area in the ischemic brain. When loaded with curcumin, suppressed inflammatory response and cellular apoptosis in lesion region was observed (Tian et al. 2018). Another study utilizing exosomes as a diagnostic tool for neuroimaging was conducted by Betzer et al. Exosomes were functionalized with 5-nm-size glucose-coated nanoparticles and administered via intranasal route. Results revealed that intranasal route led to better brain accumulation when compared with intravenous route indicating better in vivo neuroimaging. In vivo studies involving a murine model of focal brain ischemia showed that intranasally administered functionalized exosomes accumulated at the lesion site over 24 h (Betzer et al. 2017). A detailed review is provided by Khan et al. highlighting recent research for bioengineered exosomes for ischemic stroke therapy (Khan et al. 2021).

12.6.2 Theranostic in Cancer

Exosomes have shown a remarkable role in the diagnosis and therapy of cancer (Ailuno et al. 2020). They play an important role in the transmission of information by combining with receptor cells. Not only do they serve a purpose as clinical biomarkers but also they have the potential as drug delivery vectors which lead to the strong potential in the field of cancer diagnosis, prognosis, and treatment (Ma et al. 2021). Recent studies have shown that glioma-derived exosomes help in the augmentation of immunosuppressive microenvironment. Furthermore, an increase in miR-1246 of glioblastoma patients can possibly act as a biomarker in glioblastoma diagnosis and facilitate immunotherapy (Qian et al. 2020). Jia et al. loaded superparamagnetic iron oxide nanoparticles (SPIONs) and curcumin (Cur) into exosomes and then conjugated with neuropilin-1-targeted peptide (RGERPPR, RGE) for therapeutic action and imaging in glioma. The functionalized exosomes showed synergistic effect against intracranial tumors by delaying the tumor recurrence, while extending the life span of mice (Jia et al. 2018). Zou et al. developed aptamer functionalized exosomes for cell-specific delivery. A diacyl lipid-aptamer conjugate was used as a functional ligand. Exosomes were isolated from immature dendritic cell and loaded with doxorubicin by electroporation. Exosomes were then modified with diacyl lipid-DNA probes previously labeled with 5-carboxyfluorescein. Selective cellular recognition and uptake were done using CEM; a T-leukemia cell line showed that modified exosomes performed better while inducing selective therapeutic efficacy. Cellular uptake was dominated by clathrin-mediated endocytic pathway. Furthermore, a lower cytotoxicity was induced with functionalized exosomes loaded with doxorubicin when compared with free doxorubicin (Zou et al. 2019). Another study conducted by Li et al. emphasized on the importance of exosomes in cancer. The study was conducted using exosomes isolated from A33-positive LIM1215 cells and loaded with doxorubicin. Functionalization was achieved through forming a complex of A33-positive exosomes with surface carboxyl superparamagnetic iron oxide nanoparticles coated with A33 antibodies to target A33-positive colon cancer cells. In vitro cell line studies showed enhanced cellular uptake along with binding affinity. Inhibition of tumor growth and prolonged survival of mice were also observed (Li et al. 2018a). In breast tumor cells, levels of miRNA-134 are significantly downregulated. Brien et al. studied that miRNA delivery via exosomes into Hs578Ts(i)8 cells reduced cellular proliferation, signal transducer and activator of transcription 5B (STAT5B), and heat shock protein 90 (Hsp90) and enhanced sensitivity to anti-Hsp90 drugs (O'Brien et al. 2015). Kahroba et al. summarized the potential role of exosomes from tumor cells in the diagnosis and treatment of gastric cancer (Kahroba et al. 2019). In certain cancers like gliomas and adenocarcinomas, miR-21 is abnormally overexpressed which leads to the proliferation of cancer cells. Zhan et al. developed a system where exosomal membrane is manipulated by embedding doxorubicin and cholesterol-modified miRNA-21 inhibitor (miR-21i) in lipid matrix, whereas the magnetic molecules and endosomolytic peptides L17E are conjugated with the membrane. Results demonstrated that engineered exosomes effectively delivered the drug to

tumor cells and enhanced tumor accumulation as well. Downregulation of miR-21 was observed in U87 and MDA-MB-231 cell lines. In vivo studies showed inhibition of tumor growth with lesser side effects (Zhan et al. 2020). Another study involving a pH-responsive superparamagnetic nanoparticle cluster labeled with transferrin and bonded with transferrin receptor-positive (TfR+) exosomes for the delivery of chemotherapeutics to tumors was conducted by Yang et al. The resulting exosomes showed better tumor suppression when loaded with doxorubicin in comparison with free drug (Yang et al. 2019). Li et al. developed an immunoassay that could diagnose, classify, and monitor the metastasis of pancreatic cancer patients. The developed ultrasensitive polydopamine bifunctionalized surface-enhanced Raman scattering (SERS) immunoassay can detect only a single exosome in just a 2 μl sample. Not only can it distinguish between metastasized and non-metastasized tumors but also P1–2 stage from P3 stage of cancer. The SERS sensor was developed by employing a self-polymerizing polydopamine layer and encapsulating specific antibodies simultaneously. SERS tags were prepared using gold nanoparticles as core, while silver, polydopamine, antibodies, and BSA were conjugated onto the surface of gold nanoparticles (Li et al. 2018b). Phung et al. designed anti-CTLA-4 antibody-functionalized exosomes in order to target antigen-specific T-cells. Exosomes were isolated from bone marrow-derived dendritic cells loaded with ovalbumin. Functionalization of ovalbumin exosomes with anti-CTLA-4 was achieved by chemical reaction. The T-cell binding capacity functionalized exosomes showed highest ability to bind with $CD4^+$ and $CD8^+$ T-cell. Moreover, higher levels of TNF-α and IFN-γ were secreted with functionalized exosomes. Exosomes also facilitated T-cell targeting in tumor-draining lymph nodes and elevated the ratio of effector T-cells/regulatory T-cells within tumors causing inhibition in tumor growth (Phung et al. 2020). Pancreatic ductal adenocarcinomas (PDACs) are diagnosed at very advanced stages which lead to a 5-year survival rate of only 8%. PDAC is a very lethal cancer. The shortcomings of early diagnosis are due to the lack of reliable biomarkers. Studies have been conducted to identify different biomarkers for PDAC diagnosis at an early stage. Nakamura MD et al. isolated exosomes from pancreatic juice of chronic pancreatitis (CP) and PDAC patients and observed the role of exosomal microRNAs as a potential biomarker. Exosomes were isolated and characterized using ultracentrifugation and NTA, respectively. A relatively higher concentration of exo-miR-21 and exo-miR-155 was observed in PDAC patients compared with CP patients. The accuracy was improved to 91% when ex-miR profiling results were combined with pancreatic juice cytology indicating that exo-miR-21 and exo-miR-155 can serve the role of stable potential biomarkers for PDAC (Nakamura et al. 2019). Another study was conducted by Buscail et al. for the diagnosis of resectable PDAC with CD63-GPC1-positive exosomes coupled with CA19–9. GPC1-positive exosomes were isolated with a commercial kit from sera from cancer patients with the aid of anti-CD63-coupled magnetic beads. To validate the isolation exosomes were stained with anti-CD63-FITC and confirmed with flow cytometry. It was observed that the percentage of GPC1-positive beads was significantly higher in PDAC patients in comparison with the patients without cancer. The diagnostic potential of GPCI-positive exosomes was evaluated on the basis of

accuracy, sensitivity, and specificity. It was found that the resulting exosomes have high sensitivity, specificity, and diagnostic accuracy (82%, 86%, and 84%). The results were clinically relevant to the available diagnostic tools (Buscail et al. 2019). Bellavia et al. engineered HEK293T cells to express Lamp2b and human interleukin-3 (IL3L) protein via a Pinco-based construct for targeting chronic myeloid leukemia (CML). Furthermore, siRNA transfection was performed, and exosomes were isolated. These isolated exosomes were then loaded with imatinib and further tested for their efficacy in cell cytotoxicity. Outcomes of the experiment showed that IL3L exosomes loaded with imatinib showed better targeting to CML cells and inhibited in vitro and in vivo tumor growth (Bellavia et al. 2017).

12.6.3 Theranostic in CVS

Exosomes are gaining interest in cardiac protection due to the presence of various RNAs and proteins. Studies have shown a communication between cardiomyocytes, fibroblasts, and smooth muscle cells (Sluijter et al. 2014; Hergenreider et al. 2012). During cardiac tissue injury, exosomes derived from activated macrophage containing miR-155 are known to decrease the fibroblast proliferation and reduce cardiac inflammation (Wang et al. 2017a). Kore et al. studied the effect of MSC-derived exosomes on cardiac injury. The team studies the proteomic alterations in mice with left coronary artery ligation focusing on peri-infarct areas. Cell death and inflammatory marker expression were investigated using Western blot and immunofluorescence. Cell death markers were significantly reduced with MSC exosome treatment leading to better survival of mice. Survival of mice increased to 82% with MSC exosome treatment on the seventh day in comparison to the saline-treated mice. Enhanced fractional shortening and left ventricular end-systolic thickness were observed to be 2% and 0.7 mm, respectively. Improved proteomic profile was observed in infarct as well as peri-infarct regions. Cell line study on primary mouse cardiomyocytes revealed reversed inflammation-induced pro-apoptotic and inflammatory signals indicating the cardioprotective effects of exosomes (Kore et al. 2021). Exosomes are also associated with myocardial ischemia providing a fact that their levels corelate with the severity of cardiac injury. The levels of exosomes with unique miRNA have been found to be elevated in patients with cardiovascular diseases (Sluijter et al. 2018). Patients with acute myocardial infarction and unstable angina pectoris have been found to have significantly elevated serum levels of miRNA (miR)-1 and miR-133a. Kuwabara et al. utilized exosomes as a diagnostic tool to measure miRNA-133a levels by stimulating H9c2 cardiomyocytes with A23187, a divalent cation ionophore. It was observed that elevated levels of circulating miRNA-133a originated from injured myocardium since the elevated levels were observed where dead cells were detected. Exosomal levels of miRNA-133a can be used as a biomarker in cardiac diseases (Kuwabara et al. 2011). Gallet et al. isolated cardiosphere-derived cell (CDC) exosomes and studied them in a pig model of acute and convalescent myocardial infarction. Exosomes were administered by intramyocardial delivery after reperfusion. MRI

images after a month of treatment showed reduced scar size, while preserving the left ventricular ejection fraction. Histological figures revealed reduction in collagen content of left ventricle and hypertrophy in cardiomyocytes (Gallet et al. 2017). Ibrahim et al. suggested that exosomes isolated from CDCs can facilitate in the regeneration of scarred heart muscle. Exosomes secreted from CDC can facilitate the proliferation of cardiomyocytes and inhibit apoptosis. CDC exosomes also improves the viable mass after myocardial infarction. Furthermore, they contain miRNA-146a which increases the thickness of infarct wall and enhances tube formation in human umbilical cord endothelial cells which highlight the potential of exosomes as cell-free therapeutics (Ibrahim et al. 2014). Bu et al. revealed that exosome-mediated delivery can be useful in atherosclerosis treatment. IL-10 is a potent anti-inflammatory cytokine which was engineered to replace miR-122 sites with miR-155 recognition sites. These engineered IL-10 mRNAs were then encapsulated into exosomes and delivered efficiently into macrophages in the plaque of apolipoprotein E-deficient mice. It was observed that the encapsulated IL-10 mRNA was translated into protein in the cells of plaque with low systemic leaky expression. Repeated delivery of IL-10 in the plaque could alleviate atherosclerosis (Bu et al. 2021). Schindler et al. loaded doxorubicin into exosomes which is associated with severe cardiotoxicity to enhance the potency and rapid cell entry. Exosomes were isolated from HEK293 cells and loaded with doxorubicin via electroporation. Results showed an 18-fold higher intracellular accumulation of doxorubicin when compared with free doxorubicin and 65-fold increase when compared with Myocet and Doxil. Exosomal doxorubicin demonstrated superior potency in comparison with free drug or liposomal formulation (Schindler et al. 2019). Chen et al. suggested that the exosomal noncoding RNAs (exo-ncRNAs) can be a diagnostic as well as prognostic biomarker for atrial fibrillation. The team reviewed that these exosomes can be engineered, directly or indirectly, for atrial fibrillation treatment. Exo-ncRNAs can also provide a mechanism-based diagnosis of atrial fibrillation (Chen et al. 2021). Hou et al. fabricated a cardiac stent by immobilizing exosomes on 316 L stainless steel plates previously coated with polydopamine (PDA) with better pro-endothelialization function. The PDA-exosome surface enhanced the endothelial function and CD31 expression as well. The exosome-modified surface also regulated macrophages which is indicative of regeneration of endothelial tissue. In vivo studies revealed good anti-hyperplasia function of PDA-exosome coating (Hou et al. 2020).

12.6.4 Exosomes in Skin

From previously published literature, it is evident that exosomes are capable of regulating the functions and diseases of skin. Epithelial progenitor cells contain subunits of exosomes that may facilitate the proliferation of epithelial progenitor cells which plays a vital role in development of the epidermis (Liu et al. 2018; Mistry et al. 2012). Exosomes have the capability of angiogenesis, collagen synthesis, and regulating inflammation with respect to skin tissue regeneration (Yang et al. 2021).

Shafei et al. isolated exosomes from adipose-derived stem cells and loaded in alginate-based hydrogel. The hydrogel was used as a scaffold to restrict the exosomes in the wound site. In vivo studies showed significant improvement in the wound closure, collagen synthesis, and angiogenesis in the wounded area when treated with exosomal alginate-based hydrogel (Shafei et al. 2020). It has also been seen that MSC exosomes can be used in various skin conditions like wound healing, skin tissue regeneration, and repair of skin barrier (Ha et al. 2020). Wang et al. found that MSC-derived exosomes can also protect against oxidative stress-induced skin injury. The experiment was conducted on H_2O_2-stimulated epidermal keratinocytes and UV-irradiated wild-type and nuclear factor-erythroid 2-related factor 2 (Nrf2) knocked down cell and animal models. Observations from the experiments revealed that MSC exosomes reduced DNA damage and ROS generation. Boosted antioxidant activities were evident from increased ferric ion reducing antioxidant power, glutathione peroxidase, and superoxide dismutase activities mediated by Nef2 signaling pathway (Wang et al. 2020a). Wang et al. formulated a system consisting of a self-healing and antibacterial polypeptide-based hydrogel loaded with adipose-derived MSC exosomes for diabetic wound healing. In vitro results revealed enhanced migration and tube forming capability of human umbilical vein endothelial cells (HUVECs). Sustained release of exosomes from hydrogel was also observed which enhanced the proliferation of HUVECs. In vivo studies showed that the healing performance of exosomal hydrogel exhibited faster healing in comparison with exosome, hydrogel, and control. Collagen fiber deposition was also well organized in the group treated with exosomal hydrogel. Furthermore, less scarring and skin appendages were also observed in the treatment group (Wang et al. 2019). A study investigating the therapeutic effects MSC-derived exosomes in eczema was conducted by Wang et al. Eczema mice model was established using 2,4-two nitrochlorobenzene, and the effect of exosomes was evaluated using severity score and histopathological analysis. Significant differences in the score of skin injury and lymphocyte infiltration in the skin were seen between treatment and control group. Newer epidermis and dermis were formed with less scar tissue (Wang et al. 2020b). Su et al. formulated polyethylenimine (PEI)-modified electrospun fibers functionalized with mesenchymal stromal exosomes onto fibrous polyester material for tissue repair. Exosomes were immobilized in scaffold and released only when came in direct contact with the cells. Cellular uptake studies showed that PEI exosome signals were present at 50.8 ± 5.8% fluorescence remaining on day 7. Tissue repair observed in skin injury models indicated that scaffolds carrying exosomes may offer of a cell-free regenerative system by healing large skin wounds (Su et al. 2021).

12.7 Conclusion

The discovery of exosomes has provided a new outlook in the field of drug delivery and medicine. Exosome breakthrough is far from the most interesting discoveries in recent years. These extracellular vesicles are in nanometers and have an endocytic

origin. They play a vital role in intercellular communication between tumors and their corresponding cells for targeting. Exosome contents and their functions depend on the type of cells from where they originate. Apart from proteins and mRNAs specific to the parent cell, exosomes contain lipids, enzymes, nucleic acids, and transcription factors on their surface. Origin of exosomes from various cells is responsible for their different composition and functions. Exosomes can be derived from a variety of cells. Majority of these are isolated from macrophages, tumor, and mesenchymal cells. Although exosomes have proved to be a better delivery system, their isolation techniques have shortcoming in respect to their purity and effectiveness. Researchers have used ultracentrifugation for isolation of exosomes in nearly half of the reported studies. Apart from ultracentrifugation many other techniques are available. These include polymer precipitation, size-based exclusion, and microfluidic-based and immunoaffinity capture-based techniques. Nowadays commercial kits are also available in the market for isolating exosomes of highest purity in a short period of time. For analyzing the parameters of exosomes such as their size and number, DLS and NTA are commonly used. Electron microscopy is used for the morphological assessment. However, an instrument that can characterize structural and biological parameters simultaneously would be an ideal technique. Exosomes have been used as biomarkers for diagnosis and as carriers for drug delivery for targeted action. Various techniques are employed to functionalize exosomes in order to target the therapeutic agents toward specific cells. Some of these techniques are based on a chemical reaction in the presence of a catalyst (click chemistry) and some are based on gene coding for proteins and peptides (genetic engineering). Applications of exosomes in diagnosis and therapeutics are widespread in every aspect of disease. Since exosomes can be secreted from specific cells, they can be loaded with therapeutic molecules for targeted delivery, while widening the therapeutic window. In this chapter we have covered the diagnostic and therapeutic aspects in cancer, neurological, cardiovascular, and skin disorders. Despite the promising outcomes of exosomes, there is still a large gap to be filled for a low-cost large-scale production. Improved isolation techniques are also required to reduce contamination in the process and enhance the effectiveness and loading efficiency.

References

Ailuno G, Baldassari S, Lai F, Florio T, Caviglioli G (2020) Exosomes and extracellular vesicles as emerging Theranostic platforms in cancer research. Cell 9(12):2569. https://doi.org/10.3390/cells9122569

Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ (2011) Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol 29(4):341–345. https://doi.org/10.1038/nbt.1807

An Y, Jin T, Zhu Y, Zhang F, He P (2019) An ultrasensitive electrochemical aptasensor for the determination of tumor exosomes based on click chemistry. Biosens Bioelectron 142:111503. https://doi.org/10.1016/j.bios.2019.111503

Bellavia D, Raimondo S, Calabrese G, Forte S, Cristaldi M, Patinella A, Memeo L, Manno M, Raccosta S, Diana P, Cirrincione G, Giavaresi G, Monteleone F, Fontana S, De Leo G, Alessandro R (2017) Interleukin 3- receptor targeted exosomes inhibit in vitro and in vivo chronic myelogenous leukemia cell growth. Theranostics 7(5):1333–1345. https://doi.org/10.7150/thno.17092

Betzer O, Perets N, Angel A, Motiei M, Sadan T, Yadid G, Offen D, Popovtzer R (2017) In vivo neuroimaging of exosomes using gold nanoparticles. ACS Nano 11(11):10883–10893. https://doi.org/10.1021/acsnano.7b04495

Bouchareychas L, Duong P, Covarrubias S, Alsop E, Phu TA, Chung A, Gomes M, Wong D, Meechoovet B, Capili A, Yamamoto R, Nakauchi H, McManus MT, Carpenter S, Van Keuren-Jensen K, Raffai RL (2020) Macrophage exosomes resolve atherosclerosis by regulating hematopoiesis and inflammation via MicroRNA Cargo. Cell Rep 32(2):107881. https://doi.org/10.1016/j.celrep.2020.107881

Boyiadzis M, Whiteside TL (2017) The emerging roles of tumor-derived exosomes in hematological malignancies. Leukemia 31(6):1259–1268. https://doi.org/10.1038/leu.2017.91

Bu T, Li Z, Hou Y, Sun W, Zhang R, Zhao L, Wei M, Yang G, Yuan L (2021) Exosome-mediated delivery of inflammation-responsive il-10 mRNA for controlled atherosclerosis treatment. Theranostics 11(20):9988–10000. https://doi.org/10.7150/thno.64229

Bunggulawa EJ, Wang W, Yin T, Wang N, Durkan C, Wang Y, Wang G (2018) Recent advancements in the use of exosomes as drug delivery systems. J Nanobiotechnol 16(1):81. https://doi.org/10.1186/s12951-018-0403-9

Buscail E, Chauvet A, Quincy P, Degrandi O, Buscail C, Lamrissi I, Moranvillier I, Caumont C, Verdon S, Brisson A, Marty M, Chiche L, Laurent C, Vendrely V, Moreau-Gaudry F, Bedel A, Dabernat S (2019) CD63-GPC1-positive exosomes coupled with CA19-9 offer good diagnostic potential for resectable pancreatic ductal adenocarcinoma. Transl Oncol 12(11):1395–1403. https://doi.org/10.1016/j.tranon.2019.07.009

Chang YH, Wu KC, Harn HJ, Lin SZ, Ding DC (2018) Exosomes and stem cells in degenerative disease diagnosis and therapy. Cell Transplant 27(3):349–363. https://doi.org/10.1177/0963689717723636

Chen C, Chen Q, Cheng K, Zou T, Pang Y, Ling Y, Xu Y, Zhu W (2021) Exosomes and exosomal non-coding RNAs are novel promises for the mechanism-based diagnosis and treatments of atrial fibrillation. Front Cardiovasc Med 8:782451. https://doi.org/10.3389/fcvm.2021.782451

Cheruvanky A, Zhou H, Pisitkun T, Kopp JB, Knepper MA, Yuen PS, Star RA (2007) Rapid isolation of urinary exosomal biomarkers using a nanomembrane ultrafiltration concentrator. Am J Physiol Renal Physiol 292(5):F1657–F1661. https://doi.org/10.1152/ajprenal.00434.2006

Chung KY, Quek JM, Neo SH, Too HP (2020) Polymer-based precipitation of extracellular vesicular miRNAs from serum improve gastric cancer miRNA biomarker performance. J Mol Diagn 22(5):610–618. https://doi.org/10.1016/j.jmoldx.2020.01.016

Cui GH, Wu J, Mou FF, Xie WH, Wang FB, Wang QL, Fang J, Xu YW, Dong YR, Liu JR, Guo HD (2018) Exosomes derived from hypoxia-preconditioned mesenchymal stromal cells ameliorate cognitive decline by rescuing synaptic dysfunction and regulating inflammatory responses in APP/PS1 mice. FASEB J 32(2):654–668. https://doi.org/10.1096/fj.201700600R

Dudani JS, Gossett DR, Tse HT, Lamm RJ, Kulkarni RP, Carlo DD (2015) Rapid inertial solution exchange for enrichment and flow cytometric detection of microvesicles. Biomicrofluidics 9(1):014112. https://doi.org/10.1063/1.4907807

Gallet R, Dawkins J, Valle J, Simsolo E, de Couto G, Middleton R, Tseliou E, Luthringer D, Kreke M, Smith RR, Marbán L, Ghaleb B, Marbán E (2017) Exosomes secreted by cardiosphere-derived cells reduce scarring, attenuate adverse remodelling, and improve function in acute and chronic porcine myocardial infarction. Eur Heart J 38(3):201–211. https://doi.org/10.1093/eurheartj/ehw240

Gercel-Taylor C, Atay S, Tullis RH, Kesimer M, Taylor DD (2012) Nanoparticle analysis of circulating cell-derived vesicles in ovarian cancer patients. Anal Biochem 428(1):44–53. https://doi.org/10.1016/j.ab.2012.06.004

Guan S, Yu H, Yan G, Gao M, Sun W, Zhang X (2020) Characterization of urinary exosomes purified with size exclusion chromatography and ultracentrifugation. J Proteome Res 19(6): 2217–2225. https://doi.org/10.1021/acs.jproteome.9b00693

Guo QR, Zhang LL, Liu JF, Li Z, Li JJ, Zhou WM, Wang H, Li JQ, Liu DY, Yu XY, Zhang JY (2021) Multifunctional microfluidic chip for cancer diagnosis and treatment. Nano 5(1):73–89. https://doi.org/10.7150/ntno.49614

Ha DH, Kim HK, Lee J, Kwon HH, Park GH, Yang SH, Jung JY, Choi H, Lee JH, Sung S, Yi YW, Cho BS (2020) Mesenchymal stem/stromal cell-derived exosomes for immunomodulatory therapeutics and skin regeneration. Cell 9(5):1157. https://doi.org/10.3390/cells9051157

Hardij J, Cecchet F, Berquand A, Gheldof D, Chatelain C, Mullier F, Chatelain B, Dogné JM (2013) Characterisation of tissue factor-bearing extracellular vesicles with AFM: comparison of air-tapping-mode AFM and liquid peak force AFM. J Extracell Vesicles 2. https://doi.org/10.3402/jev.v2i0.21045

Hein CD, Liu XM, Wang D (2008) Click chemistry, a powerful tool for pharmaceutical sciences. Pharm Res 25(10):2216–2230. https://doi.org/10.1007/s11095-008-9616-1

Heinemann ML, Vykoukal J (2017) Sequential filtration: a gentle method for the isolation of functional extracellular vesicles. Methods Mol Biol (Clifton, NJ), 1660:33–41. https://doi.org/10.1007/978-1-4939-7253-1_4

Hergenreider E, Heydt S, Tréguer K, Boettger T, Horrevoets AJ, Zeiher AM, Scheffer MP, Frangakis AS, Yin X, Mayr M, Braun T, Urbich C, Boon RA, Dimmeler S (2012) Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. Nat Cell Biol 14(3):249–256. https://doi.org/10.1038/ncb2441

Hou R, Li Y, Sui Z, Yuan H, Yang K, Liang Z, Zhang L, Zhang Y (2019) Advances in exosome isolation methods and their applications in proteomic analysis of biological samples. Anal Bioanal Chem 411(21):5351–5361. https://doi.org/10.1007/s00216-019-01982-0

Hou YC, Li JA, Zhu SJ, Cao C, Tang JN, Zhang JY, Guan SK (2020) Tailoring of cardiovascular stent material surface by immobilizing exosomes for better pro-endothelialization function. Colloids Surf B Biointerfaces 189:110831. https://doi.org/10.1016/j.colsurfb.2020.110831

Ibrahim AG, Cheng K, Marbán E (2014) Exosomes as critical agents of cardiac regeneration triggered by cell therapy. Stem Cell Reports 2(5):606–619. https://doi.org/10.1016/j.stemcr.2014.04.006

Jabbari N, Akbariazar E, Feqhhi M, Rahbarghazi R, Rezaie J (2020) Breast cancer-derived exosomes: tumor progression and therapeutic agents. J Cell Physiol 235(10):6345–6356. https://doi.org/10.1002/jcp.29668

Jeppesen DK, Fenix AM, Franklin JL, Higginbotham JN, Zhang Q, Zimmerman LJ, Liebler DC, Ping J, Liu Q, Evans R, Fissell WH, Patton JG, Rome LH, Burnette DT, Coffey RJ (2019) Reassessment of exosome composition. Cell 177(2):428–445.e18. https://doi.org/10.1016/j.cell.2019.02.029

Jia G, Han Y, An Y, Ding Y, He C, Wang X, Tang Q (2018) NRP-1 targeted and cargo-loaded exosomes facilitate simultaneous imaging and therapy of glioma in vitro and in vivo. Biomaterials 178:302–316. https://doi.org/10.1016/j.biomaterials.2018.06.029

Jia L, Zhu M, Kong C, Pang Y, Zhang H, Qiu Q, Wei C, Tang Y, Wang Q, Li Y, Li T, Li F, Wang Q, Li Y, Wei Y, Jia J (2021) Blood neuro-exosomal synaptic proteins predict Alzheimer's disease at the asymptomatic stage. Alzheimers Dement 17(1):49–60. https://doi.org/10.1002/alz.12166

Kahroba H, Hejazi MS, Samadi N (2019) Exosomes: from carcinogenesis and metastasis to diagnosis and treatment of gastric cancer. Cell Mol Life Sci 76(9):1747–1758. https://doi.org/10.1007/s00018-019-03035-2

Khan H, Pan JJ, Li Y, Zhang Z, Yang GY (2021) Native and bioengineered exosomes for ischemic stroke therapy. Front Cell Dev Biol 9:619565. https://doi.org/10.3389/fcell.2021.619565

Khongkow M, Yata T, Boonrungsiman S, Ruktanonchai UR, Graham D, Namdee K (2019) Surface modification of gold nanoparticles with neuron-targeted exosome for enhanced blood-brain barrier penetration. Sci Rep 9(1):8278. https://doi.org/10.1038/s41598-019-44569-6

Kim H, Yun N, Mun D, Kang JY, Lee SH, Park H, Park H, Joung B (2018) Cardiac-specific delivery by cardiac tissue-targeting peptide-expressing exosomes. Biochem Biophys Res Commun 499(4):803–808. https://doi.org/10.1016/j.bbrc.2018.03.227

Konoshenko MY, Lekchnov EA, Vlassov AV, Laktionov PP (2018) Isolation of extracellular vesicles: general methodologies and latest trends. Biomed Res Int 2018:8545347. https://doi.org/10.1155/2018/8545347

Kore RA, Wang X, Ding Z, Griffin RJ, Tackett AJ, Mehta JL (2021) MSC exosome-mediated cardioprotection in ischemic mouse heart comparative proteomics of infarct and peri-infarct areas. Mol Cell Biochem 476(4):1691–1704. https://doi.org/10.1007/s11010-020-04029-6

Kuwabara Y, Ono K, Horie T, Nishi H, Nagao K, Kinoshita M, Watanabe S, Baba O, Kojima Y, Shizuta S, Imai M, Tamura T, Kita T, Kimura T (2011) Increased microRNA-1 and microRNA-133a levels in serum of patients with cardiovascular disease indicate myocardial damage. Circ Cardiovasc Genet 4(4):446–454. https://doi.org/10.1161/CIRCGENETICS.110.958975

Lan J, Sun L, Xu F, Liu L, Hu F, Song D, Hou Z, Wu W, Luo X, Wang J, Yuan X, Hu J, Wang G (2019) M2 macrophage-derived exosomes promote cell migration and invasion in colon cancer. Cancer Res 79(1):146–158. https://doi.org/10.1158/0008-5472.CAN-18-0014

Lee TS, Kim Y, Zhang W, Song IH, Tung CH (2018) Facile metabolic glycan labeling strategy for exosome tracking. Biochim Biophys Acta Gen Subj 1862(5):1091–1100. https://doi.org/10.1016/j.bbagen.2018.02.001

Li M, Li S, Du C, Zhang Y, Li Y, Chu L, Han X, Galons H, Zhang Y, Sun H, Yu P (2020) Exosomes from different cells: characteristics, modifications, and therapeutic applications. Eur J Med Chem 207:112784. https://doi.org/10.1016/j.ejmech.2020.112784

Li M, Wang T, Tian H, Wei G, Zhao L, Shi Y (2019) Macrophage-derived exosomes accelerate wound healing through their anti-inflammation effects in a diabetic rat model. Artif Cells Nanomed Biotechnol 47(1):3793–3803. https://doi.org/10.1080/21691401.2019.1669617

Li P, Kaslan M, Lee SH, Yao J, Gao Z (2017) Progress in exosome isolation techniques. Theranostics 7(3):789–804. https://doi.org/10.7150/thno.18133

Li TD, Zhang R, Chen H, Huang ZP, Ye X, Wang H, Deng AM, Kong JL (2018b) An ultrasensitive polydopamine bi-functionalized SERS immunoassay for exosome-based diagnosis and classification of pancreatic cancer. Chem Sci 9(24):5372–5382. https://doi.org/10.1039/c8sc01611a

Li Y, Gao Y, Gong C, Wang Z, Xia Q, Gu F, Hu C, Zhang L, Guo H, Gao S (2018a) A33 antibody-functionalized exosomes for targeted delivery of doxorubicin against colorectal cancer. Nanomedicine 14(7):1973–1985. https://doi.org/10.1016/j.nano.2018.05.020

Liga A, Vliegenthart AD, Oosthuyzen W, Dear JW, Kersaudy-Kerhoas M (2015) Exosome isolation: a microfluidic road-map. Lab Chip 15(11):2388–2394. https://doi.org/10.1039/c5lc00240k

Liu L, Li Y, Peng H, Liu R, Ji W, Shi Z, Shen J, Ma G, Zhang X (2020b) Targeted exosome coating gene-chem nanocomplex as "nanoscavenger" for clearing α-synuclein and immune activation of Parkinson's disease. Sci Adv 6(50):eaba3967. https://doi.org/10.1126/sciadv.aba3967

Liu S, Chen J, Shi J, Zhou W, Wang L, Fang W, Zhong Y, Chen X, Chen Y, Sabri A, Liu S (2020a) M1-like macrophage-derived exosomes suppress angiogenesis and exacerbate cardiac dysfunction in a myocardial infarction microenvironment. Basic Res Cardiol 115(2):22. https://doi.org/10.1007/s00395-020-0781-7

Liu Y, Wang H, Wang J (2018) Exosomes as a novel pathway for regulating development and diseases of the skin (review). Biomed Rep 8:207–214. https://doi.org/10.3892/br.2018.1054

Livshits MA, Khomyakova E, Evtushenko EG, Lazarev VN, Kulemin NA, Semina SE, Generozov EV, Govorun VM (2015) Isolation of exosomes by differential centrifugation: theoretical analysis of a commonly used protocol. Sci Rep 5:17319. https://doi.org/10.1038/srep17319

Luo S, Sun X, Huang M, Ma Q, Du L, Cui Y (2021) Enhanced neuroprotective effects of epicatechin gallate encapsulated by bovine milk-derived exosomes against Parkinson's disease through antiapoptosis and antimitophagy. J Agric Food Chem 69(17):5134–5143. https://doi.org/10.1021/acs.jafc.0c07658

Ma T, Fu B, Yang X, Xiao Y, Pan M (2019) Adipose mesenchymal stem cell-derived exosomes promote cell proliferation, migration, and inhibit cell apoptosis via Wnt/β-catenin signaling in cutaneous wound healing. J Cell Biochem 120(6):10847–10854. https://doi.org/10.1002/jcb.28376

Ma YS, Yang XL, Xin R, Liu JB, Fu D (2021) Power and promise of exosomes as clinical biomarkers and therapeutic vectors for liquid biopsy and cancer control. Biochim Biophys Acta Rev Cancer 1875(1):188497. https://doi.org/10.1016/j.bbcan.2020.188497

Maas SL, Broekman ML, de Vrij J (2017) Tunable resistive pulse sensing for the characterization of extracellular vesicles. Methods Mol Biol (Clifton, N.J.) 1545:21–33. https://doi.org/10.1007/978-1-4939-6728-5_2

Milane L, Singh A, Mattheolabakis G, Suresh M, Amiji MM (2015) Exosome mediated communication within the tumor microenvironment. J Control Release 219:278–294. https://doi.org/10.1016/j.jconrel.2015.06.029

Mistry DS, Chen Y, Sen GL (2012) Progenitor function in self-renewing human epidermis is maintained by the exosome. Cell Stem Cell 11(1):127–135. https://doi.org/10.1016/j.stem.2012.04.022

Mousavi S, Moallem R, Hassanian SM, Sadeghzade M, Mardani R, Ferns GA, Khazaei M, Avan A (2019) Tumor-derived exosomes: potential biomarkers and therapeutic target in the treatment of colorectal cancer. J Cell Physiol 234(8):12422–12432. https://doi.org/10.1002/jcp.28080

Nakamura S, Sadakari Y, Ohtsuka T, Okayama T, Nakashima Y, Gotoh Y, Saeki K, Mori Y, Nakata K, Miyasaka Y, Onishi H, Oda Y, Goggins M, Nakamura M (2019) Pancreatic juice exosomal MicroRNAs as biomarkers for detection of pancreatic ductal adenocarcinoma. Ann Surg Oncol 26(7):2104–2111. https://doi.org/10.1245/s10434-019-07269-z

Nordin JZ, Lee Y, Vader P, Mäger I, Johansson HJ, Heusermann W, Wiklander OP, Hällbrink M, Seow Y, Bultema JJ, Gilthorpe J, Davies T, Fairchild PJ, Gabrielsson S, Meisner-Kober NC, Lehtiö J, Smith CI, Wood MJ, El Andaloussi S (2015) Ultrafiltration with size-exclusion liquid chromatography for high yield isolation of extracellular vesicles preserving intact biophysical and functional properties. Nanomedicine 11(4):879–883. https://doi.org/10.1016/j.nano.2015.01.003

O'Brien K, Lowry MC, Corcoran C, Martinez VG, Daly M, Rani S, Gallagher WM, Radomski MW, MacLeod RA, O'Driscoll L (2015) miR-134 in extracellular vesicles reduces triple-negative breast cancer aggression and increases drug sensitivity. Oncotarget 6(32):32774–32789. https://doi.org/10.18632/oncotarget.5192

Oksvold MP, Neurauter A, Pedersen KW (2015) Magnetic bead-based isolation of exosomes. Methods Mol Biol (Clifton, NJ) 1218:465–481. https://doi.org/10.1007/978-1-4939-1538-5_27

Orr L, Adam M, Johnstone RM (1987) Externalization of membrane-bound activities during sheep reticulocyte maturation is temperature and ATP dependent. Biochem Cell Biol = Biochimie et biologie cellulaire 65(12):1080–1090. https://doi.org/10.1139/o87-141

Parisse P, Rago I, Ulloa Severino L, Perissinotto F, Ambrosetti E, Paoletti P, Ricci M, Beltrami AP, Cesselli D, Casalis L (2017) Atomic force microscopy analysis of extracellular vesicles. Eur Biophys J 46(8):813–820. https://doi.org/10.1007/s00249-017-1252-4

Patil SM, Sawant SS, Kunda NK (2020) Exosomes as drug delivery systems: a brief overview and progress update. Eur J Pharm Biopharm 154:259–269. https://doi.org/10.1016/j.ejpb.2020.07.026

Perets N, Betzer O, Shapira R, Brenstein S, Angel A, Sadan T, Ashery U, Popovtzer R, Offen D (2019) Golden exosomes selectively target brain pathologies in neurodegenerative and neurodevelopmental disorders. Nano Lett 19(6):3422–3431. https://doi.org/10.1021/acs.nanolett.8b04148

Phung CD, Pham TT, Nguyen HT, Nguyen TT, Ou W, Jeong JH, Choi HG, Ku SK, Yong CS, Kim JO (2020) Anti-CTLA-4 antibody-functionalized dendritic cell-derived exosomes targeting tumor-draining lymph nodes for effective induction of antitumor T-cell responses. Acta Biomater 115:371–382. https://doi.org/10.1016/j.actbio.2020.08.008

van der Pol E, Hoekstra AG, Sturk A, Otto C, van Leeuwen TG, Nieuwland R (2010) Optical and non-optical methods for detection and characterization of microparticles and exosomes. J Thromb Haemost 8(12):2596–2607. https://doi.org/10.1111/j.1538-7836.2010.04074.x

Qian M, Wang S, Guo X, Wang J, Zhang Z, Qiu W, Gao X, Chen Z, Xu J, Zhao R, Xue H, Li G (2020) Hypoxic glioma-derived exosomes deliver microRNA-1246 to induce M2 macrophage polarization by targeting TERF2IP via the STAT3 and NF-κB pathways. Oncogene 39(2): 428–442. https://doi.org/10.1038/s41388-019-0996-y

Qu M, Lin Q, Huang L, Fu Y, Wang L, He S, Fu Y, Yang S, Zhang Z, Zhang L, Sun X (2018) Dopamine-loaded blood exosomes targeted to brain for better treatment of Parkinson's disease. J Control Release 287:156–166. https://doi.org/10.1016/j.jconrel.2018.08.035

Ramos LT, Sánchez-Abarca LI, Muntión S, Preciado S, Puig N, López-Ruano G, Hernández-Hernández Á, Redondo A, Ortega R, Rodríguez C, Sánchez-Guijo F, del Cañizo C (2016) MSC surface markers (CD44, CD73, and CD90) can identify human MSC-derived extracellular vesicles by conventional flow cytometry. Cell Commun Signal 14:2. https://doi.org/10.1186/s12964-015-0124-8

Riazifar M, Mohammadi MR, Pone EJ, Yeri A, Lässer C, Segaliny AI, McIntyre LL, Shelke GV, Hutchins E, Hamamoto A, Calle EN, Crescitelli R, Liao W, Pham V, Yin Y, Jayaraman J, Lakey J, Walsh CM, Van Keuren-Jensen K, Lotvall J, Zhao W (2019) Stem cell-derived exosomes as nanotherapeutics for autoimmune and neurodegenerative disorders. ACS Nano 13(6):6670–6688. https://doi.org/10.1021/acsnano.9b01004

Ryu KJ, Lee JY, Park C, Cho D, Kim SJ (2020) Isolation of small extracellular vesicles from human serum using a combination of ultracentrifugation with polymer-based precipitation. Ann Lab Med 40(3):253–258. https://doi.org/10.3343/alm.2020.40.3.253

Sabapatha A, Gercel-Taylor C, Taylor DD (2006) Specific isolation of placenta-derived exosomes from the circulation of pregnant women and their immunoregulatory consequences. Am J Reprod Immunol (New York, NY: 1989) 56(5–6):345–355. https://doi.org/10.1111/j.1600-0897.2006.00435.x

Saeedi S, Israel S, Nagy C, Turecki G (2019) The emerging role of exosomes in mental disorders. Transl Psychiatry 9(1):122. https://doi.org/10.1038/s41398-019-0459-9

Schindler C, Collinson A, Matthews C, Pointon A, Jenkinson L, Minter RR, Vaughan TJ, Tigue NJ (2019) Exosomal delivery of doxorubicin enables rapid cell entry and enhanced in vitro potency. PLoS One 14(3):e0214545. https://doi.org/10.1371/journal.pone.0214545

Shafei S, Khanmohammadi M, Heidari R, Ghanbari H, Taghdiri Nooshabadi V, Farzamfar S, Akbariqomi M, Sanikhani NS, Absalan M, Tavoosidana G (2020) Exosome loaded alginate hydrogel promotes tissue regeneration in full-thickness skin wounds: An in vivo study. J Biomed Mater Res A 108(3):545–556. https://doi.org/10.1002/jbm.a.36835

Sharma S, Zuñiga F, Rice GE, Perrin LC, Hooper JD, Salomon C (2017) Tumor-derived exosomes in ovarian cancer - liquid biopsies for early detection and real-time monitoring of cancer progression. Oncotarget 8(61):104687–104703. https://doi.org/10.18632/oncotarget.22191

Sidhom K, Obi PO, Saleem A (2020) A review of exosomal isolation methods: is size exclusion chromatography the best option? Int J Mol Sci 21(18):6466. https://doi.org/10.3390/ijms21186466

Sluijter J, Davidson SM, Boulanger CM, Buzás EI, de Kleijn D, Engel FB, Giricz Z, Hausenloy DJ, Kishore R, Lecour S, Leor J, Madonna R, Perrino C, Prunier F, Sahoo S, Schiffelers RM, Schulz R, Van Laake LW, Ytrehus K, Ferdinandy P (2018) Extracellular vesicles in diagnostics and therapy of the ischaemic heart: position paper from the working group on cellular biology of the heart of the European Society of Cardiology. Cardiovasc Res 114(1):19–34. https://doi.org/10.1093/cvr/cvx211

Sluijter JP, Verhage V, Deddens JC, van den Akker F, Doevendans PA (2014) Microvesicles and exosomes for intracardiac communication. Cardiovasc Res 102(2):302–311. https://doi.org/10.1093/cvr/cvu022

Smyth T, Petrova K, Payton NM, Persaud I, Redzic JS, Graner MW, Smith-Jones P, Anchordoquy TJ (2014) Surface functionalization of exosomes using click chemistry. Bioconjug Chem 25(10):1777–1784. https://doi.org/10.1021/bc500291r

Sokolova V, Ludwig AK, Hornung S, Rotan O, Horn PA, Epple M, Giebel B (2011) Characterisation of exosomes derived from human cells by nanoparticle tracking analysis and scanning electron microscopy. Colloids Surf B Biointerfaces 87(1):146–150. https://doi.org/10.1016/j.colsurfb.2011.05.013

Su N, Hao Y, Wang F, Hou W, Chen H, Luo Y (2021) Mesenchymal stromal exosome-functionalized scaffolds induce innate and adaptive immunomodulatory responses toward tissue repair. Sci Adv 7(20):eabf7207. https://doi.org/10.1126/sciadv.abf7207

Tan CY, Lai RC, Wong W, Dan YY, Lim SK, Ho HK (2014) Mesenchymal stem cell-derived exosomes promote hepatic regeneration in drug-induced liver injury models. Stem Cell Res Ther 5(3):76. https://doi.org/10.1186/scrt465

Thakur A, Qiu G, Ng SP, Guan J, Yue J, Lee Y, Wu CL (2017) Direct detection of two different tumor-derived extracellular vesicles by SAM-AuNIs LSPR biosensor. Biosens Bioelectron 94:400–407. https://doi.org/10.1016/j.bios.2017.03.036

Théry C, Zitvogel L, Amigorena S (2002) Exosomes: composition, biogenesis and function. Nat Rev Immunol 2(8):569–579. https://doi.org/10.1038/nri855

Tian T, Zhang HX, He CP, Fan S, Zhu YL, Qi C, Huang NP, Xiao ZD, Lu ZH, Tannous BA, Gao J (2018) Surface functionalized exosomes as targeted drug delivery vehicles for cerebral ischemia therapy. Biomaterials 150:137–149. https://doi.org/10.1016/j.biomaterials.2017.10.012

Tian Y, Li S, Song J, Ji T, Zhu M, Anderson GJ, Wei J, Nie G (2014) A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. Biomaterials 35(7):2383–2390. https://doi.org/10.1016/j.biomaterials.2013.11.083

Wang C, Wang M, Xu T, Zhang X, Lin C, Gao W, Xu H, Lei B, Mao C (2019) Engineering bioactive self-healing antibacterial exosomes hydrogel for promoting chronic diabetic wound healing and complete skin regeneration. Theranostics 9(1):65–76. https://doi.org/10.7150/thno.29766

Wang C, Zhang C, Liu LAX, Chen B, Li Y, Du J (2017a) Macrophage-derived mir-155-containing exosomes suppress fibroblast proliferation and promote fibroblast inflammation during cardiac injury. Mol Ther 25(1):192–204. https://doi.org/10.1016/j.ymthe.2016.09.001

Wang J, Li W, Lu Z, Zhang L, Hu Y, Li Q, Du W, Feng X, Jia H, Liu BF (2017b) The use of RGD-engineered exosomes for enhanced targeting ability and synergistic therapy toward angiogenesis. Nanoscale 9(40):15598–15605. https://doi.org/10.1039/c7nr04425a

Wang M, Zhao Y, Zhang Q (2020b) Human mesenchymal stem cell-derived exosomes accelerate wound healing of mice eczema. J Dermatolog Treat:1–5. Advance online publication. https://doi.org/10.1080/09546634.2020.1820935

Wang T, Jian Z, Baskys A, Yang J, Li J, Guo H, Hei Y, Xian P, He Z, Li Z, Li N, Long Q (2020a) MSC-derived exosomes protect against oxidative stress-induced skin injury via adaptive regulation of the NRF2 defense system. Biomaterials 257:120264. https://doi.org/10.1016/j.biomaterials.2020.120264

Wang Z, Wu HJ, Fine D, Schmulen J, Hu Y, Godin B, Zhang JX, Liu X (2013) Ciliated micropillars for the microfluidic-based isolation of nanoscale lipid vesicles. Lab Chip 13(15):2879–2882. https://doi.org/10.1039/c3lc41343h

Witwer KW, Buzás EI, Bemis LT, Bora A, Lässer C, Lötvall J, Nolte-'t Hoen EN, Piper MG, Sivaraman S, Skog J, Théry C, Wauben MH, Hochberg F (2013) Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. J Extracell Vesicles 2. https://doi.org/10.3402/jev.v2i0.20360

Wu J, Gao W, Tang Q, Yu Y, You W, Wu Z, Fan Y, Zhang L, Wu C, Han G, Zuo X, Zhang Y, Chen Z, Ding W, Li X, Lin F, Shen H, Tang J, Zhang Y, Wang X (2021) M2 macrophage-derived exosomes facilitate HCC metastasis by transferring αM β2 integrin to tumor cells. Hepatology (Baltimore, Md) 73(4):1365–1380. https://doi.org/10.1002/hep.31432. (Retraction published Hepatology. 2021 Dec;74(6):3564)

Wu M, Ouyang Y, Wang Z, Zhang R, Huang PH, Chen C, Li H, Li P, Quinn D, Dao M, Suresh S, Sadovsky Y, Huang TJ (2017) Isolation of exosomes from whole blood by integrating acoustics and microfluidics. Proc Natl Acad Sci U S A 114(40):10584–10589. https://doi.org/10.1073/pnas.1709210114

Wunsch BH, Smith JT, Gifford SM, Wang C, Brink M, Bruce RL, Austin RH, Stolovitzky G, Astier Y (2016) Nanoscale lateral displacement arrays for the separation of exosomes and colloids down to 20 nm. Nat Nanotechnol 11(11):936–940. https://doi.org/10.1038/nnano.2016.134

Xian P, Hei Y, Wang R, Wang T, Yang J, Li J, Di Z, Liu Z, Baskys A, Liu W, Wu S, Long Q (2019) Mesenchymal stem cell-derived exosomes as a nanotherapeutic agent for amelioration of inflammation-induced astrocyte alterations in mice. Theranostics 9(20):5956–5975. https://doi.org/10.7150/thno.33872

Xunian Z, Kalluri R (2020) Biology and therapeutic potential of mesenchymal stem cell-derived exosomes. Cancer Sci 111(9):3100–3110. https://doi.org/10.1111/cas.14563

Yan B, Liu Q, Liu G, Huang X, Zhu G, Gao L, Xu Y (2020) Macrophage-derived exosomes mediate osteosarcoma cell behavior by activating AKT signaling. RSC Adv. 10- 5032-39. https://doi.org/10.1039/C9RA07332A

Yang D, Zhang W, Zhang H, Zhang F, Chen L, Ma L, Larcher LM, Chen S, Liu N, Zhao Q, Tran P, Chen C, Veedu RN, Wang T (2020) Progress, opportunity, and perspective on exosome isolation - efforts for efficient exosome-based theranostics. Theranostics 10(8):3684–3707. https://doi.org/10.7150/thno.41580

Yang GH, Lee YB, Kang D, Choi E, Nam Y, Lee KH, You HJ, Kang HJ, An SH, Jeon H (2021) Overcome the barriers of the skin: exosome therapy. Biomater Res 25(1):22. https://doi.org/10.1186/s40824-021-00224-8

Yang L, Han D, Zhan Q, Li X, Shan P, Hu Y, Ding H, Wang Y, Zhang L, Zhang Y, Xue S, Zhao J, Hou X, Wang Y, Li P, Yuan X, Qi H (2019) Blood TfR+ exosomes separated by a pH-responsive method deliver chemotherapeutics for tumor therapy. Theranostics 9(25):7680–7696. https://doi.org/10.7150/thno.37220

Ye C, Li H, Bao M, Zhuo R, Jiang G, Wang W (2020) Alveolar macrophage—derived exosomes modulate severity and outcome of acute lung injury. Aging 12(7):6120–6128. https://doi.org/10.18632/aging.103010

Zeringer E, Barta T, Li M, Vlassov AV (2015) Strategies for isolation of exosomes. Cold Spring Harb Protoc 2015(4):319–323. https://doi.org/10.1101/pdb.top074476

Zhan Q, Yi K, Qi H, Li S, Li X, Wang Q, Wang Y, Liu C, Qiu M, Yuan X, Zhao J, Hou X, Kang C (2020) Engineering blood exosomes for tumor-targeting efficient gene/chemo combination therapy. Theranostics 10(17):7889–7905. https://doi.org/10.7150/thno.45028

Zhang X, Zhou C (2019) The function of tumor-derived exosomes. J BUON: official journal of the Balkan Union of Oncology 24(3):897–904

Zheng H, Zhan Y, Liu S, Lu J, Luo J, Feng J, Fan S (2018) The roles of tumor-derived exosomes in non-small cell lung cancer and their clinical implications. J Exp Clin Cancer Res 37(1):226. https://doi.org/10.1186/s13046-018-0901-5

Zhu LP, Tian T, Wang JY, He JN, Chen T, Pan M, Xu L, Zhang HX, Qiu XT, Li CC, Wang KK, Shen H, Zhang GG, Bai YP (2018) Hypoxia-elicited mesenchymal stem cell-derived exosomes facilitates cardiac repair through miR-125b-mediated prevention of cell death in myocardial infarction. Theranostics 8(22):6163–6177. https://doi.org/10.7150/thno.28021

Zou J, Shi M, Liu X, Jin C, Xing X, Qiu L, Tan W (2019) Aptamer-functionalized exosomes: elucidating the cellular uptake mechanism and the potential for cancer-targeted chemotherapy. Anal Chem 91(3):2425–2430. https://doi.org/10.1021/acs.analchem.8b05204

Theranostic Applications of Functionalized Polymeric Micelles

13

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Abstract

Curiosity in theranostic agents has continuously been growing because of its unique ability of simultaneous detection and therapy. Polymeric micelles are used as diagnostic imaging, drug, biologics, and gene delivery carriers. These nanosized agents can be made from a variety of polymers. Polymeric micelles have some advantages over others, including the ability to load poorly soluble drugs, biocompatibility, longevity, the ability to accumulate in pathological areas with compromised vasculature, high stability, incorporation of imaging contrast agents, ability to change the release of the combined pharmaceutical ingredient, targeted delivery, and so on. In stimuli-responsive therapy, pH-thermo, ultrasound, enzyme, and light-sensitive block copolymers are used. Moreover, surface modification ability makes them suitable for targeting various diseases or targeting intracellular spaces. Additionally, the integration of imaging moiety makes them suitable for their use in in vivo biodistribution studies. Therefore, such "smart," multifunctional polymeric micelles act as a key to improvising the efficacy of current treatments. Overall, polymeric micelle theranostic nanosystems are a more personalized and effective form of treatment. In the

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current chapter, we discussed the theranostic use of polymeric micelles in various diseases and theranostic applications of functionalized polymeric micelles.

Keywords

Functionalized polymeric micelles · Theranostics · Cancer · Neurodegenerative disorder · AIDS

13.1 Introduction

The "one treatment for all" approach was used years ago to diagnose and treat diseases. The conventional method has many problems, such as drug delivery at the active sites, unknown side effect, and less bioavailability. The evolutions of systems for diagnosis and treatment have introduced many new techniques for imaging disease such as magnetic resonance imaging (MRI), computed imaging, ultrasound, and advanced therapies like gene therapy and several other advancements. However, still, surgery is a choice for many disease treatments. The conventional method of diagnosis and treatments like surgeries fails many times due to complications, such as invasiveness of treatment, the seriousness of surgeries, post-surgery adverse effect, and sometimes resistance to treatment; hence there is an emerging need for the design of a more efficient, robust, and target-specific diagnosis as well as treatment and can be a more personalized form of treatment. Theranostics can be the solution to the conventional approach (Upponi et al. 2018).

The term "theranostic" refers to the agent that serves dual roles as diagnostics and therapeutics with increased safety and efficacy. A theranostic drug delivery system can be used to overcome drug transport issues and thus increase drug concentration in the target tissue. The most dependable approach, however, will be to attach a drug molecule to a desired carrier for tissue or cell targeting. Size reduction and design can also be used to target the site of action. This approach to drug delivery works best based on nanotechnology.

Recent advancements in pharmaceutical nanotechnology and nanoformulations are acquiring greater attention as drug carriers due to physiological and biochemical properties. They can be produced in various sizes, forms, and technologies (Naqvi et al. 2020). The nanoparticle can be a conclusion over traditional medicine by giving opportunities such as more formularized synthesis, ease of functionalization, and ability to update to the desired application (e.g., diagnostic and therapeutic). It can offer more advantages as it can be encapsulated and improve solubility, specificity, and biocompatibility. The growth in nanotechnology has contributed to significant research development in the medical and pharmaceutical industries as an innovative, intelligent drug delivery system (Subramani and Ahmed 2012) (Fig. 13.1).

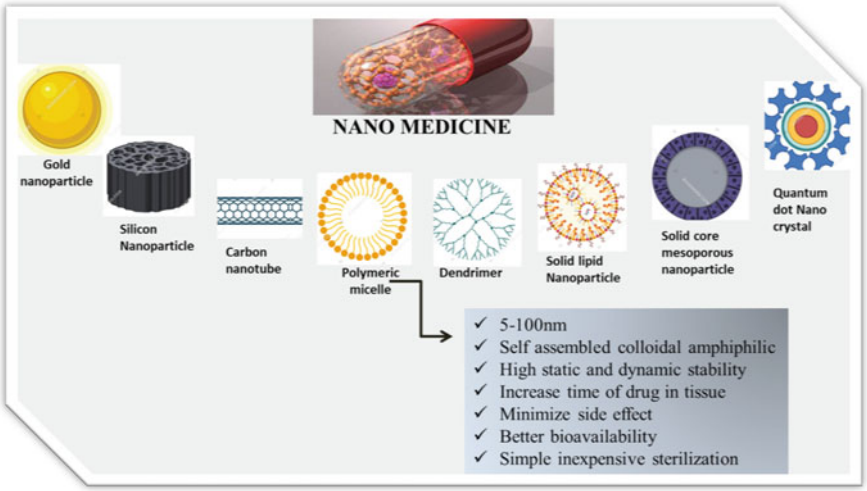


Fig. 13.1 Advantages of polymeric micelles among various nanotherapeutics

The most appealing property of nanoformulation is the nanosize of 1–100 nm (as the efficiency of the drug is directly proportional to size and dimensions) and can be prepared in various forms, such as can be designed very similar to the biological entities. Small physical dimensions can give the advantage of penetrating through the cell membrane and natural barrier, which large molecules couldn't penetrate. Nanotechnology can effectively deliver the active constituent through the blood-brain barrier (Subramani and Ahmed 2012). The pharmacokinetics and pharmacodynamics of drug nanoformulation can be easily modified using chemical techniques. Biological moieties can also attach to surfaces such as lipids, peptides, nucleic acid, and antibodies to target drugs for a specific disease. Previously only one drug could be administered, but currently, two or more drugs can be attached to the surface or loaded into the drug-bearing system. The so-called liposome can be used as a multidrug loading system without interaction between compounds. The high loading capacity is a characteristic of micelles and control release property. Micelles proved as a novel drug delivery due to high stability, high accumulation of drug, and functionalization for target ligand and can be modified as a theranostic approach (Mokhosi et al. 2022).

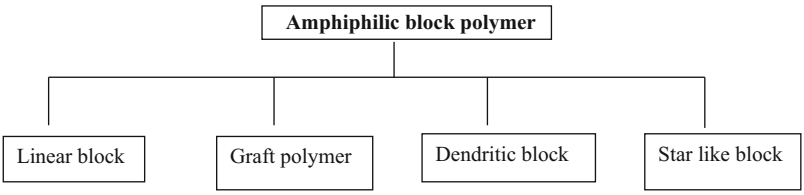
Micelles are aggregated colloidal nanoparticles of sizes 3–50 nm. Micelles are found in various shapes (sphere, elliptical, cylinder, inverted, planner) since there is no persistent shape of micelles. The structural features can be visualized by transmission electron microscopy, light scattering, and spectroscopy. The micelles are made up of di-block, tri-block, and nonlinear molecules of polymer or surfactant. Here the block means block copolymer, which is made up of macromolecules consisting of sections of different monomers (usually two or three). Block copolymer is generally made up of two or three other polymers by condensation reaction. The di-block consists of hydrophilic and hydrophobic parts. The packing of

polymers is calculated as the packing parameter (P). The packing parameter P is proportional to the volume of the hydrophobic block and inversely proportional to the surface area of the hydrophobic and hydrophilic interfaces and the length of the polymer (Owen et al. 2012).

$$P + V/al$$

where P gives packing parameter

V hydrophilic block volume
A surface area of the hydrophilic and lipophilic interface
L length of the polymer



13.1.1 Hydrophilic Block

Hydrophilic block mainly contains polyethylene glycol having a length range 1–15KDa, non-toxic. The hydrophilic block usually contains hydroxyl (-OH) or methyl ether ($-CH_3-O-$) group at the end, but when there have to attach drug molecule (NH_3) present. Mainly these end groups depend on the drug molecule to be connected.

13.1.2 Core Shell

The core is hydrophobic, acts as a drug reservoir, and protects biological pH and environment. The outer part of the micelles is called the corona. The corona is hydrophilic and can modify biodistribution, ADME characteristics, and interaction with biomolecules. The corona can be functionalized for targeting the active drug molecules to the active site. Thus interaction takes place between the target tissue and micelles (Xu et al. 2013).

13.2 Micelle Stability and Critical Micelle Concentration (CMC)

The polymer concentration above which the micelles form or the concentration at which micelle formation starts is called CMC. At a specific temperature, when the solubility graph curve bisects or crosses the CMC (concentration at which micelle formation starts), the temperature point is called the Kraft point. At the CMC, the conductivity and surface tension changes. CMC of the amphiphilic block polymer is 1–30 mg/L. The nonionic surfactant has low CMC 10^{-4}–10^{-3} M, and the ionic surfactant has high CMC 10^{-3}–10^{-2} M. The hydrophilic and lipophilic balance (HLB) of surfactant is also essential to forming micelles. The HLB balance is used to apply micelles as living organism cells or biological vesicles in a novel drug delivery system (Turchi et al. 2022).

13.3 Advantages of Micelles

Micelles are available in nanosize range $<$ 100 nm; thus, they can be injected without blockage of blood vessels, advantageous for in vivo. Primarily polyethylene glycol is used to increase the time of micelles in tissue. Polyethylene glycol helps reduce micelle's adhesion to the opsonins, which are endogenous substances that bind to foreign materials and make them prone to phagocytosis. The micelles' size helps increase vascular permeability and decrease renal excretion. Due to the target selectivity of micelles, side effects are minimized by preventing off-target accumulation. The circulation half-life of micelles depends on particle size, shape, surface chemistry, and surface charge. The hydrophobic micelles with the charged surface have a short half-life. Hydrophilic micelle with a neutral charge on the surface has a high half-life in the body. Cationic micelles are more prone to phagocytosis, and hence, they have short half-life and circulation of blood. Due to the small size, sterilization of formulation becomes easy. Micelles can be simply sterilized by filtration. Micelles have high static and structural dynamic stability. In the micelles, more hydrophobic drugs can be incorporated, and water solubility of the drug is also maintained, thus giving good bioavailability and producing less toxicity in vivo (Naqvi et al. 2020; Movassaghian et al. 2015).

13.4 Primary Classification of Micelles

Micelles can mainly be categorized into the following:

(a) Lipid-based micelles.
(b) Reverse micelles.
(c) Polymer-based micelles.

13.4.1 Lipid-Based Micelles

The lipid-based micelles have a hydrophobic core and hydrophilic outer covering. Thus, they limit the loading volume of lipoidal drugs in the core and limit the absorption and distribution of the drug in the body. There are many applications of micelles in regular life and novel drug delivery systems. In everyday life, micelles are used in cosmetics, detergent, paper industry, paint, and food preparation industry (mainly the ready-to-cook food industry). The micelles can be used as an aid to make the other material soluble in the biological system. A new material or functional group can be attached to the micelle. This can be called templating or casting (Aguilar 2013).

13.4.2 The Reverse Micelles

The reverse micelle inner core contains the water molecule mixed with a nonpolar solvent. Generally, reverse micelles are considered small water molecules entrapped by the surfactant monolayer. Reverse micelles are homogeneous, clear, and transparent. Typically, reverse micelles are prepared by dissolving surfactant (cationic, anionic, zwitterionic, and mixed) into the nonpolar solvent, and then the aqueous buffer is added in a proper amount (Naqvi et al. 2012). The characterization can be done for shape, size, the structure of the aqueous core, microviscosity, and aggregation number (aggregation number of a micelle is the average number of surfactant and monomer in a spherical micelle). The reverse micelles have a range of usage in food science, especially in the extraction of enzymes, DNA, RNA, and proteins. The reverse micelles show greater efficiency and more water solubility than prepared by alkaline extraction and isoelectric precipitation (AEIP). Currently, researchers are finding application of reverse micelle opportunities in oil extraction and finding ways to separate surfactant from oil. Researchers are also trying to discover reverse micelle preparation from edible and more biological compatible surfactants. The reverse micelles for protein extraction are expensive compared to the traditional AEIP approach, and the yield is also low. Thus, developmental modification has to be done (Sun and Bandara 2019).

13.4.3 Polymer-Based Micelles

Polymeric micelles are typically composed of a hydrophilic shell (PEG) that safeguards the drugs inside the core from enzyme degradation and a hydrophobic core. Polyethylene glycol (PEG), chitosan, dextran, and other hydrophilic polymers are also used to construct polymeric micelles (Mi et al. 2017). Polymer-based micelles contain the copolymer aggregated in water in the core, primarily hydrophobic. The copolymer should (1) be able to form micelles, (2) have adequate loading capacity and solubility, (3) be stable throughout the biological environment, and (4) be non-reactive, and (5) the production at bulk should be easy. Commonly used

core former are the copolymer of hydrophobic poly amino acid, lactic acid copolymer, and glycolic acid. The hydrophobic drugs and poorly water-soluble drugs can be given effect to increase systemic action. Drug therapy is mainly incorporated with nucleic acids, anticancer drugs, and antibodies, whereas contrast agents (CAs) can be either incorporated or conjugated on the surface for imaging. Hydrophobic biomolecules can be attached to the surface to increase bioavailability and tissue penetration. The polymer attached gives the advantage of controlling the release of drugs in the biological system. The drug can effectively cross the biological membrane because of polymer. These micelles have more stability in various environments in the body. Until 2022, the only FDA-approved micelle for treating moderate to severe vasomotor symptoms of menopause was a standard micellar formulation of estradiol (EstrasorbTM). Transdermal administration eliminates first-pass metabolism and gastrointestinal side effects, resulting in 8–14 days of steady blood levels.

13.5 Polysaccharide-Based Drug Delivery System of Polymeric Micelles

The use of naturally occurring polysaccharides in drugs targeting the colon is gaining popularity. These monosaccharide polymers are abundant, widely available, inexpensive, and available in a variety of structures with varying properties. They are chemically and biochemically modifiable, highly stable, safe, non-toxic, hydrophilic and gel-forming, and biodegradable. These include polysaccharides derived from plants (guar gum, inulin), animals (chitosan, chondroitin sulfate), algae (alginates), and bacteria (dextran). The colonic microflora can break down the polysaccharides into simple saccharine (Zhang et al. 2013a) (Table 13.1 and Fig. 13.2).

13.6 Features of Polymeric Micelles

Polymeric micelles are unique from others because of their colloidal nanoparticles, structure stability, easy synthetic route, high drug loading capacity, self-assembled (5–100 nm), excellent bioavailability, less toxicity, increased blood circulation time, and solubilize poorly water-soluble drugs or hydrophobic drugs in the core (Jhaveri and Torchilin 2014).

13.6.1 Applications of Polymeric Micelles

Polymeric micelles offer some advantages over others such as drug-loaded nanocarrier, reduced toxicity, and extend circulation time of drug in the blood; they can be used as a solubilizing agent and increase the bioavailability of the drug and targeted drug delivery.

Table 13.1 Polymers used in polymeric micelles (Yi et al. 2018)

Polymer	Properties	Application in DDS
Polyethylene glycol	Hydrophobic, biocompatible, biodegradable, non-immunogenic, and stabile in circulation	The enhanced permeability and retention (EPR) effect causes prolonged circulation and subsequent accumulation in tumor tissues
Polymer grafted chitosan	Biocompatible, non-toxic, biodegradable, mucoadhesion, anti-inflammatory, and antimicrobial	Modulate hydrophilicity, drug loading, and drug targeting
Polyester		
Poly(lactide) (PLA)	Two optically stereoisomers; thus, four different polymer formulations can be formed	Medical and food industry scaffold, in orthopedic
Poly(glycolide) (PGA)	Fast degradation, high tensile strength, high melting point (225–230 °C)	Absorbable suture, tissue regeneration, and DDS
Poly(lacide-co-glycoide) (PLGA)	Excellent biocompatibility, mechanical property	As biomaterial including membrane, DDS for several; diseases, sutures, local, and systemic treatments
Poly (e-caprolactone) (PCL)	Slow-degrading	Long-term therapy, nanocarrier for cancer
Poly(hydroxy alkanoates) (PHA)	Excellent biodegradability and biocompatibility	Preparing tissue-engineered nerve, surgical, and medical devices
Poly(butylene succinate) (PBS)	Biodegradability, processability, balanced mechanical properties	Bone repairing scaffold and drug release system
Poly(propylene fumarate) (PPF)	Cross-linking ability, form covalent polymer structure	Orthopedic implant, scaffold, drug delivery system
Poly(2-oxazoline) (pOx)		
Poly (2-oxazoline) (pOx)	Biocompatible, biodegradable, and able for functionalization	Adhesives, coating polymer, dispersant, and drug delivery systems
Dextran	Biocompatible and biodegradable non-toxic, non-immunogenic, and capable of derivatization	Avoid opsonization in the circulatory system and bioadhesive

13.6.2 Enhancement of Bioavailability of Polymeric Micelles

The bioavailability of polymeric micelles can be increased by protecting the drugs from the GIT environment, controlling the release of drugs, and increasing drug residence time in GIT (Fares et al. 2018).

13.6.2.1 Protection of Drugs from GIT

The drug should not get dissociate due to GIT enzymes, various pH levels, and bile salts. The micelle should retain the core-shell structure till it reaches the active site.

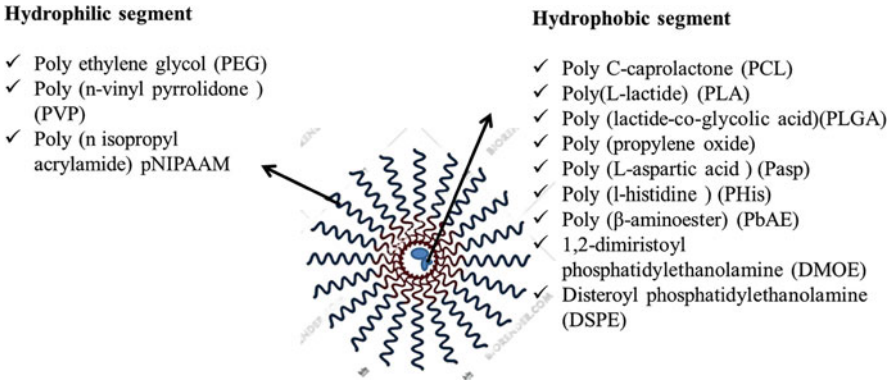


Fig. 13.2 Polymers used in constructing polymeric micelles

The CMC and HLB values are critical in this. Aside from these values, micelle kinetic stability is also required. To increase the selectivity of action and efficacy of drug delivery to the site of action, pH-sensitive polymeric micelles must be prepared. As pH-sensitive polymeric micelles, acrylic-based polymeric micelles are used. The pH-responsive micelles mainly include poly N-isopropylacrylamide and poly alkyl acrylic acid hydrophilic polymer chain.

13.6.2.2 Controlled Release of Drug

The normal polymeric micelle can get destabilized in the pH-changing environment. In this, the pH-sensitive polymer-based micelles are used to control the release. To inhibit the release of the drug, the formulation's transit time needs to be increased. This technique is easy for hydrophobic drugs in the core with the copolymer. Still, in the case of water-soluble or poor water-soluble drugs, the controlled release should not cause precipitation within the micellar phase. This may lead to a decrease in absorption.

13.6.2.3 Increasing the Drug Residence Time in GIT

The drug that acts in the stomach should not leave the stomach before getting absorbed. Thus, a mucoadhesive formulation of micelles is prepared. The mucoadhesive can better be employed with a bioadhesive polymer (Bromberg 2008).

13.6.3 Use of Polymeric Micelles

Micelles can be used in various forms because they overcome the limitations of conventional drugs, e.g., they are used as targeted drug delivery across multiple targeting therapies, types of cancers, neurodegenerative diseases, arthritis, and many more diseases.

13.6.3.1 Targeted Drug Delivery

Micelles are the potential nanocarrier for drug compounds that are hydrophilic as well as lipophilic. The drug product achieves physical and chemical protection from degradation before reaching the active site. The drug can be modified into control release, sustained release, or targeted release, a powerful strategy in the novel drug delivery system. The bioavailability of the formulation can be increased by improved absorption and utilization. Decrease the fluctuation in circulating drug levels, and decrease the incidence related to the severity of untoward effects related to high peak plasma concentration. Targeted drug delivery requires less total dose over the entire course of therapy, decreases local and systemic side effects, and reduces dosing frequency.

13.6.3.2 Colonic Drug Delivery

Azo-polymeric prodrug: More recent strategies aim to target the colon with drugs by using polymers as drug carriers. For this purpose, both synthetic and naturally occurring polymers have been used. Additionally, subsynthetic polymers were utilized to create polymeric prodrugs with an azo linkage between the polymer and the drug. They are tested for use in a colon drug delivery system (CDDS). Several azo polymers have also been assessed as coating materials for drug cores. These are also susceptible to azoreductase cleavage in the large bowel. It has been discovered that coating peptide capsules with polymers cross-linked with the azo aromatic group protects the drug from degradation in the gastrointestinal tract. These azo bonds are reduced in the colon, releasing the drug.

13.6.3.3 Ocular Targeted Drug Delivery

Polymeric micelles are used in drug delivery because they are mucoadhesive and thus can be retained in the ocular site for a longer duration. And micelles will give a clear solution when dissolved in water; therefore, the treatment does not interfere with the vision (Gegundez-Arias et al. 2016). Chetoni et al. concluded that the indomethacin with Poloxamer 407 micellar solution will give quick onset of action and more ocular bioavailability (Chetoni et al. 2000). F. Alvarez-Rivera et al. (2016) formed micelles of polyvinyl capro lactum-polyvinyl acetate-polyethylene glycol copolymer loaded with alpha lipophilic acid, which is used as an antioxidant for treating retinopathy and diabetic retinopathy. The micelle forms of the drug have shown a ten times increase in solubility, and retention time in the cornea is also increased (Francis and Kumar 2016). Mandal et al. (2017) created 10–40 nm dexamethasone micelles using D-tocopherol polyethylene glycol 1000 succinate (Vit E TPEGS) and Octoxyl-40 (OC-40) (Mandal et al. 2017). Liaco and a coworker developed a copolymer of nonionic triblock ethylene oxide and propylene oxide (PEO-PPO-PEO) for gene delivery in ocular tissue with low toxicity and better emulsifying solubilizing property.

13.6.3.4 Targeted Cancer Treatment

Polymeric micelles are used in cancer diagnosis, image-guided therapy, and monitoring the therapeutic effects. Cancer therapeutics have limitations such as severe

side effects and suboptimal therapeutic efficacy, which can be overcome by nanomedicines, which help improve drug biodistribution and target accumulation because they are stable in blood circulation, resulting in a fine balance of safety and efficacy. Liposomes, polymer-drug conjugates, and polymeric micelles are examples of nanomedicines that have been used to date and work through passive targeting, active targeting, or triggered release. Targeting moieties such as growth factors, phenylboronic acid, sugar, antibodies, and aptamer can also be used to achieve cancer-specific targeting. Furthermore, tumors and metastases are highly heterogeneous, which requires a non-invasive and quantitative assessment of targeting efficiency. Such nanotheranostics would be helpful for personalized medicines and clinical translation (Zhang and Mi 2019). In cancer, polymeric micelles are used in optical imaging, MRI, and multifunctional imaging by fusing them with treatment functionalities (Mi et al. 2017). Curcumin solubility is increased by targeting tumors with polyethylene glycol poly(lactic acid) PEG-PLA-loaded curcumin.

13.6.3.5 Diagnostics of Polymeric Micelles

Diagnostics are the agent that helps in imaging disease or disorder without producing harmful effects on the body. Diagnostics will give molecular, physiological, and anatomical information about the disease. In the case of radiopharmaceuticals given as diagnostics, their degradation should happen within time, as we are also offering the diagnostics to the patient and healthy person. Radiopharmaceutical diagnostics and imaging substances should reach the cell and be in the required concentration to diagnose the tissues or the cells. The nanoparticle can give targeted delivery of diagnostics and can act as a vehicle. This can be achieved by using micelles, as several reports have suggested that micelles can be used to produce targeted delivery of the diagnostic agent. The multifunctional micelles can be used. With the diagnostic treatment, the molecule can also be introduced, and tracing of treatment can be done easily with the radiopharmaceutical. All other studied micelles like pH-responsive, thermal-responsive, photoresponsive, and targeted modified micelles can be used to achieve the delivery of diagnostic and therapeutic.

13.7 Theranostics

Nanotheranostics overcomes the limitations of existing treatment modalities, such as diagnosing and treating the diseases at their earliest stage, thus providing personalized therapy with a bright prognosis. Diseases like cancer, cardiovascular diseases, and AIDS are most likely curable or at least treatable. Nanotheranostic pluronic and tetronic-like polymeric micelles occur in a sol-gel transition, making them intelligent nanosystems that improve patient compliance through the oral route and prolonged depots (Domingues et al. 2019). They have numerous advantages, such as stimuli-responsive release, escape from intracellular autophagy, synergetic and combinatory therapy, tumor biology, tracing nanoparticles in organisms, identification of predictive markers, and monitoring the therapeutic effects of nanomedicines, siRNA co-delivery, multimodality therapies, oral delivery,

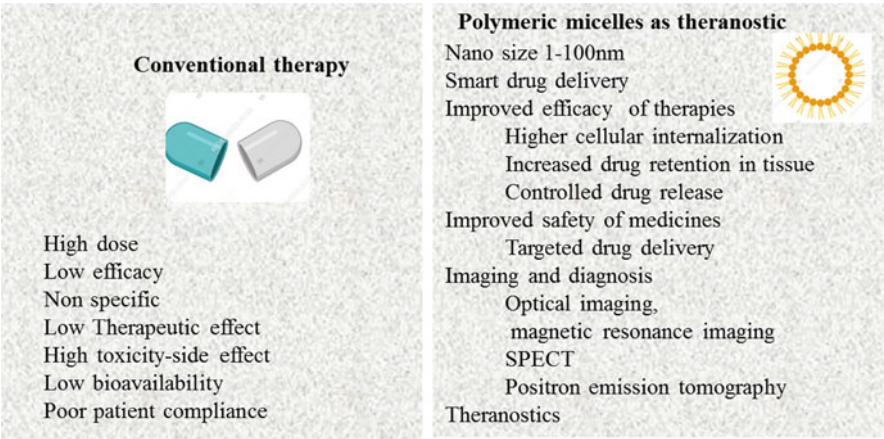


Fig. 13.3 Theranostic applications of polymeric micelles over conventional therapy

image-guided therapy, patient stratification, delivery across the blood-brain barrier, and so on (Mi et al. 2017) (Fig. 13.3).

Classical imaging modalities for diagnosing diseases include optical imaging, MRI, SPECT, PET, and CT. Molecular imaging is more advantageous than a biopsy. It can be further improved by administrating probes or contrast agents (CAs) that emit signals which help acquire anatomical or molecular information. Still, some clinically available probes lack selectivity for achieving an accurate diagnosis. As a result, a carrier that allows for targeting and increasing the intensity of reporting signals was required. Nanocarriers have advantages such as small size, high loading capacity, and the ability to include stimuli-responsive elements in their structure, allowing for effective probe and drug delivery in tumors. Dendrimers, conjugates, liposomes, emulsions, and polymeric nano-assemblies are some of the nanocarriers that have been developed. Nonetheless, polymeric nanocarriers have benefits such as high stability, biocompatibility, ease of formulation, co-loading multiple payloads, and manageable size and morphology for tumor-targeted drug delivery (Mi et al. 2017).

13.8 Theranostic Use of Polymeric Micelles in Various Diseases

Polymeric micelles are used in various diseases such as cancer, neurodegenerative disorder, diabetes, AIDS, atherosclerosis, arthritis, etc.

13.8.1 Cancer Theranostics

It is too challenging to deliver the drug to the tumor cell with conventional drug delivery in cancer. Traditional multidrug therapy also has so many side effects

besides the active site of the tumor. Thus, scientists are developing novel drug delivery systems that will load different drugs and give targeted drug delivery to the active tumor-growing cells. The polymeric micelles will penetrate or leak from the blood vessel through enhanced penetration and retention due to the tumor-growing cells and debilitated lymphatic drain. These micelles are assigned as the passive targeting micelles. The micelles were found to integrate with the surface protein, receptors that are predominantly found on tumor cells. These interactions are successful only because of surface modification of micelles for selective targeting; these properties of micelles are referred to as "active targeting."

13.8.1.1 Passive Targeting

Passive targeting can be said as the passive diffusion of large macromolecules, lipids, and the therapeutic agent. There is a high intracellular concentration of the nanoparticles/micelles due to passive targeting (Pradeep et al. 2017). Nanoparticles exhibit prolonged systemic circulation followed by passive accumulation in cells, even in the absence of targeting ligands, suggesting that drugs and drug-loaded polymeric micelles may be passively retained. For passive targeting, micelles should have a high distribution volume and smaller size. Enhanced permeability and retention (EPR) effect also plays an important role in passive targeting (Maeda 2001).

Enhanced Permeability and Retention (EPR) Effect

Matsumura and Maeda first reported the EPR effect in 1986. Maeda et al. expanded it further and proved it. Their findings demonstrated that most solid tumors have abnormally formed blood arteries that frequently produce a wide range of vascular endothelial variables. As a result, vascular permeability in solid tumors has risen. These will ensure that the tumor receives enough nutrients and oxygen to stimulate rapid growth. This is taken into account by the EPR effect. Malignant blood arteries' architectural and pathological characteristics encourage macromolecule diffusion into tumor tissues. The proteins released by macromolecules and tumor arteries are having molecular weight of more than 42 kDa, thus gets accumulated in tumor tissue. Inflammation or asphyxia causes tumor infract, and kinin and vascular permeability factors damage the blood vessel wall, resulting in leaky vessels. Polymeric micelles act as a solubilizing agent for hydrophobic drugs (class II and IV). Micelles increase the solubility by 10–8400 folds. PTX (paclitaxel)-poly (D-lactide) ME PEG diblock copolymer gives 5000 fold better water solubility (Matsumura and Maeda 1986). There are various porosities of the membranes that increase the efficacy in the biologics and the employed like antibodies, small interfering RNA which are also called silencing RNA, and therapeutic genes. There are some drawbacks of passive targeting, like the following:

1. Some tumor cells don't allow heterogeneous substances to pass.
2. Due to PEG on amphiphilic structure, intracellular intake of micelles gets affected. Thus, the passive targeting approach fails, and targeting needs to be done.

13.8.1.2 Active Targeting

Active targeting can be done by surface modification with a ligand specific to the antigen, multitargeting for the peptide carbohydrates and targeting the tumor's particular cells. By the process of active targeting, transferrin receptor overexpression and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) can be targeted.

Carbohydrate-based targeting: The functionalized micelles will target the overexpressed asialoglycoprotein receptor (ASGPR) in hepatocellular carcinoma. These functionalized micelles contain (polyhydroxyethyl aspartamide-ethylenediamine polylactic acid copolymer) galactose on the surface.

Monoclonal antibody targeting: Polyethylene glycol-phosphatidylethanolamine loaded with doxorubicin targeted for monoclonal antibody mAb2C5 is employed in an ovarian cancer cell model.

Folate-based targeting: Curcumin functionalized with amphiphilic polymer (polyethylene glycol) poly(lactic acid) increases the solubility and targets the tumor.

Peptide protein targeting: Proteins are small, which helps increase the in vivo solubility of drugs. Arginylglycylaspartic acid (Arg-Gly-Asp) is attached to the peptide, which helps in cell adhesion by attaching cell surface protein and integrin. This integrin adhesion will help in the attachment of the cell to the substrate (Kaur et al. 2022).

13.8.2 Theranostic Applications of Polymeric Micelles in Cancer

Guan et al. (2019) studied and reported that dual photoacoustic or near IR fluorescent imaging (NIRF) could detect the anatomical location of the tumor by incorporating cyanine dye with photosensitizer-loaded micelles (Guan et al. 2019). Gong et al. created a polyethylene glycol (PEG) nanomicelle that incorporated the PDT drug IR825. The nanomicelle was modified to include chlorin 6 Ce6, a porphyrin capable of encapsulating Gd3+ ions. This resulted in developing a nanoparticle that could perform PTT/PDT and multimodal imaging (fluorescent, MR, and photoacoustic imaging) while inhibiting tumor growth. Kim et al. (2012) described an anticancer theranostic system based on HA (MW 100 kDa)-DOX conjugates. The HA-DOX conjugates were formed through a chemical reaction between the carboxylic groups of HA and the amine groups of DOX, and they self-assembled into micelle-like NPs. In vitro anti-proliferation tests of cancer cells demonstrated the antitumor effect of HA-DOX micelles, and the advantage of the Au half-NIR-absorbing shells and electromagnetic properties are useful for theranostic applications to cancer treatment (Choi et al. 2012; Kim et al. 2012). Yang et al. (2020) investigated how to administer Pt1Ag28@ACD micelles effectively, passive and charge-focused. Pt1Ag28@ACD fluorescence imaging was improved by aggregation. A micelle aggregation effect was used to provide enhanced phototherapy of Pt1Ag28@ACD. They used hydrophobic interaction to solubilize structurally precise oil-soluble bimetallic NCs (Pt1Ag28). Positively charged Pt1Ag28@ACD of 60 nm may provide effective passive targeted delivery while being easily untaken by negatively charged cancer

cells, thereby increasing the targeting impact. Furthermore, the established in vitro and in vivo experimental results of micelle solubilization of ACD micelles to Pt1Ag28 NCs are finally nicely achieved by aggregation-induced emission fluorescence imaging (AIE FI)-guided improved photothermal/photodynamic treatment (PTT/PDT) on the tumor. This theranostic platform solves the problem of lipophilic NCs penetrating water-phase organisms; thus the mechanism between organisms and oil-soluble NCs can be studied in future precision medicine research (Yang et al. 2020).

Jeyamogan et al. (2021) reported the accumulation of micelle in 4 T1 breast cancer cells using fluorescence under near-infrared illumination. Carboxylated poly (styrene-co-chloromethyl styrene)-graft-poly(ethylene glycol) (PS-g-PEG-COOH) micelles were co-loaded with paclitaxel and combined with folic acid as a targeting agent against folate receptors overexpressed on 4 T1 murine breast cancer cells. Surprisingly, the development rate of 4 T1 murine breast cancer was reduced in 20 days of treatment, demonstrating the usefulness of the aforementioned polymeric micelle for simultaneous theranostic of cancer patients (Jeyamogan et al. 2021).

13.8.2.1 In Hepatocellular Carcinoma

Researchers Yongjun Liu et al. (2011) synthesized micelles of triblock polymer of poly-lactic-acid–(poly-ethylene-glycol)-poly (L-lysine) hydrophobic drug and Gd-DTPA (Gd (III) diethylenetriaminepentaacetate) as an imaging agent, at low CMC concentration 3.76×10^{-7} Mole/L. This multifunctional polymer chain (PLA-PEG-PLL) has the potential to functionalize micelles. It was predicted that this polymer could be made as a theranostic with the hydrophobic drug in the core Gd-DTPA as an imaging agent. This technique has proven high-resolution 3D mapping and can give morphological features without ionization. This can potentially target the AFP protein, an essential diagnostic protein in hepatocellular carcinoma. In the subsequent study in 2015, they used the same micelles of group PLA-PEG-PLL and attached targeting agent AFP antibodies to the amino group of PLL amino acid. The drug PTX (paclitaxel) is loaded inside the core. The imaging agent Gd-DTPA is also attached to the targeting agent. Self assembeled TGPM micelles helps in scanning the tumor growth of cells in real time (Liu et al. 2011). Polymeric micelles containing gold nanorods (GNRs) and adriamycin were synthesized and tested as a new therapeutic and diagnostic tool. In this study, the active targeting agent was epithelial cell adhesion molecule (EpCAM), a critical CSC surface marker. Photoacoustic imaging was used for GNR individuation and tissue recognition.

13.8.2.2 In Breast Cancer

In breast cancer, resveratrol is the highly referred drug in cancer developing cells. Resveratrol (trans-3, 4′, 5-trihydroxy stilbenes) is a plant resultant potent polyphenolic phytoalexin. Most breast cancers are created due to the expression of estrogen, progesterone, and growth factor receptors. Resveratrol acts by various pathways and results in cytotoxicity by apoptosis, inhibiting the development of cancer and decreasing the new blood vessel formation in tumor. Thus resveratrol is used as a

chemopreventive and chemotherapeutic agent. Besides the protective property, resveratrol shows chemical instability in vivo, less water solubility, and a short half-life. Gregoriou et al. (2021) prepared and studied resveratrol loaded with coumarin-6, and micelles are prepared with pluronic-127 and vitamin E TPEG (a synthetic derivative of tocopherol polyethylene glycol 1000 succinate). The derived micelles have shown selective toxicity for cancerous cells and show high solubility of anticancer drugs. The TPEG is primarily used in cancer therapy to improve anticancer drugs' pharmacokinetics and decrease multidrug resistance (Gregoriou et al. 2021).

13.8.2.3 In Colorectal Cancer

Colorectal cancer has traditionally been treated with surgery and chemotherapy. Patients with T 3–4/Nx pathology scores should get chemotherapy, according to the newest NCCN (National Comprehensive Cancer Network) guidelines. Furthermore, regardless of whether resection is possible, individuals with metastatic disease should get chemotherapy. The use of neoadjuvant chemotherapy and adjuvant chemotherapy has been proven to benefit patients who live longer. Irinotecan (IRI) is a chemotherapeutic medication that is commonly used to treat malignancies, including colorectal cancer (CRC) IRI has shown some degree of therapeutic and anti-metastatic effectiveness due to the intricacy of CRC pathogenesis and metastasis behavior. As a result, developing techniques to improve IRI therapeutic efficacy and metastasis inhibition would benefit CRC treatment. It's worth mentioning that micelle co-assembly can result in hierarchical nanostructures capable of carrying various payloads. As a result, polymeric micelles (PHMs) offer a unique and adaptable polymeric substrate for co-delivery. For example, Sun et al. described using PCL-PEG and PCL-PEI PHMs as small molecule inhibitors in combination with miR-34a delivery to combat melanoma. Diverse PCL-PEI and PCL-PEG ratios can provide the best drug/gene delivery qualities to PHMs. They developed polymeric hybrid micelles based on the PEI-PLA and DSPE-PEG copolymers, into which the IRI and tumor-suppressive miR-34a genes were co-loaded to provide chemo-miR-34a replenishment therapy for CRC. IRI and miR-34a could be encapsulated into MINPs to control miR-34a-related genes and boost miR-34a expression, inhibiting tumor genesis and increasing apoptosis in cancer cells. The constructed MINPs showed a particular excitation-dependent multi-wavelength emission characteristic and may be used to image lysosomes. MINPs aggregated at the tumor site, causing unusual anticancer activity while maintaining good biocompatibility. As a result, combined chemo-gene therapy using MINPs may be a potential technique for further development as a standard CRC treatment strategy (Sawant and Torchilin 2010).

A public health priority right now is the discovery of effective colorectal cancer therapy. The authors propose a multifunctional theranostic micellar drug delivery system based on electrostatic interaction PDMA-block-poly (−caprolactone) (PDMA-b-PCL) micelles as nanocarriers for SN-38 (7-ethyl-10-hydroxycamptothecin), ultra-small super paramagnetic nanoparticles (USPIO), and VEGF small interfering RNA (siRNA). Before complexation with the micelles, the

VEGF siRNA was attached to polyethylene glycol (PEG) (siRNA-PEG) to improve stability and extend the siRNA's stay in circulation. They developed mixed micelles containing mPEG-PCL and PDMA-b-PCL copolymer to improve in vivo biosafety. The SN-38/USPIO-loaded siRNA-PEG mixed micelleplexes passively targeted tumor areas and allowed VEGF silencing and chemotherapy to work together to effectively reduce VEGF. In T2-weighted imaging, the SN-38/USPIO-loaded siRNA-PEG mixed micelleplexes worked as a negative magnetic resonance imaging (MRI) contrast agent, resulting in a powerful tool for diagnosis and therapeutic outcome tracking. The combination therapeutic impact of the chemotherapeutic SN-38 and the VEGF siRNA, which operated cooperatively to reduce tumor growth during multi-dose treatment, was demonstrated in vivo with the SN-38/USPIO-loaded mixed micelleplexes (Lee et al. 2016).

13.8.2.4 Lung Carcinoma

The 15–20 nm PEG-PE micelles have shown effective protein drug delivery to the tumor. Paclitaxel (PTX) loaded with immunological cells 2C5 or 2G4 showed higher drug accumulation while experimenting in mice (Howell et al. 2013). Kong et al. (2012) have demonstrated that the lipids utilized in this formulation, DC-Chol and DOPE, can condense hydrophobically coated gold nanoparticles while also allowing them to distribute siRNA to target cells efficiently. These micelles contain the PEG-2000-PE lipid, which confers biocompatibility and provides for prolonged blood circulation durations (Kong et al. 2012) to provide MRI contrast as well as DNA/drug delivery to target cells. The researchers discovered that combining DOPE, DC-cholesterol, and PEG-2000-PE resulted in high efficiency of gene transfection and drug absorption (Kong et al. 2012).

13.8.3 Biodegradable Polymeric Micelles in Cancer Theranostic

Itaka et al. (2003) developed polymeric biodegradable micelles for systemic drug delivery having block copolymer polyethylene glycol–ply N`-N (2-amino ethyl) 2-amino ethyl aspartame and plasmid DNA via electrostatic interaction. Xiao et al. (2012) used H40, peptides (cRGD), and NOTA to create a multifunctional unimolecular micelle (to tag 64Cu and PET imaging). A microPET/microCT scanner was used to get the in vivo PET/CT pictures. Through the tail vein, the tumor-xenografted animals were given 5–10 MBq (megabecquerel) of the appropriate H40 unimolecular micelles. PET imaging revealed that cRGD nanomicelles accumulated in the tumor and were more cytotoxic than unconjugated nanomicelles. DOX localization in U87MG tumors was also confirmed using ex vivo fluorescence imaging (using the IVIS spectrum). The researchers discovered a significant difference between targeted and nontargeted nanomicelles. For cancer-targeted MRI, attempts have been undertaken to build multifunctional nanomicelles of H40 polyesters as an assimilated approach (Xiao et al. 2012). Yang et al. (2018) developed fluorescent APMs from poly (fluorene-alt-(4, 7-bis (hexylthien)-2, 1, 3-benzothiadiazole))-graft-polycaprolactone-block-poly [oligo (ethylene glycol)

methyl ether methacrylate] for fluorescence imaging. They showed a high fluorescence quantum yield and improved photostability because of the biodegradable polycaprolactone segment (Yang et al. 2018). Chen et al. (2015) created self-fluorescent PMs using cRGD peptide linked to Boltron®H40, a biodegradable photoluminescent polymer. These photos exhibited blue fluorescence images of a human glioblastoma cell line with tumor-targeting capability (Chen et al. 2015).

13.8.4 Multifunctional Micelles in Cancer Theranostic

Multifunctional micelles have two characteristics: (1) targeting agent and (2) imaging agent. They triggered the release of the drug. The study performed by Movassaghian et al. (2015) concluded that multifunctional micelles could be made of PEG-PE base with the tissue-specific target having specificity for tumor and infarct tissue using ligand monoclonal antibodies mAb 2C5 and mAb (monoclonal antibodies) G4, respectively. The secondary targeting ligand TATP can be added to PEG-PE and result in TATP-PEG-PE. The PEG-PE can be made pH-sensitive by combining with hydrazone PEG-Hz-PE. The secondary ligand will give an advantage as it takes time to degrade and thus increase the intracellular delivery of the drug (Movassaghian et al. 2015) (Fig. 13.4).

Kumar et al. (2012) created a multimodal theranostic formulation of CdSe quantum dots (QDs) and the anticancer drug doxorubicin (DOX) co-encapsulated

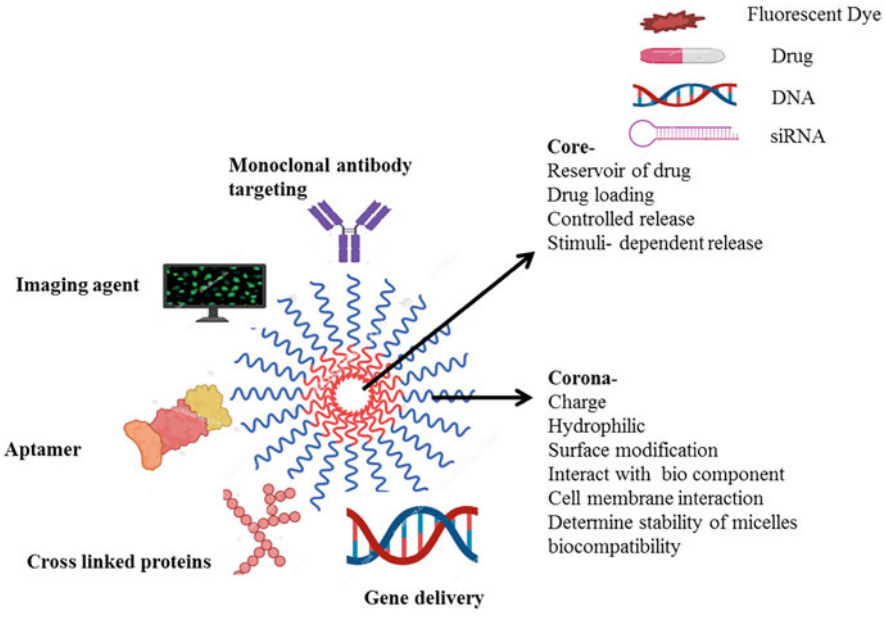


Fig. 13.4 Surface modification of polymeric micelles for multifunctional theranostics

in a hydrophobic core. Weihua Zhuang et al. developed a novel mPEG-SS-Poly (AEMA-co-TBIS) (mPEATss) copolymer to create multifunctional polymeric micelles with pronounced AIE properties for cancer therapy and active two-photon bio-imaging. pH and redox dual responsive theranostics were demonstrated for the antitumor drug DOX and AIE active two-photon cell and tissue imaging (Amini et al. 2018). CdSe quantum dots (QDs) and the anticancer drug doxorubicin (DOX) were co-encapsulated into phospholipid-based polymeric micelles for optical fluorescence imaging and controlled drug delivery, which can be used to treat a variety of diseases, including cancer (Thomas et al. 2013). Polymeric polyethylene glycol-phosphatidylethanolamine-based micelles were prepared as a magnetic resonance imaging contrast agent that showed apoptotic activity in breast and melanoma tumor mouse models and is thus suitable for simultaneous cancer detection and therapy (Naqvi et al. 2010; Upponi et al. 2018).

The researchers created self-quenchable indocyanine green (ICG)-encapsulated micelles with folic acid (FA)-targeting specificity (FA-ICG-micelles), which have the potential to be used in photodynamic theranostics in cancer. Both antitumor drugs (doxorubicin) and gold core-shell quantum dot nanoparticles (Au-SiO2/QDs) are present in polymeric micelles. The synthetic derivative (PHEA-LA-PEG-FA) was obtained by functionalizing -poly(N-hydroxyethyl)-dl-aspartamide (PHEA) with lipoic acid (LA), polyethylene glycol (PEG), and folic acid (FA).

Guo et al. (2014) formulated a multifunctional unimolecular micelle based on a new brush-shaped amphiphilic block copolymer for tumor-targeting and non-invasive PET (positron emission tomography). Doxorubicin contained inside unimolecular micelles was released over time in a pH-dependent manner. TRC105-conjugated targeted unimolecular micelles (PHEMA-PLLA-PEG-TRC105) were found to have significantly better cellular absorption than nontargeted micelles in CD105-positive cells. PET imaging and ex vivo biodistribution studies revealed that 4 T1 murine breast tumor-carrying animals exposed to Cu-labeled targeted micelles had more tumor accumulation than those treated with nontargeted micelles. These multifunctional tumor-targeting unimolecular micelles have pH-controllable drug release patterns and PET imaging capacity, making them attractive dosage form nanocarriers for cancer theranostics (Guo et al. 2014).

Polymeric micelles containing paclitaxel (PM-DTPA/PTX) were created for the theranostic treatment of solid tumors. Nanotheranostics with a fluorophore and paclitaxel-loaded polymeric micelles were used for various tumor types via computed tomography-fluorescence molecular tomography imaging (Oda et al. 2020). Non-invasive imaging indicated active targeting of FOL-bearing micelles and a significant amount of DOX reaching the tumor to produce a therapeutic impact.

13.8.5 Theranostic Applications of Polymeric Micelles in Diabetes

Synthetic polymeric micelle nanoparticles offer a lot of potential to improve the therapeutic efficacy of drug delivery methods due to their benefits, such as chemical

structural diversity, functional design ability, controllable sizes, and lengthy clearance time. Nanomaterial for the diagnosis and treatment of diabetes has been explored in recent years, and several new uses for diabetes detection and treatment have been planned using these nanomaterials. Nanomaterial-assisted biomedicine has demonstrated considerable benefits in diabetes diagnosis and treatment, including diabetic biomarker detection, glucose management, insulin mimicking, and complication prevention. Liu et al. developed FQS-coated insulin-loaded poly (lactide-co-glycolide)-monomethoxy-poly(polyethylene glycol) micelle cores for oral insulin delivery (targeting ligand FQSIYPpIK) trimethyl CS chloride, modified (Liu et al. 2015). Shan et al. (2015) created a self-assembled nanoparticle with a CPP (penetratin) core and a dissociable hydrophilic poly(N- (2-hydroxypropyl) methacrylamide) copolymer (pHPMA) layer for oral insulin delivery.

13.8.6 Theranostic Applications of Polymeric Micelles in Neurodegenerative Disorder

Curcumin, polyphenolic compounds, rosmarinic acid, tannic acid, and its derivatives are examples of amyloid theranostics that have been documented in the literature. These materials work admirably as theranostic agents, inhibiting Aβ via stacking interactions between polyphenolic rings and aromatic amino acid residues in the amyloid protein. To keep the polyphenolic molecule-protein complex stable, other structural units form hydrogen bonds. Zhang et al. developed CRANAD-17 and NIR-based curcumin derivatives for use as theranostics. Aβ CRANAD-17 can break down copper-induced Aβ crosslinking and bind to both soluble and insoluble forms of Aβ (Zhang et al. 2013b).

The BBB permeability and biocompatibility of (E)-4-(4-(dimethylamino)styryl)-1-(2-hydroxyethyl) quinolin-1-ium chloride (DMA-SLOH) molecule was enhanced by the addition of lipophilic alkyl chains. Simultaneously, the DBA-SLOH molecule demonstrated a binding affinity for both Aβ monomers and aggregates, limiting the aggregation of amyloid peptide monomers and preventing the formation of dangerous oligomers, indicating that it holds great promise as a theranostic agent for Aβ detection (Li et al. 2016). Phenothiazine-based drugs and the NIR probes discussed above may also operate as a theranostic for Aβ aggregation. Phenothiazine-based has a donor-acceptor design that allows it to inhibit and detect Aβ. The fluorescence intensity of the probe was increased after interaction with amyloid fibrils; additionally, this probe exhibited great stability in mice serum and less toxicity in human neuronal cells (Dao et al. 2017). Metal-Aβ aggregates could be precisely targeted by a novel fluorescence-based theranostic chelator (BTTA). BTTA effectively identifies metal-Aβ aggregates in vivo and in vitro using fluorescence variations, and mitigation of aggregation is validated by reduced neurotoxicity (Yang et al. 2016). In addition to fluorescence-based theranostics, MRI detection-based theranostic drugs have also been described. As amyloid theranostics, lipophilic compounds similar to Congo red (CR) are also available; one contains 19F as an MRI active agent. These

compounds, like CR, can attach to amyloid plaques and can be used to identify amyloid plaques in the brains of living mice (Higuchi et al. 2005).

Chibhabha et al. developed anionic, water-soluble DSPE-PEG2000 curcumin polymeric micelles capable of detecting Aβ plaques in the brain and retina. Another study found that the curcumin-Ni electrode has good sensitivity and a wide detection range of 0.001 to 10 nM Aβ oligomer. This electropolymerized curcumin ensures the identification of an oligomer even at low concentrations, making it a promising tool for the initial detection of Alzheimer's disease (Chibhabha et al. 2020).

13.8.7 Theranostic Application of Polymeric Micelles in AIDS

Long-term faithfulness to antiretroviral therapy is a critical unmet requirement in treating human immunodeficiency virus type one (HIV-1) infection (ART). Long-acting ART regimens would increase drug adherence, reduce secondary toxicity, and avoid new infections if they will be implemented in clinical practice. These could eventually help to bring forth an AIDS-free planet. To accomplish this job, they increased the hydrophobicity of existing antiretroviral medicines (ARVs), resulting in the production of cell- and tissue-penetrant nanocrystals encased in biodegradable polymers. These were created to create drug stores in monocytes and macrophages. However, the capacity to quickly analyze drug formulation, tissue biodistribution, and pharmacokinetics (PKs) to achieve meaningful pharmacodynamic (PD) improvements remains a substantial barrier. This is especially important because medication tissue dispersion is limited, even though the half-lives of the rare available prolong acting medicines are measured in weeks to months. Multimodal decorated nanoparticles encapsulating hydrophobic ARVs and bio-imaging agents in a single nanoformulation were created to address these technological and biologic obstacles. They were combined into a single "multimodal imaging theranostic nanoparticle using core-shell building methods." The drug and imaging contrast agents were integrated into a polymeric core, and the particle's surface was covered with lipids adorned with targeting moieties. Macrophages quickly absorbed the produced particles. The reticuloendothelial system was the site of tissue dispersion, indicating HIV-1's target tissues. A polycaprolactone (PCL) core was used for packaging europium (Eu3+)-doped cobalt ferrite (CF, EuCF) crystals and the hydrophobic medication dolutegravir (DTG). The particle's "shell" was covered with a lipid coating. PC, DSPE-PEG2000, and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) lipids improved particle biocompatibility and lipophilicity, making macrophage targeting easier.

13.8.8 Theranostic Application of Polymeric Micelles in Atherosclerosis

Cardiovascular diseases are the leading cause of morbidity and mortality around the world, and nanomedicine can aid in targeting, primary diagnosis, and

resident delivery of diagnostic chemicals. To accomplish this, Yoo et al. (2016) synthesized fibrin-binding peptide amphiphile micelles (PAMs) by combining the targeting peptide cysteine-arginine-glutamic acid-lysine-alanine (CREKA) with two types of amphiphilic molecules containing the gadolinium (Gd) chelator diethylenetriaminepentaacetic (MRI). In formulations containing DSPE-PEG2000-DTPA (Gd), transmission electron micrographs revealed a homogeneous population of spherical micelles, whereas DTPA-BSA (Gd) and CREKA amphiphilic produced both spherical and cylindrical micelles. Clot-binding assays revealed that DSPE-PEG2000-DTPA (Gd)-based CREKA micelles targeted clots 8 times more effectively than nontargeting (NT) counterpart micelles. CREKA and NT and DTPA-BSA (Gd) micelles thus showed no difference. In vivo MRI and optical imaging of the aortas and hearts revealed that the peptide ligand provided fibrin selectivity without any variation in nanoparticle formulations or morphologies. All micelles were removed via the reticuloendothelial system and renal clearance, according to biodistribution studies, and histology revealed no evidence of necrosis. Finally, their findings exhibit the effective synthesis and molecular imaging capabilities of two distinct types of molecules, DTPA-BSA (Gd) and CREKA-Gd PAMs (Yoo et al. 2016).

13.8.9 Theranostic Application of Polymeric Micelles in Arthritis

Angiogenesis is critical for rheumatoid arthritis progression (RA). The arginine-glycine-aspartate (RGD) peptide has great empathy and specificity for integrin $\alpha v\beta_3$, the most studied angiogenesis target. Methotrexate's anti-rheumatic characteristics could be improved by nimesulide. However, the practical applicability of methotrexate and nimesulide was incomplete due to their water insolubility and lack of targeting ability.

The thin-film hydration method successfully synthesizes RGD-functionalized polymers, RGD-PEG3400-PLA2000, which are then used to create RGD-modified drug-loaded micelles. R-M/N-PMs considerably reduced the viability of inflammatory RAW264.7 cells in the CAM assay while also suppressing angiogenesis in chick embryos. They also performed real-time fluorescence imaging by using an in vivo small animal imaging system. They discovered that R-M/N-PMs labeled with DiD were primarily disseminated to arthritic joints and that RGD improved micelle targeting and thus stimulated micelle maintenance in arthritic joints. Most essentially, an in vivo study of arthritic rats revealed that R-M/N-PMs reduced joint swelling, bone erosion, immunological organ index, and inflammatory cytokines in the blood, improving rheumatoid arthritis treatment efficacy (Wang et al. 2019).

In an in vitro cell viability assay, RGD-containing micelles considerably increased the inhibitory influence on cell growth of LPS-stimulated Raw264.7 cells compared to RGD-free micelles, indicating the importance of RGD in the treatment of inflammation-related disorders. In the in vivo assay of an arthritic rat model, both R-M/N-PMs and M/N-PMs were primarily distributed to the arthritic joints. Because of RGD's high affinity and specificity for integrin v3, the R-M/N-

PMs group retained a stronger fluorescence signal than the M/N-PMs group 24 hours after injection, enhancing micelle retention in arthritic joints.

13.9 Diagnostic and Imaging Application of Micelles

Polymeric micelles should be used for multimodal imaging by optical-MRI, optical-SCPET, optical-CT, PET-MRI, SCPET-MRI, and other methods that demonstrate good performance for precise tumor imaging when loaded with appropriate CAs. Despite significant efforts to formulate polymeric micelles for multifunctional molecular imaging, their translation to clinical applications has been limited due to the challenges of incorporating each imaging mode into the systems.

13.9.1 Optical Imaging

Because of its convenience, ease of use, high sensitivity, a large number of optical dyes, and multi-channel imaging capability, optical imaging is the most commonly used imaging modality in oncology research. Optical imaging can be performed using a dye-infused probe. Optical imaging has the advantage of being able to select dyes, label them easily, and obtain authentic images. For in vivo imaging, tissue penetration is required, while imaging background signals should be avoided. Polymeric micelles are used to increase the sensitivity and specificity of optical imaging. Polymeric micelles are necessary for in vivo imaging to probe lesions specifically without generating a background signal. Tsai et al. (2010) used mPEG-b-PLA, Cy5.5–PEG–PLA, FOL–PEG–PLA, and poly (2-hydroxyethyl methacrylate)m(Poly (HEMA)-co-His-g-PLA as a polymeric carrier for tumor-targeted delivery. Micelle biodistribution and tumor accumulation were studied using in vivo optical imaging. Tumor size was also measured for therapy efficacy (Tsai et al. 2010).

13.9.2 Fluorescent Imaging

Tang and his colleagues first describe the aggregation-induced emission approach. Bio-imaging with fluorescent compound-induced quenching provides high efficacy, sensitivity, and strong fluorescent and is accomplished using two techniques: photon excited aggregation-induced emission (AIE) and two-photon excited aggregation-induced emission (AIE). The disadvantage of this technique is its limited application as well as tissue and cell toxicity. In fluorescent imaging takes place excitation at the longer wavelength near IR. The two-photon fluorescent imaging (TPFI) has more advantages than MRI and CT for dual optical and micro single-photon emission computed tomography (SCPET); polymeric micelles loaded with additional CAs, such as radioactive rhenium-188 (188Re) combined with NIR dye of IR-780 iodide, could be used; two-photon fluorescent imaging is a more powerful weapon than any

other method in bio-imaging. In combination with TPFI, the drug delivery system will provide ease in finding the location of the tumor by increasing permeability and retention. In two photon imaging, drug release process happens by active aggregation-induced emission. The fluorescence can be best seen in the diluted solutions; as the concentration increases, the aggregation and fluorescence emission weaken (Peng et al. 2011).

In fluorescence imaging, near-infrared fluorescent (NIRF) dyes (general emission wavelength of 650–900 nm) could reduce tissue absorption and autofluorescence. In a current study, NIR fluorescence imaging was used to create a form of pH-activatable PEG-PLA micelles encapsulating photosensitizer for cancer PDT. Zhuang et al. (2019) have made polymeric micelles of polymer MPEG-SS poly (AEMA-coTBIS) (mPEATss) with two-photon emission AIE probe that will increase the cell internalization of therapeutic drug. The further modification to the polymer causes the release of drug triggered by pH, redox reaction, and charge conversion, leading to enhancement of cell internalization for theranostic. Biocompatibility of two excited photon fluorescence can be studied by MTT assay against 4 T1 cells. The efficacy of antitumor drug doxorubicin can be increased by two-photon bio-imaging, and drug-associated side effects can be decreased (Zhuang et al. 2019). Liu et al. used confocal laser scanning microscopy to create dual responsive PICs encapsulated with two fluorescent dyes, green 5-aminofluorescin and red rhodamine B, that demonstrated intense pH and thermoresponsive fluorescence. Yang et al. prepared fluorescent APMs from poly (fluorene-alt-(4, 7-bis (hexylthien)-2, 1, 3- benzothiadiazole))-graft-polycaprolactone-blockpoly [oligo (ethylene glycol) methyl ether methacrylate] for fluorescence imaging. The results showed a high fluorescence quantum yield and improved photostability due to the biodegradable polycaprolactone segment (Ge et al. 2013).

13.9.3 The Magnetic Resonance Contrast Agent

In the clinic, MRI is a broadly adopted molecular imaging modality used for anatomical imaging with fine spectral resolution (1 mm) and soft tissue imaging capability. However, low intensity/sensitivity and extended signal acquisition time severely limit its use. Thus the contrast agent can be used to increase the magnification of the image. Polymeric micelles could also be aimed to react to pathological parameters in tumor tissues, such as low pH, enzymes, redox potential, and hyaluronidase, to improve contrast and even magnify and convert pathological signals into visible contrast enhancement. Polyethylene glycol B-poly (L-histamine) amphiphilic block copolymer with methoxy polyethylene glycol b-poly L-lactic acid provides long-term imaging in the IR of vascular tumors. Micelles loaded with the dye carbocyanine will provide an enhanced image. Liu et al. used poly (lactic acid)-polyethylene glycol-poly(L-lysine)-diethylenetriamine penta-acetic acid (PLA-PEG-PLL-DTPA) and PLA-PEG-PLL-biotin to formulate theranostic micelles for simultaneous magnetic resonance imaging (MRI) and treatment of HCC (Liu et al. 2015).

Desser et al. (1994) created pH-responsive mixed APMs composed of (PEGP (L-His)) and (PEG-P(L-LA)-DTPA-Gd) as a cancer-recognizable contrast agent that sensed small tumors by displaying T1 MR contrast augmentation at tumor sites under acidic tumor environment-induced micellar disruption due to protonation of imidazole group of histidine blocks. A nanosized combination agent of the poorly soluble chemotherapeutic agent is polymeric polyethylene glycol-phosphatidylethanolamine-based micelles loaded with paclitaxel and super paramagnetic iron oxide nanoparticles (SPION). A hydrophobic magnetic resonance imaging contrast agent for cancer detection and therapy has been developed. It has been proposed that such a combination enables diagnostic imaging as well as apoptotic anti-tumor activity, resulting in a more personalized and effective form of treatment (Desser et al. 1994). Jiang et al. (2022) investigated the potential effect of superparamagnetic iron oxide nanoparticles (SPIO), which have been used for decades in the development of theranostic polymeric micelles for targeted cancer therapy and diagnostics. They discovered that clustered SPIO in micelles had a significant impact on MR imaging things and biophysical properties of polymeric micelles by influencing micelle veracity concerning micelle size, doxo loading, critical micelle concentration (CMC), and in vitro dissociation (Jiang et al. 2022).

13.9.4 X-Ray Computed Tomography

Differential X-ray absorption by different tissues enables X-ray computed tomography to differentiate between different anatomical features. As contrast agents for high spatial and temporal resolution, X-ray absorbing heavy metals such as iodine, barium, and bromine are used. The main disadvantage of this imaging method is that it necessitates a higher concentration of contrast agents (1022 M). This can be overcome by using micelles as carrier systems to target the contrast agent (Oerlemans et al. 2010). Long-circulating micelles containing iodine and poly (L-lysine)-PEG have shown improved contrast in CT scans of the aorta, heart, liver, and spleen (Torchilin et al. 1999).

13.9.5 Imaging and Radionuclide-Based Therapy Agents

A radioisotope is an unsteady radionuclide that decays by emitting gamma rays or alpha or beta particles. For clinical use, radionuclides with desirable properties can be labeled as various compounds. A radiopharmaceutical is a radionuclide formulation with sufficient purity and pharmaceutical safety for administration for diagnostic or therapeutic purposes (Bodei et al. 2004). Diagnostic imaging techniques such as SPECT and PET use gamma emitters, whereas treatment uses alpha- and beta-emitting radioisotopes (Velikyan 2013). The tumor microenvironment-responsive polymeric micelles might be used for tumor-specific optical imaging by producing dyes only in cancerous tissue. Recently, organic dye (IR780 iodide)-loaded polyester micelles (IR780 micelles) labeled with radioactive rhenium-188 (188Re) for dual

optical and PET imaging-guided tumor PTT have been explored (Zhu et al. 2018). The radionuclide chosen for radiotheranostics must have the following characteristics: (1) a suitable half-life; (2) the presence of various decay schemes or isotope pairs; (3) the absence of high-energy gamma emission; (4) suitable radiochemistry; and (5) isotope accessibility. Because of these requirements, metal radioisotopes are becoming progressively popular for theranostic applications among the various radionuclides.

13.10 Polymeric Micelles as a Prodrug Theranostic

The polymeric micelles as a theranostic have the disadvantage of drug release in the bloodstream before it reaches the site of action, disappointing endocytosis, and we cannot find in vivo distribution. Thus the prodrug is the new procedure to enhance drug stability in systemic circulation. The micelle with charge conversion ability can be used for this purpose. In a study done by Xu et al. (2020), the drug release and surface charge of micelles are triggered because of acidic pH, doxorubicin (DOX) labeled with photon fluorophore (TP), and copolymer TP-PEI (DA/DOX)-PEG. This gives fluorescent imaging of deep tissue and removes the tumor. In the micelles of the prodrug, the pH-sensitive bond would be broken. The outer PEG (polyethylene glycol) attached demethylmaleic anhydride (DA) on the polyethylenimine (PEI) section. Thus, the charge of micelles converts from negative to positive, causing endocytosis of micelle. The DOX starts releasing, indicating active site release of drug and disruption of tumor cell. The ability to image and target the tumor cells shows these prodrug micelles are the potential approach for theranostic tumors (Xu et al. 2020).

13.11 Polymeric Micelles in Clinical Trial till 05/04/2022

Several polymeric micelles as cancer theranostics are under clinical trials (Table 13.2).

13.12 Conclusion and Future Perspectives

Theranostics creates a different path for improving from “trial and error” treatment to customized medicine. Even though several studies have shown that polymeric micelles outperform other nanoparticles as a theranostic agent in cancer and other disorders, micelle production is difficult. To accomplish optimum theranostic effects, bioactive medicinal compounds and imaging agents must be compatible. To avoid unfavorable chemical activity that could be harmful to the neighboring cells, bioactive chemicals and imaging agents should be carefully chosen. Second, because micelles are small in size, the very less concentration of drug within the core results in early drug release even before the micelle reaches its target site. As a result,

Table 13.2 Polymeric micelles under clinical trials as cancer theranostics https://www.clinicaltrials.gov/

Study name	Action	Status	Drug	Phase of study
Genexol PM was administered to a patient with advanced urothelial cancer who had formerly been treated with gemcitabine and platinum	Bladder cancer Ureter cancer	Completed	Genexol PM	Phase 2
PM in a patient with progressive urothelial cancer who had formerly been treated with gemcitabine and platinumGenexol PM safety and efficacy in gynecologic cancer	Head and neck squamous cell carcinoma	Unknown status	Docetaxel PM	Phase 2
Paclitaxel in a patient treated with advanced or metastatic pancreatic cancer	Pancreatic cancer	Completed	Paclitaxel-loaded PM	Phase 2
In a patient with advanced solid tumors, docetaxel was administered	Used in advanced solid tumor	Not yet recruiting	Docetaxel polymeric micelles for injection	Phase 2
Paclitaxel-loaded polymeric micelle in a taxane-treated breast cancer patient	Recurrent breast cancer	Unknown status	Paclitaxel-loaded polymeric micelles	Phase 4
Paclitaxel (Genexol) cisplatin versus paclitaxel-loaded polymeric micelles (Genexol-PM) cisplatin in advanced non-small cell lung cancer	Non-small cell lung cancer	Completed	Paclitaxel (Genexol) Paclitaxel-loaded polymeric micelles (Genexol-PM)	Phase 1 Phase 2
Paclitaxel-loaded PM and carboplatin in treating advance ovarian cancer	Ovarian cancer	Unknown status	Carboplatin Paclitaxel-loaded polymeric micelle	Phase1 Phase2
Docetaxel-PM and oxaliplatin in esophageal carcinoma	Esophagus squamous cell carcinoma Metastatic cancer	Unknown status	Docetaxel-PM Oxaliplatin	Phase 2
After sorafenib failure, a phase 2 study of weekly Genexol-PM was conducted in patients with hepatocellular carcinoma	Carcinoma, hepatocellular	Terminated	Genexol PM	Phase 2

(continued)

Table 13.2 (continued)

Study name	Action	Status	Drug	Phase of study
Genexol-PM and gemcitabine phase 2 trial in advanced small cell lung cancer	Non-small cell lung cancer	Completed	Genexol-PM/ gemcitabine	Phase 2
Study of NC-6004 in combination with 5-FU and cetuximab in head and neck cancer	Head and neck neoplasms	Terminated	1.Nc-6004 2. Cetuximab 3. 5-FU	Phase 1
Analyze the safety and efficacy of Genexol-PM in the treatment of gynecologic cancer	Gynecologic cancer	Unknown status	Genexol-PM	Phase 1
IG-001 versus nab-paclitaxel bioequivalence study in metastatic or locally recurrent breast cancer	Breast cancer that really has propagate Breast cancer that reappears locally	Completed	1.Nab-paclitaxel 2.Ig-001	Completed

to ensure effective drug delivery to the target region, the compatible drug concentration with the core size should be cautiously considered. In recognition of the outstanding layout of polymeric micelles to be employed as a theranostic device, the pharmacological features counterpart between imaging agents, targeting agents, and bioactive therapeutic chemicals should be sensibly designated and investigated.

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References

Aguilar ZP (2013) Chapter 2 - Types of nanomaterials and corresponding methods of synthesis in nanomaterials for medical applications. In: Aguilar ZP (ed). Elsevier, pp 33–82

Amini MA, Abbasi A, Cai P, Lip H, Gordijo C et al (2018) Combining tumor microenvironment modulating nanoparticles with doxorubicin to enhance chemotherapeutic efficacy and boost antitumor immunity. J Natl Cancer Inst 111:399–408

Bodei L, Handkiewicz-Junak D, Grana C, Mazzetta C, Rocca P et al (2004) Receptor radionuclide therapy with 90Y-DOTATOC in patients with medullary thyroid carcinomas. Cancer Biother Radiopharm 19:65–71

Bromberg L (2008) Polymeric micelles in oral chemotherapy. J Control Release 128:99–112

Chen G, Wang L, Cordie T, Vokoun C, Eliceiri KW, Gong S (2015) Multi-functional self-fluorescent unimolecular micelles for tumor-targeted drug delivery and bioimaging. Biomaterials 47:41–50

Chetoni P, Panichi L, Burgalassi S, Benelli U, Saettone M (2000) Pharmacokinetics and anti-inflammatory activity in rabbits of a novel indomethacin ophthalmic solution. J Ocular Pharmacol Ther: Off J Assoc Ocular Pharmacol Ther 16:363–372

Chibhabha F, Yang Y, Ying K, Jia F, Zhang Q et al (2020) Non-invasive optical imaging of retinal Aβ plaques using curcumin loaded polymeric micelles in APPswe/PS1ΔE9 transgenic mice for the diagnosis of Alzheimer's disease. J Mater Chem B 8:7438–7452

Choi KY, Jeon EJ, Yoon HY, Lee BS, Na JH et al (2012) Theranostic nanoparticles based on PEGylated hyaluronic acid for the diagnosis, therapy and monitoring of colon cancer. Biomaterials 33:6186–6193

Dao P, Ye F, Liu Y, Du ZY, Zhang K et al (2017) Development of phenothiazine-based theranostic compounds that act both as inhibitors of β-amyloid aggregation and as imaging probes for amyloid plaques in Alzheimer's disease. ACS Chem Neurosci 8:798–806

Desser TS, Rubin DL, Muller HH, Qing F, Khodor S et al (1994) Dynamics of tumor imaging with Gd-DTPA-polyethylene glycol polymers: dependence on molecular weight. J Magn Reson Imaging: JMRI 4:467–472

Domingues C, Alvarez-Lorenzo C, Concheiro A, Veiga F, Figueiras A (2019) Nanotheranostic pluronic-like polymeric micelles: shedding light into the dark shadows of tumors. Mol Pharm 16:4757–4774

Fares AR, ElMeshad AN, Kassem MAA (2018) Enhancement of dissolution and oral bioavailability of lacidipine via pluronic P123/F127 mixed polymeric micelles: formulation, optimization using central composite design and in vivo bioavailability study. Drug Delivery 25:132–142

Francis R, Kumar DS (2016) Biomedical applications of polymeric materials and composites. John Wiley & Sons

Ge J, Li M, Zhang Q, Yang CZ, Wooley PH et al (2013) Silica aerogel improves the biocompatibility in a poly caprolactone composite used as a tissue engineering scaffold. Int J Polym Sci 2013:402859

Gegundez-Arias ME, Ortega C, Garrido J, Ponte B, Alvarez F, Marin D (2016) International conference on bioinformatics and biomedical engineering. Springer, pp 369–379

Gregoriou Y, Gregoriou G, Yilmaz V, Kapnisis K, Prokopi M et al (2021) Resveratrol loaded polymeric micelles for theranostic targeting of breast cancer cells. Nanotheranostics 5:113–124

Guan Y, Zhang Y, Zou J, Huang L-P, Chordia MD et al (2019) Synthesis and biological evaluation of genistein-IR783 conjugate: cancer cell targeted delivery in MCF-7 for superior anti-cancer therapy. 24:4120

Guo J, Hong H, Chen G, Shi S, Nayak TR et al (2014) Theranostic unimolecular micelles based on brush-shaped amphiphilic block copolymers for tumor-targeted drug delivery and positron emission tomography imaging. ACS Appl Mater Interfaces 6:21769–21779

Higuchi M, Iwata N, Matsuba Y, Sato K, Sasamoto K, Saido TC (2005) 19F and 1H MRI detection of amyloid β plaques in vivo. Nat Neurosci 8:527–533

Howell M, Mallela J, Wang C, Ravi S, Dixit S et al (2013) Manganese-loaded lipid-micellar theranostics for simultaneous drug and gene delivery to lungs. J Control Release: Off J Control Release Soc 167:210–218

Itaka K, Yamauchi K, Harada A, Nakamura K, Kawaguchi H, Kataoka K (2003) Polyion complex micelles from plasmid DNA and poly(ethylene glycol)-poly(L-lysine) block copolymer as serum-tolerable polyplex system: physicochemical properties of micelles relevant to gene transfection efficiency. Biomaterials 24:4495–4506

Jeyamogan S, Khan NA, Siddiqui R (2021) Application and importance of theranostics in the diagnosis and treatment of cancer. Arch Med Res 52:131–142

Jhaveri AM, Torchilin VP (2014) Multifunctional polymeric micelles for delivery of drugs and siRNA. Front Pharmacol 5:77

Jiang Y, Lee J, Seo J-M, Davaa E, Shin K-J, Yang S-G (2022) Enhanced thermodynamic, pharmacokinetic and theranostic properties of polymeric micelles via hydrophobic core-clustering of superparamagnetic iron oxide nanoparticles. Biomater Res 26:8

Kaur J, Gulati M, Kapoor B, Jha NK, Gupta PK et al (2022) Advances in designing of polymeric micelles for biomedical application in brain related diseases. Chem Biol Interact 361:109960

Kim KS, Park S-J, Lee M-Y, Lim K-G, Hahn SK (2012) Gold half-shell coated hyaluronic acid-doxorubicin conjugate micelles for theranostic applications. Macromol Res 20:277–282

Kong WH, Bae KH, Jo SD, Kim JS, Park TG (2012) Cationic lipid-coated gold nanoparticles as efficient and non-cytotoxic intracellular siRNA delivery vehicles. Pharm Res 29:362–374

Kumar R, Kulkarni A, Nagesha D, Sridhar S (2012) In vitro evaluation of theranostic polymeric micelles for imaging and drug delivery in cancer. Theranostics 2:714–722

Lee SY, Yang CY, Peng CL, Wei MF, Chen KC et al (2016) A theranostic micelleplex co-delivering SN-38 and VEGF siRNA for colorectal cancer therapy. Biomaterials 86:92–105

Li Y, Xu D, Ho SL, Li HW, Yang R, Wong MS (2016) A theranostic agent for in vivo near-infrared imaging of β-amyloid species and inhibition of β-amyloid aggregation. Biomaterials 94:84–92

Liu Y, Chen Z, Liu C, Yu D, Lu Z, Zhang N (2011) Gadolinium-loaded polymeric nanoparticles modified with Anti-VEGF as multifunctional MRI contrast agents for the diagnosis of liver cancer. Biomaterials 32:5167–5176

Liu Y, Li J, Liu F, Feng L, Yu D, Zhang N (2015) Theranostic polymeric micelles for the diagnosis and treatment of hepatocellular carcinoma. J Biomed Nanotechnol 11:613–622

Maeda H (2001) The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. Adv Enzym Regul 41:189–207

Mandal A, Bisht R, Rupenthal ID, Mitra AK (2017) Polymeric micelles for ocular drug delivery: from structural frameworks to recent preclinical studies. J Control Release 248:96–116

Matsumura Y, Maeda H (1986) A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res 46:6387–6392

Mi P, Wang F, Nishiyama N, Cabral H (2017) Molecular cancer imaging with polymeric nanoassemblies: from tumor detection to theranostics. Macromol Biosci 17

Mokhosi SR, Mdlalose W, Nhlapo A, Singh M (2022) Advances in the synthesis and application of magnetic ferrite nanoparticles for cancer therapy. Pharmaceutics 14

Movassaghian S, Merkel OM, Torchilin VP (2015) Applications of polymer micelles for imaging and drug delivery. Wiley interdisciplinary reviews. Nanomed Nanobiotechnol 7:691–707

Naqvi S, Maitra AN, Abdin MZ, Akmal M, Arora I, Samim M (2012) Calcium phosphate nanoparticle mediated genetic transformation in plants. J Mater Chem 22:3500–3507

Naqvi S, Panghal A, Flora SJS (2020) Nanotechnology: a promising approach for delivery of neuroprotective drugs. Front Neurosci 14:494

Naqvi S, Samim M, Abdin M, Ahmed FJ, Maitra A et al (2010) Concentration-dependent toxicity of iron oxide nanoparticles mediated by increased oxidative stress. Int J Nanomedicine 5:983–989

Oda CMR, Silva JO, Fernandes RS, Braga AV, Machado RR et al (2020) Encapsulating paclitaxel in polymeric nanomicelles increases antitumor activity and prevents peripheral neuropathy. Biomed Pharmacother 132:110864

Oerlemans C, Bult W, Bos M, Storm G, Nijsen JF, Hennink WE (2010) Polymeric micelles in anticancer therapy: targeting, imaging and triggered release. Pharm Res 27:2569–2589

Owen SC, Chan DPY, Shoichet MS (2012) Polymeric micelle stability. Nano Today 7:53–65

Peng C-L, Shih Y-H, Lee P-C, Hsieh TM-H, Luo T-Y, Shieh M-J (2011) Multimodal image-guided photothermal therapy mediated by 188Re-labeled micelles containing a cyanine-type photosensitizer. ACS Nano 5:5594–5607

Pradeep P, Kumar P, Choonara YE, Pillay V (2017) Targeted nanotechnologies for cancer intervention: a patent review (2010–2016). Expert Opin Ther Pat 27:1005–1019

Sawant R, Torchilin V (2010) Polymeric micelles: polyethylene glycol-phosphatidylethanolamine (PEG-PE)-based micelles as an example. Methods Mol Biol. (Clifton, N.J.) 624:131–149

Shan W, Zhu X, Liu M, Li L, Zhong J et al (2015) Overcoming the diffusion barrier of mucus and absorption barrier of epithelium by self-assembled nanoparticles for oral delivery of insulin. ACS Nano 9:2345–2356

Subramani K, Ahmed W (2012) Nanoparticulate drug delivery systems for oral cancer treatment. In: Emerging nanotechnologies in dentistry. Elsevier, pp 333–345

Sun X, Bandara N (2019) Applications of reverse micelles technique in food science: a comprehensive review. Trends Food Sci Technol 91:106–115

Thomas D, Lonappan L, Rajith L, Cyriac ST, Girish Kumar K (2013) Quantum Dots (QDs) based fluorescent sensor for the selective determination of nimesulide. J Fluoresc 23:473–478

Torchilin VP, Frank-Kamenetsky MD, Wolf GL (1999) CT visualization of blood pool in rats by using long-circulating, iodine-containing micelles. Acad Radiol 6:61–65

Tsai HC, Chang WH, Lo CL, Tsai CH, Chang CH et al (2010) Graft and diblock copolymer multifunctional micelles for cancer chemotherapy and imaging. Biomaterials 31:2293–2301

Turchi M, Karcz AP, Andersson MP (2022) First-principles prediction of critical micellar concentrations for ionic and nonionic surfactants. J Colloid Interface Sci 606:618–627

Upponi JR, Jerajani K, Nagesha DK, Kulkarni P, Sridhar S et al (2018) Polymeric micelles: theranostic co-delivery system for poorly water-soluble drugs and contrast agents. Biomaterials 170:26–36

Velikyan I (2013) Prospective of ^{68}Ga-radiopharmaceutical development. Theranostics 4:47–80

Wang Y, Liu Z, Li T, Chen L, Lyu J et al (2019) Enhanced therapeutic effect of RGD-modified polymeric micelles loaded with low-dose methotrexate and nimesulide on rheumatoid arthritis. 9:708

Xiao Y, Hong H, Javadi A, Engle JW, Xu W et al (2012) Multifunctional unimolecular micelles for cancer-targeted drug delivery and positron emission tomography imaging. Biomaterials 33:3071–3082

Xu H, Ma B, Jiang J, Xiao S, Peng R et al (2020) Integrated prodrug micelles with two-photon bioimaging and pH-triggered drug delivery for cancer theranostics. 7:171–180

Xu W, Ling P, Zhang T (2013) Polymeric micelles, a promising drug delivery system to enhance bioavailability of poorly water-soluble drugs. 2013

Yang H, Guo J, Tong R, Yang C, Chen JK (2018) pH-sensitive micelles based on star copolymer Ad-(PCL-b-PDEAEMA-b-PPEGMA)$_4$ for controlled drug delivery. Polymers 10

Yang T, Yang L, Zhang C, Wang Y, Ma X et al (2016) A copper–amyloid-β targeted fluorescent chelator as a potential theranostic agent for Alzheimer's disease. Inorg Chem Front 3:1572–1581

Yang Y, Wang S, Zhou Y, Wang X, Liu X et al (2020) Structurally accurate lipophilic Pt(1)Ag(28) nanoclusters based cancer theranostic micelles for dualtargeting/aggregation enhanced fluorescence imaging and photothermal/photodynamic therapies. Colloids Surf B: Biointerfaces 196:111346

Yi Y, Lin G, Chen S, Liu J, Zhang H, Mi P (2018) Polyester micelles for drug delivery and cancer theranostics: current achievements, progresses and future perspectives. Mater Sci Eng C 83:218–232

Yoo SP, Pineda F, Barrett JC, Poon C, Tirrell M, Chung EJ (2016) Gadolinium-functionalized peptide amphiphile micelles for multimodal imaging of atherosclerotic lesions. ACS Omega 1:996–1003

Zhang H, Mi P (2019) 12 - Polymeric micelles for tumor theranostics in theranostic bionanomaterials. In: Cui W, Zhao X (eds). Elsevier, pp 289–302

Zhang N, Wardwell PR, Bader RA (2013a) Polysaccharide-based micelles for drug delivery. Pharmaceutics 5:329–352

Zhang X, Tian Y, Li Z, Tian X, Sun H et al (2013b) Design and synthesis of curcumin analogues for in vivo fluorescence imaging and inhibiting copper-induced cross-linking of amyloid beta species in Alzheimer's disease. J Am Chem Soc 135:16397–16409

Zhu H, Cheng P, Chen P, Pu K (2018) Recent progress in the development of near-infrared organic photothermal and photodynamic nanotherapeutics. Biomater Sci 6:746–765

Zhuang W, Yang L, Ma B, Kong Q, Li G et al (2019) Multifunctional two-photon AIE luminogens for highly mitochondria-specific bioimaging and efficient photodynamic therapy. ACS Appl Mater Interfaces 11:20715–20724

Functionalized Nanocrystals and Theranostic Applications

14

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Abstract

Nanocrystals are pure drug crystals with sizes in the nanometer range. Due to the advantages of high drug loading, platform stability, and ease of scaling up, nanocrystals have been widely used to deliver poorly water-soluble drugs. They boost the drug's saturation solubility due to nanometer-sized particle size and improved dissolving rate due to larger surface area. Higher passive diffusion is produced by the increased concentration gradient between the epidermal membrane and the topically applied formulation due to the increased saturation solubility. Nanocrystal suspensions can be made using the most cost-effective and advantageous technique. In order to create capsules and tablets, the generated nanocrystal suspension can either be employed as a liquid dosage form or converted into dry solid powders. In the present review, we discuss several types of nanocrystals, preparation of nanocrystals, stability, functionalization of nanocrystals, theranostic applications of nanocrystals in various diseases, advantages of theranostic nanocrystals, and challenges and future goals

Keywords

Drug crystals · Stabilization · Surface area · Polymers · Surfactants · Functionalization

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14.1 Introduction

Despite having remarkable efficacy, nanocrystals were originally conceptualized in the nineteenth century, and their marketability is still constrained (Junyaprasert and Morakul 2015). In order to effectively transport the drug into or across the cells, nanocrystals of a parent drug with submicron particle size (100–1000 nm) are used (Müller et al. 2011; Chen et al. 2011a). They boost the drug's saturation solubility due to nanometer-sized particle size and improved dissolving rate due to larger surface area. Higher passive diffusion is produced by the increased concentration gradient between the epidermal membrane and the topically applied formulation due to the increased saturation solubility (Mishra et al. 2009; Al Shaal et al. 2011). Targeting of hair follicles was seen in particles of sizes in the nanometer range. Additionally, they showed higher cell surface or membrane adhesion, which can lengthen the period that a formulation remains in the skin (Müller et al. 2016). In the current medication development process, safe formulation, and pharmacoeconomic value, a growing number of poorly soluble pharmaceuticals have drawn attention as a problematic approach. Pharmaceutical firms may potentially profit by utilizing nanocrystal technology to redesign a product line for an already-existing formulation (Raghava Srivalli and Mishra 2014). The several methods used to create nanocrystals can be divided into bottom-up, top-down, and combination methods. According to the available literature, producing commercial nanocrystals does not frequently use the bottom-up technique. This approach comes with a number of issues, including the necessity to remove the solvent, difficulties with process optimization, and numerous medicines that are poorly soluble in both aqueous and organic mediums (Joshi et al. 2019; Liu et al. 2019).

The use of an organic solvent in precipitation procedures in bottom-up techniques, which must be entirely eliminated from the formulation and also results in the high manufacturing process, is their main drawback. Therefore, the creation of nanocrystals has not utilized the bottom-up method. An alternative method for producing nanocrystals is to use top-down technology (Junyaprasert and Morakul 2015). In the case of top-down technology, nanocrystals are made by the rapid wet media milling process. Nanocrystal suspensions can be made using the most cost-effective and advantageous technique. In order to create capsules and tablets, the generated nanocrystal suspension can either be employed as a liquid dosage form or converted into dry solid powders (Van Eerdenbrugh et al. 2009). Sols are typically used to obtain nanocrystals of different materials. Sols containing nanocrystals exhibit the same behaviour as traditional colloids. For instance, a dispersion's stability is influenced by the medium's ionic strength. Optical clarity is exceptionally good in nanocrystalline sols. The existence of a ligand shell, a layer of molecular species adsorbed on the surface of the particles, is a crucial component that contributes to the stability of nanocrystal sols. Without the ligand shell, the particles have a propensity to group together to create bulk species that settle or flocculate in the medium. The ligands contribute to particle stability in two distinct ways, depending on the dispersion medium. As a result, in an aqueous medium, coulomb interactions between charged ligand species create an electrical double layer, which

acts as a repulsive force to balance the attractive van der Waals attraction between the small grains. The requisite repulsive force is produced in an organic medium by the ligands' loss of conformational freedom and the apparent rise in solute concentration. Nanocrystals that are disseminated in liquids are either sterically or charge stabilized (Rao et al. 2007). In the drug development process, more than 40% of drug candidates have poor solubility, which results in poor and variable bioavailability (Ranjita 2013). Most medications have internal side effects due to their non-specific dispersion throughout the body, which further restricts their clinical usage (Trapani et al. 2012). Nanocarrier-based targeting methods are significant remedies for these issues. Selective delivery of poorly soluble medications to damaged tissues, organs, or cells has been successfully accomplished using nanocarriers, such as liposomes, nanoparticles, micelles, and nano-emulsions (Hollis et al. 2013a). However, the inherent disadvantages, such as unstable platforms, restricted drug loading, expensive production, scale-up challenges, and quality control challenges, limit the acceptability of these nanocarriers in clinic (Lu et al. 2015a). Only a few nanocarrier-based products, such as Doxils, Dauno Xomes, and Abraxanes, are commercially viable. The creation of nanocrystals came about as a result of many shortcomings in the targeted therapeutic delivery methods that were already in use. While pure drug crystals may occasionally be physically stabilized by surfactants and/or polymers (Keck and Muller 2006; Rabinow 2004; Xu et al. 2012), nanocrystals are drug crystals with particle sizes ranging from dozens to a few hundreds of nanometers. Absence of any carrier molecules provides a theoretical drug loading range of 50% to 90% (w/w) (Fuhrmann et al. 2013), resulting in therapeutic concentrations that are acceptable at modest doses (Muller et al. 2011). Excipients used for encapsulating and solubilizing medicines may no longer have any negative side effects. Most notably, the nanocrystal formulation generally avoids the physical instability problems associated with previous nanocarriers (Chen et al. 2011b; Junghanns and Muller 2008; Guo and Huang 2014). A dozen commercial products demonstrate the simplicity of scaling up for nanocrystals, and both top-down and bottom-up technologies have been well developed to create nanocrystals with required particle size and size distribution (Nagarwal et al. 2011).

14.2 Types of Nanocrystals

14.2.1 Chitin and Chitosan

The most prevalent amino polysaccharide polymer found in nature, chitin serves as the foundation for the exoskeletons of crustaceans, insects, and fungal cell walls. Chitin can be deacetylated chemically or enzymatically to produce its most well-known by-product, chitosan. Shrimp and crab shells, which are a plentiful by-product of the food processing industry and produce significant amounts of this biopolymer for use in biomedical applications, are the primary natural sources of chitin. Chitin production and breakdown in living chitin-synthesizing organisms must be strictly regulated enzymatically in order to preserve equilibrium. The key

enzyme in the chitin production process, chitin synthase, creates the chitin polymer using UDP-N-Acetylglucosamine (UDPGlcNAc), whereas chitinase enzymes break down chitin. The primary natural mediators of chitin breakdown are thought to be bacteria. Due to their distinct biochemical characteristics, such as biocompatibility, biodegradability, nontoxicity, and the capacity to form films, chitin and chitosan have found a wide range of interesting biomedical applications. In its most recent developments, nanotechnology has also increasingly used chitin- and chitosan-based products. A lot of chitin and chitosan have been used to create polymer scaffolds. Additionally, there is growing interest in using chitosan to create tailored nanocarriers and enable microencapsulation techniques for the delivery of pharmaceuticals, biologics, and vaccines. It is expected that each application will require specially created chitosan-based nano- and microparticles with certain size and cargo-release properties. Chitosan nano−/microparticles with high loading efficiencies can encapsulate protein cargos; however their consistent production is still difficult to achieve. With varying degrees of deacetylation, chitosan can be successfully employed in solutions, as hydrogels, nano- or microparticles, and to create an infinite variety of derivatives with specific biological capabilities (Elieh-Ali-Komi and Hamblin 2016).

14.2.2 Quantum Dot

Colloidal fluorescent semiconductor nanocrystals known as quantum dots are roughly spherical; have distinctive optical, electronic, and photophysical properties; and have exciting potential for use in biological labelling, imaging, and detection as well as effective donors of fluorescence resonance energy (William et al. 2006). The most often used quantum dots are made of a mixture of II-VI elements, specifically CdS and CdSe. Along with oxides, halides, and tellurides, additional sulphides and selenides have also been described (Wang and Herron 1991; Murray et al. 1993). It has also been used to combine III-V elements (InP and InAs) (Mićić and Nozik 1996; Pötschke et al. 2004).

A quantum dot has a diameter that ranges from 2 to 10 nm. Fluorescence cannot be seen after the quantum confinement effect is lost above this scale. Quantum dots have higher quantum yields and are brighter than typical small molecule luminophores. Additionally, because quantum dots of various compositions exhibit emission spectra with distinctive nuances (Bailey and Nie 2003), taking use of these properties would make it possible to do multiplexed assays like those suggested by Soman and Giorgio (Soman and Giorgio 2009). According to Chan and Nie (Chan and Nie 1998), a single crystal of 5 nm in diameter may function as a solid support for two to five molecules since a quantum dot surface can be activated with a wide variety of reactive moieties. Quantum dots are excellent contrast agents for imaging and labels for bioassays due to all of these features. Recently, several reviews on the creation, characteristics, and uses of QDs have been published (Mazumder et al. 2009; Jamieson et al. 2007; Huo 2007).

14.2.3 Colloidal Nanocrystals

Colloidal nanocrystals (or crystalline nanoparticles) have emerged as a significant class of materials with a wide range of potential uses, from electronics and optoelectronics to medicine. The enormous advancements in nanocrystal synthesis have led to the current substantial research emphasis on these compounds. For a wide variety of inorganic compounds, it is now possible to achieve impressively small size distributions of just a few percent, rational shape-engineering, compositional modification, electrical doping, and customized surface chemistries. Inorganic nanocrystal-based photovoltaic and light-emitting devices can now compete in performance with other cutting-edge materials (Kovalenko et al. 2015).

14.2.4 Cellulose Nanocrystal and Nanofibres

Two major categories can be used to classify nanocellulose: cellulose nanocrystals (CNCs) and cellulose nanofibres (CNFs). Based on its characteristics, sizes, techniques of extraction, and uses, nanocellulose was categorized. The cellulosic sources and the processing conditions have a significant impact on the CNMs' properties. Although the physical properties of the mechanically extracted CNFs and acid hydrolysed CNCs are different, their chemical properties are similar (Li et al. 2019a). CNFs are typically extracted from cellulose fibres using a mechanical technique, whereas CNCs are often obtained by a chemical acid hydrolysis procedure.

The CNFs are typically produced mechanically from the natural fibres that have already been treated. In this procedure, a greater shear stress is produced, which causes CNFs to be extracted from the pretreatment fibres in a longitudinal orientation. However, this manufacturing technique typically involves a high energy consumption, which results in the delamination of the fibre. Up to 98% less energy is used during the chemical preparation procedure (Li et al. 2019a). This section has a detailed discussion of the advantages and disadvantages of the most popular mechanical methods for the extraction of CNFs.

14.3 Preparation of Nanocrystals

14.3.1 Top-Down Techniques

The top-down procedures utilize high-energy mechanical forces, which are used to comminute big crystals either through medium milling (MM) (nanocrystals) or high-pressure homogenization (HPH) (IDD-Ps, DissoCubess, and Nanopures) (Shegokar and Müller 2010; Merisko-Liversidge et al. 2003). The top-down method's major benefit is that it is a universal method for creating crystalline nanoparticles (Rabinow 2004) and is adaptable in terms of production scale (Müller et al. 2001). As a result, the method has been widely used to create commercial nanocrystals. With the

exception of Triglides, almost all commercial items were created by nanocrystals. High energy and time requirements, as well as contamination from the grinding media, are drawbacks of this technology. For instance, even at high pressures of up to 1700 bar, it takes 50–100 cycles of homogenization to produce the correct particle size and size distribution (Keck and Muller 2006; Junghanns and Muller 2008). Similarly, milling times range from a few hours to several days, depending on the characteristics of the medication, the milling medium, and the degree of particle size reduction (Gao et al. 2008; Peltonen and Hirvonen 2010). The top-down method may not be the best option for preparing nanocrystals for intravenous injection since contamination from the grinding media causes unanticipated side effects.

14.3.1.1 Media Milling (Nanocrystals)

The media mill's main parts are a milling chamber, motor, recirculating chamber, coolant, and milling material. During the procedure, a crude slurry including the medication, water, and stabilizers is supplied into the milling chamber and stirred by the motor. The milling chamber is typically 2–30% (w/v) filled with slurry, with the milling medium occupying the remaining 10–50% (w/v) of the slurry. In order to lower the particle size during agitation, the milling medium rotates inside the chamber, creating high energy forces by shearing and hitting with the medicines. Depending on the scale, the operation can be carried out either in batch (discontinuous mode) or recirculation (continuous mode). Recirculation is beneficial for speeding up milling and reducing particle size. Depending on the scale, the operation can be carried out either in batch (discontinuous mode) or recirculation (continuous mode). Recirculation is beneficial for speeding up milling and reducing particle size. If recirculation mode is used, media separators can keep the milling medium in the chamber. Due to the tremendous energy produced during milling and long-term operation, the thermogenesis is intense, raising stability difficulties. As a result, the coolant is required to regulate the temperature when milling (Lu et al. 2016).

14.3.1.2 High-Pressure Homogenization (IDD-Ps, DissoCubess, and Nanopure)

Drug suspensions are delivered into a high-pressure homogenizer during the HPH process, where they are compelled to transit through an extremely short homogenization channel in a quick burst under high pressure. Drug particle fracture is caused by cavitation, high-shear forces, and particle collisions. The process typically consists of three steps: (1) dispersing the raw drug powders in a pure solution or a solution containing a stabilizer; (2) reducing the particle size by high-speed homogenization or shearing under low pressures; and (3) applying high-pressure homogenization to produce the desired particle size and size distribution. HPH can be further separated into three patented technologies based on the tools and substance used: the microfluidizer for IDD-Ps technology, the piston gap homogenizer for DissoCubess (water), and Nanopures (non-aqueous media) (Lu et al. 2016).

14.3.2 Bottom-up Techniques

The bottom-up method, which involves the two key phases of nucleation and subsequent crystal formation, produces nanocrystals from solutions. In contrast, nucleation is crucial for producing uniformly tiny nanocrystals. With a higher nucleation rate, more nuclei are produced from the supersaturated solution, which results in less supersaturation. As a result, it is possible to forecast that each nucleus will develop less in the long run (Hollis and Li 2010). Additionally, an arrow particle size distribution is obtained (Hollis and Li 2010) if several nuclei are formed simultaneously in the nucleation stage. Thus, it is crucial to encourage quick and uniform nucleation during the bottom-up process. Either combining with an antisolvent or removing the solvent can cause nucleation to occur (Hollis and Li 2010; de Waard et al. 2011). Common mixing tools, such as magnetic stirring and an agitator blade (Xia et al. 2014), are typically used to combine medication solution and antisolvent. Sonication can be used to create cavitation effects in order to promote the nucleation (Dalvi and Yadav 2015). It is known as sonoprecipitation. Nanocrystal preparation has also been carried out using confined impinging jet reactors (D'Addio and Prud'homme 2011), multiple inlet vortex mixers (Liu et al. 2008), and static mixers (Alvarez and Myerson 2010). With these tools, the two fluids are thoroughly mixed at the microscopic level in a matter of milliseconds. Even before nucleation begins, a homogenous solution with high supersaturation may be attained, promoting tiny nanocrystals with narrow size dispersion. Common techniques for removing solvent include freeze-drying and spray-drying. Additionally, controlled crystallization during freeze-drying techniques and spray-freezing into liquid have recently been developed to prepare nanocrystals by removing solvent. By utilizing the special physical characteristics of supercritical fluid (SCF), which combine diffusivity like a gas and solubilization like a liquid, SCF can be used to create nanocrystals. Additionally, the precipitation of nanoparticles can be substantially facilitated by quick and simple removal of SCF without extensive drying. The most preferred SCF is supercritical carbon dioxide (SCO2) because of its mild critical point (31 1C and 73.8 bar) and minimal environmental impact. SCO2 can be rapidly expanded from a medication solution, or it can be precipitated using SCO2 as an antisolvent, depending on how well a molecule dissolves in SCO2. Nanocrystals are a category of solid dosage forms that take advantage of the drug's crystal structure and the nanoscience idea to improve solubility, dissolution, and physicochemical qualities. When compared to other solid dosage forms, nanocrystal frequently presents a lot of difficulties in terms of chemical and physical stability during production and storage. As a result, crucial steps in the formation of nanocrystals include their physicochemical characteristics, hazardous effects on humans, and use in medication delivery via diverse routes of administration. To guarantee solid state consistency in the nanocrystals and its effect on therapeutic effectiveness, a variety of approaches are used (Lu et al. 2016).

14.4 Stability

Due to the lack of particle aggregation and the Ostwald ripening process, the nanocrystal suspension was shown to be stable (Im et al. 2020). A suitable stabilizer can be added, as well as other stabilizers, surfactants, and amphiphilic copolymers, to increase stability (Muller and Jacobs 2002a; Muller and Jacobs 2002b). The surfactants soon disperse, adhere to the crystal's surface, and stabilize the system by forming both a static and an electrostatic barrier between the crystals as homogenization progresses (Lee et al. 2005). For the purpose of stabilizing nanocrystals, the surfactant must meet certain requirements regarding its affinity for the particle surface (Kipp 2004). The second need is that a surfactant produces an adequate high diffusion rate during homogenization. In order to maintain the necessary steric or electronic repulsion between the particles, the stabilizer content should be sufficient to completely cover the particle surface. However, more stabilizers do not necessarily imply a better outcome (Mantzaris 2005).

14.4.1 Functionalization of Nanocrystals

The size range of nanocrystals is comparable to that of biomolecular and cellular systems, and this is coupled with their unique physical features. With these characteristics, these materials are very desirable for use in therapeutic and diagnostic scenarios. However, regulated interactions with biomacromolecules are necessary for these materials to be useful in biomedicine. Enhanced cellular internalization ability, non-cytotoxicity, and better payload binding capacity are all characteristics of well-designed nanocrystal monolayer architectures that are essential for efficient intracellular delivery. Surface functionality may also be modified to offer the selective or specific identification necessary for biosensing. Non-interacting "stealth" qualities, which inhibit immune system capture of particles, demonstrate that non-interaction itself may be a significant quality.

Customizing particle interfaces is difficult, but chemists have a wide array of tools at their disposal to synthesize useful materials. Small molecules, surfactants, dendrimers, polymers, and biomolecules are only some of the ligands that have been used in various methods to functionalize nanocrystals. Multiple medicinal medications or bio-macromolecules may be included by covalent or non-covalent conjugation using the various multivalent surface structures created using small molecules and polymeric ligands. Nanocrystals with attached biomolecules may also provide their host with desirable characteristics, such as enhanced specific recognition or biocompatibility. Due to the simplicity of such functionalization, chemists may easily produce the needed functionalities for their use in clinical applications.

Functional cellulose nanocrystals were generated by Boujemaoui et al. by combining acid hydrolysis and Fischer esterification with different organic acids. ATRP initiator, allyl, alkyne, and thiol functional groups were integrated as functionalities (Boujemaoui et al. 2015). Recent research has shown that elongated nanoparticles offer specific benefits over their spherical equivalents in drug delivery applications. Guo et al. developed functionalized cellulose nanocrystals with PEG-metal-chelating block copolymers in aqueous media via controlled conjugation. mPEG-PGlu (DPTA)18-HyNic and PEG-PGlu (DPTA)25-HyNic are coupled to CNCs for the first time to allow the chelation of radionuclides for diagnostic and therapeutic purposes. Preliminary testing on a human ovarian cancer cell line (HEYA8) revealed that these CNCs are nontoxic and that their penetration characteristics may be easily evaluated in multicellular tumour spheroids (MCTSs) using optical imaging (Guo et al. 2016). Pinheiro et al. created PBAT nanocomposites enhanced with nanocrystals containing functionalized cellulose. Functionalized cellulose nanocrystals (CNC) offer greater temperature resistance and enhance dispersion and polymer matrix interactions. These effective dispersion and interactions result in nanocomposites with enhanced characteristics (Pinheiro et al. 2017). Qiao et al. developed a new carboxylate-functionalized adsorbent (CNM) based on cellulose nanocrystals (CNC) to study the adsorptive removal of various cationic dyes (such as crystal violet, methylene blue, malachite green, and basic fuchsin). CNM demonstrated extensive adsorption capabilities for cationic dyes, as well as a quick adsorption rate and effective adsorption capacities for crystal violet. At least four times, CNM could be regenerated and employed for crystal violet adsorption (Qiao et al. 2015).

It is well recognized that the poor mechanical and thermal characteristics of biodegradable polymers (poly(3-hydroxybutyrate-co-3-hydroxyvalerate), polylactic acid, and polycaprolactone) limit their extensive usage as environmentally friendly and biomedical products. Since a result, intensive efforts have been undertaken to produce innovative green materials used to make on biodegradable polymers and renewable polysaccharide materials (cellulose, starch, and chitin), as these nanocomposites might replace certain petrochemical polymers and combat "white pollution". Particularly, cellulose nanocrystals (CNCs) derived from natural cellulose sources may serve as reinforcing agents for several biodegradable polymeric matrices. Functionalized cellulose nanocrystals (PHCNs) were created by Yu et al. by grafting poly (3 hydroxybutyrate-co-3 hydroxyvalerate) (PHBV) onto cellulose nanocrystals (CNCs). The nanocomposites demonstrated a larger window for melt processing than PHBV alone. In addition, the crystallinity and hydrophilic characteristics of the nanocomposites may be modified by varying the PHCN concentration. In addition, human MG-63 cells were not harmed by the nanocomposites. These high-performance bio-nanocomposites have the potential to increase the use of CNCs derived from natural resources and their practical use as PHBV-based bioplastics and biomedical materials (Yu et al. 2014).

14.5 Theranostic Applications of Nanocrystals in Various Diseases.

14.5.1 Cancer

According to 2008 research from the International Agency for Research on Cancer, incidence rates have been quadrupled since 1975, leading to an estimated 13–17 million cancer deaths globally by 2030. While novel medicines are being developed and approved for many forms of cancer, the primary method of preventing cancer mortality is by early identification, detection, and treatment of malignant cellular proliferation. Fluorescence imaging and magnetic resonance imaging (MRI) are two emerging nanoimaging techniques that may one day allow for the early detection and diagnosis of cancer, something that is presently impossible with traditional imaging methods. The unique features of nanocrystals result in their theranostic potential, which enables disease diagnosis and therapy simultaneously (Fig. 14.1) (Lu et al. 2015b).

Radiation and chemotherapy are currently used to treat cancer – however, their treatments are inaccurate, nonspecific, and often given at excessive doses, causing significant side effects that are detrimental to the patient's well-being. There is now a wide range of cancer-targeted nano-therapy systems designed to tackle the drawbacks of conventional treatments. These systems combine therapy, imaging, and tumour targeting capabilities in one platform.

Magnetic resonance imaging (MRI) is among some of the imaging technologies used in cancer treatment since this technique provides superior imaging of deep tissues and is combined with certain other imaging techniques to provide additional diagnostic data for identifying tumours accurately and guiding cancer therapy. A broad variety of medical imaging approaches may benefit from the use of inorganic

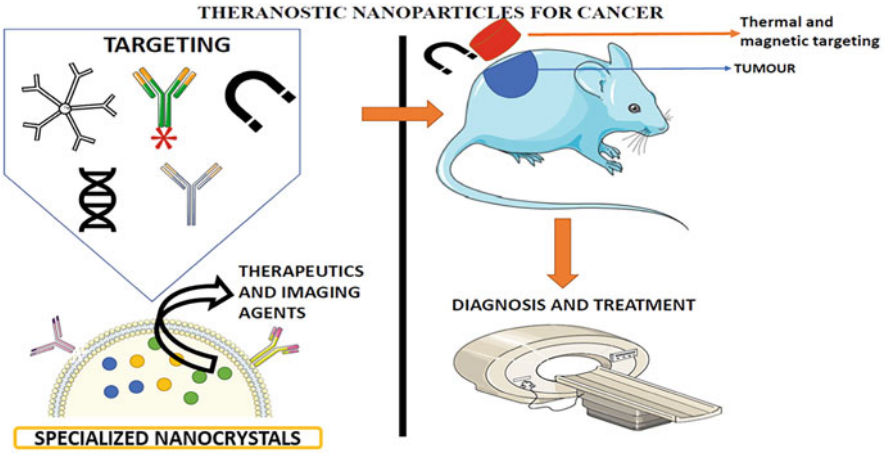

Fig. 14.1 Theranostic nanocrystals for cancer

nanocrystals, including drug or gene delivery complexes, therapeutic hyperthermia agents, and diagnostic systems. Nanocrystals may either create their own contrast, such as iron oxides, or gold nanoparticles covered with gadolinium chelates. It is possible to employ these MR-active nanocrystals for a variety of imaging applications, including the imaging of blood vessels, the liver, the brain, and other organs. Nanocrystals with water-soluble and biocompatible properties are consequently widely sought after because of their many uses (Cormode et al. 2013). Hollis et al. created unique hybrid paclitaxel (PTX) nanocrystals with bioactivatable (MMPSense® 750 FAST) plus near-infrared region (Flamma Fluor® FPR-648) fluorophores that were tested for anticancer effectiveness and diagnostic characteristics in a mouse breast cancer xenograft model. The anticancer activity of hybrid nanocrystals was shown, as well as the possibility of multi-modular biological imaging for diagnostic investigations. Furthermore, this nanocrystal formulation was shown to be equally effective as Taxol® but with lower toxicity when injected (Hollis et al. 2013b). Recently Hollis et al. also prepared paclitaxel (PTX) nanocrystals (200 nm) by crystallization. A near-infrared fluorescent dye was physically incorporated into the crystal lattice to make PTX nanocrystals tagged with tritium. A 20 mg/kg intravenous dosage was shown to accumulate in the tumour at a rate of less than 1%. According to the results of a survival trial, the nanocrystal treatments had equivalent anticancer efficacy to that of the standard solubilization formulation (Taxol®) (Hollis et al. 2013c). Fluorescent optical probes based on near-infrared quantum dots have received a lot of interest recently. Water-dispersible, highly luminous AgInS2 nanocrystals were successfully fabricated by Liu et al. as near-infrared probes for tumour targeting and imaging in vivo. An emission in the near-infrared band with a QY of up to 35% was achieved using this micelle-capsulated nanocrystal formulation. Liu and colleagues were able to passively administer AgInS2 NCs formulation to the tumour location by using an optical imaging equipment that allowed them to see the whole body of the tiny animal subjects being studied. The ultra-small crystal size, near-infrared emission luminescence, and high quantum yield of AgInS2 NCs make them a promising option for use as a biological contrast agent for tumour sensing and imaging (Liu et al. 2013).

Cancer patients may also benefit from heating tumours as a treatment method. There has been a resurgence of interest in thermal therapy in recent decades due to several scientific papers showing significant improvements in cancer therapies, a deeper understanding of how temperatures cause cell death, and the development of new technologies for localized and controlled heating of tumours. Traditional thermal treatments have focused on whole-body warming, but tailored thermal techniques may provide precise thermal therapy at the cellular or tissue level. Cancer theranostics may be made more efficient and precise by combining MRI with photothermal treatment (PTT) (Chu et al. 2018). For example, to establish an effective MNC (magnetic nanocrystal)-mediated theranostic technique, the work of Xie et al. has focused on developing a combination of simultaneous diagnostics and heating therapy of tumours utilizing MRI and alternating current magnetic field (ACMF). They synthesized PEG-phospholipid-coated Mn–Zn ferrite MNCs which were able to achieve a magnetism coefficient (r2) of 338 mM^{-1} s^{-1} and specific

absorption rate (SAR) of 324 W g^{-1} Fe, respectively. It has been shown that they significantly reduce macrophage phagocytosis while simultaneously enhancing their biocompatibility in vitro. Because of their increased permeability and retention (EPR) properties, the passive targeting of tumours and magnetically induced heating of tumours was made possible. The tumour surface could be heated to roughly 43 °C in 30 minutes with an ACMF of 12 A at 390 kHz based on intravenous injections of MNCs. Long-term hyperthermia may efficiently cause tumour cell death, block tumour angiogenesis, and eventually reduce tumour development within a certain time frame (Xie et al. 2014). Liu et al. presented an effective synergistic treatment for tumours. They effectively generated tumour neovascular-targeted Mn–Zn ferrite MNCs (magnetic nanocrystals) that contained paclitaxel (PTX) inside a biocompatible PEG-phospholipid (DSPE-PEG2000) film and interface, connected with a tripeptide of arginine-glycine-aspartic acid (RGD). The high-performance modified nanocrystals exhibited exceptional magnetic features, such as magnetic resonance imaging (MRI) with great contrast and outstanding magnetically induced heat generating capability. MNCs-PTX@RGD were capable of efficiently penetrating tumour tissue from tumour-fenestrated vascular networks in order to acquire a sufficient temperature (about 43 °C) when subjected to an alternate current magnetic field (ACMF, 2.58 kA m-1, 390 kHz), resulting in a successful TMH effect (Liu et al. 2021).

Developing gene therapies for a number of illnesses, including cancer, has sparked tremendous interest. However, the effectiveness of nucleic acid transport to tumour cells and in vivo efficacy are challenging to predict. These nanoparticles may be employed for gene transfer by combining anticancer therapy with imaging or diagnostic skills, to monitor the efficacy and activity of therapeutic substances in tumours (Lu 2014). To deliver siRNAs to human neural cells for gene therapy, Law et al. used quantum rod (QR)-based formulations. Imaging and gene delivery were integrated into a single nanoparticle technology that showed minimal toxicity, high transfection efficiency, and great biocompatibility (Law et al. 2012).

The pH differences between the tumour microenvironment (pH 6–7), endosomes (pH 5–6), and lysosomes (pH 4–5) have also been used to deliver chemotherapy to tumours. Several strategies have been proposed, including the utilization of ionizable chemical groups (e.g., carboxylic acids and tertiary amines), acid-labile chemical bonds (e.g., acetal and acyl hydrazine linkages), anionic and cationic pH-responsive polymers, and pH-sensitive peptides (e.g., GALA peptide). Zhou et al. created DOX-encapsulated PLGA with Ce6 (PDT and fluorescence imaging), Gd-DTPA (T1 MRI tracking), and folate (targeting) as a tumour-targeted, charge-switchable nanomaterial with multistage pH-sensitive behaviour for combined chemotherapy-PDT of cancer. At pH 6.5, the nanomaterial's charge reverses to positive without releasing DOX or Ce6, and it then progressively releases DOX into the nucleus of MGC-803 cancer cells through the endolysosome (pH 4.5–5.5). This multistage pH-sensitive characteristic resulted in increased tumour penetration (as measured by MRI and NIRF imaging), a prolonged retention duration, and good chemotherapy-PDT synergistic anti-tumour activity against MGC-803 tumours in mice, with total tumour eradication reported after 15 days (Wang et al. 2017; Chen et al. 2017; Zhou

et al. 2018). Liu et al. designed ultra-small pH-reactive Nd-doped NaDyF4 nanoagents for enhanced cancer theranostics through in situ aggregation. NaDyF4: 10% Nd was further combined with GA-Fe in consideration of its theranostic capabilities. The resulting NaDyF4:10% Nd-Fe-GA demonstrated a high R^2, significant NIR II DCL, remarkable photothermal conversion efficiency (60.12%), and no detectable toxicity. In addition, the efficient pH-responsibility features cause nanoagents to assemble in acidic conditions. Furthermore, both in vitro and in vivo investigations indicated improved contrast in tumour regions, indicating that ultra-small NaDyF4:10% Nd-Fe-GA has the potential to be used as a pH-responsive in situ aggregation-based cancer theranostic.

14.5.2 Cardiovascular Disease

Cardiovascular disease (CVD) is a term that refers to a collection of disorders that affect the blood vessels and the heart. It is a leading cause of morbidity and mortality worldwide. Despite great advancements in research on CVDs, they continue to be the major cause of mortality globally. As healthcare interventions, a range of techniques have been used. As medical technologies advance, more concise and targeted strategies are required to further enhance treatment outcomes. Among them, theranostic materials are a relatively new and promising technique that relates to the coupling of therapeutic elements with imaging agents, allowing for early identification and treatment of disease (Fig. 14.2). Additionally, since theranostic agents are capable of tracking medications and monitoring treatment, they provide a very efficient method for focused, safe pharmacotherapy that is patient centred. Theranostics is a modern discipline of medicine in which specialized targeted medication is provided in response to particular targeted diagnostic tests. Although theranostic research on CVDs is still in its infancy, theranostic nanoparticles have grown in popularity over the past decade owing to the benefits of integrating detection and therapy in a single agent. There are a few studies describing the emergence of the diagnostic or therapeutic nanoparticles (Organization WH 2020; Krumholz et al. 2005; Mendis et al. 2011).

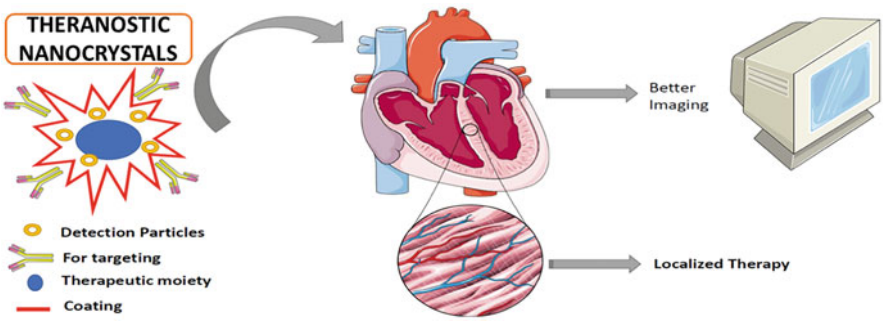

Fig. 14.2 Theranostic nanocrystals for cardiovascular disease

Limited and regulated drug delivery to the heart still remains a concern giving numerous off-target events along with limited accumulation of pharmaceuticals in the heart. There is a need to create and enhance technologies to enable for better creation of pharmaceutical candidates for management of cardiac disorders. Over the past decade, innovative pharmacological platforms and nanomaterials were devised to localize bioactive molecules to the heart. Yet, the research continues in its inception, not only in the creation of instruments but also in the knowledge of impacts of these materials on heart function and tissue viability. Upconverting nanocrystals are nanomaterials that lately stimulated interest in theranostic nanomedicine technology. Their unique photophysical features enable for sensitive in vivo imaging that may be paired with spatio-temporal control for selective release of encapsulated medicines. Recent research by Kermorgant et al. on the potential upconverting NaYF4:Yb, Tm nanocrystals demonstrates for the first time their safety when injected into the myocardial or pericardial region of mice. The accumulation and upconversion of nanoparticles in the cardiac zone did not affect heart rate variability or cardiac function even 15 days after a single injection. Overall, these nanoparticles demonstrate nontoxicity in the pericardial area and are safe for regulated spatiotemporal drug delivery. These findings suggested the use of upconverting nanocrystals as prospective theranostic instruments capable of overcoming a number of the most significant constraints of traditional experimental cardiology techniques (Kermorgant et al. 2019). For the evaluation and diagnosis of pressure-related disorders, Li et al. manufactured implantable pressure biosensors. This approach was used to create core/shell polyvinylidene difluoride (PVDF)/hydroxylamine hydrochloride (HHE) organic piezoelectric nanofibres with well-controlled and self-orientated nanocrystals in the spatial uniaxial orientation (SUO) of β-phase-rich fibres. Piezoelectric efficiency, wear resistance, consistency, and biocompatibility were all much improved by these nanocrystals. Li and colleagues implanted PVDF/HHE OPNs sensors into the pig's heart, which demonstrated ultrahigh detecting precision and accuracy to record micropressure variations at the exterior cardiovascular walls, cardiovascular flexibility, and the occurrence of atrioventricular cardiovascular blockage as well as thrombus formation (Li et al. 2019b).

14.5.2.1 Atherosclerosis

Atherosclerosis is a deadly disease that damages the heart and brain among other vital organs. The endothelium and smooth muscle of the arterial wall are damaged when noxious substances associated with factors such as high levels of cholesterol, high blood pressure, diabetes, smoking, and homocysteinaemia interfere with the homeostatic state of the artery wall. We term this disease process atherosclerosis because of the excessive inflammatory, fibroproliferative, and defensive response that ensues as a consequence (atherosclerosis) (Ross 1995).

Heart and brain problems caused by atherosclerosis are very harmful to human health. However, nano-photothermal technologies has already been proved to reduce vascular inflammation, but existing photothermal agents are ineffective in monitoring the disease's progression. For the treatment of arterial inflammation, Lu et al.

devised and manufactured a highly effective bifunctional nanoplatform. A straightforward hydrothermal process was used to create Cu3BiS3 nanocrystals with a diameter of around 12 nm. It was found that the Cu3BiS3 nanocrystals displayed an incredible photothermal effect because of their strong NIR absorption. Atherosclerosis can be prevented by using Cu3BiS3 nanocrystals to kill macrophages in vitro and in vivo, which play a critical role in the development of the atherosclerosis. Furthermore, Cu3BiS3 nanocrystals may be employed as a CT contrast agent for the detection of inflammation of the carotid arteries (Lu et al. 2020). Furthermore, gold, iron oxide, or quantum dot nanocrystals were used in the development of multimodality HDL-mimicking nanoparticles by Cormode et al. for computed tomography, magnetic resonance, and fluorescence imaging, respectively. Multimodal characteristics were obtained by including gadolinium and rhodamine in the nanosystem. Macrophages were able to effectively ingest the HDL-like nanoparticles confirmed utilizing confocal microscopy (CM), TEM, CT, T1-weighted MRI, T2-weighted MRI, and fluorescence imaging. Multimodality scanning of atherosclerosis utilizing targeted HDL nanocrystals was also performed in vivo (Cormode et al. 2008).

14.5.2.2 Thrombosis

Haemostasis is the process that preserves the viability of a sealed, high-pressure circulatory system following vascular injury. Vessel wall damage and the efflux of blood from the circulation swiftly trigger actions in the vessel wall and in blood that close the gap. Circulating platelets are drawn to the site of injury, where they constitute a key component of the growing thrombus; blood coagulation, triggered by tissue factor, culminates in the synthesis of thrombin and fibrin (Shibata et al. 1979).

Ma et al. investigated the viability and effectiveness of target thrombolysis using recombinant tissue plasminogen activator (rtPA) covalently linked to a magnetic nanoparticle (MNP) and held in vivo at the target location by an external magnet. They produced and studied polyacrylic acid-coated magnetite (PAA–MNP, 246 nm); rtPA was bound to PAA–MNP through carbodiimide-mediated amide bond formation. In conclusion, covalent immobilization of rtPA to PAA–MNP resulted in a stable rtPA formulation and a predictable quantity of rtPA in the vicinity of the target site under magnetic direction; this strategy may result in repeatable and effective target thrombolysis with more than 20% of a standard dosage of rtPA (Ma et al. 2009). Recently, Jung et al. designed thrombus-specific theranostic (T-FBM) nanoparticles that might be used as an antithrombotic nanomedicine by amplifying photoacoustic signals caused by H2O2. These T-FBM nanoparticles were engineered to specifically target fibrin-rich thrombi and were triggered with H2O2 to create CO2 bubbles that amplified the photoacoustic output. Inside endothelial cells, these T-FBM nanoparticles also exhibited antioxidant, anti-inflammatory, and antiplatelet activity when they are activated with H2O2. Also, these T-FBM nanoparticles greatly increased photoacoustic contrast in thrombosed arteries and significantly inhibited thrombus formation in animal models of carotid artery damage. Based on their research findings, T-FBM nanoparticles will have

significant translational potential as nanotheranostics treating cardiovascular disorders connected with H2O2 (Jung et al. 2018).

Nanocrystals of EP-2104R, a fibrin-targeting Gd-based MR CA with six amino acid cyclic polypeptide linked to four Gd (DOTA) chelates, were produced by Uppal et al. Dual PET-MR probe EP-2104R was partly depleted of Gd3+ and subsequently chelated with 64Cu to produce a fibrin-targeting dual PET-MR probe enabling concurrent PET and MR scanning of thrombus. By using combined PET and MR imaging, we could clearly identify the thrombosis in a rat artery thrombus model (Uppal et al. 2012; Yang et al. 2018).

14.5.2.3 Myocardial Infarction

Myocardial infarction (MI) is a kind of heart muscle damage caused by a restriction of the heart's blood supply, most often owing to thrombosis. Myocardial infarction (MI) may be identified clinically by electrocardiographic (ECG) abnormalities, high levels of biochemical markers (biomarkers) indicating myocardial necrosis, and imaging, or it can be characterized pathologically. MI may develop as the initial symptom of coronary artery disease (CAD), or it might recur in people with preexisting disease. Between 60% and 80% of MI induced by thrombosis are initiated by the fracture of susceptible atherosclerotic plaques. The remaining 20%–40% is due to intimal surface erosion. MI may result in major complications such as arrhythmia, heart failure, cardiogenic shock, or cardiac arrest, resulting in increased disability and mortality worldwide (Zia et al. 2020; Ta et al. 2017; Thygesen et al. 2012).

TNF-α, a pro-inflammatory cytokine, is thought to be involved in irreparable cardiac damage at the outset of MI. TNF-α-mediated MI is caused by a number of mechanisms, including the stimulation of proinflammatory cytokine networks and the generation of RNS and ROS. As a result, efficient TNF-α inhibition in the course of inflammatory phase seems to be critical for MI therapy (Bozkurt et al. 1998; Kleinbongard et al. 2011; Pagani et al. 1992). Somasuntharam et al. employed gold nanoparticles (AuNPs) functionalized with deoxyribozyme (DNAzyme) to catalytically suppress tumour necrosis factor-α (TNF-α) in vivo as a possible treatment for myocardial infarction in this work (MI). They achieved 50% of TNF-α silencing utilizing primary macrophages as a model, which was not possible using lipofectamine-based techniques. Local injection of DNAzyme coupled to gold nanoparticles (AuNPs) into the rat myocardium inhibited TNF-α by 50%, resulting in considerable anti-inflammatory actions and improved acute cardiovascular output after MI (Somasuntharam et al. 2016).

Ischemic heart disease is the initial stage of myocardial infarction (MI). The atherosclerotic plaque inside the coronary artery causes stenosis resulting in inadequate blood flow and decreased supply of blood to the myocardium. This plaque may also break inside the vessels as the condition advances, causing thrombosis and finally blockage. Therefore, treatment of ischemic heart illness in its early stages may be an effective technique for the treatment of myocardial infarction (Anderson et al. 2017; Manfroi et al. 2002; Nabel and Braunwald 2012). Feiner et al. revealed a synthetic cardiac patch that merges cardiac cells with elastic, independent electronics

and a three-dimensional nanocomposite framework to provide a more effective treatment for heart failure. In addition to exhibiting strong electronic features, the patch was also capable of monitoring cellular electrical activity and providing on-demand electrical stimulation to synchronize cell contraction. Moreover, they have shown that electroactive polymers comprising biological components may be coated on specific electrodes in order to deliver medications into the patch microenvironment upon demand (Feiner et al. 2016).

14.5.2.4 Vascular Injury and Restenosis

Vascular damage produced by stenting and restenosis may result in a range of cardiovascular problems, including thrombosis, myocardial infarction, and ischemic heart disease if not treated promptly.

In the study done by Lanza and fellow investigators, they have incorporated paclitaxel or doxorubicin into perfluorocarbon nanoparticle which was attached to the anti-recombinant porcine tissue factor to target smooth muscle cells. The nanosystem developed provides unique and quantitative drug delivery system through MRI (Lanza et al. 2002). Cyrus et al. have also prepared perfluorocarbon nanoparticle with αvβ3-target and loaded with rapamycin. The result obtained demonstrates that αvβ3-targeted nanoparticles may quickly block the restenosis of New Zealand white (NZW) rabbit. This will offer MRI imaging with accuracy within few minutes after giving injection (Cyrus et al. 2008). In another study Gu et al. have synthesized theranostic layered double hydroxide nanoparticles which contain low molecular weight heparin (used for therapy) and CdTe quantum dots (required for imaging). The results showed that the nanoparticles synthesized were targeting wounded arteries and can minimize the injured area for rats (Gu et al. 2012). The shape of the particle is anticipated to be important in the targeted delivery of therapeutic medications or imaging compounds to wounded blood arteries using nanocarriers. He et al. and colleagues proved that cuboidal cyclodextrin frameworks outperform spherical counterparts in terms of haemostasis and wounded vascular targeting. Cuboidal and biocompatible cyclodextrin metal-organic frameworks (CD-MOFs) were created and coupled with GRGDS peptide by bridging and surface modification (GS5-MOFs). Cuboidal nanocrystals exhibited increased adherence and aggregation with activated platelets in vitro under static and biologically relevant flow conditions. They also demonstrated effective haemostatic effects, with bleeding duration and blood loss reduced by 90% and robust damaged vascular targeting in vivo, much outperforming spherical-CD nanosponges with about the same chemical makeup (He et al. 2019).

14.5.3 Neurodegenerative Disorders

There are a range of diseases known as neurodegenerative disorders that affect different parts of the brain, such as the hippocampus, the cerebellum, and the brain stem, and are caused by protein build-up in the cells, gene alterations, synaptic degeneration, and mitochondrial malfunction. Inconsistent neuronal performance

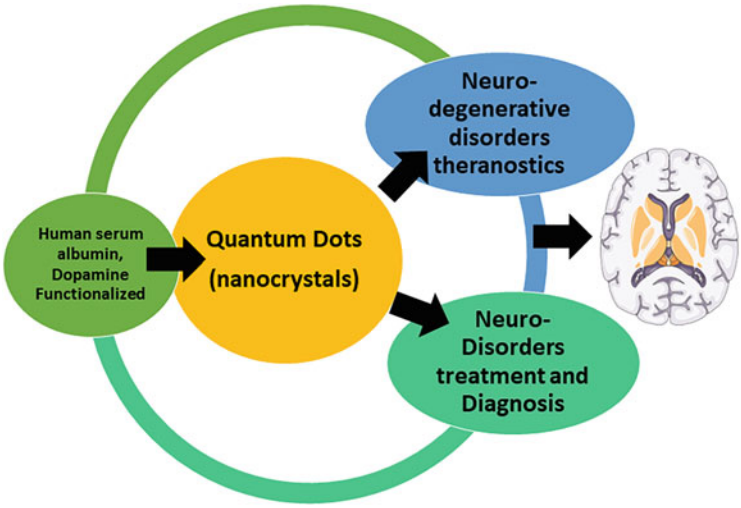

Fig. 14.3 Quantum dots (QDs) as theranostics for neurodegenerative disorders

and disruptions between neuronal cells or any region of the brain result in neuronal cell death, leading to ND-related disorders. Neurodegeneration refers to the destruction of neuronal cells or the degradation of the brain. AD, Parkinson's disease (PD), and prion disease are all examples of neuronal impairments induced by a combination of numerous variables. The blood-brain barrier (BBB) acts as a protective barrier to the central nervous system (CNS) by regulating homeostasis. Besides reducing the passage of toxic xenobiotic compounds and endogenous compounds to CNS, BBB also inhibits the entry of therapeutic substances. Nanotechnology gives the opportunity to deliver smaller molecules/drugs against CNS ailments through BBB (Nirale et al. 2020a; Sweeney et al. 2018; Norrara et al. 2017; Sharma et al. 2019; Barnham et al. 2004; Lin and Beal 2006). Quantum dots (QDs) are most prominently used for the treatment of neurodegenerative disorders (Fig. 14.3).

QDs are semiconductor type of nanocrystals with exceptional and unique features such as fluorescence, greater photostability, and electrochemical capabilities. These features of Qds are a result of their zero-dimensional construction, in which the quantum confinement effect among bulk and discrete molecules confines them. Due to their size-dependent qualities, they possess adjustable fluorescence properties: big QDs emit red light because of their tiny energy gap, whereas small QDs emit blue light because of their wider energy gap. In the biological and pharmaceutical areas, they are particularly useful for pharmaceutical targeting, cell diagnostics, and visualization. They are also utilized in the diagnosis and treatment of NDs because of their small particle size and high permeability. QDs are nanostructures that exhibit low cytotoxicity, cell proliferation, cell differentiation, and cellular metabolic activity (Singh et al. 2021).

14.5.3.1 Alzheimer's Disease (AD)

This progressive neurodegenerative ailment, Alzheimer's disease (AD), is one of the most frequent causes of dementia in the elderly and ultimately results in death. Initial minor memory impairment leads to a fully devastating loss of physically and mentally abilities, which is defined as a degenerative dementing illness. Following the commencement of symptoms, the disease's course may range from a few years to more than 20 years, with an average survival time of around 8 years. Ten to fifteen percent of people over 65 and up to 47% of those over the age of 80 are affected by Alzheimer's disease. In Alzheimer's, the -amyloid peptide builds up in the brain's nerve cells (neurons), causing memory loss and deteriorating cognitive abilities (Smith 1998; Cai et al. 2020; Cummings and Cole 2002). To improve treatment outcomes, drugs needed to be targeted across the blood-brain barrier (BBB) and into the central nervous system (CNS) at the ideal therapeutic dosage. Diagnostic tools, drug carriers, and theranostics based on nanotechnology provide very sensitive molecular detection, efficient drug targeting, and their combination. Significant research has been conducted in this field over the last decade, and we have witnessed great results in AD treatment. Numerous nanoparticles from the organic and inorganic nanomaterial categories have been successfully explored for their anti-AD properties (Ahmad et al. 2017).

Numerous publications have examined various tactics and approaches for successfully delivering drugs to the CNS while also diagnosing (Amiri et al. 2013). Wang et al. synthesized a multifunctional theranostic nanocomposite (HSA-BFP@CDs) by conjugating triple-functionalized human serum albumin (HSA-BFP) with carbon dots (CDs) as a ROS scavenger. When HSA-BFP@CDs interact with Aβ aggregates, a fluorescence "off-on" effect at 700 nm was seen, demonstrating the capacity to detect Aβ plaques and the possibility for earlier diagnosis of Alzheimer's disease. It also efficiently suppresses Aβ aggregation, resulting in an increase in cell. Moreover, numerous ROS, including hydroxyl radicals, superoxide radicals, hydrogen peroxide, and Aβ-Cu2 + −induced-ROS, may be scavenged by using HSA-BFP@CDs, thereby leading to the reduction of cellular oxidative damages. It has been shown that HSA-BFP@CDS may be used to detect and treat amyloid plaques, reduce Aβ deposition, and alleviate the effects of oxidative stress in the brain of *Caenorhabditis* worms, an animal model of Alzheimer's disease (AD). A new understanding of protein-carbon dot conjugate design and the development of AD multi-target treatment were gained via this research (Pansieri et al. 2017). Chen et al. devised a quick, ultrasensitive detection approach for tau protein and other neurological biomarkers. They synthesized CuInS2/ZnS quantum dots functionalized using dopamine to detect tau protein utilizing a new tyrosinase (TYR)-induced tau aptamer–tau–tau antibody (anti-tau) sandwich fluorescence immunoassay. Quantum dots with high brightness, low toxicity, and great biocompatibility were successfully synthesized and decked with dopamine using amide conjugation. The fluorescence intensity of the immunoassay based on DA-functionalized CuInS2/ZnS quantum dots demonstrates excellent performance in terms of linearity with the logarithm of tau protein concentration (Chen et al. 2019).

14.5.3.2 Parkinson's Disease (PD)

Parkinson's disease is the second most prevalent neurodegenerative condition, affecting 2–3% of the 65-year-old population. Parkinson's disease is characterized by neuronal death in the substantia nigra, which results in striatal dopamine insufficiency, and intracellular inclusions containing clumps of α-synuclein. Parkinson's disease is a diverse illness with variants that advance swiftly and slowly. Deep brain stimulation and therapy with levodopa-carbidopa enteral suspension may assist persons with drug-resistant tremor, symptoms that increase after the medicine wears off, and dyskinesias. However, poor brain transfers and limited bioavailability during traditional therapy pose a formidable obstacle in the treatment and diagnosis. In response to these shortcomings, drug delivery nanocrystals (NP) may serve as an exceptional tool for enhancing the therapeutic efficacy of anti-Parkinson's medications for simultaneous therapy and diagnostics (Poewe et al. 2017; Armstrong and Okun 2020; Ghazy et al. 2021).

Theranostic nanocarriers based on liposomes, quantum dots (QDs), and an anti-Parkinson's medication have been created by Wen et al. for long-term monitoring and drug delivery. In this research, apomorphine was employed as a model drug. It has been licenced for use in the treatment of "off" phases of Parkinson's disease by injection as a rescue medicine. However, its short half-life (approximately 41 minutes) and intrinsic instability have limited its clinical use. Incorporating it into nanoparticles may help to mitigate these drawbacks (Wen et al. 2012). Liposomal QD/apomorphine-integrated multifunctional liposomes have emerged as useful diagnostic and therapeutic tools. Cancer, Alzheimer's, Parkinson's, amyotrophic lateral sclerosis, diabetes, and heart failure are all triggered by mitochondrial malfunction. Hua et al. used chitosan, ethylenediamine, and mercaptosuccinic acid as carbon sources to make a new form of luminous CDs (carbon dots). High mitochondrial targeting specificity, wash free and long-term imaging, great photostability, and multicolour FL imaging are only some of the advantages of CDs manufactured in this way. Aside from that, the synthetic process for CDs is easy, inexpensive, and environmentally benign, making it an excellent choice for large-scale manufacture and potential biological uses in the future. CDs may enter cells through temperature-dependent transport and caveolae-mediated endocytosis and then precisely target mitochondria (Hua et al. 2017).

14.5.3.3 Prion Disease

Prion disorder is known as spongiform encephalopathy (SE) because of its sponge-like development in the brain. In the case of the prion's sickness, the brain becomes a sponge-like structure because healthy tissues are replaced by cysts or vacuoles. Prion disease is caused by misfolded prion proteins aggregating in the cerebrum and cerebellum, mostly. The infectious proteins are called prions, which are found in the cell membranes of certain neurons and contain 253 amino acids in the form of three helices and two pleated sheets that are found in the cell membranes of some neurons (Forloni et al. 2019; Hagiwara et al. 2019; Nirale et al. 2020b). Xiao et al. constructed a technique using two aptamers that recognize two distinct epitopes from PrPSc to differentiate it from PrPc in serum and brain tissue homogenate. The

aptamers are bound to the surface of magnetic microparticles and quantum dots. It forms a sandwich structure with strong fluorescence in aqueous media in the presence of PrPSc, which may be separated by an external magnetic field (Xiao et al. 2010). Xie et al. have shown that QD-PEG dispersed in nitrile acetic acid may serve as a site-specific marker for the in vitro PrP expressed at the cell surface (Furmanski et al. 2009).

14.6 Advantages of Theranostic Nanocrystals

Although the discipline of theranostics is still in its infancy, significant research and development efforts have been made toward theranostic nanocrystals for cancer and cardiovascular-guided imaging and treatment. Biodegradable and metabolizable polymeric nanoparticles have been created and evaluated in small animal models using a variety of theranostic nanoplatforms. These particles have a long circulation life, are biosafe, can be decorated with targeted moieties, and can be loaded with medicinal and contrast chemicals. These multifunctional polymer nanocrystals containing anatomical information may be employed for noninvasive diagnostics of some disorders. Additionally, the pharmacokinetics, biodistribution, and targeting efficacy of conjugated or encapsulated medicinal compounds, as well as therapeutic responses, may be determined. These capabilities may aid in the preclinical and clinical stages of drug development. When it comes to accomplishing the objective of theranostic activity, the nanocrystal semiconductors known as quantum dots (QDs) are by far the most efficient and effective medicine delivery methods. This is because they are an intermediate between bulk semiconductors and discrete entities in terms of qualities like luminescence, photostability, electrical properties, high excitation capacity, and size adjustable emission. They also have remarkable optical and electrochemical properties. There are a wide range of uses for these devices, including diagnostics, bioimaging, and bio-sensory applications as well as therapeutic treatments.

Nanocrystal formulation allows for the delivery of poorly soluble pharmaceuticals and provides significant benefits over current methods. In addition to oral and intravenous drug administration, nanocrystal formulations have demonstrated promising outcomes for transdermal, ophthalmic, and pulmonary drug delivery. With the continuous development of much more poorly soluble pharmaceuticals, the demand for nanocrystal-based products will become crucial for the development of innovative ways to assess and comprehend the in vivo destiny of these substances. The hybrid nanocrystal idea offers a platform for nanocrystal bioimaging in vivo. Embedding fluorescent probes in drug nanocrystals does not need additional stages in the manufacturing of nanocrystals, which is an additional benefit of using hybrid nanocrystals as an innovative theranostic tool (Lu et al. 2019).

14.7 Challenges and Future Goals

Evidently, theranostic nanocrystals are being used to combine diagnostic and therapeutic capabilities, resulting in much enhanced individualized illness treatment. Many important hurdles must be solved in order for clinical translation to occur, including selecting the optimum nanoplatform, improving ligand conjugation efficiency, and developing an ideal synthesis process with fewer steps, greater repeatability, and reduced costs.

Despite significant technology advancements in prevention of disease, diagnostics, and treatment, nanotheranostics, which is emerging as a new paradigm for disease diagnosis and treatment in clinics, is still in its early stages. The nanobiointeraction is one of the most difficult aspects of bringing theranostic nanomedicine to clinics. The hazardous impact of nanoformulations is highly reliant on numerous aspects such as size, ζ-potential, and solubility. According to experimental research, nanoparticles with high therapeutic qualities may not always be appropriate diagnostic tools. Anti-EGFR-coated gold nanoparticles with a 20 nm size exhibited excellent tumour uptake, whereas gold nanoparticles with a 50 nm size demonstrated the best CT contrast enhancement. This review demonstrates the importance of nanoparticle size in their use as medicinal agents or diagnostic instruments. Another important issue with clinical application of theranostic nanomedicines is the complexity in developing a repeatable and controlled production process. Large-scale nanoparticle synthesis suffers from poor batch-to-batch repeatability, a wide range of altered physical and chemical properties, and insufficient yield. Good manufacturing practices, as well as more accurate manufacture, control, and chemistry, are required for the development of multifunctional theranostic nanoparticles that can be transferred from the laboratories to health centres, which is a tough undertaking to do on a big scale. The large divide between the research community and regulatory agencies is a third important problem that must be addressed. Many government rules based on regulatory variables linked to quality management, manufacturing methods, safety profile, and intellectual property protection are being used to oversee the commercialization of nanomedicine. The lack of defined regulatory and safety criteria has a significant impact on the timely and successful applicability of the theranostics to market.

Theranostic nanoagents have the potential to significantly improve disease detection and treatment by incorporating several capabilities, including those used for targeting, imaging, and therapy, into a single nanoscaffold. However, many concerns must be addressed before the discipline can progress beyond its infancy, including whether to use nanoagents and how to effectively match the dose of diagnostic and therapeutic components. Most critically, scientists creating nanoparticle preparations must reconcile their synthetic approaches with the nanoagents' eventual therapeutic value. This can only be accomplished via the establishment of productive relationships with scientists and physicians working in domains unrelated to their own. Given these obstacles, the field of theranostics is fast expanding and, like a toddler, is certain to have a few bumps and bruises along the way.

Diagnostic imaging using disease-related molecular targets, downstream molecular targets influenced by therapy, and a nanostructure analogous to a therapeutic nanostructure might be utilized to assess therapeutic response and effectiveness during or after treatment. Unfortunately, theranostic technology for medication response monitoring is still in the early stages of research; few studies have focused on combining treatment and response imaging into a single theranostic system. However, the need for a more individualized approach to medical care, as well as considerable continuing breakthroughs in nanotechnology and diagnostic imaging technologies, may soon change theranostic systems. However, certain critical difficulties must be addressed first in order to achieve effective assimilation into theranostic systems. Theranostic systems, in particular, must address the apparent mismatch between the ideal concentrations of imaging and therapeutic agents for prospective clinical application, since the optimal concentration for a desired treatment is often considerably greater than that necessary for imaging. Furthermore, a theranostic system might incorporate numerous functions inside a single system, which can add to the system's complexity. As a result, developing repeatable and straightforward approaches for constructing theranostic systems may aid in the effective integration of imaging and treatment. Furthermore, using clinically proven nanoparticles to hasten the clinical translation of theranostic NP might reduce the risk of translation. Diagnostic imaging has been demonstrated to play a significant role in several aspects of personalized medicine, including monitoring medication effectiveness. The development of diagnostic imaging after treatment, ideally in a single system, might enable customized medicine by allowing therapy selection, treatment planning, objective response monitoring, and follow-up therapy planning based on the individual molecular features of a disease, thereby laying the groundwork for personalized medicine.

References

Ahmad J, Akhter S, Rizwanullah M (2017) Nanotechnology based theranostic approaches in Alzheimer's disease management: current status and future perspective. Current Alzheimer [Internet]. [cited 2022 Jun 29]

Al Shaal L et al (2011) Production and characterization of antioxidant apigenin nanocrystals as a novel UV skin protective formulation. Int J Pharm 420(1):133–140

Alvarez AJ, Myerson AS (2010) Continuous plug flow crystallization of pharmaceutical compounds. Cryst Growth Des 10(5):2219–2228

Amiri H, Saeidi K, Borhani P, Manafirad A, Ghavami M, Zerbi V (2013) Alzheimer's disease: pathophysiology and applications of magnetic nanoparticles as MRI theranostic agents. ACS Chem Neurosci 4(11):1417–1429

Anderson J, Morrow D, Medicine NEJ of, Medical M, Soc undefined (2017) Acute myocardial infarction. Campion EW, editor. New Engl J Med [Internet]. 376(21):2053–64

Armstrong MJ, Okun MS (2020) Diagnosis and treatment of Parkinson disease: a review. JAMA [Internet] 323(6):548–560. [cited 2022 Jun 29]

Bailey RE, Nie S (2003) Alloyed semiconductor quantum dots: tuning the optical properties without changing the particle size. J Am Chem Soc 125(23):7100–7106

Barnham K, Masters C, Bush A (2004. [cited 2022 Jun 29]) Neurodegenerative diseases and oxidative stress. Nat Rev Drug Discov [Internet] 3:205

Boujemaoui A, Mongkhontreerat S, Malmström E, Carlmark A (2015) Preparation and characterization of functionalized cellulose nanocrystals. Carbohydr Polym 115:457–464
Bozkurt B, Kribbs SB, Clubb FJ, Michael LH, Didenko VV, Hornsby PJ et al (1998) Pathophysiologically relevant concentrations of tumor necrosis factor- α promote progressive left ventricular dysfunction and remodeling in rats. Circulation 97(14):1382–1391
Cai J, Dao P, Chen H, Yan L, Li YL, Zhang W et al (2020) Ultrasmall superparamagnetic iron oxide nanoparticles-bound NIR dyes: novel theranostic agents for Alzheimer's disease. Dyes Pigments 173:107968
Chan WC, Nie S (1998) Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science 281(5385):2016–2018
Chen B, Dai W, He B, Zhang H, Wang X, Wang Y (2017) Current multistage drug delivery systems based on the tumor microenvironment. Theranostics [Internet]. [cited 2022 Jun 27]
Chen H, Khemtong C, Yang X, Chang X, Gao J (2011b) Nanonization strategies for poorly water-soluble drugs. Drug Discov Today 16:354–360
Chen H et al (2011a) Nanonization strategies for poorly water-soluble drugs. Drug Discov Today 16(7–8):354–360
Chen L, Lin J, Yi J, Weng Q, Zhou Y, Han Z et al (2019) A tyrosinase-induced fluorescence immunoassay for detection of tau protein using dopamine-functionalized CuInS2/ZnS quantum dots. Analytic Bioanalytic Chem 411(20):5277–5285
Chu Z, Wang Z, Chen L, Wang X, Huang C, Cui M et al (2018) Combining magnetic resonance imaging with Photothermal therapy of CuS@BSA nanoparticles for cancer Theranostics. ACS Appl Nano Mater [Internet] 1(5):2332–2340
Cormode DP, Sanchez-Gaytan BL, Mieszawska AJ, Fayad ZA, Mulder WJM (2013) Inorganic nanocrystals as contrast agents in MRI: synthesis, coating and introduction of multifunctionality. NMR in Biomed[Internet] 26(7):766–780
Cormode DP, Skajaa T, van Schooneveld MM, Koole R, Jarzyna P, Lobatto ME et al (2008) Nanocrystal core high-density lipoproteins: a multimodality contrast agent platform. Nano Lett 8(11):3715–3723
Cummings JL, Cole G (2002) Alzheimer Disease. JAMA [Internet] 287(18):2335–2338
Cyrus T, Zhang H, Allen JS, Williams TA, Hu G, Caruthers SD et al (2008) Intramural Delivery of Rapamycin With αvβ3-Targeted Paramagnetic Nanoparticles Inhibits Stenosis After Balloon Injury, and vascular biology [Internet]. Arteriosclerosis Thrombosis Vascular Biology 28(5): 820–826
D'Addio SM, Prud'homme RK (2011) Controlling drug nanoparticle formation by rapid precipitation. Adv Drug Deliv Rev 63(6):417–426
Dalvi SV, Yadav MD (2015) Effect of ultrasound and stabilizers on nucleation kinetics of curcumin during liquid antisolvent precipitation. Ultrason Sonochem 24:114–122
Elieh-Ali-Komi D, Hamblin MR (2016) Chitin and chitosan: production and application of versatile biomedical nanomaterials. Int J Adv Res 4(3):411
Feiner R, Engel L, Fleischer S, Malki M, Gal I (2016) Engineered hybrid cardiac patches with multifunctional electronics for online monitoring and regulation of tissue function. Nat Mater [Internet]
Forloni G, Roiter I, Tagliavini F (2019) Clinical trials of prion disease therapeutics. Curr Opin Pharmacol [Internet] 44:53. Available from: https://www.sciencedirect.com/science/article/pii/S1471489219300141
Fuhrmann K, Polomska A, Aeberli C, Castagner B, Gauthier MA, Leroux JC (2013) Modular design of redox-responsive stabilizers for nanocrystals. ACS Nano 7:8243–8250
Furmanski J, Anderson M, Bal S, Greenwald A (2009. [cited 2022 Jun 30]) Clinical fracture of cross-linked UHMWPE acetabular liners. Biomaterials [Internet]. 30:5572
Gao L, Zhang D, Chen M (2008) Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system. J Nanopart Res 10(5):845–862
Ghazy E, Rahdar A, Barani M, Kyzas GZ (2021 May) Nanomaterials for Parkinson disease: recent progress. J Mol Struct 5(1231):129698

Gu Z, Rolfe BE, Xu ZP, Campbell JH, Lu GQM, Thomas AC (2012) Antibody-targeted drug delivery to injured arteries using layered double hydroxide nanoparticles. Adv Healthc Mater 1(5):669–673

Guo M, Her S, Keunen R, Zhang S, Allen C, Winnik MA (2016) Functionalization of cellulose nanocrystals with PEG-metal-chelating block copolymers via controlled conjugation in aqueous media. ACS Omega 1(1):93–107

Guo S, Huang L (2014) Nanoparticles containing insoluble drug for cancer therapy. Biotechnol Adv 32:778–788

Hagiwara K, Sato Y, Yamakawa Y, Hara H, Tobiume M, Okemoto-Nakamura Y et al (2019) Tracking and clarifying differential traits of classical- and atypical L-type bovine spongiform encephalopathy prions after transmission from cattle to cynomolgus monkeys. PLoS One 14(5)

He Y, Xu J, Sun X, Ren X, Maharjan A, York P et al (2019) Cuboidal tethered cyclodextrin frameworks tailored for hemostasis and injured vessel targeting. Theranostics [Internet]. 9(9): 2489

Hollis CP, Li T (2010) Nanocrystals production, characterization, and application for cancer therapy. Pharmaceut Sci Encyclopedia: Drug Discov Develop Manufactur 15:1–26

Hollis CP, Weiss HL, Evers BM, Gemeinhart RA, Li T (2013b) In vivo investigation of hybrid paclitaxel nanocrystals with dual fluorescent probes for cancer theranostics. Pharm Res 31(6): 1450–1459

Hollis CP, Weiss HL, Leggas M, Evers BM, Gemeinhart RA, Li T (2013c) Biodistribution and bioimaging studies of hybrid paclitaxel nanocrystals: lessons learned of the EPR effect and image-guided drug delivery. J Control Release 172(1):12–21

Hollis CP, Zhao R, Li T (2013a) Hybrid nanocrystal as a versatile platform for cancer theranostics. In: Park K (ed) Biomaterials for cancer therapeutics: diagnosis, prevention and therapy. Wood- head Publishing, Cambridge, pp 186–204

Hua XW, Bao YW, Chen Z, Wu FG (2017 Aug) Carbon quantum dots with intrinsic mitochondrial targeting ability for mitochondria-based theranostics. Nanoscale 9(30):10948–10960

Huo Q (2007) A perspective on bioconjugated nanoparticles and quantum dots. Colloids Surf B: Biointerfaces 59(1):1

Im SH, Jung HT, Ho MJ, Lee JE, Kim HT, Kim DY et al (2020) 2020. Montelukast nanocrystals for transdermal delivery with improved chemical stability. Pharmaceutics 12(1):1–18

Jamieson T, Bakhshi R, Petrova D, Pocock R, Imani M, Seifalian AM (2007) Biological applications of quantum dots. Biomaterials 28(31):4717–4732

Joshi K, Chandra A, Jain K, Talegaonkar S (2019) Nanocrystalization: an emerging technology to enhance the bioavailability of poorly soluble drugs. Pharm Nanotechnol 7(4):259–278. https://doi.org/10.2174/2211738507666190405182524

Jung E, Kang C, Lee J, Yoo D, Hwang DW, Kim D et al (2018) Molecularly engineered Theranostic nanoparticles for thrombosed vessels: H2O2-Activatable contrast-enhanced photoacoustic imaging and antithrombotic therapy. ACS Nano 12(1):392–401

Junghanns JU, Muller RH (2008) Nanocrystal technology, drug delivery and clinical applications. Int J Nanomedicine 3:295–309

Junyaprasert VB, Morakul B (2015) Nanocrystals for enhancement of oral bioavailability of poorly water-soluble drugs. Asian J Pharm Sci. 10:13–23

Keck CM, Muller RH (2006) Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. Eur J Pharm Biopharm 62:3–16

Kermorgant M, Ben Salem J, Santelli J, Calise D, Oster AC, Lairez O et al (2019) Evaluation of upconverting nanoparticles towards heart theranostics. PLOS One [Internet] 14(12):e0225729

Kipp J (2004) The role of solid nanoparticle technology in the parenteral delivery of poorly water-soluble drugs. Int J Pharm 284:109–122

Kleinbongard P, Schulz R, Heusch G (2011) TNFα in myocardial ischemia/reperfusion, remodeling and heart failure. Heart Fail Rev 16(1):49–69

Kovalenko MV, Manna L, Cabot A, Hens Z, Talapin DV, Kagan CR, Klimov VI, Rogach AL, Reiss P, Milliron DJ, Guyot-Sionnnest P (2015) Prospects of nanoscience with nanocrystals. ACS Nano 9(2):1012–1057

Krumholz HM, Peterson ED, Ayanian JZ, Chin MH, Debusk RF, Goldman L et al (2005) Report of the National Heart, Lung, and Blood Institute working group on outcomes research in cardiovascular disease. Circulation 111(23):3158–3166

Lanza GM, Yu X, Winter PM, Abendschein DR, Karukstis KK, Scott MJ et al (2002) Targeted antiproliferative drug delivery to vascular smooth muscle cells with a magnetic resonance imaging nanoparticle contrast agent: implications for rational therapy of restenosis. Circulation 106(22):2842–2847

Law WC, Mahajan SD, Kopwitthaya A, Reynolds JL, Liu M, Liu X et al (2012) Gene silencing of human neuronal cells for drug addiction therapy using anisotropic nanocrystals. Theranostics [Internet] 2(7):695

Lee J, Lee S, Choi J (2005) Amphiphilic amino acid copolymers as stabilizers for the preparation of nanocrystal dispersion. Eur J Pharm Sci 24:441–449

Li H, Yan Z, Xiong Q, Chen X, Lin Y, Xu Y, Bai L, Jiang W, Zheng D, Xing C (2019a) Renoprotective effect and mechanism of polysaccharide from Polyporus umbellatus sclerotia on renal fibrosis. Carbohydr Polym 212:1

Li T, Feng ZQ, Qu M, Yan K, Yuan T, Gao B et al (2019b) Core/Shell piezoelectric nanofibers with spatial self-orientated β-phase nanocrystals for real-time micropressure monitoring of cardiovascular walls. ACS Nano [Internet] 13(9):10062–10073

Lin MT, Beal MF (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443(7113):787–795

Liu L, Hu R, Roy I, Lin G, Ye L, Reynolds JL et al (2013) Synthesis of luminescent near-infrared AgInS2 nanocrystals as optical probes for in vivo applications. Theranostics [Internet] 3(2):109

Liu S, Shi D, Chen L, Yan Y, Wang X, Song Y et al (2021) Paclitaxel-loaded magnetic nanocrystals for tumor neovascular-targeted theranostics: an amplifying synergistic therapy combining magnetic hyperthermia with chemotherapy. Nanoscale [Internet] 13(6):3613–3626

Liu T, Yu X, Yin H, Möschwitzer JP (2019) Advanced modification of drug nanocrystals by using novel fabrication and downstream approaches for tailor-made drug delivery. Drug Del 26(1): 1092–1103

Liu Y, Cheng C, Prud'homme RK, Fox RO (2008) Mixing in a multi-inlet vortex mixer (MIVM) for flash nano-precipitation. Chem Eng Sci 63(11):2829–2842

Lu R, Zhu J, Yu C, Nie Z, Gao Y (2020) Cu3BiS3 nanocrystals as efficient Nanoplatforms for CT imaging guided Photothermal therapy of arterial inflammation. Front Bioeng Biotechnol 8:981

Lu Y, Chen Y, Gemeinhart RA, Wu W, Li T (2015a) Developing nanocrystals for cancer treatment. Nanomedicine (Lond) 10:2537–2552

Lu Y, Chen Y, Gemeinhart RA, Wu W, Li T (2015b) Developing nanocrystals for cancer treatment. Nanomedicine 10(16):2537–2552

Lu Y, Li Y, Wu W (2016) Injected nanocrystals for targeted drug delivery. Acta Pharm Sin B 6(2): 106–113

Lu Y, Lv Y, Li T (2019) Hybrid drug nanocrystals. Adv Drug Deliv Rev 15(143):115–133

Lu ZR (2014) Theranostics: Fusion of Therapeutics and Diagnostics. Pharmaceut Res 31(6): 1355–1357

Ma Y, Wu S, Wu T, Chang Y, Hua M, Chen J (2009) Magnetically targeted thrombolysis with recombinant tissue plasminogen activator bound to polyacrylic acid-coated nanoparticles. Biomaterials [Internet]. 30:3343

Manfroi W, Peukert C, Berti C, Noer C (2002) Acute myocardial infarction: the first manifestation of ischemic heart disease and relation to risk factors. Arquivos Brasileiros 78:392

Mantzaris N (2005) Liquid-phase synthesis of nanoparticles: particle size distribution dynamics and control. Chem Eng Sci 60:4749–4770

Mazumder S, Dey R, Mitra MK, Mukherjee S, Das GC (2009) Biofunctionalized quantum dots in biology and medicine. J Nanomater 1:2009

Mendis S, Puska P, Norrving B (2011) Global atlas on cardiovascular disease prevention and control [Internet]. World Health Organization

Merisko-Liversidge E, Liversidge GG, Cooper ER (2003) Nanosizing: a formulation approach for poorly-water-soluble compounds. Eur J Pharm Sci 18(2):113–120

Mićić OI, Nozik AJ (1996) Synthesis and characterization of binary and ternary III–V quantum dots. J Lumin 70(1–6):95–107

Mishra PR et al (2009) Production and characterization of hesperetin nanosuspensions for dermal delivery. Int J Pharm 371(1–2):182–189

Muller R, Jacobs C (2002a) Buparvaquone mucoadhesive nanosuspension: preparation, optimisation and long-term stability. Int J Pharm 237:151–161

Muller RH, Jacobs C (2002b) Production and characterization of a budesonide nanosuspension for pulmonary administration. Pharm Res 19:189–194

Muller RH, Gohla S, Keck CM (2011) State of the art of nanocrystals—special features, production, nanotoxicology aspects and intracellular delivery. Eur J Pharm Biopharm 78:1–9

Müller RH, Jacobs C, Kayser O (2001) Nanosuspensions as particulate drug formulations in therapy: rationale for development and what we can expect for the future. Adv Drug Deliv Rev 47(1):3–19

Müller RH et al (2011) State of the art of nanocrystals-special features, production, nanotoxicology aspects and intracellular delivery. Eur J Pharm Biopharm 78(1):1–9

Müller RH et al (2016) Nanocrystals for passive dermal penetration enhancement. In: Dragicevic N, Maibach HI (eds) Percutaneous penetration enhancers chemical methods in penetration enhancement: nanocarriers. Springer-Verlag, Berlin Heidelberg, pp 283–295

Murray C, Norris DJ, Bawendi MG (1993) Synthesis and characterization of nearly monodisperse CdE (E= sulfur, selenium, tellurium) semiconductor nanocrystallites. J Am Chem Soc 115(19): 8706–8715

Nabel EG, Braunwald E (2012) A tale of coronary artery disease and myocardial infarction. N Engl J Med 366(1):54–63

Nagarwal RC, Kumar R, Dhanawat M, Das N, Pandit JK (2011) Nanocrystal technology in the delivery of poorly soluble drugs: an overview. Curr Drug Deliv 8:398–406

Nirale P, Paul A, Yadav KS (2020a) Nanoemulsions for targeting the neurodegenerative diseases: Alzheimer's, Parkinson's and Prion's. Life Sci. 245:117394

Nirale P, Paul A, Yadav KS (2020b) Nanoemulsions for targeting the neurodegenerative diseases: Alzheimer's, Parkinson's and Prion's. Life Sci 15:245

Norrara B, Doerl JG, Guzen FP, Cavalcanti JRLP, Freire MAM (2017) Commentary: localized vs. systematic neurodegeneration: a paradigm shift in understanding neurodegenerative diseases. Front Syst Neurosci 11

Organization WH (2020) WHO package of essential noncommunicable (PEN) disease interventions for primary health care. [cited 2022 Jun 28]

Pagani FD, Baker LS, Hsi C, Knox M, Fink MP, Visnert MS (1992. [cited 2022 Jun 28]) Left ventricular systolic and diastolic dysfunction after infusion of tumor necrosis factor-alpha in conscious dogs. J Clin Investig [Internet] 90:389

Pansieri J, Plissonneau M, Stransky-Heilkron N, Dumoulin M, Heinrich-Balard L, Rivory P et al (2017) Multimodal imaging Gd-nanoparticles functionalized with Pittsburgh compound B or a nanobody for amyloid plaques targeting. Nanomedicine [Internet] 12(14):1675–1687. https://doi.org/10.2217/nnm-2017-0079. [cited 2022 Jun 30]

Peltonen L, Hirvonen J (2010) Pharmaceutical nanocrystals by nanomilling: critical process parameters, particle fracturing and stabilization methods. J Pharm Pharmacol 62(11):1569–1579

Pinheiro IF, Ferreira FV, Souza DH, Gouveia RF, Lona LM, Morales AR, Mei LH (2017) Mechanical, rheological and degradation properties of PBAT nanocomposites reinforced by functionalized cellulose nanocrystals. Eur Polym J 97:356–365

Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J et al (2017) Parkinson disease. Nat Rev Dis Primers 3(1):1–21

Pötschke K, Müller-Kirsch L, Heitz R, Sellin RL, Pohl UW, Bimberg D, Zakharov N, Werner P (2004) Ripening of self-organized InAs quantum dots. Physica E 21(2–4):606–610
Qiao H, Zhou Y, Yu F, Wang E, Min Y, Huang Q, Pang L, Ma T (2015) Effective removal of cationic dyes using carboxylate-functionalized cellulose nanocrystals. Chemosphere 141:297–303
Rabinow BE (2004) Nanosuspensions in drug delivery. Nat Rev Drug Discov 3:785–796
Raghava Srivalli KM, Mishra B (2014) Drug nanocrystals: a way toward scale-up. Saudi Pharm J 24(4):386–404
Ranjita S (2013) Nanosuspensions: a new approach for organ and cellular targeting in infectious diseases. J Pharm Invest 43:1–26
Rao CN, Thomas PJ, Kulkarni GU (2007) Nanocrystals. In: Synthesis, properties and applications. Springer, Berlin
Ross R (1995) Cell biology of atherosclerosis. Ann Rev Physiol [Internet] 57:791–804
Sharma G, Sharma AR, Lee SS, Bhattacharya M, Nam JS, Chakraborty C (2019) Advances in nanocarriers enabled brain targeted drug delivery across blood brain barrier. Int J Pharm [Internet] 559:360–372
Shegokar R, Müller RH (2010) Nanocrystals: industrially feasible multifunctional formulation technology for poorly soluble actives. Int J Pharm 399(1–2):129–139
Shibata N, Okamoto K, Tatsumi N (1979) Mechanism of thrombus formation. Rinsho Byori Suppl 40:211–229
Singh A, Mallika TN, Gorain B, Yadav AK, Tiwari S, Flora SJS et al (2021) Quantum dot: heralding a brighter future in neurodegenerative disorders. J Drug Deliv Sci Technol 65:102700
Smith MA (1998 Jan) Alzheimer Disease. Int Rev Neurobiol 1(42):1–54
Soman C, Giorgio T (2009) Sensitive and multiplexed detection of proteomic antigens via quantum dot aggregation. Nanomedicine 5(4):402–409
Somasuntharam I, Yehl K, Carroll S, Maxwell J (2016. [cited 2022 Jun 28]) Knockdown of TNF-α by DNAzyme gold nanoparticles as an anti-inflammatory therapy for myocardial infarction. Biomaterials [Internet] 83:12
Sweeney MD, Sagare AP, Zlokovic BV (2018) Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nature reviews. Neurology 14(3):133–150
Ta H, Li Z, Hagemeyer C, Cowin G (2017) Self-confirming molecular imaging of activated platelets via iron oxide nanoparticles displaying unique dual MRI contrast. Atherosclerosis *263*:e146
Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD (2012) Third universal definition of myocardial infarction. J Am Coll Cardiol 60(16):1581–1598
Trapani G, Denora N, Trapani A, Laquintana V (2012) Recent advances in ligand targeted therapy. J Drug Target 20:1–22
Uppal R, Ciesienski KL, Chonde DB, Loving GS, Caravan P (2012) Discrete bimodal probes for thrombus imaging. J Am Chem Soc [Internet] 134(26):10799
Van Eerdenbrugh B, Vermant J, Martens JA, Froyen L, Van Humbeeck J, Augustijns P et al (2009) A screening study of surface stabilization during the production of drug nanocrystals. J Pharm Sci 98(6):2091–2103
de Waard H, Frijlink HW, Hinrichs WL (2011) Bottom-up preparation techniques for nanocrystals of lipophilic drugs. Pharm Res 28(5):1220–1223
Wang C, Zhao T, Li Y, Huang G, White M (2017) Investigation of endosome and lysosome biology by ultra pH-sensitive nanoprobes. Adv Drug Deliv [Internet] 113:87–96
Wang Y, Herron N (1991) Nanometer-sized semiconductor clusters: materials synthesis, quantum size effects, and photophysical properties. J Phys Chem 95(2):525–532
Wen CJ, Zhang LW, Al-Suwayeh SA, Yen TC, Fang JY (2012) Theranostic liposomes loaded with quantum dots and apomorphine for brain targeting and bioimaging. Int J Nanomed [Internet] 7:1599
William WY, Chang E, Drezek R, Colvin VL (2006) Water-soluble quantum dots for biomedical applications. Biochem Biophys Res Commun 348(3):781–786

Xia D, Gan Y, Cui F (2014) Application of precipitation methods for the production of water-insoluble drug nanocrystals: production techniques and stability of nanocrystals. Curr Pharm Des 20(3):408–435

Xiao SJ, Hu PP, Wu XD, Zou YL, Chen LQ, Peng L et al (2010) Sensitive discrimination and detection of prion disease-associated isoform with a dual-aptamer strategy by developing a sandwich structure of magnetic microparticles and quantum dots. Anal Chem 82(23): 9736–9742

Xie J, Zhang Y, Yan C, Song L, Wen S, Zang F et al (2014) High-performance PEGylated Mn–Zn ferrite nanocrystals as a passive-targeted agent for magnetically induced cancer theranostics. Biomaterials 35(33):9126–9136

Xu Y, Liu X, Lian R, Zheng S, Yin Z, Lu Y et al (2012) Enhanced dissolution and oral bioavailability of aripiprazole nano suspensions prepared by nanoprecipitation/homogenization based on acid-base neutralization. Int J Pharm 438:287–295

Yang CT, Ghosh KK, Padmanabhan P, Langer O, Liu J, Eng DNC et al (2018) PET-MR and SPECT-MR multimodality probes: development and challenges. Theranostics [Internet] 8(22): 6210

Yu HY, Qin ZY, Yan CF, Yao JM (2014) Green nanocomposites based on functionalized cellulose nanocrystals: a study on the relationship between interfacial interaction and property enhancement. ACS Sustain Chem Eng 2(4):875–886

Zhou J, Li T, Zhang C, Xiao J, Cui D, Cheng Y (2018. [cited 2022 Jun 27]) Charge-switchable nanocapsules with multistage pH-responsive behaviours for enhanced tumour-targeted chemo/photodynamic therapy guided by NIR/MR imaging. Nanoscale [Internet]. 10:9707

Zia A, Wu Y, Nguyen T, Wang X, Peter K (2020) The choice of targets and ligands for site-specific delivery of nanomedicine to atherosclerosis. Cardiovasc Res [Internet] 116(13):2055–2068

Theranostics Applications of Functionalized Magnetic Nanoparticles

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15.1 Introduction

The amalgamation of nanotechnology, molecular biology, and medicine has taken a lead in great evolution in the field of diagnosis, therapeutics, and nanobiotechnology. This newer approach has resulted in the development of novel materials and processes (Pankhurst et al. 2003). The last few decades have reported the application of nanotechnology-based nanomedicine in various medical fields such as therapy, imaging, and diagnosis (Gobbo et al. 2015). The goals of theranostics nanomedicine are comprised of precised detection, superior tissue accumulation, higher payload at the delivery site, prolonged retention at the desired site, improved therapeutic efficacy, efficient site-specific targeting, reduced dose-associated systemic toxicity that are outcomes in satisfactory therapeutic response. Literatures have reported application of theranostics nanomedicine in cancer therapy in terms of personalized medicine where we can cater the therapy according to individual's need (Yoo et al. 2011).

The steady development of nanomedicine has resulted in early diagnosis, precise and effective treatment assisting in curing various diseases efficiently. Early diagnosis of disease and subsequent therapy are the utmost prerequisite for a favorable prognosis and successful treatment; thus, biomedical researchers are continuously taking immense efforts in developing and improving imaging techniques and treatment methods. One of the revolutionary delivery systems consists of surface engineered magnetic nanoparticles (MNPs) which inherit the properties of site-specific delivery facilitated through magnetic attributes (Xie et al. 2011). Such

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MNPs have been reported to act as vectors for in gene delivery facilitating gene directional transportation activated under the influence of the magnetic field (Kami et al. 2011). Further, such MNPs can be utilized for diagnosis as they possess unique magnetic sensitivity, which can thus be applied in magnetic resonance imaging (Guo et al. 2018). Subsequently the MNPs were gradually exploited for drug delivery, enzyme immobilization, and numerous biotechnological applications (Vaghari et al. 2016).

The MNPs are endows with homogenous nanosized range, biocompatibility, non-immunogenicity, non-toxicity, superparamagnetism, etc. (Bilal et al. 2018). Further they can be easily tailored for facile fabrication, adsorption kinetics, and magnetic moment during formulation for specific applications (Materón et al. 2021). Surface engineered MNPs have two important roles in the field of biomedical applications, viz., as image enhancing agents for MRI and the other as drug-delivery wagons for xenobiotics and pharmacologically/biologically active substances. Another stream that has emerged in field of nanomedicine is known as theranostics which is an amalgamation of therapeutic and diagnostic agent that can concomitantly deliver the drug as well as the diagnostic at the specific site. Regardless of the numerous literature suggesting in vitro and in vivo assessment of functionalized MNPs and predicting their probable biomedical applications, only few formulations have made it to clinical trials and even fewer as commercial products (Gudovan et al. 2015).

The basic mechanism involved in the theranostics action of MNPs is that in the presence of an external magnetic field (externally created), they act by aligning in the direction of magnetization along the field direction. Further, on terminating the magnetic field, their magnetization direction gets randomized ascribing to temperature-induced thermal relaxation resulting in zero overall magnetic moment, thus stabilizing the MNPs (Trahms 2009). When such stable and strongly superparamagnetic MNPs are introduced into biological systems, they act as suitable probes for diagnosis/biological imaging, drug delivery, and therapeutic applications (Shin et al. 2015). The MNPs composed of iron oxide, specifically magnetite (Fe_3O_4), have been reported for their extensive application in the biomedical field in terms of clinical imaging owing to facile fabrication, nanosize, biocompatibility, and high magnetic moments (Shin et al. 2015). USFDA has approved a few number of such MNPs for biomedical application, and Feridex® (iron oxide cluster of 120–180 nm size) is a commercially available MNP employed for clinical liver imaging (Sun and Sun 2017).

This chapter mainly discuss about the synthesis, recent advancement of MNPs in terms of both imaging and therapeutic applications, and their capabilities in giving satisfactory clinical outcome. An amalgamation of property of deep tissue permeation, capability of the magnetic field generation, and MNPs attribute to enhance magnetic resonance imaging sensitivity along with magnetic heating efficiency make MNPs a promising candidate for successful future theranostics (Yoo et al. 2011; Ho et al. 2011).

15.2 Synthesis and Functionalization of MNPs

To be efficient as theranostics agents, the MNPs (Fig. 15.1) should be bestowed with numerous properties, namely, superparamagnetic, biocompatible, non-toxic, targeting ability, facile surface engineering, high entrapment efficiency, deliver high payload at desired site, and in some cases ability to dissipate heat at the site vicinity (Cohen and Shoushan 2013). During synthesis the formulation scientist needs to take care on optimization of important properties, viz., homogenous particle size distribution with reproducibility, desirable morphology (shape and crystallinity), and long-term in vitro and in vivo stability. The MNPs can be formulated in various forms such as nanorods, nanowires, nanocubes, and iron oxide MNPs employing diverse fabrication methods. The commonly employed method comprises bottom-up (wet chemistry) and top-down approach techniques (Fig. 15.1). The bottom-up techniques include hydrothermal, laser pyrolysis solvothermal, sol-gel, co-precipitation, electrochemical, and flow injection synthesis

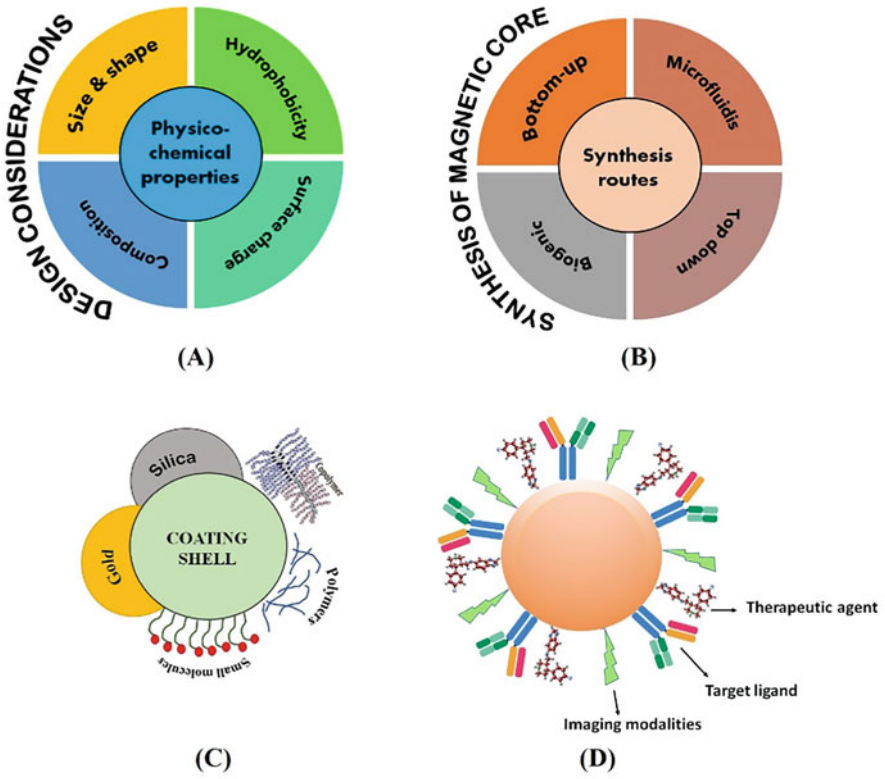

Fig. 15.1 Synthesis and functionalization of theranostics nanoparticles, (**a**) design considerations; (**b**) manufacturing techniques of magnetic core; (**c**) surface modifications with metallic and non-metallic materials, (**d**) targeted delivery of nanoparticles via functionalized surface

techniques (Materón et al. 2021). Other important design strategies are aimed on developing a combination of MNPs and AuNPs and advanced engineered MNPs for fabrication of shells/containers (Gauger et al. 2020).

Regardless of the progress made so far, techniques like aqueous phase synthesis conferred certain limitations specifically in getting homogenous size distribution, desired surface morphology, and magnetic performance of MNPs. Thus, it is need of the hour to develop more sensitive medical detection devices/diagnostic agents having high accuracy, multi-modality probes. Such sensitive diagnostics can be fabricated by coupling Fe_3O_4 MNPs with other nanocarriers having different functionalities, which is a challenging task to achieve via aqueous phase methods (Wei et al. 2012). Fabrication of MNPs through organic phase reactions can address the aforementioned challenges. However, stability issues of MNPs manufactured by this technique occur ascribing to nonpolar hydrocarbon solvent or a weakly polar ether solvent and can be stabilized by addition of a long chain bipolar surfactant such as oleic acid and oleylamine. They act by passivation through COO and/or -NH_2-bonding to the MNP surface and coat the particles with thick hydrocarbon coating that provides stability. Further these coated MNPs can be specifically catered to exhibit desired bio-circulation, bio-distribution, and bio-elimination properties (Tartaj et al. 2003).

15.2.1 Superparamagnetic Iron Oxide Nanoparticles (SPIONs)

SPIONs have attained huge considerations owing to their unique properties such as biodegradability and biocompatibility which make them suitable platforms for developing the theranostics agents. Such magnetic cores are bestowed with the ability to cater theranostics agents according to the specific need of mapping and guiding them to the specific site of disease (Mahmoudi et al. 2011). One of the latest advances in the field of SPIONs works on microfluidic reactors which are comprised of generation of supermoments (i.e. desired magnetic moment) due to intraparticle spin net magnetic interactions. Such particles have been extensively employed for the fabrication of MNPs that hold suitable physicochemical properties required for biomedical applications (Mosayebi et al. 2017). Further, MNPs obtained through this method have intermediate strength of magnetic interactions, narrow size distribution, and randomness in positions of particles. However, if the concentration is enhanced further, the inter-particle interactions become more stronger and can result in formation of ferromagnetic domain state. These ferromagnetic-like correlations will occur between the supermoments of the nanoparticles as well as between the atomic moments within the particles (Bedanta et al. 2013).

15.2.2 Coated MNPs

The MNPs are quite unstable in nature; thus coating their surface is a prerequisite to stabilize them. The coating of surface in the colloidal state not only provides

stabilization but also increases their interactions with the biological system. The surface modification can be carried out through organic/inorganic coating materials such as silica, gold, small protein/peptide molecules, aptamers, and synthetic and block copolymers/polymers. However, literature have evidenced the use of polysaccharides, and synthetic polymers, lipids, for coating owing to the biocompatible nature of aforesaid (Demirer et al. 2015).

15.2.3 MNPs with Improved Magnetic Response

The improvement in magnetic power/magnitude basically focusses on varying a magnetic phase composition. A suggestively increased magnetic response in respect of MRI and hyperthermia can be accomplished by merging soft magnetic phase ($MnFe_2O_4$ NPs) and hard magnetic phase ($CoFe_2O_4$ NPs) in bi-magnetic NP clusters, further stabilized through a biocompatible polymer such as sodium dodecyl sulfate (Gauger et al. 2020). In the same context, Fetiveau et al. formulated paramagnetic ultrasmall NPs of $[Fe^{3+}(Fe^{2+}(CN)_6)]$ (Prussian blue) decorated with $Gd3^+$ ions. The exterior surface of $Gd3^+$ ion-decorated MNPs exhibited excellent longitudinal relativities of more than 40 mM^{-1} s^{-1} along with a significantly higher photothermal effect (Fétiveau et al. 2019). In addition, to improve photothermal effect, a deeper, clearer photoacoustic imaging of tumors was seen. These MNPs formulated with M = Mn, Fe, Co, or Ni (formula MFe_2O_4,) were found approximately twofold more efficient than Fe_3O_4 NPs for MRI applications (Mohapatra et al. 2013). Concomitantly, when $MnFe_2O_4$ NPs, conjugated with antibodies such as herceptin (cancer-targeting moieties), resulted in a very precise and sensitive detection of HER2/neu cancer cells in vivo that aids in efficient diagnosis. Recent publications have suggested that the addition of zinc to $MnFe_2O_4$ NPs holds even superior diagnostic efficiency (Cohen and Shoushan 2013).

15.2.4 Combination of Magnetic and Au NPs

Janus particles are a kind of NPs that hold two distinctive surface properties. They are fabricated with trioctahedral magnetite NPs and AuNPs coated with polymer-L-lysine (Su et al. 2019). These MNPs yielded a single nanoprobe bestowed with considerable in vivo stability and capability for efficient photothermal tumor ablation ascribing to gold along with superior MRI contrast attributes credited to the polymer layer (Gauger et al. 2020). Thus, such MNPs having variable component offer multiple roles credited to multicomponent nature that have been utilized for theranostics applications (Mourdikoudis et al. 2021). Another class of such MNPs consists of Au-Fe_3O_4 NPs prepared through coating of $Fe(CO)_5$ over the surface of the AuNPs followed by oxidation.

The AuNPs can be formulated in two ways, firstly in situ through injecting chloroauric acid (trichlorogold, $H[AuCl_4]\cdot H_2O$) solution into the reaction mixture and secondly can be pre-formulated in the presence of oleylamine. Lately literatures

have reported the formulation of AuNPs via reduction of $HAuCl_4 \cdot 3H_2O$ employing TBAB (tert-butylamine borane complex) in the presence of tetralin (1,2,3,4-tetrahydronaphthalene) and oleylamine. Another method reported for synthesis of Au-Fe_3O_4 NPs is via injecting $Fe(CO)_5$ into 1-octadecene solution containing the AuNPs where Fe nucleates and grows onto Au NPs. Further on the exposure with air, the oxidation of Fe NPs occurs, yielding Fe_3O_4 NPs, resulting in the formation of Au-Fe_3O_4 NPs. The advantage of this technique is the control the over size through adjustment of $Fe(CO)_5$ and Au ratio (Peng et al. 2008).

15.2.5 Monodisperse MNPs

Fe-loaded MNPs carry high magnetizations and are considered as important agents for sensitive and specific diagnosis. However, metallic FeNPs are chemically unstable ascribing to prompt oxidation to iron oxide NPs that hold low magnetization amplitude. Consequently, to prevent the MNPS from oxidation and to ensure their stability are the biggest challenges during synthesis of MNPs (Ho et al. 2011). Literatures have reported formulation of monodispersed MNPs employing various precursors through thermal decomposition methodology. One such investigation was executed by Hyeon et al. where $Fe(CO)_5$ was employed as a precursor for synthesizing mono-dispersed g-Fe_2O_3 NPs with magnetic core. The optimization of particle size was done by varying the molar ratio of Fe $(CO)_5$:oleic acid that yields particles in a size range of 4–11 nm (Hyeon 2003). Taking into consideration the above approach, Sun et al. were the first to prepare Fe_3O_4 NPs through pyrolyzation of Tris(acetylacetonato) (Fe)(III)$(acac)_3$)] in the presence of oleylamine, oleic acid, and 1,2-hexadecanediol. The research group also synthesized metal-doped iron oxide nanocrystals in a size range of 3–20 nm holding magnetic core using the same approach (Cohen and Shoushan 2013).

The surface modification of MNPs with suitable polymers/targeting moieties is required for numeral reasons such as stability, targeting specificity, and size that can be implicated for various biomedical applications such as cancer therapy, brain targeting, and imaging (Tripathi et al. 2020). One of such applications includes immunoassays where MNPs are surface decorated through immobilizing proteins, enzymes, and antibodies that act as targeting ligands holding selectivity for particular biomolecules/overexpressed factor of concern. Employing the same approach, Yang et al. formulated biofunctionalized dextran-coated Fe_3O_4 NPs decorated with targeting moieties having binding specificity to CD-34 (anti-CD-34) on stem cells, CK-MB infarcted myocardium (anti-CK-MB), and troponin I infarcted myocardium (anti-troponin I). These NPs act as a biovector for delivery of stem cell to the infarcted myocardium. The successful surface engineered MNPs can be achieved through maintaining a perfect balance between intermolecular forces responsible for interaction and the outermost layer of the magnetic NPs (Frimpong and Hilt 2010). A schematic representation of common functionalization methods is shown in Fig. 15.2.

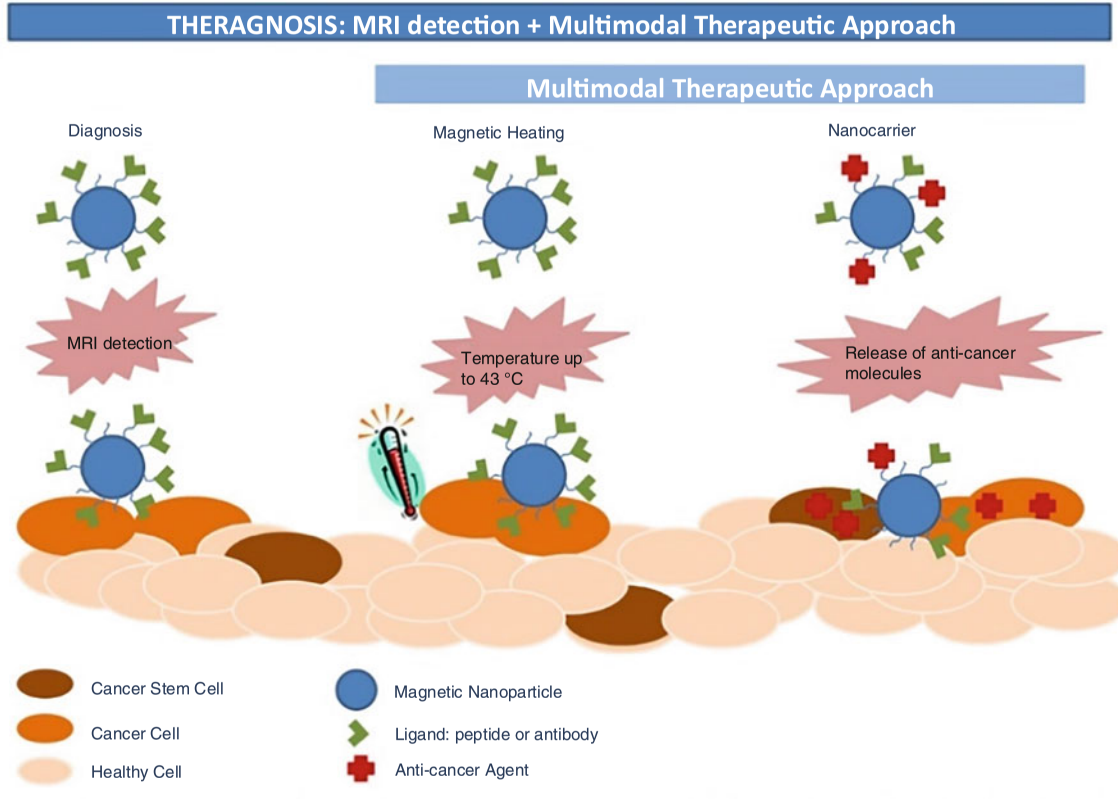

Fig. 15.2 Representation of theranostics applications of MNPs [reproduced with permission (Gobbo et al. 2015)]

15.2.6 Controlling Surface Functionality Via Surface-Initiated Polymerization

The surface engineering of MNPs can be executed employing a variety of ligands/polymers, but they result in an overall reduction in magnetization (Bohara et al. 2016). This reduction in magnetization on surface engineering is the major limitation ascribing to a thick shell coating that significantly impedes with the magnetic attributes of the core material. The limitation can be conquered by using surface-initiated polymerization techniques that involve layering of NP surface with an initiator-bearing ligand followed by initiating the polymerization from the surface, where the initiator can be attached to the surface of NPs via in situ reactions or through ligand exchange. Surface functionalization through initiators makes them macroinitiators that aid in the polymerization while offering a complementary advantage of optimizing and maintaining the anchoring functionality of NPs to the desired entities as well as magnetization. The commonly employed techniques for surface-initiated polymerization of surface engineered MNPs comprise ring-opening polymerization, nitroxide-mediated polymerization, reversible addition-fragmentation chain-transfer polymerization, and ATRP (atom-transfer radical polymerization) (Frimpong and Hilt 2010).

15.3 Applications of Theragnostic Nanoparticles

15.3.1 Applications in Cancer

Cancer is a fatal disease and has numerous challenges, viz., MDR, off-targeting, dose-dependent adverse effects, lack of early diagnosis, and many more, that lead to unsatisfactory clinical outcome. Thus, it is need of the hour to develop the effective drug delivery system that can address the abovementioned challenges (Jain and Jain 2016; Pathak and Jain 2023). The literature has evidenced the potential of MNPs as theranostics that can counter the cancer therapy-associated challenges (Materón et al. 2021). The developed MNPs should hold the attributes to identify the changes at the molecular level (that occurs due to cancer), diagnose and clearly depict the targeted cancer, specifically deliver the payload at tumor vicinity, and can be easily monitored for tracing the accumulation and the therapeutic efficacy (Gobbo et al. 2015).

Various pieces of literature have reported the capability of gold NPs and quantum dots for detection of nearly hundreds of cancer biomarkers in blood assays and tissue biopsies. Nevertheless, they were found to be unsuitable for human in vivo diagnostics applications owing to toxicity and other safety issues. On the contrary, MNPs have been extensively employed in clinical diagnosis as contrast agent in MRI (magnetic resonance imaging) technique. Among various types of MNPs, the most popular are ferromagnetic iron oxide NPs and ultrasmall superparamagnetic iron oxide NPs that have widely utilized in clinical imaging owing to formation of a sharper and brighter image. These MNPs are bestowed with superparamagnetic properties and can change the spin-spin relaxation time of the nearby aqua

molecules, resulting in detection of altered gene expression and tumors and, thus, cancer/other diseases (Jia et al. 2017). Concomitantly, they can be utilized for active/passive targeting to deliver high payload at desired site making them suitable for in vivo theranostics application (Nicolás et al. 2013). Further they carry several favorable attributes such as modified drug release, facile surface modification, non-toxicity, non-immunogenicity, reduced adverse effect, and superior therapeutic efficacy that make them a preferrable drug cargo over other NPs. Additionally, these drug-loaded magnetic cargos can be guided to the anticipated target site employing an external magnetic field to deliver the payloads, parallelly tracking the biodistribution alteration/pattern in vivo (Gl et al. 2012; Guardia et al. 2012; Das et al. 2010).

The surface engineering/coating of MNPs with polymers like dextran and polyethylene glycol makes them more stable and provides linker for binding of anticancer drugs/antibodies/ targeting ligands, to their surface (Mukerjee and Vishwanatha 2009). On the other hand, surfactants and polymers also facilitate opsonization (increasing circulation half-life) and enhance biocompatibility (Feng et al. 2013). One important application of MNPs is magnetofection (Fig. 15.2) which comprises the employment of an external magnetic field to accumulate and retain MNPs at the specific area and has been practiced most often to improve site-specific therapy (Plank et al. 2011). However, this technique is not appropriate for non-accessible tumors. However, the non-specific internalization by the cells is the major limitations encountered by MNPs during transit before reaching the desired site (Moore et al. 2000; Mikhaylova et al. 2004).

Recently MNPs have been recognized for their application in a new technique, namely, nano-cryosurgery, proposed for improving freezing efficiency of the conventional cryosurgery (utilizing artificially generated extreme cold conditions to destroy cancer cells and abnormal tissue), although is in very initial stages. The external application of magnetic field is an alternative practice to treat cancer with magnetic NPs. This therapy involves magnetic heating at marginally low intensity (invasive) utilizing superparamagnetic iron oxide MNPs (heat generating particles) specifically at the tumor vicinity (Fig. 15.2). Till date, various types of cancers such as brain, breast, prostate, and liver have been treated using this technique (Dilnawaz et al. 2010). The most noteworthy benefit of this approach is that MNPs can be injected directly into the tumor vicinity where they showed a significantly longer retention available for repeated therapeutic session. Recently, gene therapy has attained attention as an alternative treatment for numerous diseases including cancer ascribing to potential to selectively target the apoptotic/anti-apoptotic genes directly, resulting in regulating the altered gene expression responsible for carcinogenesis (Plank et al. 2011). In the same context, Gorgannezhad et al. fabricated RNA-based graphene oxide-loaded MNPs for detection of FGFR2:FAM76A fusion gene (Gorgannezhad et al. 2018). The results demonstrated a very high sensitivity for detection as low as 1.0 fM and great specificity and reproducibility as supported by qRT-PCR analysis.

15.3.2 Theranostics Applications of Magnetic Nanoparticles in Neurodegenerative Disorders

Theranostics MNPs have been recognized for their aptitude to deliver the drugs across the blood-brain barrier (BBB) for the therapy of CNS (central nervous system)-associated disorders such as neurodegenerative disorder, viz., Alzheimer's disease, Parkinson's disease, epilepsy, and Huntington's disease (Fig. 15.3). Neurodegenerative disorders denote conditions that are inconsistent and hereditary characterized by the gradual loss of perception in thinking activities, memory loss, and loss of skilled movements. Currently used various types of NPs composed of lipids, polymers, carbon, or metals hold the potential for addressing the limitations offered by conventional drug delivery systems (Moore et al. 2000; Ramanathan et al. 2018).

The available theranostics techniques comprise of MRI (magnetic resonance imaging), PET (positron-emission tomography), and CT (single-photon emission computed tomography). As theranostics agents MNPs can encase various drugs/protein molecules and genes and, thus, are employed as successful drug delivery systems (Mikhaylova et al. 2004). Further specific endogenous BBB transporters (amino acids, peptides, glucose, insulin, and proteins) are surface functionalized over these MNPs that facilitate permeation across the BBB.

The mechanism involves carrier-mediated transcytosis that can deliver therapeutics/diagnostic utilizing nutrients such as amino acids, purine bases, and glucose. These nutrients act as substrate-selective inhibitors and can deliver the therapeutics/diagnostic which imitate endogenous carrier substrates. On the other hand, transportation of large molecules (lipoprotein and antibodies) is achieved through the receptor−/adsorptive-mediated endocytosis that involves binding of receptor-specific ligand (Ramanathan et al. 2018). These MNPs can be delivered either through nasal/olfactory bulb or through sensory nerve endings that present at the airway epithelia (Kosari and Vafai 2021). Taking this approach into consideration, Qiao et al. developed lactoferrin-modified PEG-coated Fe_3O_4 nanoparticle for brain delivery (Qiao et al. 2012). The results demonstrated significantly enhanced potential of NPs to cross BBB (in vitro in porcine BBB model and in vivo in animal model) via receptor-mediated transcytosis mechanism when compared with PEG-coated Fe_3O_4 NPs.

15.3.3 Treatment of Secondary Interventions and Infectious Diseases

Literatures have evidenced the tumoricidal potential of MNPs in terms of reduced tumor volume and number (Dennis et al. 2009). Concomitantly, the potential can be further enhanced by coupling magnetic hyperthermia with classical therapeutic/immunotherapy (CD3+, CD4+, CD8+, and natural killer) that results in improved clinical outcome. In this system MNPs stimulate a host immune response against cancer cells concomitantly generating a localized thermo-ablative effect leading to

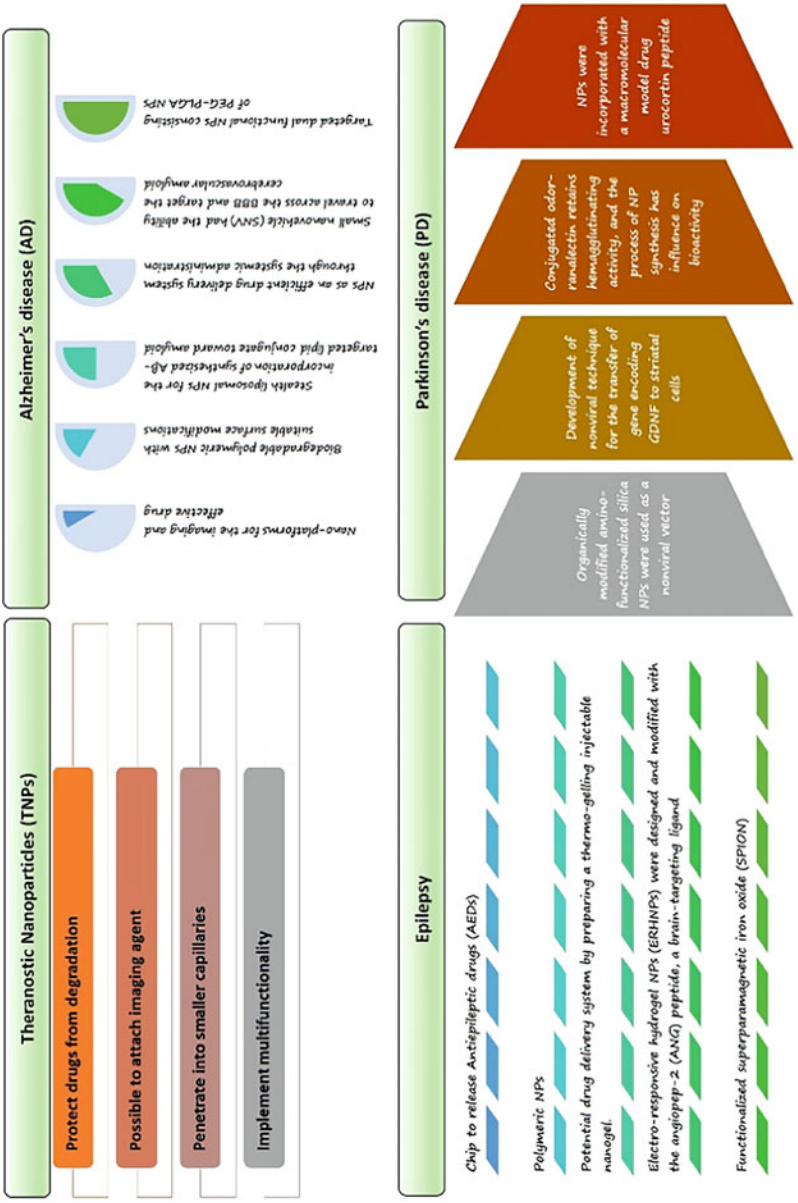

Fig. 15.3 Theranostics nanoparticles' functions and application in the treatment of neurodegenerative disorders

partial/complete tumor regression (Pan et al. 2020). Likewise, MNPs can be conjugated with immunotherapeutic agents such as IL-2 (interleukin-2) and GM-CSF (granulocyte macrophage colony stimulating factor). These surface engineered MNPs have shown substantial degeneration of melanoma tumors in animal models (Nicolás et al. 2013). On the another hand studies have reported that, such MNPs are found to be promising therapy for infectious diseases.

The hyperthermia generated because of MNPs results in physical destruction of pathogenic organisms, even in drug-resistance pathogen. Iron oxide MNPs can effectively eradicate bacterial biofilms in vitro. Additionally, literatures have suggested the efficiency of magnetic hyperthermia (20–60 mg/ml SPN solutions) in disrupting the membrane integrity of *P. aeruginosa* biofilms in vitro (Rodrigues et al. 2013). Further, if some of the bacteria survive of magnetic hyperthermia, they get killed by exposure of bactericidal that has been encased into MNPs leading to successful destruction and improved therapeutic efficacy. Another study reported significant elimination of *P. aeruginosa* biofilms from 8.68 to 2.58 CFUs when subjected to thermal shocks in a temperature range of 50 °C and 80 °C in vitro. Parallel to this study, Singh and Bahadur formulated magnetite-zinc oxide MNPs and evaluated their bactericidal potential against various bacteria. The results of the experiment revealed significant bactericidal activity of MNPs against a variety of bacteria suggesting their potential application in infectious diseases (Singh et al. 2015).

15.3.4 MNPs and Targeted Drug Delivery

MNPs have been widely utilized for biomedical applications specifically for diagnosis and treatment and as a drug carrier for targeted drug delivery. Further, the theranostics potential of MNPs can be enhanced by their surface functionalization with a variety of organic and inorganic materials that facilitate stealth effect preventing from RES uptake, long circulation half-life, specific targeting, and reducing toxicity. Further, the surface can be decorated with pH- or temperature-sensitive materials/polymers to optimize drug loading and get modified drug release. All these lead to enhanced therapeutic efficacy, specific targeting, high payload at desired site and low off-targets, superior internalization, and reduced dose-dependent toxicity (Mou et al. 2015). The loading/conjugation of therapeutic cells, proteins, and nucleic acids further enhances targeting diagnostic and therapeutic potential of MNPs and thus employed for biotherapeutics (Mok and Zhang 2013).

Recently, biotherapeutics involving therapeutic cells, proteins, and genes has been extensively studied and is under consideration as an alternative therapy for various diseases. Regardless of substantial target specificity and minimal adverse effects, clinical translation of biotherapeutics has been compromised ascribing to inadequate stability and poor delivery proficiency when compared with drugs of chemical origin. These limitations can be successfully addressed through SPION-based drug delivery systems owing to remarkable biocompatibility and excellent paramagnetism that facilitates long-term retention and payload at the target sites

achieved through suitable magnet. Furthermore, technologies that yield altered, homogeneous SPIONs are employed that can promise the possibility of successful clinical translation (Chomoucka et al. 2010). In the same context, microparticles loaded with chemotherapeutic agents containing iron oxide NPs designated as NIMs (nano-in-microparticles) were effectively employed for magnetic-derived delivery to the lungs under influence of an applied magnetic field. The experiment involves endotracheal administration of fluorescently labeled NIMs to mice as a dry powder under influence of an external magnet positioned over one lung. The result revealed doxorubicin-loaded NIMs under the magnetic activated lung showed improved therapeutic efficacy and targeted delivery with greater payload at desired site (Price et al. 2017).

15.3.4.1 Modification for Tumor Targeting

Surface engineering of core-shell iron oxide NP-polymer with folic acid facilitates improved targeting and cellular uptake into tumor cells through folate receptor-mediated endocytosis (Huang et al. 2016; Roy et al. 2016; Gholibegloo et al. 2019). On the other hand, decoration with lysine and cysteine derivatives improves tumor cell ligand binding and targeting as well as enhanced internalization. Another example states utilization of tissue-type plasminogen activator peptide for surface modification of MNPs for targeting pancreatic cancer cells (Dobiasch et al. 2016).

15.3.4.2 Therapeutic Viruses

Literatures have reported the application and potential of therapeutic viruses for cancer therapy among which adenovirus is regarded as extremely effective gene delivery systems. However, the viruses have limited therapeutic application and are still under development owing to challenges such as non-specific immune responses and poor transfection efficiency that don't make them clinically viable. The MNP conjugation of adenoviral particles to the MNP surface via electrostatic interactions can shield the virus from getting inactivated through the immune cells credited to MNP stealth effect. Concomitantly, in the application of an external magnetic field, the virus-conjugated MNPs can be specifically targeted to the desired site resulting in improved gene transfection efficiency coined as magnetofection (Cho et al. 2018).

Recently magnetosomes commonly known as biomimetic magnetic microparticles have attained attention in specific gene delivery. Magnetosomes are synthesized through MNC (magnetic nanocluster) core azide-membrane engineering and electrostatic assembly followed by surface engineering through RGD peptide (arginine-glycine-aspartic) macrophage shell (Wang et al. 2013). These magnetosomes showed successful delivery of siRNA with superior stealth effect, RGD targeting, clearer MRI imaging and magnetic accumulation through an external magnetic field, and significant cytoplasm trafficking (Zhang et al. 2018). The result also revealed three- to fourfold higher transfection in adenovirus-conjugated magnetosome-exposed NIG-3 T3 cells (in vivo) when compared with SPN-deficient functionalized adenovirus particles (Saatchi et al. 2017; Sun et al. 2018).

15.3.4.3 Nucleic Acid and Protein Delivery

The therapies based on nucleic acid/protein molecules such as (siRNA) are drastically dependent on the cellular internalization/cellular uptake. Further, taking into consideration the concerns of safety, stability, economic viability, and methodology, nonviral vectors, such as MNPs, are found to be suitable as delivery wagons (Lee et al. 2009). Additionally, application of external magnetic fields can alter the accumulation and retention of SPNs in nucleic acid-based therapies. This alteration facilitates and improves the potential of loaded molecules in recommitting the down-regulation/up-regulation of gene toward normal levels (Lee et al. 2017). Applying this approach, Wang et al. formulated polyethylenimine-coated MNPs conjugated with lipofectamines and plasmid DNA. The short-hairpin RNA was used as targeting moiety that holds a good affinity toward IGF-1R (type 1 insulin-like growth factor receptor). The results demonstrated a two-fold increased gene suppression rate subsequent to in vivo magnetofection over a 72 h incubation period (Wang et al. 2018).

Concomitantly, the SPNs exhibited improved delivery of nucleic acid-based vaccines suggesting potential of SPNs as vectors. One such experiment demonstrated the potential of SPNs where nucleic acid base vaccine-conjugated NPs were formulated. These conjugated SPNs were encoded for MSP119 surface protein (Plasmodium yoelii merozoite). The SPNs showed transfection in African green monkey kidney cells at significantly higher frequency when compared with chemical-based methods in vitro (Al-Deen et al. 2011; Dziegiel 2016).

In continuation with above experiment, Nan et al. designed an improved experiment to further improve the transfection of MSP119 along with dendritic cell maturation. The results revealed that the MSP119 protein-loaded MNPs hold the potential to confer malaria resistance and can aid in prevention of such infectious diseases as well as offer improved therapy (Nan et al. 2017). Also, they can efficiently deliver the therapeutic proteins at target sites with improved accumulation and retention (Ge et al. 2017). Another study by Niemirowicz et al. demonstrated conjugation of CLL-37 (cathelicidin LL-3, inhibit proliferation in cancer cell) to MNPs that results in considerable depression in viability in DLD-1 and HT-29 cells (colon cancer cells) and superior apoptosis when compared with free CLL-37 exposed cells (Niemirowicz et al. 2017). Likewise, chlorotoxin-coated SPNs have shown a significant inhibitory potential up to 98% in glioma cells when a comparison was executed with free chlorotoxin that showed only 48% inhibition and reduction in cellular proliferation. The results of above studies give auxiliary evidence of MNPs in significantly improved delivery of therapeutic peptides (Veiseh et al. 2009).

15.3.4.4 Cell-Based Therapies

Stem cell- and immune cell-based therapies have been investigated since long for the therapy of critical conditions, such as ischemia. However, their application is limited owing to poor cell retention and stability. However, MNPs have been reported to address the issues. The MNPs can be conjugated over the surface of stem cells and immune cells to improve stability and retention resulting in an overall improved

gene transfection (Riemer et al. 2004). In the same context, Kyrtatos et al. established effective delivery of $CD133^+$ endothelial progenitor cells at the sites of vascular injury in carotid artery injury animal models. Concurrently, the application of an external magnetic field facilitated accumulation of MNPs at catheterization sites resulting in a 5.4-fold enhanced $CD133^+$ cell engraftment. Also, a marked reduction in restenosis and fewer scar tissue formation were observed (Kyrtatos et al. 2009). Another experiment executed by Cheng et al. showed the improved therapeutic potential of MNP-conjugated cardiospheres. The MNP-conjugated cardiospheres exhibited fourfold higher cell retention over a period of 24 h and 3 weeks, respectively (short-term and long-term studies), subsequent to parenteral administration into the left ventricular cavity of female Wistar Kyoto rats (Cheng et al. 2012).

15.3.5 Targeted Magnetic Nanoparticle for Multimodal Diagnostics

Owing to one or more limitations, individual imaging modality has limited applications. Thus, the development of a multimodal combination of imaging agent/modalities, each with its own advantages and properties, may offer more precise and specific diagnostic information of a particular disorder. Recently MNPs are employed in MRI credited to non-invasive imaging technique that has substantial permeation potential in live tissues/organs with an excellent 3D/4D resolution (Thomas et al. 2013; Tomitaka et al. 2019). The concept of multimodal contrast agent based on SPION is shown in Fig. 15.4.

15.3.6 Imaging

Iron oxide NPs (MIONPs) are the most preferred applicant for polysaccharide coatings. Recently, FDA has approved dextran-coated iron oxide NPs as MRI contrast agent owing to excellent spatial resolution, long circulation half-life, and substantial penetration into tissues without any damage to tissue under magnetic field, i.e., safe for human use. The human body naturally lacks any magnetic moment, i.e., nearly zero, but when magnetic field is applied externally, it showed significant magnetic moment. The MRI is executed over a period of 40 min subsequent to MIONP infusion. The MIONPs afterward get eliminated out of the body through phagocytosis indicating non-toxicity aspect of the same. Sensitive and delicate vascular structures such as cardiac cavities/chambers, aorta, and pulmonary arteries can be seamlessly imaged through MRI using MIONPs. These studies prove the MIONP potential for sensitive imaging through MRI as well as MRI-guided delivery of drugs/genes (Byrne et al. 2008).

15.3.6.1 Photoacoustic Imaging

Photoacoustic imaging (PAI) is a relatively innovative imaging sensory system that works with an amalgamation of optical laser excitation and ultrasound imaging.

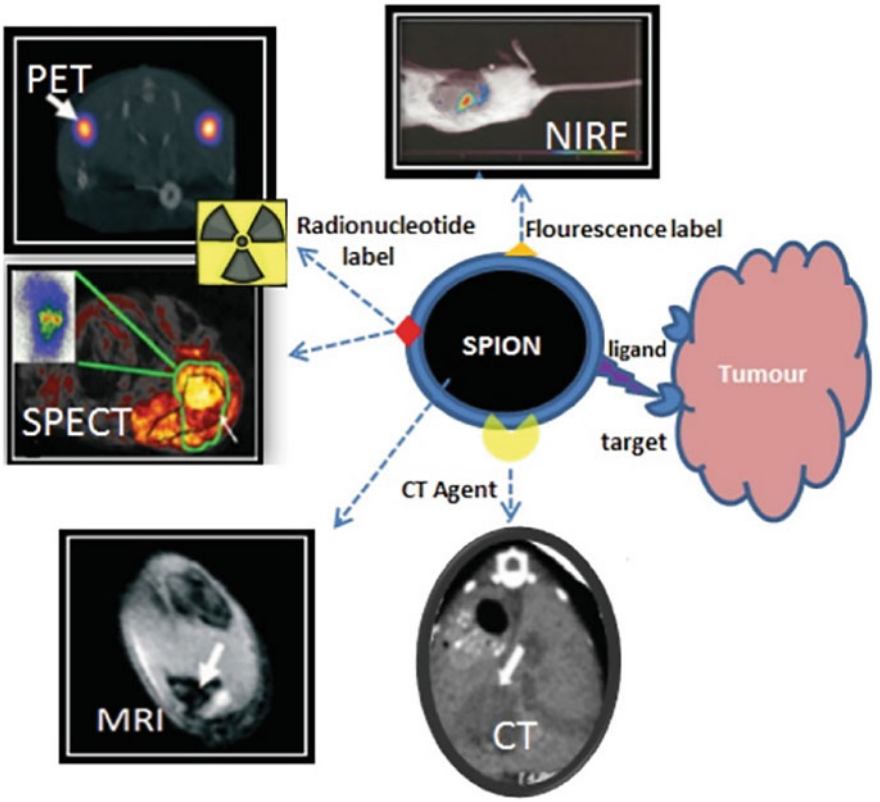

Fig. 15.4 Concept of multimodal contrast agent based on SPION. Reproduced with permission (Thomas et al. 2013)

Further, it can be combined with other imaging methods to formulate a multimodality bestowed with superior imaging accuracy. An experiment executed by Fetiveau et al. evidenced a strong intratumoral PAI signal consequent to irradiation at ~808 nm through gadolinium-conjugated $Fe^{3+}[Fe^{2+}(CN)_6]$ NPs (Fétiveau et al. 2019). Likewise, Lu et al. fabricated dendrimer-steadied nanoflowers employing Au and ultrasmall iron oxide NPs. Further, the prepared nanoflowers were made for multimodality approach combining with PAI, MRI, and computer tomography (Lu et al. 2018). The multimodal nanoflower showed a high relaxivity value of 3.22 mM^{-1} s^{-1} and 82.7% PCE (photothermal conversion efficiency), evidencing their application as theranostics that hold translational possibility for clinical applications.

15.3.6.2 Fluorescence Imaging

A combination of fluorescence imaging and MRI has gained success in the field of diagnosis (Vijayan et al. 2019). The fluorescence imaging is executed accompanying

MRI through fluorescent-labeled polymer (Etrych et al. 2019). It involves Fe_2O_3 NP functionalization with fluorescent dye-labeled polymer that yields a nanoprobe. This nanoprobe is competent in imaging and quantification of pancreatic beta cell mass that can be utilized in diagnosis/indication of type 2 diabetes onset (Xin et al. 2020). The presence of acidic environment at the site of beta pancreatic cells results in pH-assisted release of dye generating fluorescence, and finally beta cell detection and quantification (based on intensity) via confocal microscopy are achieved. Additionally, the nanoprobe presented strong inhibition of the toxic accumulation of human islet amyloid polypeptide that is culprit behind beta cell degeneration in type 2 diabetes.

15.4 Recent Medicinal Applications of Gold-Coated Iron Oxide Nanoparticles

Recently the research has focused on probability of biomedical application of gold MNPs in terms of prerequisite properties, viz., biocompatibility, targeting efficiency, precise imaging as diagnostics, and binding specificity with biological factors as well as coating materials. Currently their use as a magnetic carrier has been explored for drug targeting. Kayal and Ramanujan fabricated and evaluated doxorubicin-loaded Au-Fe_3O_4 (gold-coated iron oxide) MNPs (Kayal and Ramanujan 2010). The prepared gold-coated MNPs were exposed to magnetic fields of variable intensity (increasing order of strength). The experimental result revealed a substantial proportion of accumulation of MNPs within the magnetic fields, suggesting their drug delivery potential. The other application of $AuFe_3O_4$ has been recognized in photothermal therapy. Bhana et al. executed the cytotoxicity evaluation of core-shell system against breast cancer and head and neck cancer cell lines (SK-BR-3 and KB-3-1, respectively). The results demonstrated about 36% cell death in both KB-3-1 and SK-BR-3 cell lines on exposure to a combination of photothermal and photodynamic therapy, compared with each modality individually (Anderson et al. 2019).

Photothermal therapy is another effective therapy used for theranostics, in which gold NPs are coated with molecules like PEG, followed by irradiation with a laser, in a wavelength range matching with UV-vis λ-max of the AuNPs. This brings about high vibration in the AuNPs generating heat resulting in death of the adjacent tissue, while the superparamagnetic core facilitates precise targeting leading to satisfactory therapeutic outcome. Likewise, Kirui et al. formulated gold hybrid NPs and evaluated them over colorectal cancer SW1222 cells. The result showed significant decline in viability of SW1222 cells credited to photothermal effect that fastens cellular apoptosis (Kirui et al. 2010).

15.5 Conclusion

MNPs have been recognized as suitable wagons for targeting and multimodal imaging, i.e., theranostics. They are bestowed with numerous merits such as biocompatibility, stability, high-resolution imaging, precise binding and targeting, significant penetration into tissues, etc. The most frequent application of MNPs is in the field of cancer therapy as theranostics, neurodegenerative diseases as delivery wagons competent of crossing BBB, and treating resistant bacterial infection. However, despite abovementioned advantages, MNPs have limited clinical use, and only a few could make it to clinical trials. Thus, continuous research is going on in improving the reproducibility, reliability, and safety (in terms of toxicity) of MNPs for scale-up and clinical translation. Working on this line of thought and research idea, we believe that MNP-based theranostics will endure to flourish and will take up the MNPs from bench to bedside.

References

Al-Deen FN, Ho J, Selomulya C, Ma C, Coppel R (2011) Superparamagnetic nanoparticles for effective delivery of malaria DNA vaccine. Langmuir 27(7):3703–3712

Anderson SD, Gwenin VV, Gwenin CD (2019) Magnetic functionalized nanoparticles for biomedical, drug delivery and imaging applications. Nanoscale Res Lett 14(1):1–16

Bedanta S, Barman A, Kleemann W, Petracic O, Seki T (2013) Magnetic nanoparticles: a subject for both fundamental research and applications. J Nanomater 2013:1

Bilal M, Zhao Y, Rasheed T, Iqbal HM (2018) Magnetic nanoparticles as versatile carriers for enzymes immobilization: a review. Int J Biol Macromol 120:2530–2544

Bohara RA, Thorat ND, Pawar SH (2016) Role of functionalization: strategies to explore potential nano-bio applications of magnetic nanoparticles. RSC Adv 6(50):43989–44012

Byrne JD, Betancourt T, Brannon-Peppas L (2008) Active targeting schemes for nanoparticle systems in cancer therapeutics. Adv Drug Deliv Rev 60(15):1615–1626

Cheng E, Vaisica JA, Ou J, Baryshnikova A, Lu Y, Roth FP et al (2012) Genome rearrangements caused by depletion of essential DNA replication proteins in Saccharomyces cerevisiae. Genetics 192(1):147–160

Cho H-Y, Lee T, Yoon J, Han Z, Rabie H, Lee K-B et al (2018) Magnetic oleosome as a functional lipophilic drug carrier for cancer therapy. ACS Appl Mater Interfaces 10(11):9301–9309

Chomoucka J, Drbohlavova J, Huska D, Adam V, Kizek R, Hubalek J (2010) Magnetic nanoparticles and targeted drug delivering. Pharmacol Res 62(2):144–149

Cohen Y, Shoushan SY (2013) Magnetic nanoparticles-based diagnostics and theranostics. Curr Opin Biotechnol 24(4):672–681

Das RK, Kasoju N, Bora U (2010) Encapsulation of curcumin in alginate-chitosan-pluronic composite nanoparticles for delivery to cancer cells. Nanomedicine 6(1):153–160

Demirer GS, Okur AC, Kizilel S (2015) Synthesis and design of biologically inspired biocompatible iron oxide nanoparticles for biomedical applications. J Mater Chem B 3(40):7831–7849

Dennis C, Jackson A, Borchers J, Hoopes P, Strawbridge R, Foreman A et al (2009) Nearly complete regression of tumors via collective behavior of magnetic nanoparticles in hyperthermia. Nanotechnology 20(39):395103

Dilnawaz F, Singh A, Mohanty C, Sahoo SK (2010) Dual drug loaded superparamagnetic iron oxide nanoparticles for targeted cancer therapy. Biomaterials 31(13):3694–3706

Dobiasch S, Szanyi S, Kjaev A, Werner J, Strauss A, Weis C et al (2016) Synthesis and functionalization of protease-activated nanoparticles with tissue plasminogen activator peptides as targeting moiety and diagnostic tool for pancreatic cancer. J Nanobiotechnol 14(1):1–18

Dziegiel N (2016) Nanoparticles as a tool for transfection and transgenesis-a review. Ann Anim Sci 16(1):53

Etrych T, Janoušková O, Chytil P (2019) Fluorescence imaging as a tool in preclinical evaluation of polymer-based nano-DDS systems intended for cancer treatment. Pharmaceutics 11(9):471

Feng R, Zhu W, Song Z, Zhao L, Zhai G (2013) Novel star-type methoxy-poly (ethylene glycol) (PEG)–poly (ε-caprolactone)(PCL) copolymeric nanoparticles for controlled release of curcumin. J Nanopart Res 15(6):1–12

Fétiveau L, Paul G, Nicolas-Boluda A, Volatron J, George R, Laurent S et al (2019) Tailored ultra-small Prussian blue-based nanoparticles for MRI imaging and combined photothermal/ photoacoustic theranostics. Chem Commun 55(98):14844–14847

Frimpong RA, Hilt JZ (2010) Magnetic nanoparticles in biomedicine: synthesis, functionalization and applications. Nanomedicine 5(9):1401–1414

Gauger AJ, Hershberger KK, Bronstein LM (2020) Theranostics based on magnetic nanoparticles and polymers: intelligent design for efficient diagnostics and therapy. Front Chem 8:561

Ge J, Zhang Y, Dong Z, Jia J, Zhu J, Miao X et al (2017) Initiation of targeted nanodrug delivery in vivo by a multifunctional magnetic implant. ACS Appl Mater Interfaces 9(24):20771–20778

Gholibegloo E, Mortezazadeh T, Salehian F, Forootanfar H, Firoozpour L, Foroumadi A et al (2019) Folic acid decorated magnetic nanosponge: an efficient nanosystem for targeted curcumin delivery and magnetic resonance imaging. J Colloid Interface Sci 556:128–139

Gl B, Di Corato R, Kolosnjaj-Tabi J, Dupuis V, Clément O, Gazeau F et al (2012) Ultra magnetic liposomes for MR imaging, targeting, and hyperthermia. Langmuir 28(32):11834–11842

Gobbo OL, Sjaastad K, Radomski MW, Volkov Y, Prina-Mello A (2015) Magnetic nanoparticles in cancer theranostics Theranostics 5(11):1249

Gorgannezhad L, Umer M, Kamal Masud M, Hossain MSA, Tanaka S, Yamauchi Y et al (2018) Detection of FGFR2: FAM76A fusion gene in circulating tumor RNA based on catalytic signal amplification of graphene oxide-loaded magnetic nanoparticles. Electroanalysis 30(10): 2293–2301

Guardia P, Di Corato R, Lartigue L, Wilhelm C, Espinosa A, Garcia-Hernandez M et al (2012) Water-soluble iron oxide nanocubes with high values of specific absorption rate for cancer cell hyperthermia treatment. ACS Nano 6(4):3080–3091

Gudovan D, Balaure PC, Eduard Mihaiescu D, Fudulu A, Radu M (2015) Functionalized magnetic nanoparticles for biomedical applications. Curr Pharm Des 21(42):6038–6054

Guo T, Lin M, Huang J, Zhou C, Tian W, Yu H et al (2018) The recent advances of magnetic nanoparticles in medicine. J Nanomater 2018:1

Ho D, Sun X, Sun S (2011) Monodisperse magnetic nanoparticles for theranostic applications. Acc Chem Res 44(10):875–882

Huang Y, Zhang B, Xie S, Yang B, Xu Q, Tan J (2016) Superparamagnetic iron oxide nanoparticles modified with tween 80 pass through the intact blood–brain barrier in rats under magnetic field. ACS Appl Mater Interfaces 8(18):11336–11341

Hyeon T (2003) Chemical synthesis of magnetic nanoparticles. Chem Commun 8:927–934

Jain K, Jain NK (2016) Chapter 8: Dendrimers as nanomedicine in cancer therapy. In: Dendrimers in nanomedicine. Pan Stanford Publishing Pte. Ltd., Singapore, pp 293–310

Jia F, Xu L, Yan W, Wu W, Yu Q, Tian X et al (2017) A magnetic relaxation switch aptasensor for the rapid detection of Pseudomonas aeruginosa using superparamagnetic nanoparticles. Microchim Acta 184(5):1539–1545

Kami D, Takeda S, Itakura Y, Gojo S, Watanabe M, Toyoda M (2011) Application of magnetic nanoparticles to gene delivery. Int J Mol Sci 12(6):3705–3722

Kayal S, Ramanujan R (2010) Doxorubicin loaded PVA coated iron oxide nanoparticles for targeted drug delivery. Mater Sci Eng C 30(3):484–490

Kirui DK, Rey DA, Batt CA (2010) Gold hybrid nanoparticles for targeted phototherapy and cancer imaging. Nanotechnology 21(10):105105

Kosari E, Vafai K (2021) Transport and dynamic analysis of magnetic nanoparticles in brain microvascular vessels. Phys Fluids 33(8):081907

Kyrtatos PG, Lehtolainen P, Junemann-Ramirez M, Garcia-Prieto A, Price AN, Martin JF et al (2009) Magnetic tagging increases delivery of circulating progenitors in vascular injury. J Am Coll Cardiol Intv 2(8):794–802

Lee JH, Lee K, Moon SH, Lee Y, Park TG, Cheon J (2009) All-in-one target-cell-specific magnetic nanoparticles for simultaneous molecular imaging and siRNA delivery. Angew Chem Int Ed 48(23):4174–4179

Lee K, David AE, Zhang J, Shin MC, Yang VC (2017) Enhanced accumulation of theranostic nanoparticles in brain tumor by external magnetic field mediated in situ clustering of magnetic nanoparticles. J Ind Eng Chem 54:389–397

Lu S, Li X, Zhang J, Peng C, Shen M, Shi X (2018) Dendrimer-stabilized gold nanoflowers embedded with ultrasmall iron oxide nanoparticles for multimode imaging–guided combination therapy of tumors. Adv Sci 5(12):1801612

Mahmoudi M, Sant S, Wang B, Laurent S, Sen T (2011) Superparamagnetic iron oxide nanoparticles (SPIONs): development, surface modification and applications in chemotherapy. Adv Drug Deliv Rev 63(1–2):24–46

Materón EM, Miyazaki CM, Carr O, Joshi N, Picciani PH, Dalmaschio CJ et al (2021) Magnetic nanoparticles in biomedical applications: a review. Appl Surf Sci Adv 6:100163

Mikhaylova M, Kim DK, Berry CC, Zagorodni A, Toprak M, Curtis AS et al (2004) BSA immobilization on amine-functionalized superparamagnetic iron oxide nanoparticles. Chem Mater 16(12):2344–2354

Mohapatra J, Mitra A, Bahadur D, Aslam M (2013) Surface controlled synthesis of MFe 2 O 4 (M= Mn, Fe, Co, Ni and Zn) nanoparticles and their magnetic characteristics. CrystEngComm 15(3): 524–532

Mok H, Zhang M (2013) Superparamagnetic iron oxide nanoparticle-based delivery systems for biotherapeutics. Expert Opin Drug Deliv 10(1):73–87

Moore A, Marecos E, Bogdanov A Jr, Weissleder R (2000) Tumoral distribution of long-circulating dextran-coated iron oxide nanoparticles in a rodent model. Radiology 214(2):568–574

Mosayebi J, Kiyasatfar M, Laurent S (2017) Synthesis, functionalization, and design of magnetic nanoparticles for theranostic applications. Adv Healthc Mater 6(23):1700306

Mou X, Ali Z, Li S, He N (2015) Applications of magnetic nanoparticles in targeted drug delivery system. J Nanosci Nanotechnol 15(1):54–62

Mourdikoudis S, Kostopoulou A, LaGrow AP (2021) Magnetic nanoparticle composites: synergistic effects and applications. Adv Sci 8(12):2004951

Mukerjee A, Vishwanatha JK (2009) Formulation, characterization and evaluation of curcumin-loaded PLGA nanospheres for cancer therapy. Anticancer Res 29(10):3867–3875

Nan X, Zhang X, Liu Y, Zhou M, Chen X, Zhang X (2017) Dual-targeted multifunctional nanoparticles for magnetic resonance imaging guided cancer diagnosis and therapy. ACS Appl Mater Interfaces 9(11):9986–9995

Nicolás P, Saleta M, Troiani H, Zysler R, Lassalle V, Ferreira ML (2013) Preparation of iron oxide nanoparticles stabilized with biomolecules: experimental and mechanistic issues. Acta Biomater 9(1):4754–4762

Niemirowicz K, Durnaś B, Tokajuk G, Piktel E, Michalak G, Gu X et al (2017) Formulation and candidacidal activity of magnetic nanoparticles coated with cathelicidin LL-37 and ceragenin CSA-13. Sci Rep 7(1):1–12

Pan J, Hu P, Guo Y, Hao J, Ni D, Xu Y et al (2020) Combined magnetic hyperthermia and immune therapy for primary and metastatic tumor treatments. ACS Nano 14(1):1033–1044

Pankhurst QA, Connolly J, Jones SK, Dobson J (2003) Applications of magnetic nanoparticles in biomedicine. J Phys D Appl Phys 36(13):R167.s

Pathak A, Jain K (2023) Chapter 13 - Dendrimer–drug conjugates. In: Madan J, Baldi A, Chaudhary M, Chopra N (eds) Polymer-drug conjugates: linker chemistry, protocols and applications. Elsevier, pp 315–345

Peng S, Lee Y, Wang C, Yin H, Dai S, Sun S (2008) A facile synthesis of monodisperse au nanoparticles and their catalysis of CO oxidation. Nano Res 1(3):229–234

Plank C, Zelphati O, Mykhaylyk O (2011) Magnetically enhanced nucleic acid delivery. Ten years of magnetofection—Progress and prospects. Adv Drug Deliv Rev 63(14–15):1300–1331

Price DN, Stromberg LR, Kunda NK, Muttil P (2017) In vivo pulmonary delivery and magnetic-targeting of dry powder nano-in-microparticles. Mol Pharm 14(12):4741–4750

Qiao R, Jia Q, Huwel S, Xia R, Liu T, Gao F et al (2012) Receptor-mediated delivery of magnetic nanoparticles across the blood–brain barrier. ACS Nano 6(4):3304–3310

Ramanathan S, Archunan G, Sivakumar M, Selvan ST, Fred AL, Kumar S et al (2018) Theranostic applications of nanoparticles in neurodegenerative disorders. Int J Nanomedicine 13:5561

Riemer AB, Klinger M, Wagner S, Bernhaus A, Mazzucchelli L, Pehamberger H et al (2004) Generation of peptide mimics of the epitope recognized by trastuzumab on the oncogenic protein Her-2/neu. J Immunol 173(1):394–401

Rodrigues D, Bañobre-López M, Espiña B, Rivas J, Azeredo J (2013) Effect of magnetic hyperthermia on the structure of biofilm and cellular viability of a food spoilage bacterium. Biofouling 29(10):1225–1232

Roy E, Patra S, Madhuri R, Sharma PK (2016) Stimuli-responsive poly (N-isopropyl acrylamide)-co-tyrosine@ gadolinium: iron oxide nanoparticle-based nanotheranostic for cancer diagnosis and treatment. Colloids Surf B: Biointerfaces 142:248–258

Saatchi K, Tod SE, Leung D, Nicholson KE, Andreu I, Buchwalder C et al (2017) Characterization of alendronic- and undecylenic acid coated magnetic nanoparticles for the targeted delivery of rosiglitazone to subcutaneous adipose tissue. Nanomedicine 13(2):559–568

Shin T-H, Choi Y, Kim S, Cheon J (2015) Recent advances in magnetic nanoparticle-based multi-modal imaging. Chem Soc Rev 44(14):4501–4516

Singh S, Barick K, Bahadur D (2015) Inactivation of bacterial pathogens under magnetic hyperthermia using Fe3O4–ZnO nanocomposite. Powder Technol 269:513–519

Su H, Price C-AH, Jing L, Tian Q, Liu J, Qian K (2019) Janus particles: design, preparation, and biomedical applications. Materials today bio 4:100033

Sun W, Fan J, Wang S, Kang Y, Du J, Peng X (2018) Biodegradable drug-loaded hydroxyapatite nanotherapeutic agent for targeted drug release in tumors. ACS Appl Mater Interfaces 10(9): 7832–7840

Sun X, Sun S (2017) Preparation of magnetic nanoparticles for biomedical applications. Springer, Biomedical Nanotechnology, pp 73–89

Tartaj P, del Puerto MM, Veintemillas-Verdaguer S, González-Carreño T, Serna CJ (2003) The preparation of magnetic nanoparticles for applications in biomedicine. J Phys D Appl Phys 36(13):R182

Thomas R, Park I-K, Jeong YY (2013) Magnetic iron oxide nanoparticles for multimodal imaging and therapy of cancer. Int J Mol Sci 14(8):15910–15930

Tomitaka A, Kaushik A, Kevadiya BD, Mukadam I, Gendelman HE, Khalili K et al (2019) Surface-engineered multimodal magnetic nanoparticles to manage CNS diseases. Drug Discov Today 24(3):873–882

Trahms L (2009) Biomedical applications of magnetic nanoparticles. Springer, Colloidal Magnetic Fluids, pp 327–358

Tripathi CB, Parashar P, Arya M, Singh M, Kanoujia J, Kaithwas G, Saraf SA (2020) Biotin anchored nanostructured lipid carriers for targeted delivery of doxorubicin in management of mammary gland carcinoma through regulation of apoptotic modulator. J Liposome Res 30(1):21–36

Vaghari H, Jafarizadeh-Malmiri H, Mohammadlou M, Berenjian A, Anarjan N, Jafari N et al (2016) Application of magnetic nanoparticles in smart enzyme immobilization. Biotechnol Lett 38(2): 223–233

Veiseh O, Sun C, Fang C, Bhattarai N, Gunn J, Kievit F et al (2009) Specific targeting of brain tumors with an optical/magnetic resonance imaging nanoprobe across the blood-brain barrier. Cancer Res 69(15):6200–6207

Vijayan VM, Beeran AE, Shenoy SJ, Muthu J, Thomas V (2019) New magneto-fluorescent hybrid polymer nanogel for theranostic applications. ACS Appl Bio Mater 2(2):757–768

Wang F, Li Y, Shen Y, Wang A, Wang S, Xie T (2013) The functions and applications of RGD in tumor therapy and tissue engineering. Int J Mol Sci 14(7):13447–13462

Wang X, Hu C, Schurz L, De Marco C, Chen X, Pané S et al (2018) Surface-chemistry-mediated control of individual magnetic helical microswimmers in a swarm. ACS Nano 12(6):6210–6217

Wei Y, Han B, Hu X, Lin Y, Wang X, Deng X (2012) Synthesis of Fe3O4 nanoparticles and their magnetic properties. Proc Eng 27:632–637

Xie J, Liu G, Eden HS, Ai H, Chen X (2011) Surface-engineered magnetic nanoparticle platforms for cancer imaging and therapy. Acc Chem Res 44(10):883–892

Xin F, Li Y, Fu C, Javed I, Huang X, Schaschkow A et al (2020) Multimodal nanoprobe for pancreatic beta cell detection and amyloidosis mitigation. Chem Mater 32(3):1080–1088

Yoo D, Lee J-H, Shin T-H, Cheon J (2011) Theranostic magnetic nanoparticles. Acc Chem Res 44(10):863–874

Zhang F, Zhao L, Wang S, Yang J, Lu G, Luo N et al (2018) Construction of a biomimetic Magnetosome and its application as a SiRNA carrier for high-performance anticancer therapy. Adv Funct Mater 28(1):1703326

Functionalized Mesoporous Silica-Based Nanoparticles for Theranostic Applications

16

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Abstract

Mesoporous silica nanoparticles (MSNs) have gained huge attention among scientific groups by virtue of its distinct features. The monodispersity nature, tunable pore size, and surface engineering made it versatile nanocarriers in the field of pharmaceutical sciences and biomedical arena. MSNs have been exploited in bio-imaging by means of its greater stability and inherent photoluminescence features. Apart from bio-imaging, smart stimuli-responsive nanomaterials have been explored in the management of several devastating diseases specifically cancers and neurological disorders. The applicability of surface-modified MSNs provides the site-specific targeting while reducing the off-target noxious effects. The current chapter majorly covers the MSN-mediated nanotechnological approaches to showcase the significant theranostic applications.

Keywords

Mesoporous silica nanoparticles · Nanoparticles · MSN · Drug delivery

Abbreviations

5-FU	5-Fluorouracil
AMF	Alternating magnetic field
APTES	(3-Aminopropyl) triethoxysilane

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ATO	Arsenic trioxide
ATP	Adenosine tri-phosphate
CAP	Capecitabine
CMC	Critical micelle concentration
CTAB	Cetyl trimethyl ammonium bromide
DDS	Drug delivery systems
DNA	Deoxyribonucleic acid
DOX	Doxorubicin
EphA2	Ephrin type-A receptor 2
EPR	Enhanced permeability and retention
FA	Folic acid
GEM	Gemcitabine
HCC	Hepatocellular cancer
LCST	Lower critical solution temperature
MDR	Multidrug resistance
MSNs	Mesoporous silica nanoparticles
NIR	Near infrared
O/W	Oil in water
ORMOSIL	Organically modified silica
PLAA	Phospholipase A2 activating protein
PMO	Periodic mesoporous organosilica
PNIPAM	Poly(N-isopropylacrylamide)
PSMA	Anti-prostrate-specific membrane antigen
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SBA	Santa Barbara Amorphous
TAT	Trans-activator of transcription
TEOS	Tetraethyl orthosilicate

16.1 Introduction

Cancer is a devastating disease to human health, and both the prevalence and mortality of cancer are endlessly mounting in the recent era. The use of chemotherapy is one of the principal therapeutic strategies enforced to treat cancer in clinics. Unfortunately, general treatment of cancer disease remains elusive for several shortcoming and complications, and one of the main reasons for such complication is detrimental intricacy of the pharmaceutical molecules or conventional drug transport nano-system. It is mainly due to the washout of the therapeutic molecules prior to the cancerous tissues. Nevertheless, the bottleneck of conventional chemotherapies, namely, insignificant pharmacokinetics, irrelevant bio-distribution, and off-target delivery, leads to less exploitation of the drugs and long-term adverse effects (Soni et al. 2015; Gauro et al. 2021; Narayan et al. 2021).

A higher recurrence tendency and the shortcomings of chemotherapy to control cancer will be attributable to multidrug resistance (MDR). However, the majority of malicious tumor patients lose their lives because of some degree of MDR. Thus, MDR in several cancer became one of the utmost hindrances to chemotherapeutic treatment (Zhao et al. 2018). To tackle these problems, nanotechnology-based drug delivery systems have been introduced and developed rapidly, in recent decades. To address these shortcomings, anticancer therapeutic-based delivery platforms have recently showcased remarkable outcomes in cancer therapy. Several drug delivery platforms specifically liposomes, polymeric micelles, and inorganic nanoparticles, namely, mesoporous silica nanoparticles (MSNs), are already approved by FDA for clinical use in cancer treatment. While the drug could not reach the preferred diseased site alone, the carriers have the ability to deliver the therapeutics by virtue of their unique properties. A "carrier" is considered as a polymer or intelligent molecule that exploits the delivery of therapeutic molecules at the preferred diseased location of cancer tissues and reduces the off-targeted delivery burden of drug alone. Thus, the nanoscale drug delivery platforms assist in penetrating tumor tissues as a result of enhanced permeability and retention (EPR) effect (Jain et al. 2014; Khan et al. 2019; Pan et al. 2017). Nanocarriers have a tendency to improve the pharmacokinetics and biodistribution of drugs while reducing toxic adverse effects. Commercial therapeutics, such as liposomal doxorubicin, can sometimes improve the quality of life for cancer patients, though they can only modestly enhance the survival rate overall. It is therefore necessary to develop more advanced drug delivery systems (DDS) to overcome the chemotherapy-based side effects. Among the newly described DDS, the properties of controlled drug release based on stimuli sensitivity and ligand-mediated active targeting are preferred at the diseased site with superior therapeutic potential.

Nanotechnological approaches composed of MSNs have attracted plausible attention from scientists around the globe during the past era. MSNs exhibit high drug loading ability and excellent biocompatibility. It can be easily synthesized in the variety of sizes, shapes, pore sizes, and volumes and can also be easily surface functionalized. Moreover, the high drug loading ability and excellent biocompatibilities result MSNs as an attractive option for designing safe and effective drug delivery platform (Narayan et al. 2018). This approach mediated fascinating features that have capacity to markedly modulate the bioavailability and targeted delivery of therapeutics at the site of action for prolonged period of time.

16.2 Basic Structure and Properties

Mesoporous materials are classified on the basis of their pore size mainly microporous (less than 2 nm), mesoporous (2−50 nm), and macro-porous (greater than 50 nm). MSNs are the honeycomb-like silica-based inorganic porous nanocarriers made up of empty channels with medium-sized pores having diameter in a range of 2–50 nm as shown in Fig. 16.1 (Beltrán-Osuna and Perilla 2016). There are a number

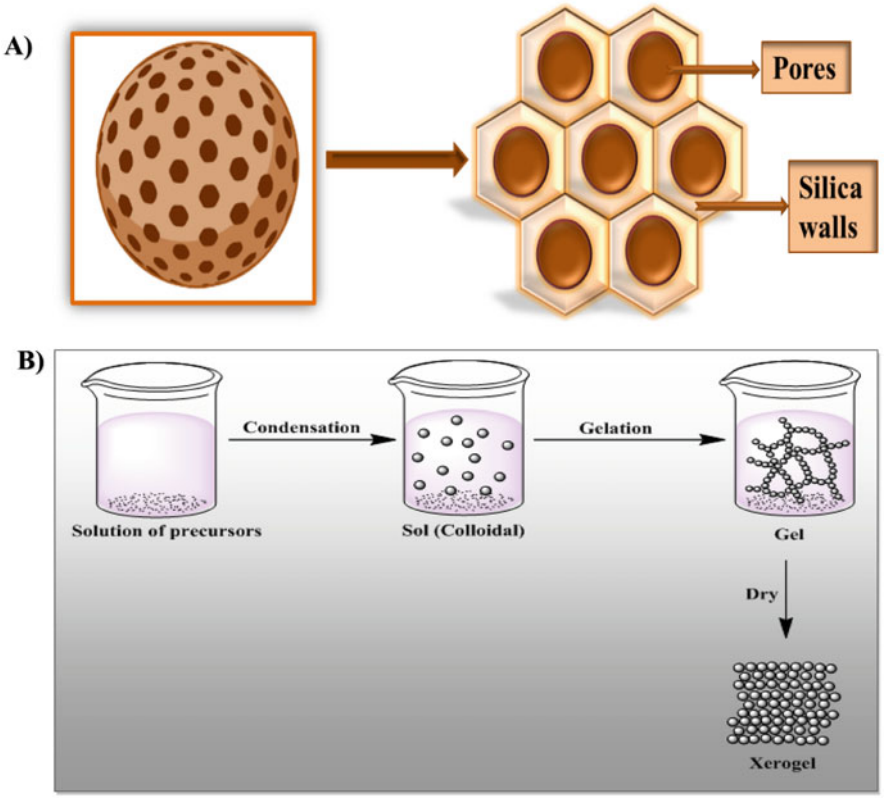

Fig. 16.1 (**a**) Structure of MSNs (mesoporous silica nanoparticles) having pores for cargo loading and controlled release and silica walls for surface functionalization. (**b**) Systematic illustration of preparation of nanoparticles (MSN) from sol-gel technique which is typically called as stober process in which hydrolysis, condensation, and gelation processes take place

of nanoparticles which make them unique among each other like liposomes, gold nanoparticles, dendrimers, and selenium nanoparticles. However, MSN shows more uniqueness as compared to other nanocarriers in terms of biocompatibility, fabrication, and surface modification. In addition, MSNs also have distinct properties like greater surface area (>800 m^2/g) with high pore volume (>0.9 cm^2/g) with the help of which they are able to capture or encapsulate several kinds of bioactive molecules. The biodegradable nature of MSNs converts silanol into silicic acid which gets easily eliminated from the body, whereas their fine-tuned pore size allows trouble-free endocytosis with negligible cytotoxicity (Liu et al. 2020). Drug molecule adsorption on mesoporous silica was linked to "surface electrochemistry," whereas drug molecule release is influenced by surface area and diffusion via pores. They are resistant to the external environment including high pH, mechanical stress, heat, and hydrolysis-induced degradation which makes them more rigid and stable. MSNs have additional benefits such as rapid mass transport, excellent substrate adherence,

and good solution dispersity. Protection from premature metabolism and degradation of the therapeutics carrying cargoes permits a site-specific control release at preferred diseased tissues (Slowing et al. 2008), thus improving the circulation time and drug stability (Watermann and Brieger 2017). The surface modification offers the delayed release of various drugs from MSNs that enhances therapeutic effect and bioavailability (Biswas 2017).

16.3 Introduction of Mesoporous Families

16.3.1 M41S Family

The MCM stands for Mobil Composition of Matter, which comes under the M41S family. It is the best known and fastest-growing mesoporous family in terms of research. The hexagonal phase of MCM-41 which has significant therapeutic potential in drug delivery is undoubtedly the most well-known and studied nanocarrier. A cubic form (MCM-48) and a lamellar form (MCM-50) are other discrete members of the M41S family. The unique properties of MCM-41 and MCM-48 make them superior to MCM-50.

16.3.1.1 MCM-41

MCM-41 is the most popular inorganic silica nanoparticle, which was initially developed as a drug delivery matrix in the M41S family and features hexagonal arrangements in 2D. This space group contains 6-mm-sized pores that are well defined and uniformly sized, spanning a range of 1.5–10 nm. Usually, these types of materials are prepared by self-aggregation of surfactant where inorganic species like silica concomitantly condense. This process gives rise to meso-ordered composites or MSNs. It exhibits a unique morphology that is influenced by the method of preparation, whereas the pore diameter depends on the cationic surfactant chain length (Kresge et al. 1992). They exhibit consistent mesopore size distribution, as well as good thermal and mechanical stability**.** The high surface area enables them to encapsulate a large amount of therapeutic moiety in nanoparticle meso-structure. On the other hand, pores on the external surface facile the release of bioactive in controlled manner (Horcajada et al. 2004). The cylindrical morphology with fine symmetrical mesopores makes them a fascinating system for adsorption of various types of therapeutic molecules**.** MCM-41 comprises negative potential due to the presence of silanol and siloxane groups as the terminal functionalities. They permit positively charged molecules adsorbed on the surface. However, this individual property is not enough to describe the whole properties of MCM-41. Generally, the ordered silica mesopores with proper self-assembly of template are formed from the silica precursor in basic conditions (pH 8–11). An acidic or basic catalyst is usually needed to assist the hydrolysis of the alkoxy group of mesoporous silica, which is mainly produced from silicon alkoxides. The surface of MCM-41 can be easily modified for better pore size to increase its hydrophobic nature, to protect it

from unintended external factors (such as heat, hydrolysis, etc.), for efficient drug loading, and to extend drug release.

16.3.1.2 MCM-48

MCM-48 is the second most used crystalline mesoporous material with a 3D cubic structural arrangement having pore diameter up to 3.6 nm and space group *Ia3d* (Izquierdo-Barba et al. 2005; Kaneda et al. 2002). It catches attention due to its penetrating bi-continuous channel networks that aid in easy molecular transport and controlled drug release. It also exhibits a bi-directional mesoporous channel with a diameter range of ~3–6 nm. The large pore size can be synthesized from triblock co-polymers in acidic nature. As compared to MCM-41, this product produced a thicker wall and appeared like a microspore**.** The drug release from MCM-48 is much easier than the hexagonal MCM-41 influenced by the interconnected voids**.** It requires a longer duration reaction for synthesis, whereas, conjugating MCM-48 with some natural polymers like chitosan, starch, and cyclodextrin, the chemotherapeutic moieties with biocompatibility and solubility can be modulated (Abukhadra et al. 2020).

16.3.1.3 SBA-15

After MCM-41, SBA-15 (Santa Barbara Amorphous) is considered for higher drug loading inorganic carrier. The structural appearance, synthesis method, as well as mechanism of product formation are similar to MCM-41. Because of its large surface area, pores up to 5–30 nm in size, and thick walls, it is regarded as a potential drug carrier (Zhao et al. 1998a, 1998b). The different types of co-polymer are involved as a template in SBA-15, mostly tri-block copolymers as discussed in Table 16.1. In order to synthesize SBA-15, it is necessary to maintain appropriate environmental conditions that allow copolymers to exhibit an array of pore sizes. Probably, tri-block copolymers are the best for producing hexagonally and cubically ordered silica nanoparticles in acidic media. On the other side, combined acidic and basic media guide the encapsulation of molecules, namely, heteroatoms. It possesses a great hydrothermal stability. The formation of self-assembled micelles based on the use of a tri-block copolymer (EO_m-PO_n-EO_m) and water at a constant temperature of about 308–373 K shows a core and mantle structures. It comprises a core of PO (propylene oxide) having hydrophobic nature and a mantle of EO (ethylene oxide). EO blocks increase their hydrophobicity with increasing temperature (Wanka et al. 1994). Additionally, ethanol and water washing also contributes to pore size reduction. By washing with water and ethanol, not only impurities are removed, but the amount of EO blocks in silica walls is reduced, resulting in voids and structural shrinkage**.** As a result of impregnation and diffusion-directed controlled mechanisms, SBA-15 can entrap various chemotherapeutic substances (Song et al. 2005).

16.3.1.4 SBA-16

SBA-16 is a 3D cubic mesoporous silica microsphere, preferred nanocarrier specifically for lipophilic drugs. It is comprised of abundant space group of *Im3m*. Each

Table 16.1 Some examples of copolymers and the nature of media that can be used to synthesize SBA-15 are as follows

S. no.	Type of co-polymer	Examples	Nature of media	Morphology	Reference
1.	Oligomers	• Alkyl poly (ethylene oxide) • Alkyl phenyl poly-(ethylene oxide)	Acidic	Hexagonal and cubic	(Attard et al. 1995)
2.	Di-block copolymers (cationic and anionic) Di-block copolymers (non-ionic)	• Polystyrene-polybutadiene • Polybutadiene-poly (ethylene oxide) poly(butylene oxide)- poly (ethylene oxide)	Acidic Dilute acidic media	Hexagonal and cubic Cubicmesoporous silicas and various oxides	(Antonietti et al. 1998) (Yang et al. 1999)
3.	Tri-block copolymers	• Poly(ethylene oxide)-poly (propylene oxide)-poly(ethylene oxide)	Neutral media	Disordered mesoporous silicas and aluminas	(Bagshaw and Pinnavaia 1996)
			Acidic Media	Periodic mesoporous silicas (hexagonal structure)	
			Non-aqueous media	Periodic mesoporous oxide	

pore behaves as a bridge with its eight nearby neighbors to form a multidirectional system of mesoporous network (Sakamoto et al. 2000). The limitless benefits of this carrier have been explored such as facilitation of mass transfer, reduction in pore blockage, and multi-directional pore convenience. The most preferred template for SBA-16 is Pluronic F127 with TEOS as silica precursor under acidic conditions. The micro-mesoporous structure forms due to the presence of PEO and PPO chain in F127 (EO_{100}-PO_{65}-EO_{100}) (Zhao et al. 1998a, 1998b). Apart from that, the time taken for synthesis is also shorter (approx. 2 days) due to only one step of heating. Functionalization of silanol with organic groups on external surfaces enhances drug-surface interactions and also boosts drug loading capacity (Sharma and Asefa 2007). However, functionalization on the internal surface changes its loading capacity and behavior.

16.3.2 PMO Family

PMO stands for periodic mesoporous organosilicas having uniform cubic structure and size range above micrometers. They possess a space group *Pm-3n*, highest surface area with 1880 m^2/g, and diameter of 150–600 nm. They also prefer

sol-gel method for the synthesis**.** There are a number of interesting advantages of PMO such as ease of surface engineering, improved biocompatibility, excellent hydrothermal and mechanical stability, and hydrophobic properties of the pore wall (Asefa et al. 2000).

16.3.3 ORMOSIL Family

A fascinating class of hybrid materials is ORMOSIL nanoparticles, which can be applied in many fields especially in biochemistry. It consists of lipophilic core, adjustable mesopores with optical transparency for which it is highly preferred for multimodal imaging. They also have distinct properties like systemic safety (Roy et al. 2008), controlled drug release (Vivero-Escoto et al. 2010), and maximal clearance rate from the body. They can be fabricated by O/W micro-emulsion method using Aerosol-OT as surfactant and 1-butanol as co-surfactant. In addition, vinyl-triethoxysilane (VTES) acts as a silica precursor (Roy et al. 2014). MSN diameters can be tailored between 10 and 100 nm based on the micro-emulsion composition, with a greater level of monodispersity. They are unique in terms of storage which can be stored at 4 °C for future use. They can serve to anchor the functionalized molecule to the surface for site-specific targeting. Apart from this, they have a wide number of applications in gene therapy, photodynamic therapy (Roy et al. 2003), as well as diagnostic imaging (Kumar et al. 2008). It undergoes a biodegradation through decomposition of the silica-carbon bond.

16.3.4 Hollow Family (H-MSN)

Hollow-type MSNs are characterized by hollow core-mesoporous shell structures and homogeneous morphology with pore size range up to 20–35 nm. They represent intelligent hollow MSN-based nanotechnological approaches for delivery of pharmaceutical molecules at the site of interest. Using a dual template approach, a dense template is used for hollow interiors and a swampy template is used to form mesopores in the shell (Lou et al. 2008). They had received much attention because of their unusual nanoscale hollow structures, which can operate as vast pools for host loading (Chen et al. 2011a, 2011b). A sol-gel method is also preferred in their growth stages. Due to their smart framework design and mesoporous surface, H-MSNs have greater loading capacity, a high permeability for mass movement, low density, large surface area, and high pore volume (Han et al. 2010).

16.4 Composition and Method of Preparation

Generally, MSN is composed of a surfactant acting as a template and silica precursor as pore generating agent in the presence of catalyst as discussed in Table 16.2. They prefer temperature condition at about 80 °C to form self-assembly of surfactant and also the interaction between organic-inorganic molecules.

Table 16.2 List of the materials with their distinct properties used to fabricate MSNs

Parameters	Surfactants (templates)	Catalysts	Precursors	Method of surfactant removal	References
Type	Surfactant Cationic surfactant, Anionic surfactant, Non-ionic surfactant	Mostly basic	Silicate precursors	–	(Yamada et al. 2014) (Gai et al. 2016)
Role	Structure directing agent (to give a specific shape)	To enhance rate of reaction	Pore generating agent	To remove template from the surface	(Pal and Bhaumik 2013)
Properties	• Amphiphilic compounds. • Capacity of self-assembly. • Diverseness of their head groups ensures chemical modification. • Improved biocompatibility, solubility, bioavailability, and biodegradability of therapeutics.	• To enhance rate of reaction. • To maintain pH (alkali). • To restrict unwanted growth.	• Tetrahedron-shaped anionic group. • Have ordered pores. • Pore generating property. • Being oil-like monomer showed phase separation under static condition.	To prevent MSN from unnecessary contaminants	–
Examples (mostly preferred ingredients)	• CTAB (cetyl trimethyl ammonium bromide). • CTAC (cetyl trimethyl ammonium chloride). • DTAB (dodecyl trimethyl ammonium bromide).	• NaOH. • Ammonium hydroxide. • Ammonia.	• TEOS (tetraethoxy orthosilicate) 99%. • TMOS (tetramethoxy orthosilicate). • Sodium metasilicate	• Calcination. • Vaccum drying. • Lyophilization. • Ammonium nitrate reflux. (in mild acidic condition) • Acidic reflux (HCl + methanol).	(Cui et al. 2013) (Hanafi-Bojd et al. 2015) (Mozafarinia et al. 2021)

16.4.1 Sol-Gel Method

The sol-gel method/stober method also termed as wet chemical technique or chemical solution deposition method and is a widely preferred technique to synthesize MSNs. In this process, alkoxy silicates are hydrolyzed with alcohol and ammonia that act as catalysts. The process involves transformation of sol to gel as illustrated in Fig. 16.1. The gelation is considered as a sign of bridging comprised of inorganic silica network and forming a continuous network of lipid phase. Both the processes take place at 80 °C in order to maintain the shape of the micelles. Increasing the temperature beyond the specified level makes disturbance in the shape of micelles that results in lamellar shape (Schmidt et al. 1998). Finally, the calcination process is used to remove templates from gel to produce oxides specifically at temperature 550 °C resulting in the formation of mesoporous material. A variety of templates are used as structure directing agents for the synthesis of MSNs such as cationic surfactants, triblock co-polymers, and organic small molecules with varying pore sizes between 10 and 300 Å (Niesz et al. 2005). The rate of addition of silica precursor (TEOS) plays an important role in order to obtain controlled particle size (Nozawa et al. 2005). Hence, optimization of solvent and TEOS ratio is an important factor to obtain a tunable size of MSNs.

16.4.2 Microwave-Assisted Technique

The microwave-assisted combustion of chemical reactions to fabricate nanomaterials, particularly metal oxides, has been of great interest in the field of nanomedicine. Compared to conventional methods, this technique offers several advantages, including rapid heating to a temperature suitable for crystallization and superfast saturation caused by the dissolution of precipitated gels and short crystallization times (Newalkar et al. 2000). It is a thermally stable silica that can withstand temperatures up to 200 °C, also considering rapid heating that results in reduction in pore structure. Mesoporous organosilica with disulfide groups is synthesized using this technique and also mesoporous hectorites (Sánchez et al. 2013).

16.4.3 Chemical Etching Technique

A hollow interior is created by forming hollow mesopores between the core and the shell of silica. Hollow mesopores with a controlled pore size and higher drug loading can be synthesized using this method. Additionally, it is related to pore structures that are directed by etchants. The method is also applicable to construct heterogeneous hollow-type nanostructure with inorganic nanocrystal like silver and ferrous compound as core and silica as shell. This homogeneous templating path, i.e., "structural difference-build selective etching," can design porous structures along with a unique shell structure. When a suitable etching agent is worn, selective

etching occurs at the inner surface, while the outer surface remains intact, resulting in hollow structures (Chen et al. 2010).

16.4.4 Template-Assisted Technique

It is the most economical technique to construct ordered mesoporous silica-based nanoparticles as illustrated in Fig. 16.2. They can be synthesized via two methods: hard templating method and soft templating method.

16.4.4.1 Hard Templating Method

Templating with hard materials, such as mesoporous carbon, is an uncommon technique called exo-template or nano-casting. Non-metallic nonsiliceous carbons fall into this category. Its surface shows a hydrophobic nature, chemical inertness, and mechanical stability (Pal and Bhaumik 2013).

16.4.4.2 Soft Templating Method

It is the most widely used technique, also known as liquid crystal template approach or simply endo-template. In this technique, a surfactant or amphiphilic block co-polymer is generally used as a template for the development of MSNs. It has hydrophilic nature on the surface and is used to synthesize mesoporous structure (Haynes et al. 2020).

16.4.5 Effect of Physical Parameters on the Formation of MSNs

The rate of hydrolysis on silane group and condensation of siloxane group mainly depends on the charge state and pH of silica. In basic conditions, a portion of a silanol group (Si–OH) is converted to a silanolate (Si–O-). The negatively charged surface of silica (TEOS) is typically addressed with cationic surfactants (CTA^+). It occurs via electrostatic interaction, hydrogen bonding of silicates, and surfactant at pH 2–7. The critical micelle concentration (CMC) is the necessary factor to obtain micelles from surfactant. The process of condensation with silica occurs on the head region of the micelles. Several parameters, such as pH, silica source, additives, and temperature, influence the hydrolysis and condensation rate of silica sources where pH has a notable effect. In this scenario, the varioust hydrolysis rates of the silica sources and subsequent nucleation and grain development reactions were predicted to cause the particle size variation as depicted in Fig. 16.2. As the pH in a basic solution increases, TEOS hydrolysis increases, but there is null effect on condensation. If the pH value is below or above 8.4, the condensation rate is the highest, whereas lower pH leads to reduced condensation rate continuously. MSN particle size was found to be influenced by pH more than the amount of silica source or reaction time as shown in Fig. 16.3.

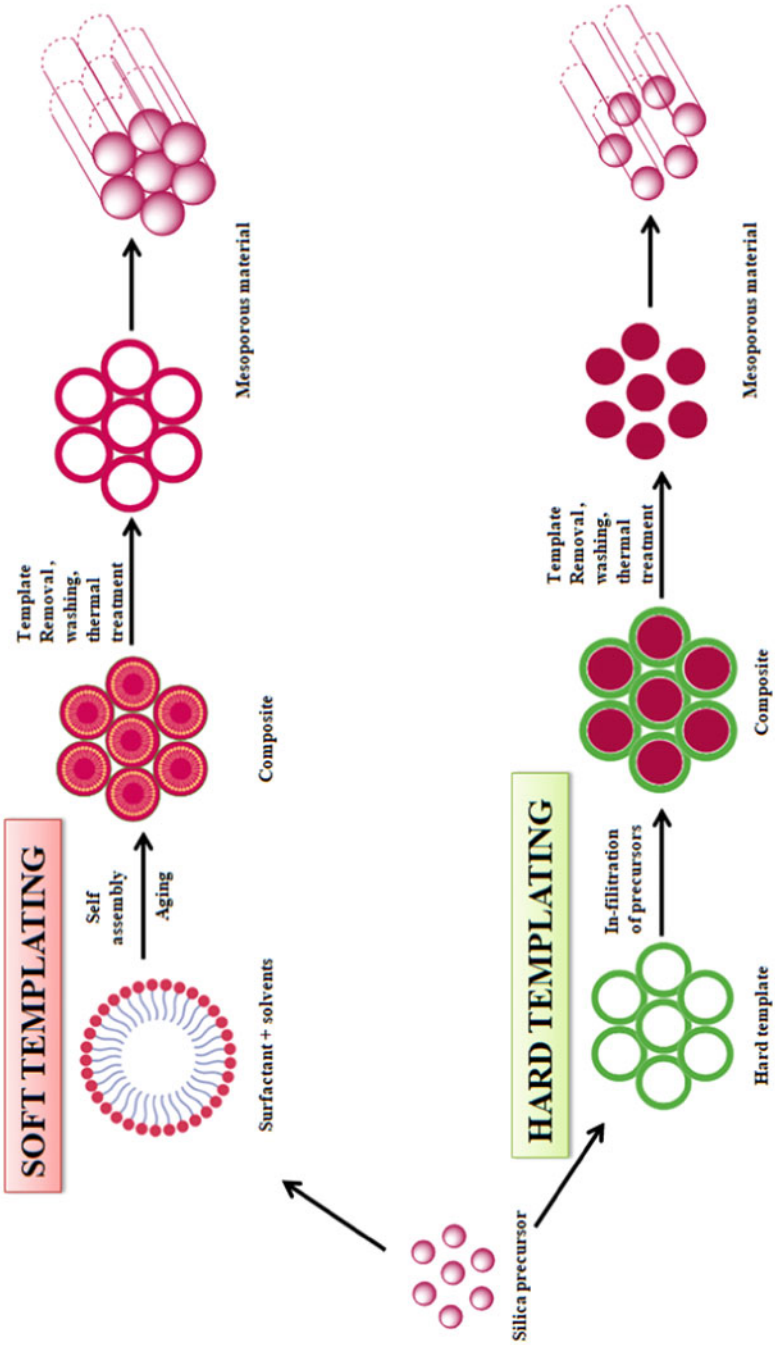

Fig. 16.2 The synthesis of mesoporous silica nanoparticles can be achieved through soft or hard techniques, but in soft templating, a surfactant is used to direct the surface, whereas in hard templating, a hard template and surface directing agent are utilized

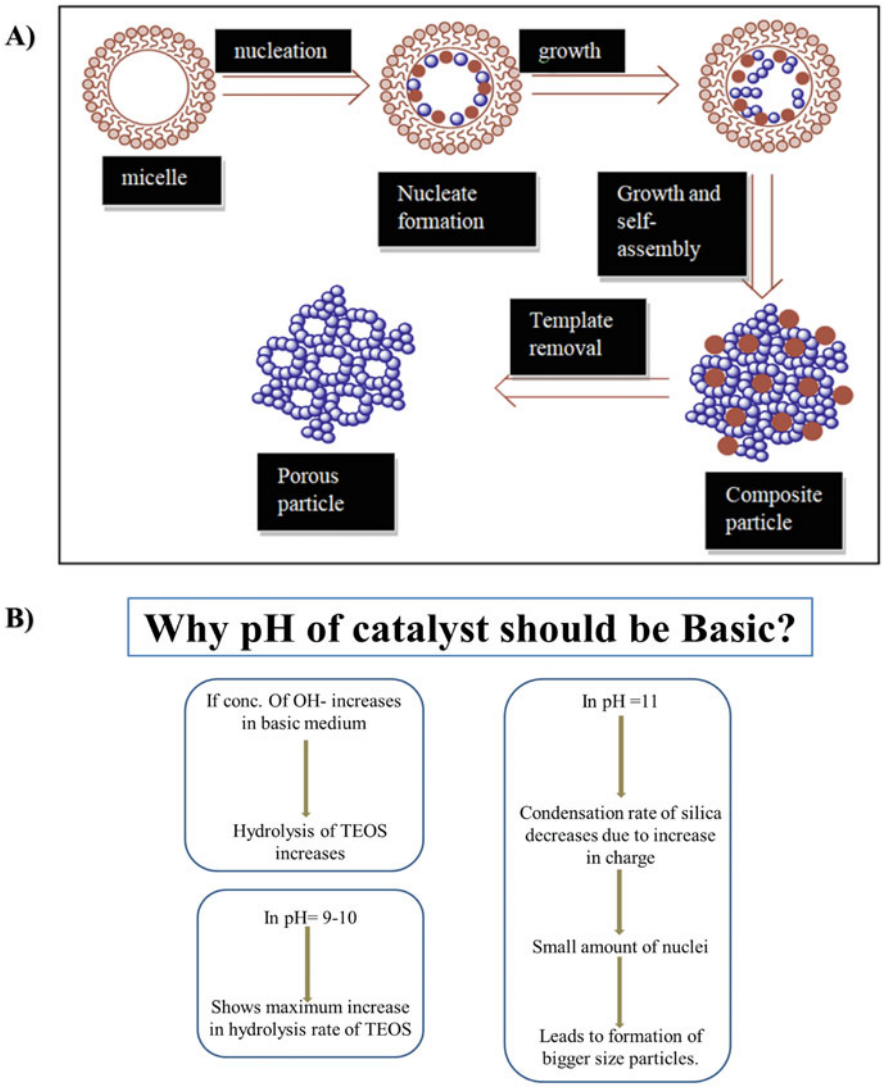

Fig. 16.3 (**a**) The stages of growth during the formation of MSNs, (**b**) the effects of physical parameters (such as temperature and pH) on the growth of MSNs

16.5 Surface Modification of MSNs

Surface modification is the tailored-made engineering of nanocarriers such as functional groups, proteins, antibodies, ligands, etc. to make them versatile drug delivery nanoplatform as shown in Fig. 16.5. Surface modification could modify the distinct

properties and functions, such as hydrophilicity, lipophilicity, surface charge properties, roughness, and biocompatibility. Different variables including morphology and size of mesostructures, electrostatic interactions between MSNs and the cell membrane, and the surface functionalization can impact the uptake mechanisms of MSNs. Apart from this, it prevents the chances of membranolysis that can occur through silanol and siloxane group which is highly unstable (Natarajan and Selvaraj 2014). Due to the presence of silanol groups on the surface of MSNs, external organic functional entities can be covalently bonded to the nanoparticles for surface modulation, hence resulting in in vivo biological action. There are several chemically modified groups such as thiol, organic chain, and amine modification, to design a surface-engineered MSN. The amine terminated MSNs functionalized with therapeutic molecules results in a higher adsorption capacity with drugs like ibuprofen, making them an efficient drug delivery (Wang et al. 2009). These changes increase biocompatibility while inhibiting particular adsorption and offering functional groups for further molecule conjugation purposes. Therefore, it is important to design the surface-engineered MSNs for gaining stimuli-responsive controlled drug release or sustained drug release, improved drug loading, target-specific drug delivery, and enhanced lipophilicity (Otsuka et al. 2000). In addition, it increases time of bio-functionality (Jokerst et al. 2011), reduces sensitivity to immune response, improves cytotoxicity (Chang et al. 2016), and obtains higher stability. Many of the research articles perform biological and histological studies like total bilirubin, creatinine, plasma protein binding, and hematological studies, and H&E staining of major internal organs demonstrates a good biocompatibility. After predicting, the analysis tells that it is necessary to perform in vivo studies like embryotoxicity, neurotoxicity, nephrotoxicity, genotoxicity, etc. to assure biosafety of nanocarriers. The functionalization includes two methods: co-condensation method and post-grafting method.

16.5.1 Co-Condensation Method

It is a single-stage method that consists organic molecules being covalently attached to the inorganic groups via silanol groups. In this process, the organic group, typically a silane accommodating reactive group, such as methoxy, chloride, or ethoxy groups, is affixed during the mesoporous synthesis. The organic groups are grafted to the outer and inner surfaces of the silica walls during synthesis process, resulting in functionalized materials. It leads to offer benefits like high loading efficiency and homogeneous surface coverage over post-grafting method. This method comprises more bioactive molecule adsorption compared to post-grafting (Datt et al. 2012). In addition, they are less likely to cause pore blocking due to homogenous mixing of organic groups (Hoffmann et al. 2006). The type of interaction such as electrostatic interaction and covalent and hydrogen bonding between silane precursor and surfactant decides the morphology of particles. The amount of functional groups accessible at the surface can be minimized due to chemical

properties and internal linkages between the precursor and the micelle (Soto et al. 2016).

16.5.2 Post-Grafting Method

The grafting process is similar to co-condensation in which the attachment occurs as a result of post-fabrication. It is mostly accomplished by the process of silylation which occurs on free and germinal silanol groups. Despite the fact, this approach produces a diverse functional group distribution, with a well-defined structure controlled by the silica matrix. This process ensures that organic groups are put on the mesopore's wall outer surface, resulting in higher functionalization degrees than the co-condensation method (Lim and Stein 1999). The amine group (NH_2) is widely employed functional group to initiate a basic characteristic, majorly using APTES. The grafting process has no effect on the structure of mesoporous nanoparticles. But it has certain limitations, such as poor charge management and poor control over the dispensation of the desired organic groups.

16.5.3 Imprint Coating Strategy

16.5.3.1 Physical Targeting or Passive Targeting

MSNs' size, shape, surface area, pore size, and surface functional groups are major physicochemical factors that influence their biodegradability. The passive targeting is designed on the basis of physicochemical properties. It is also based on the EPR effect which is the predominant approach to target cancerous or tumor cells. The benefit of passive targeting is also set on by time of blood circulation and the nanoparticles' power to overcome biological barriers. As a result, MSN features have a pivotal impact on tissue accumulation and cellular uptake.

Composition and Surface Charge

The surface composition and charge of nanoparticles are essential factors that influence the interaction with their surroundings and the negatively charged cell membrane. Mostly, the positive or neutral charged MSNs can bound efficiently with the cell membrane compared to negatively charged NPs. Surface functionalization is important to improve the cellular internalization and uptake. The greater internalization is showed by MSNs after modifying with positively charged (like MSN-NH_2) molecule(s) than the negatively charged due to electrostatic interaction (Chen et al. 2011a, 2011b). The surface alteration of MSNs can control the environment's reaction, prolonged bio-fate in the biological milieu to assure tumor targeting. It also eases the endocytosis process, guided by enhanced permeation and retention time of nanotechnological approaches (Slowing et al. 2006).

Size and Shape

The maximum therapeutic effect can be achieved with size up to 300 nm (Barbe et al. 2004). The reduced size of the nanoparticles u pto 50 nm results in increased surface area for cellular interaction and broad dispensation throughout the body, whereas an optimal effect can be seen at 100 nm size (Etheridge et al. 2013). A number of investigators researched on this factors and concluded that not only cellular uptake but also variables like cytotoxicity (Li et al. 2012), bio-distribution, clearance, and biosafety (Lin and Haynes 2010) get affected by the NP size. Whether they are similar in size, surface area, and pore diameter, they still differ in transport mechanism due to shape of the nanoparticles. The influence of shape on cellular uptake is in a specified order: "rods > spheres >cylinders >cubes" (Gratton et al. 2008)**.** Apart from cellular uptake, it also affects other behaviors such as cell viability, early apoptosis, adhesion, migration, and cytoskeleton formation (Huang et al. 2010).

16.5.3.2 Functionalization of MSNs for Active Targeting

Functionalized MSNs with Antibodies

Antibodies (Ab) are the widely explored ligands being functionalized as per the targeted receptor sites. They have the longest size range up to 10 kDa with highest selectivity (Kim et al. 2018). Ab and its fragments are gaining acceptance as a root of targeted ligand for strapping to over-expressed receptors on cancer cells. NPs functionalized with Ab manifest enhanced therapeutic efficacy while avoiding severe adverse effects. Ab can be connected to the MSN surface via electrostatic interaction or covalent attachment to target-specific antigens on the membrane of cells such as cancerous cells (Deng et al. 2011). Zhuang et al. developed DOX (doxorubicin)-filled MSNs conjugated with Ab (Anti-HER2 ScFv) MSN surface and externally functionalized by N-isopropylacrylamide (NIPAm) and methacrylic acid (MAA). It shows greater cytotoxicity via receptor-mediated endocytosis in SK-BR-3 cells (HER2-positive cancerous cells) and also ensures therapeutic efficacy and biosafety via histological studies (Zhuang et al. 2021).

Functionalized MSNs with Peptides and Transferrins

Peptides are among the most appealing type of ligand because of their biocompatibility, degradability, and low cytotoxicity. The RGD (Arg-Gly-Asp) peptide family is an ideal and regularly utilized peptide family. It specifically binds to endothelium αvβ3 integrins and has been widely exploited for tumor-specific active targeting. There are two different types of peptides, a cyclic (RGDFK) chemically conjugated to the MSNs and a linear RGD peptide sequenced with seven consecutive lysine residues (K7RGD). This conformation shows a major effect on cellular internalization through in vitro studies, hence suggesting that the RGDFK exhibits stronger bond as compared to K7RGD due to vast number of conformations in solution of K7RGD that results in reduced binding strength (Fang et al. 2012). Li et al. developed tirapazamine (TPZ)-loaded MSNs encapsulated with super magnetic nanoparticle conjugated with Ab through azo linker TAT peptide to achieve nucleus-targeted drug delivery system. The whole experiment demonstrates that developed

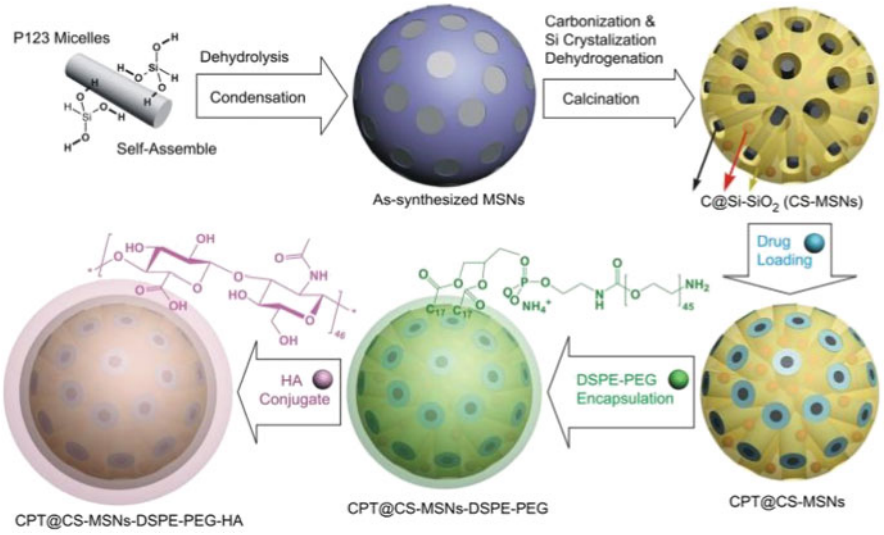

Fig. 16.4 MSN-based heterogeneous structure with hyaluronic acid (HA) conjugation for targeting to CD44 overexpressed on cancer cells. Schematic illustration for the synthesis, drug (camptothecin, CPT) loading, PEG encapsulation, and HA conjugation of mesoporous carbon@silicon-silica nanocomposite (CS-MSNs). Reproduced with permission from He et al. (2012a), copyright 2012, Elsevier Ltd

nanoformulations enhance the better antitumor efficiency under alternating magnetic field (AMF) compared to without AMF (Li et al. 2019). He et al. designed functionalized vesicular nanocarrier as an innovative theranostic nano-platform carrying chemotherapeutic agents for NIR luminescence imaging. In situ carbon measurement in mesopores is anticipated to greatly enhance the solubility of lipophilic drugs as demonstrated in Fig. 16.4 (He et al. 2012a, 2012b). MSNs can be functionalized with other proteins along with peptides, like transferrins (Tfs). It is a glycoprotein that attaches to iron and transports it throughout the body. It shows an excellent permeability factor by conjugating with NPs. Hegannawar et al. developed DOX-loaded magnetic silica counterfeit with Pluronic F-127 and conjugated with Tfs to encourage its penetration across BBB (Heggannavar et al. 2018). The experiment concludes higher cellular uptake and lower IC_{50} value in conjugated NPs as compared to blank. Also, it exhibits enhanced anticancer activity and permeability across BBB in the presence of magnetic field than free drug.

Functionalized MSNs with Aptamers

Aptamers are small RNA or single-stranded DNA molecules that can detect a specific target molecule. In comparison with Ab, it has several advantages, namely, low immunogenicity, modest size, low complexity, and ease to produce and modify. They also offer high affinity and specificity for their targets and structural flexibility. Interaction between aptamer and nanoparticles enables greater accumulation in tumor cells as well as helps in defeating in vivo tumor growth (Duo et al. 2018).

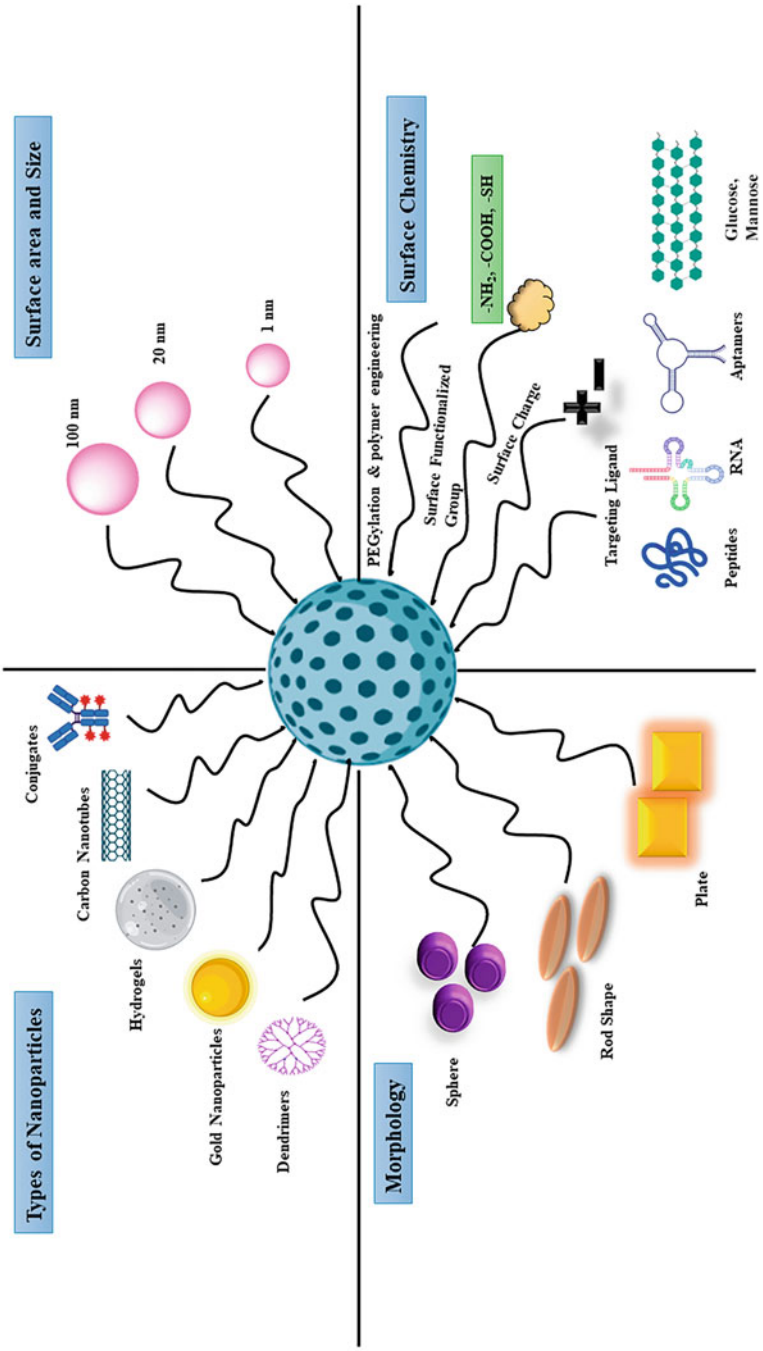

Fig. 16.5 Using MSN pores with varied surface alterations, controlled drug delivery, and release can be achieved using smart MSNs, which can be used across a wide range of therapeutic and diagnostic applications

Some examples of aptamers are AS-1411, GBI-10, DNA aptamer, TBA (thrombin binding aptamer), and so on. Xie et al. fabricated DOX-loaded MSN modified with EpCAM aptamer to achieve targeted drug delivery in colon cancer cells. The study concludes enhanced cytotoxicity and cellular uptake of hybrid nanoparticles in SW620 cells as compared to EpCAM negative Ramos cells (Xie et al. 2016).

Functionalized MSNs with Vitamins

Vitamin B9, commonly known as folic acid (FA), used to kill damaged cells, including cancer cells. It is a compound with low molecular weight and plays a very crucial role in cell survival. Additionally, it has higher affinity toward folate receptors. The receptors on human cancer cells are overexpressed several times. A functionalized FA with MSNs has better biocompatibility than a non-functionalized FA. It also displays a higher level of cytotoxicity toward cancerous cells by means of receptor-mediated cellular uptake (Wang et al. 2015)**.** The MSNs functionalized with FA also ensure a better pH-responsive drug release.

Functionalized MSNs for Stimuli-Sensitive Drug Delivery

It was discussed in the beginning that systemic administration of high concentration of drugs can cause serious side effects, especially chemotherapeutic drugs due to their non-specific attacks. Most likely, this dose affects primary organs such as the heart, liver, kidney, and bone marrow because of their rapid distribution, metabolism, and clearance. The drug adsorption on surface of MSNs capping with biomolecules prevents premature release. As a result, it helps in the designing of a stimulus-responsive novel nanoparticle. A chemical bond or molecule that undergoes a chemical or physical change in response to a stimulus can be exploited to provide a stimulus-responsive behavior (Zhang et al. 2014a, 2014b)**.** Polymeric functional coating or encapsulation can allow drug release to be controlled mechanically via switching on or off of mesopores in response to physicochemical conditions. When a polymer is lipophilic, it prefers to attach to the MSNs, and when it is hydrophilic, it swells with the surrounding fluid, allowing the cargo to escape. Depending on non-covalent interactions, the immense therapeutic functional groups are attached in response of stimuli, permitting the drug to be released.

16.6 Stimuli-Responsive Drug Release

16.6.1 Internal Stimuli-Responsive Drug Delivery

16.6.1.1 pH as Endogenous Stimuli

It is the most preferable stimuli around all of them. Each and every organ of the body has its specific pH range like normal tissue has a pH range of 7.2–7.4, inflamed tissues with 5.4 to 7.2, and tumor tissues with 6.8**.** The pH-sensitive gatekeepers are utilized to restrict medication release in response to transpose in pH. The gatekeeper at first blocks the pore. Orthoester (Liu et al. 2014), acetal (Yang et al. 2014), hydrazone, ester, and boronate (Gan et al. 2011) are some acid-labile linkages to

be applied. In the case of cancer, when the pH in the endosomes is reduced to somewhat acidic level, the gatekeeper gets hydrolyzed. Hence, cleavage of bonds occurs, resulting in controlled drug release. Narayan et al. developed capacitabine (CAP)-loaded chitosan and glucuronic acid-functionalized MSNs for treating colorectal cancer. It found the sustained release of drug in the presence of rat cecal contents by virtue of presence of cleaving of amide bond (Narayan et al. 2021). Yang et al. developed rhodamine-loaded MSNs conjugated with polymer hyper-branched polyglycerol (hPG) through Schiff base bond by layer by layer technique. It exhibited the controlled drug release at acidic environment by means of breaking a Schiff base (Yang et al. 2016a, 2016b).

16.6.1.2 Redox Potential as Internal Stimuli

The large quantity of redox potential changes among intracellular and extracellular environment in tissues confers a distinctive chance to deploy redox-responsive delivery methods. It is demonstrated that the concentration of reduced glutathione (GSH) inside the cells is 100–1000 times higher than in the external environment. This results in a natural redox potential between the oxidizing extracellular and reducing intracellular areas (Meng et al. 2012)**.** In vivo research demonstrates that the tumor tissues had at least four times greater GSH concentrations than normal tissues that help to cleave the disulfide bond (Kuppusamy et al. 2002). Chen et al. fabricated DOX-loaded MSNs conjugated by Tf as capping and targeting agent through disulfide bond. Upon high concentration of GSH, it results in breakage of bond that leads to site-specific drug delivery. However, Zhang et al. developed DOX-loaded MSNs conjugated by immobilizing cytochrome C (Cyt C) as a stopper through the same bond using AS1411 aptamer as targeting agent. They also followed the similar mechanism to deliver drug at targeted site and induced in vitro tumor cell apoptosis (Zhang et al. 2014a, 2014b).

16.6.1.3 Enzymatic Degradation as Internal Stimuli

In the body, every biological process cannot happen without enzymes. They are large macromolecules that are involved in the biological process. There are several processes associated specifically to the growth, development, metabolism, aging, disease, and immunity. Dysregulation of enzyme expression is associated with a variety of devastating diseases, such as cancer caused by protease, esterase, or glycosidase. Due to specificity of enzymatic activity to certain tissues, enzyme stimuli response is very valuable. As peptide link breaks among non-terminal amino acids are broken by enzymes like metalloproteinase, hyaluronidase (HAase), and cathepsin B, these enzymes act as successful triggers (Meenakshi Upreti and Sethi 2013). In an interesting experiment, Kumar et al. developed 5-fluorouracil (5-FU)-loaded MSNs conjugated with guar gum as a capping agent to treat colonic cancer. This experiment demonstrates improved drug release and higher anticancer activity in the presence of colonic enzyme (Kumar et al. 2017)**.**

16.6.2 External Stimuli-Responsive Drug Delivery

The process of insertion of a contrast agent to substantiate the agglomeration of nanoparticles in the targeted cells, tissues, or organ as a result of activation of MSNs to release the chemotherapeutic molecules. This is the cornerstone for this type of stimulus responsiveness. The externally applied external stimulus is light, temperature, magnetic field, ultrasound, etc. The advantages like manual adjustability and self-controllability make them smart stimulus. But on the other hand, it has a disadvantage of using special equipments and techniques to produce stimulus.

16.6.2.1 Thermoresponsive as Stimuli

Thermo- or temperature-sensitive drug delivery systems outperform their challenges due to their variegation in outline and adjust ability of phase transition temperatures, compliant selecting capability, and in situ form transitions. Due to their simplicity of control and preparation, as well as their practical applications, this system is the most intensively researched family of environmentally sensitive polymers. You et al. developed fluorescein-loaded MSNs conjugated with pyridyl-PNIPAM through thiol bond. As the temperature increases, the coil changes to globule conformation, resulting in pore blocking of hybrid. This shows controlled drug release and improved cellular uptake when the temperature is below LCST (diffusion) (You et al. 2008).

16.6.2.2 ATP-Responsive Drug Delivery

In this technique, the "molecular unit of currency" ATP is used as an activator for medicinal administration. ATP-responsive nanosystems can be enhanced using ATP-attaching aptamer which specifically identify and are activated by ATP, thus enhancing the release of drug via conformational transition (Mo et al. 2014)**.** He et al. developed MSN-functionalized aptamers to form sandwich-like DNA structure. The surface grafting of MSNs using a click chemistry approach results in pore blockage. The experiment demonstrates improved capping efficiency and high selectivity under ATP and prevents burst release of bioactive molecules (He et al. 2012a, 2012b).

16.6.2.3 H_2O_2-Responsive Drug Delivery

Reactive oxygen species (ROS) are involved in many physiological and pathological processes. At low concentration, they promote cell cycle development but inhibit the same at high concentration. ROS stress is mostly present in many types of cancerous cells (Trachootham et al. 2009). H_2O_2 is a key element of ROS and a typical indicator of oxidative stress. In the case of cancerous cells, controlled drug delivery is achieved predominantly by the accumulation of ROS. Yang et al. developed clioquinol (metal chelator)-loaded MSNs capped with β-D-glucose gold nanoparticles by forming boronate ester bond to treat Alzheimer's disease. The experiment suggests the cleavage of bond at high concentration of H_2O_2 results in efficient brain drug delivery (Yang et al. 2016a, 2016b).

16.6.2.4 Magnetic Field-Responsive Drug Delivery

Nanoparticles with magnetic stimuli-responsive compounds are potential controlled delivery methods as extrinsic magnetic fields. It can be used to accumulate the nanoparticles in the targeted delivery site. For example, a novel nanocarrier ($MSN@Fe_3O_4$) that possesses chemical linkages allows the magnetic nanoparticles to cover the mesoporous pores on their matrix efficiently as nanocaps.

16.7 Therapeutic Applications of MSNs

16.7.1 Neurodegenerative Disorder

Neurodegenerative diseases (Alzheimer's and Parkinson's disease) have become a devastating health concern affecting the elder people globally. Neurodegenerative diseases are considered as multifactorial and slowly progressive disease that causes major hurdles to the successful design and development of therapeutics-based nanosystem. The multifactorial process involves the inflammation, escalated level of iron and reactive oxygen species, protein dysfunction and accumulation, and also appearance of pro-apoptotic proteins that ultimately lead to the degeneration of neurons. Neurodegenerative disorder is an illness involving neural dysfunction/death attributed to complex pathological processes, which are recognized through features such as mitochondrial dysfunction, protein aggregation, oxidative stress, metal ion dyshomeostasis, membrane potential change, neuro-inflammation, and neurotransmitter impairment (Suthar et al. 2021, 2023). MSNs have become increasingly popular due to their unique features such as large porosity and high surface area, large surface area, low toxicity, adjustable sizes, and a broad range of applications. They have an ability to interfere with different new molecular entity, which ensures to enhance the diagnostic and therapeutic efficacy of MSNs. Mo et al. fabricated DOX-loaded MSNs functionalized with PEI-c RGD polymer to improve anti-glioblastoma characteristics. It shows quick infiltration into cancer cells and hard excretion out of cells which results in reducing toxic effects and improving anti-glioblastoma efficacy (Mo et al. 2016). In another example, organic selenium drug is loaded in MSN nanosystems conjugated with RGD peptide and evaluated as a delivery vehicle to treat human brain glioma. The delivery platform was able to prolong circulation time and shows significant antiproliferative activity against U-87 cancer cells (You et al. 2016). Lopez et al. reported a study using SBA-15 ordered mesoporous silica particle carrying valproic acid and sodic phenytoin by impregnation in which they conclude it as a novel brain delivery to treat temporal lobe epilepsy (López et al. 2006).

16.7.2 Cancer Therapy

Despite recent advancements in cancer treatment, cancer is still one of the devastating diseases, which causes mortality. Chemotherapy, radiation therapy,

surgery, laser therapy, and combinational therapies are current treatment options in the treatment of cancer. However, it is also associated with consequential complications that lower the patient's standard of living (Suthar et al. 2022). MSNs are established as a promising nanocarrier in the application of cancer therapy. In a recent experiment, Chi et al. prepared ATO-loaded magnetic large-pore MSNs integrated with magnetic iron oxide nanoparticles based on prodrug approach for superior effects in hepatocellular cancer. In another protocol, a hybrid nanosystem modified with FA that demonstrated increased drug efficacy and controlled drug release directed the prolonged cytotoxic effect in SMMC-7721 cells (Chi et al. 2019). In another example, DOX encapsulated MSNs coated with $CaCO_3$ carried in camouflaged nanosystem for better pharmacokinetics behavior. The experiment exhibited the MSNs to significant accumulation in tumor tissues. Thus, developed nanosystem exhibited a higher anticancer efficacy compared to the free DOX (Liu et al. 2019). In another investigation, Taratula et al. developed cocktail drug transport platform for a management of cancer disease. A fascinating nanotechnological approach consists of the targeting functional molecules conjugated to MSNs that carry dual drugs, doxorubicin and cisplatin. The experiment evaluates the targeting MSN-mediated nanoformulations administered through inhalation route for the treatment of lung malignancy. Furthermore, an in vitro evaluation of novel nanomaterials revealed increased cytotoxicity and excellent apoptotic activity in NSCLC cells by means of a non-pump resistance mechanism (Taratula et al. 2011). Another interesting protocol, a biomolecule grafted with MSNs embedded with pharmacologically active moiety, was exploited for selective transport to EphA2, which is an epithelial site of interest. Also, MSN-mediated nanoplatforms exhibited selective tumor targeting via EphAr2 receptors in preclinical studies. EphA2-targeted MSNs containing doxorubicin showed significant proliferative effects in breast cancer cells when compared with mesoporous silica nanoparticles containing doxorubicin (Liu et al. 2018).

16.7.3 MSN-Mediated Nanofiber Scaffolds

MSN-based electrospun nanofibers have an immense potential for treatment of devastating diseases exclusively cancers and Alzheimer's disease. In the recent investigations, MSNs have been utilized for bone tissue engineering applications. It is due to the greater encapsulation of chemotherapeutics agents and also inorganic constituent. Qiu et al. prepared PLLA nanofibers carrying a doxorubicin-loaded MSNs for effective antitumor activity against HeLa cells. The developed PLLA/MSN composite nanofibrous mats thus provide a potentially implantable framework for the treatment of cancer (Qiu et al. 2013). Mehrasa et al. used a smart nanofibrous-mediated innovative strategy to prepare polylactic-co-glycolic acid-encased MSNs using a smart nanofiber for better brain delivery. In PC-12 cells, a nanoformulation displays an increase in hydrophilicity, ameliorated cell attachment, and augmented cellular processes (Mehrasa et al. 2016). Chen et al. developed GEM-loaded MSNs combined with poly-caprolactone-mediated nanofibrous delivery platform that

showed a sustained release of drug with enhanced anti-microbial activity. In the preclinical experiments, nanoformulations proved to be a better candidate for tissue regeneration (Chen et al. 2019).

16.8 Theranostic Applications

Theranostic nanomedicine employs a combination of diagnostic and therapeutic agents to monitor and treat the overwhelming diseases. A molecular probe or contrast agent is suitable for the investigation of cellular and subcellular processes using MRI (magnetic resonance imaging), CT (computed tomography) scanners, optimal imaging, and radionuclide imaging. Theranostic nanomedicines have intrinsic optical and photochemical properties, along with the capacity to transport drugs, which are more attractive to cancer researchers.

16.8.1 Diagnostic Application of MSNs

MSN-based nanotechnological approach became an intelligent tool with a wide range of diagnostic potential, such as MRI, fluorescent/luminescent imaging, positron emission tomography (PET), and so on. Thus, it is possible to detect early-onset diseases or to guide therapeutic approaches to improve the outcome of treatment. The focus of this section was on the recent advancements of MSN-mediated diagnostic probes, as well as the integration of therapy and diagnosis (theranostic), which is a key component of ever-greater medical advances.

16.8.2 Magnetic Resonance Imaging

The MRI is a widely used intraoperative diagnostic technique, which is largely utilized in clinics. It is due to its limitless benefits, excellent soft tissue contrast, and lack of ionizing radiation (Caltagirone et al. 2015). MSN-mediated nanotechnological approaches could increase cell survival by means of theranostic application. The principle of MRI lies in inducing the generation of a pulse by causing protons to relax with an externally applied magnetic field. There are two types of relaxations: first one is spin-lattice (relaxation time T1, longitudinal relaxivity), while another one is spin-spin relaxation (relaxation time T2, transverse relaxivity). Magnetite (Fe_3O_4) has significant T2 relaxation effects and, as shown in the figure, is used as a T2-weighted MRI contrast agent. Manganese (Mn^{2+}) and gadolinium (Gd^{3+}) nanoprobes include the most common T1 contrast agent due to its greater magnetic moment, whereas the T2-weighed MRI signals were significantly imaged by the deposition of nanoparticles in lumps and later administered intravenously into nude mice. Similarly, gadolinium carrying silane anchored to the surface of MSNs exhibited an extremely effective MRI contrast mediator. The in vivo experiment exhibited the Gd-MSNs as a highly efficient intravascular T1 along

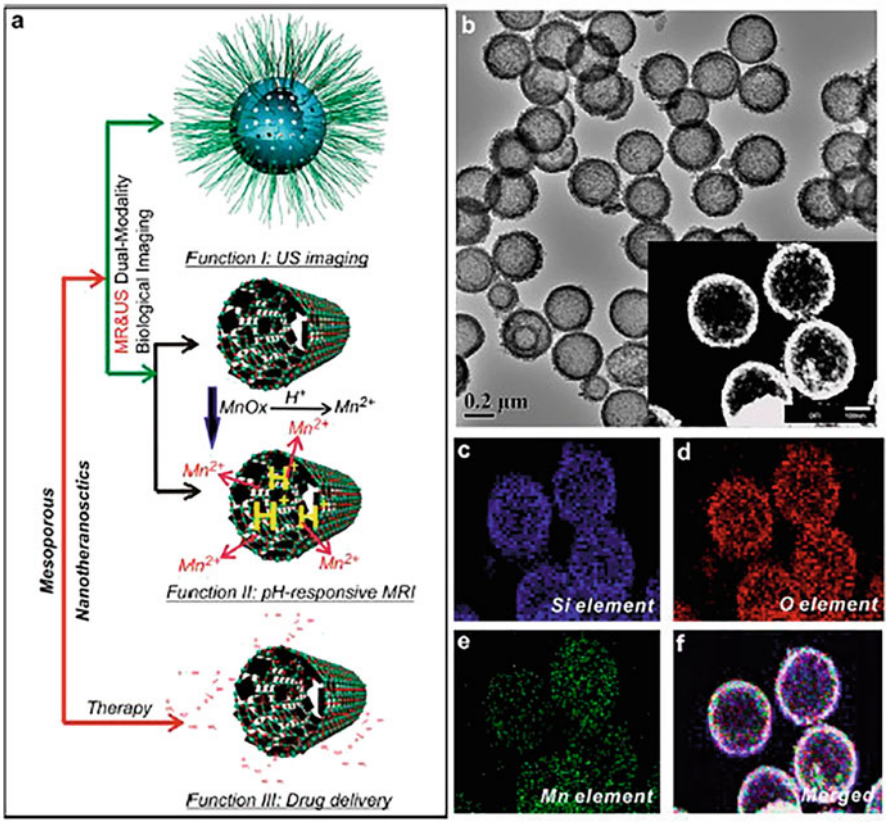

Fig. 16.6 (**a**) Schematic illustration of the microstructure and structure-related theranostic functions of HMCNs; (**b**) TEM image of HMCNs (inset, the STEM image with scale bar ¼ 100 nm); (**c**, **e**, **f**) Element mapping of Si (**c**), O (**d**), and Mn (**e**) in HMCNs (**f** color merged image of **c**, **d**, and **e**). Reproduced with permission from Chen et al. (2012), copyright 2012, Elsevier Ltd

with T2-weighed MRI contrast agents for soft tissues when used in greater dosages. As the Gd^{3+} ion becomes chelated within the networks of silica-shelled magnetite, transverse relaxivity increases, resulting in a more actual MRI for lymph nodes. MSNs with Gd_2O_3 in the mesopores also showed improved in vitro relaxation to commercial magnevist, suggesting a potential T1 contrast agent for tumor MRI. An MRI possesses a contrast agent like Gd^{3+}, which is cytotoxic, and the FDA states that it has been associated with nephrogenic systemic fibrosis and impaired kidney function. Chen et al. developed a manganese oxide-based embedded MSN as a contrast agent for pH-responsive drug cargoes exhibiting significant anti-proliferative action. MnOx nanoparticles have potential tool for MRI-T1 applications due to the presence of ionized Mn^{II} that engages with surrounding water molecules as illustrated in Fig. 16.6. The MnOx nanoparticles showcased

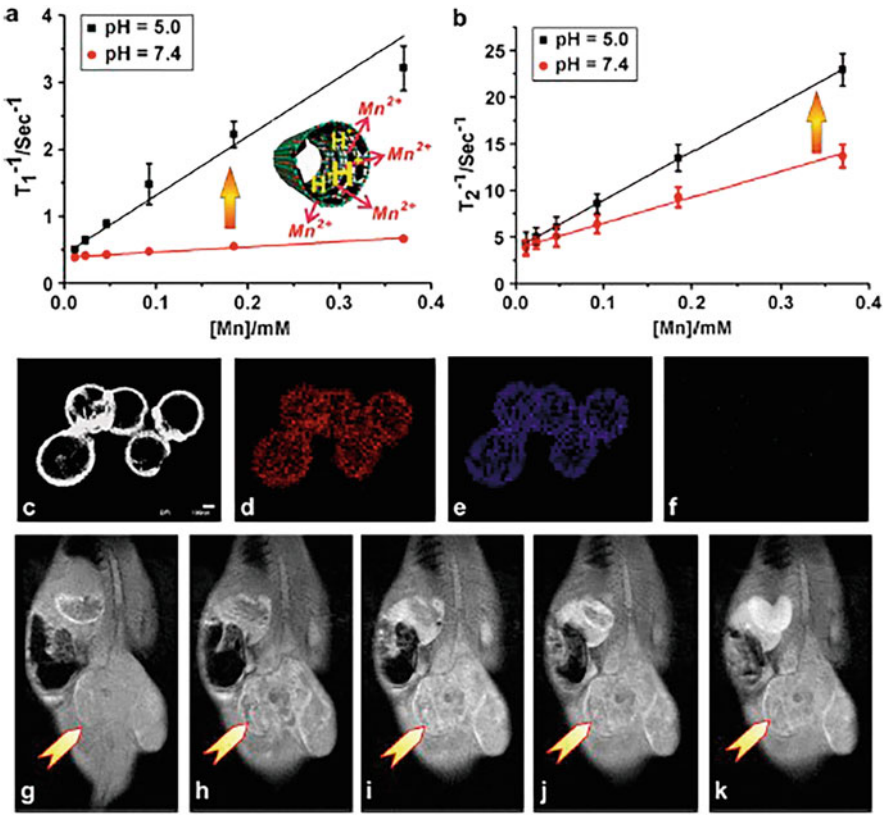

Fig. 16.7 T1 (**a**) and T2 (**b**) relaxivity of aqueous suspension of HMCNs after 4 h soaking in buffer solutions of different pH values (7.4 and 5.0) at 37 _C; STEM image (**c**, scale bar ¼ 100 nm) and corresponding element mappings of O (**d**), Si (**e**), and Mn (**f**) of HMCNs after the soaking in pH ¼ 5.0 buffer solution for 4 h; in vivo T1-weighted MRI of tumor before (**g**) and after intravenous administration of HMCNs (**h** 5 min, **i** 15 min, **j** 30 min, **k** 60 min; arrows indicate the tumor tissue). Reproduced with permission from Chen et al. (2012), copyright 2012, Elsevier Ltd

several functions such as the ability to release a weak acidic contrast agent when pH changes and active MRI capabilities. To treat cancer, mesopores could be used as reservoirs for encapsulating and releasing anticancer drugs. Energy dispersive X-ray spectroscopy (EDS) is used to measure the proportion of Mn in the complete matrix as shown in Fig. 16.7. A pH-based HMCN's nanomaterial is designed for better MRI performance for the detection of tumor environment. An increase in contrast enhancement was observed in the peripheral tumor vasculature, suggesting an enhanced permeability and retention (EPR) effect. As a result, the tumor interior demonstrates an augmentation of T1 contrast over time, which is attributed to manganese ion leakage. This leads to improved T1-weighted MRI in the weak acidic tumor microenvironment, which suggests that HMCNs could be used to deliver manganese ions for in vivo pH-sensitive imaging of tumors. The biocompatible

mesoporous silica shells carrying photothermally sensitive hydrophilic copper sulfide-mediated nanoparticles for a variety of potential theranostic applications. To clearly prove the theranostic potential of multifunctional nanoparticles, a variety of experiments were conducted, including in vitro targeting, preclinical safety profiling, photothermal ablation evaluation, in vivo vascular organ-specific imaging, bio-distribution, and histopathology.

16.8.3 Optical Imaging

Optical imaging provides a number of benefits over MRI, including a large number of probe options, excellent sensitivity, and non-ionization safety. Both in vitro and in vivo imaging of tumors can be achieved utilizing a fluorescent molecule like rare-earth-doped up-conversion, organic fluorescence, and quantum dots. Because the silica matrix is highly transparent, it has little effect on light excitation and emission as it passes through it. Trimethine cyanines (Cyc3), pentamethine cyanines (Cyc 5), heptamethine cyanines (Cy7), and indocyanine green (ICG) are some of the most regularly used cyanine dyes in optical imaging. A trimethylammonium modified MSNs carrying a ICG NIR contrast agent by an electrostatic attraction (Song et al. 2015), and a rat model with improved biodistribution and higher fluorescent intensity was observed. In vivo performance of PEGylated liposome-coated QDs with mesoporous silica shells was superior. It revealed the fluorescence and x-ray CT imaging taken at 4 h post-injection into the nude mice (Derfus et al. 2004). It also improved the biocompatibility and durability of QDs while demonstrating great optical imaging effectiveness in identifying cancer cells (Xiong et al. 2010). Gadolinium was used as the core, and mesoporous silica was used as the shell in a rattle-nanostructure-based nanotheranostic platform. An intravenous infusion of the core-shell nanoparticle resulted in a high NIR UCL signal in the tumor tissue (Huang et al. 2012). As shown in Fig. 16.8, an inherent technique of utilizing oxygen flaws in the MSN structure for optical imaging is also investigated. On the other hand, up-conversion fluorescent nanoparticles are gaining popularity as new optical imaging techniques. As a result, the combination of fluorescent molecules tagged to the surface of MSNs demonstrated the best imaging capability as well as biocompatibility (Chen et al. 2012).

16.8.4 Other Imaging Modalities

In addition to MRI and fluorescent agents, radionuclide is introduced to MSNs for PET (positron emission tomography). Clinically, PET provides an early diagnosis of tumors due to its high sensitivity. A broad range of probes and high penetration depth make it superior to other techniques. Heavy metals ^{64}Cu, ^{68}Ga, ^{99}mTc, and ^{111}In labelling medications, as well as radioisotopes ^{11}C, ^{18}F, and ^{15}O labelling molecules, are frequent PET agents. MSNs chelated with the radioisotope ^{64}Cu have recently been created for long-term PET imaging (Wang et al. 2017). Moreover,

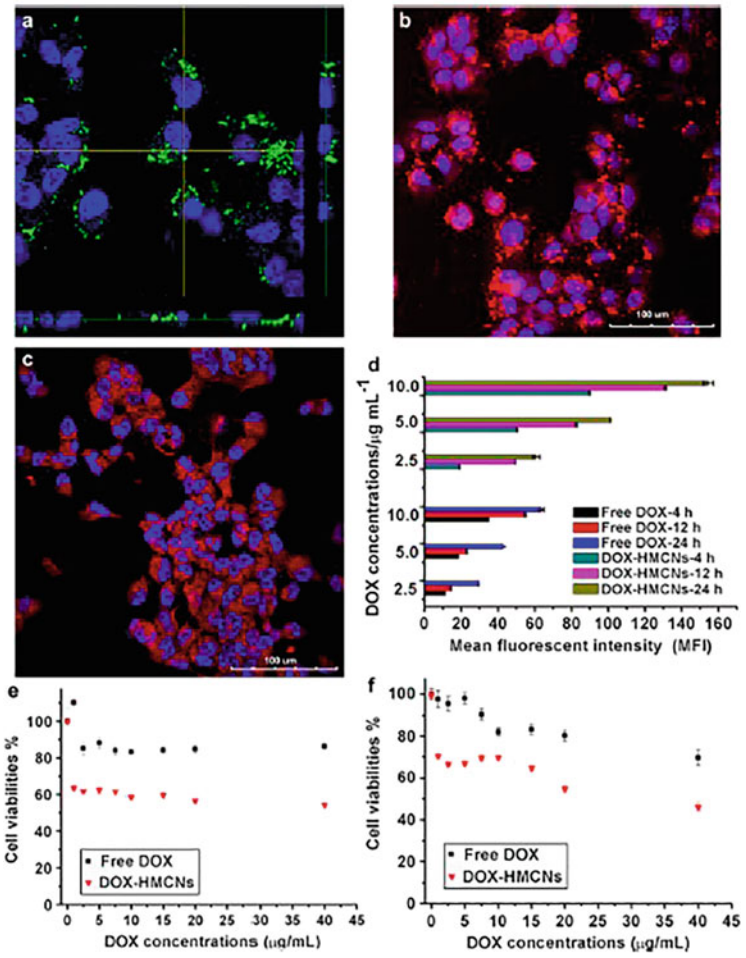

Fig. 16.8 CLSM images of MCF-7/ADR cancer cells after the uptake of FITC-HMCNs nanocapsules (**a**), DOX-HMCNs (**b**) and free DOX (**c**) at the equivalent DOX concentration of 10 mg/mL for 4 h; (**d**) The mean fluorescence intensity of DOX within MCF-7/ADR cells at three different co-incubation DOX concentrations and three different incubation durations by the introduction of free DOX and DOX-HMCNs; MCF-7/ADR cell viabilities of after the incubation with free DOX and DOX-HMCNs for different time durations (**e**: 24 h and **f**: 48 h), Reproduced with permission from Chen et al. (2012), copyright 2012, Elsevier Ltd

theranostic nanoparticles have been created for in vivo PET imaging of tumors. PET imaging showed a time-dependent uptake of hybrid nanoparticles by 4 T1 breast tumor cells post-injection. As a result, it appears that enhanced tumor accumulation is most likely the result of TRC105 conjugation. In another example silica-based nanoparticles have been extensively developed to tag with radioisotopes such as ^{18}F, ^{64}Cu, and ^{89}Zr for in vivo PET imaging. In order to assess their in vivo integrity and

biological potential, researchers prepared ^{89}Zr-coupled MSNs using chelator-free labeling. It was shown in in vivo experiments that the amount of ^{89}Zr detachment from the MSN mesopores remained 20 times slower than the rate of ^{89}Zr detachment from crushed silica. Also, MSN-hybrid derivatives played a crucial role in ^{89}Zr stabilization. Due to the compliance of MSNs, it is expected that there will be more radiolabeled mediated MSN design in the near future. Also, other therapeutic agents for cancer theranostic are allowing for a more advanced diagnostic tool. Ultrasound imaging techniques have some distinct properties such as non-invasive, non-ionizing, real-time, and cost-effective method. On the other hand, it has demerits including low sensitivity and resolution of ultra-sonography as compared to MRI, CT, or PET. The mesoporous hollow nanostructure exhibited better ultrasonographic resolutions by virtue of its hollow space regions for modulating ultrasound imaging along with theranostic applications in tumor model. It was shown in in vivo VX-2 rabbit model that exhibited the clear imaging guided by ultrasound at tumor site (Liberman et al. 2012). Other imaging applications are feasible when silica NP is mixed with unique nanoparticles, such as gold. MSNs carrying gold nanorods substantially showcased the photoacoustic signals to a significantly higher amount targeted tissues than bare counterparts. Also, gold-silica nano-hybrid nanoparticles were used as a multi-model contrast agent particularly in Raman molecular imaging. The MSN-based imaging probes were reported to be multi-model imaging approaches by using the integrative approach of imaging agents and nanoparticles in the significant tumor site imaging.

16.9 Conclusions and Future Outlook

In cancerous treatment and neurological disease, traditional drug delivery systems have disadvantages such as multidrug resistance (MDR), toxicity, and inappropriate drug distribution. Reflecting this need, the nanotechnological approaches have immense potential in the arena of pharmaceutical technology as well as theranostic applications. MSN-based nanostructures are potential candidates in the drug delivery and cancer therapy. Mesoporous scaffolds and their hybrid derivatives have explored various downstream biomedical applications due to their mesoporous structure, greater specific surface area, ease of surface engineering, and intelligent delivery platforms. In this chapter, we have discussed the cutting edge of advances within this field and elucidated the significance of targeted functionalities derived from MSNs in the forthcoming biomedical applications. Several preclinical reports establish the safety profile of MSNs in the treatment of various cancers. The tailor-made MSN-based nanosystems have exhibited the stimuli-responsive drug release of therapeutics molecules, tissue targeting, and excellent pharmacokinetic profile. Preclinical investigations assure the better bioavailability and biosafety of MSN-based smart drug delivery nanocarriers. We have summarized the in vivo biological effects of MSNsmediated nanoplatform, hopefully leading to the translation of these emerging systems to the clinical use through the integrated efforts of researchers and engineers. While MSN-based biomaterials have already achieved

numerous accomplishments, there are several apprehensions yet to be resolved, for further evolution in the biomedical arena.

References

Abukhadra MR, Refay NM, El-Sherbeeny AM, El-Meligy MA (2020) Insight into the loading and release properties of MCM-48/biopolymer composites as carriers for 5-fluorouracil: equilibrium modeling and pharmacokinetic studies. ACS omega 20:11745–11755

Antonietti M, Berton B, Göltner C, Hentze HP (1998) Synthesis of mesoporous silica with large pores and bimodal pore size distribution by templating of polymer latices. Adv Mater 2:154–159

Asefa T, MacLachlan MJ, Grondey H, Coombs N, Ozin GA (2000) Metamorphic channels in periodic mesoporous methylenesilica. Angew Chem Int Ed 10:1808–1811

Attard GS, Glyde JC, Göltner CG (1995) Liquid-crystalline phases as templates for the synthesis of mesoporous silica. Nature 6555:366–368

Bagshaw SA, Pinnavaia TJ (1996) Mesoporous alumina molecular sieves. Angew Chem Int Ed Engl 10:1102–1105

Barbe C, Bartlett J, Kong L, Finnie K, Lin HQ, Larkin M, Calleja S, Bush A, Calleja G (2004) Silica particles: a novel drug-delivery system. Adv Mater 21:1959–1966

Beltrán-Osuna ÁA, Perilla JE (2016) Colloidal and spherical mesoporous silica particles: synthesis and new technologies for delivery applications. J Sol-Gel Sci Technol 2:480–496

Biswas N (2017) Modified mesoporous silica nanoparticles for enhancing oral bioavailability and antihypertensive activity of poorly water-soluble valsartan. Eur J Pharm Sci 99:152–160

Caltagirone C, Bettoschi A, Garau A, Montis R (2015) Silica-based nanoparticles: a versatile tool for the development of efficient imaging agents. Chem Soc Rev 14:4645–4671

Chang D, Gao Y, Wang L, Liu G, Chen Y, Wang T, Tao W, Mei L, Huang L, Zeng X (2016) Polydopamine-based surface modification of mesoporous silica nanoparticles as pH-sensitive drug delivery vehicles for cancer therapy. J Colloid Interface Sci 463:279–287

Chen J, Qiu X, Ouyang J, Kong J, Zhong W, Xing MM (2011b) pH and reduction dual-sensitive copolymeric micelles for intracellular doxorubicin delivery. Biomacromolecules 10:3601–3611

Chen X, Xu C, He H (2019) Electrospinning of silica nanoparticles-entrapped nanofibers for sustained gentamicin release. Biochem Biophys Res Commun 4:1085–1089

Chen Y, Chen H, Guo L, He Q, Chen F, Zhou J, Feng J, Shi J (2010) Hollow/rattle-type mesoporous nanostructures by a structural difference-based selective etching strategy. ACS Nano 1:529–539

Chen Y, Chen H, Sun Y, Zheng Y, Zeng D, Li F, Zhang S, Wang X, Zhang K, Ma M, He Q (2011a) Multifunctional mesoporous composite nanocapsules for highly efficient MRI-guided high-intensity focused ultrasound cancer surgery. Angew Chem Int Ed 52:12505–12509

Chen Y, Yin Q, Ji X (2012) Manganese oxide-based multifunctionalized mesoporous silica nanoparticles for pH-responsive MRI, ultrasonography and circumvention of MDR in cancer cells. Biomaterials 33:7126–7137

Chi X, Zhang R, Zhao T, Gong X, Wei R, Yin Z, Lin H, Li D, Shan H, Gao J (2019) Targeted arsenite-loaded magnetic multifunctional nanoparticles for treatment of hepatocellular carcinoma. Nanotechnology 17:175101

Cui Y, Xu Q, Chow PK, Wang D, Wang CH (2013) Transferrin-conjugated magnetic silica PLGA nanoparticles loaded with doxorubicin and paclitaxel for brain glioma treatment. Biomaterials 33:8511–8520

Datt A, El-Maazawi I, Larsen SC (2012) Aspirin loading and release from MCM-41 functionalized with aminopropyl groups via co-condensation or postsynthesis modification methods. J Phys Chem C 34:18358–18366

Deng Z, Zhen Z, Hu X, Wu S, Xu Z, Chu PK (2011) Hollow chitosan–silica nanospheres as pH-sensitive targeted delivery carriers in breast cancer therapy. Biomaterials 21:4976–4986

Derfus AM, Chan WC, Bhatia SN (2004) Probing the cytotoxicity of semiconductor quantum dots. Nano Lett 1:11–18

Duo Y, Yang M, Du Z, Feng C, Xing C, Wu Y, Xie Z, Zhang F, Huang L, Zeng X, Chen H (2018) CX-5461-loaded nucleolus-targeting nanoplatform for cancer therapy through induction of pro-death autophagy. Acta Biomater 79:317–330

Etheridge ML, Campbell SA, Erdman AG, Haynes CL, Wolf SM, McCullough J (2013) The big picture on nanomedicine: the state of investigational and approved nanomedicine products. Nanomedicine 1:1–4

Fang IJ, Slowing II, Wu KC, Lin VS, Trewyn BG (2012) Ligand conformation dictates membrane and endosomal trafficking of arginine-glycine-aspartate (RGD)-functionalized mesoporous silica nanoparticles. Chemistry–a. European Journal 25:7787–7792

Gai F, Zhou T, Chu G, Li Y, Liu Y, Huo Q, Akhtar F (2016) Mixed anionic surfactant-templated mesoporous silica nanoparticles for fluorescence detection of Fe 3+. Dalton Trans 2:508–514

Gan Q, Lu X, Yuan Y, Qian J, Zhou H, Lu X, Shi J, Liu C (2011) A magnetic, reversible pH-responsive nanogated ensemble based on Fe3O4 nanoparticles-capped mesoporous silica. Biomaterials 7:1932–1942

Gauro R, Nandave M, Jain VK, Jain K (2021) Advances in dendrimer-mediated targeted drug delivery to the brain. J Nanopart Res 23:76. https://doi.org/10.1007/s11051-021-05175-8

Gratton SE, Ropp PA, Pohlhaus PD, Luft JC, Madden VJ, Napier ME, DeSimone JM (2008) The effect of particle design on cellular internalization pathways. Proc Natl Acad Sci 33:11613–11618

Han P, Ma N, Ren H, Xu H, Li Z, Wang Z, Zhang X (2010) Oxidation-responsive micelles based on a selenium-containing polymeric superamphiphile. Langmuir 18:14414–14418

Hanafi-Bojd MY, Jaafari MR, Ramezanian N, Xue M, Amin M, Shahtahmassebi N, Malaekeh-Nikouei B (2015) Surface functionalized mesoporous silica nanoparticles as an effective carrier for epirubicin delivery to cancer cells. Eur J Pharm Biopharm 89:248–258

Haynes T, Bougnouch O, Dubois V, Hermans S (2020) Preparation of mesoporous silica nanocapsules with a high specific surface area by hard and soft dual templating approach: application to biomass valorization catalysis. Microporous Mesoporous Mater 306:110400

He Q, Ma M, Wei C, Shi J (2012a) Mesoporous carbon@ silicon-silica nanotheranostics for synchronous delivery of insoluble drugs and luminescence imaging. Biomaterials 17:4392–4402

He X, Zhao Y, He D, Wang K, Xu F, Tang J (2012b) ATP-responsive controlled release system using aptamer-functionalized mesoporous silica nanoparticles. Langmuir 35:12909–12915

Heggannavar GB, Hiremath CG, Achari DD, Pangarkar VG, Kariduraganavar MY (2018) Development of doxorubicin-loaded magnetic silica–pluronic F-127 nanocarriers conjugated with transferrin for treating glioblastoma across the blood–brain barrier using an in vitro model. ACS omega 7:8017–8026

Hoffmann F, Cornelius M, Morell J, Fröba M (2006) Silica-based mesoporous organic–inorganic hybrid materials. Angew Chem Int Ed 20:3216–3251

Horcajada P, Ramila A, Perez-Pariente J, Vallet-Regı M (2004) Influence of pore size of MCM-41 matrices on drug delivery rate. Microporous Mesoporous Mater 1-3:105–109

Huang X, Teng X, Chen D, Tang F, He J (2010) The effect of the shape of mesoporous silica nanoparticles on cellular uptake and cell function. Biomaterials 3:438–448

Huang X, Zhang F, Lee S, Swierczewska M, Kiesewetter DO, Lang L, Zhang G, Zhu L, Gao H, Choi HS, Niu G (2012) Long-term multimodal imaging of tumor draining sentinel lymph nodes using mesoporous silica-based nanoprobes. Biomaterials 17:4370–4378

Izquierdo-Barba I, Martinez Á, Doadrio AL, Pérez-Pariente J, Vallet-Regí M (2005) Release evaluation of drugs from ordered three-dimensional silica structures. Eur J Pharm Sci 5:365–373

Jain K, Gupta U, Jain NK (2014) Dendronized nanoconjugates of lysine and folate for treatment of cancer. Eur J Pharm Biopharm 87(3):500–509. https://doi.org/10.1016/j.ejpb.2014.03.015

Jokerst JV, Lobovkina T, Zare RN, Gambhir SS (2011) Nanoparticle PEGylation for imaging and therapy. Nanomedicine 4:715–728

Kaneda M, Tsubakiyama T, Carlsson A, Sakamoto Y, Ohsuna T, Terasaki O, Joo SH, Ryoo R (2002) Structural study of mesoporous MCM-48 and carbon networks synthesized in the spaces of MCM-48 by electron crystallography. J Phys Chem B 6:1256–1266

Khan MA, Jain VK, Rizwanullah M, Ahmad J, Jain K (2019) PI3K/AKT/mTOR pathway inhibitors in triple-negative breast cancer: a review on drug discovery and future challenges. Drug Discov Today 24(11):2181–2191. https://doi.org/10.1016/j.drudis.2019.09.001

Kim KS, Kim J, Kim DH, Hwang HS, Na K (2018) Multifunctional trastuzumab–chlorin e6 conjugate for the treatment of HER2-positive human breast cancer. Biomater Sci 5:1217–1226

Kresge AC, Leonowicz ME, Roth WJ, Vartuli JC, Beck JS (1992) Ordered mesoporous molecular sieves synthesized by a liquid-crystal template mechanism. Nature 6397:710–712

Kumar B, Kulanthaivel S, Mondal A, Mishra S, Banerjee B, Bhaumik A, Banerjee I, Giri S (2017) Mesoporous silica nanoparticle-based enzyme responsive system for colon specific drug delivery through guar gum capping. Colloids Surf B: Biointerfaces 150:352–361

Kumar R, Roy I, Ohulchanskyy TY, Goswami LN, Bonoiu AC, Bergey EJ, Tramposch KM, Maitra A, Prasad PN (2008) Covalently dye-linked, surface-controlled, and bioconjugated organically modified silica nanoparticles as targeted probes for optical imaging. ACS Nano 3: 449–456

Kuppusamy P, Li H, Ilangovan G, Cardounel AJ, Zweier JL, Yamada K, Krishna MC, Mitchell JB (2002) Noninvasive imaging of tumor redox status and its modification by tissue glutathione levels. Cancer Res 1:307–312

Li H, Yan W, Suo X, Peng H, Yang X, Li Z, Zhang J, Liu D (2019) Nucleus-targeted nano delivery system eradicates cancer stem cells by combined thermotherapy and hypoxia-activated chemotherapy. Biomaterials 200:1–4

Li Z, Barnes JC, Bosoy A, Stoddart JF, Zink JII (2012) Mesoporous silica nanoparticles in biomedical applications. Chem Soc Rev 7:2590–2605

Liberman A, Martinez HP, Ta CN, Barback CV, Mattrey RF, Kono Y, Blair SL, Trogler WC, Kummel AC, Wu Z (2012) Hollow silica and silica-boron nano/microparticles for contrast-enhanced ultrasound to detect small tumors. Biomaterials 20:5124–5129

Lim MH, Stein A (1999) Comparative studies of grafting and direct syntheses of inorganic−organic hybrid mesoporous materials. Chem Mater 11:3285–3295

Lin YS, Haynes CL (2010) Impacts of mesoporous silica nanoparticle size, pore ordering, and pore integrity on hemolytic activity. J Am Chem Soc 13:4834–4842

Liu CM, Chen GB, Chen HH, Zhang JB, Li HZ, Sheng MX, Weng WB, Guo SM (2019) Cancer cell membrane-cloaked mesoporous silica nanoparticles with a pH-sensitive gatekeeper for cancer treatment. Colloids Surf B: Biointerfaces 175:477–486

Liu J, Huang Y, Kumar A, Tan A, Jin S, Mozhi A, Liang XJ (2014) pH-sensitive nano-systems for drug delivery in cancer therapy. Biotechnol Adv 4:693–710

Liu Y, Yang G, Jin S, Xu L, Zhao CX (2020) Development of High-Drug-Loading Nanoparticles ChemPlusChem 85:2143–2157

Liu Z, Tao Z, Zhang Q, Wan S, Zhang F, Zhang Y, Wu G, Wang J (2018) YSA-conjugated mesoporous silica nanoparticles effectively target EphA2-overexpressing breast cancer cells. Cancer Chemother Pharmacol 4:687–695

López T, Basaldella EI, Ojeda ML, Manjarrez J, Alexander-Katz R (2006) Encapsulation of valproic acid and sodic phenytoin in ordered mesoporous SiO2 solids for the treatment of temporal lobe epilepsy. Opt Mater 1:75–81

Lou XW, Archer LA, Yang Z (2008) Hollow micro−/nanostructures: synthesis and applications. Adv Mater 21:3987–4019

Meenakshi Upreti AJ, Sethi P (2013) Tumor microenvironment and nanotherapeutics. Transl Cancer Res 4:309–319

Mehrasa M, Asadollahi MA, Nasri-Nasrabadi B, Ghaedi K, Salehi H, Dolatshahi-Pirouz A, Arpanaei A (2016) Incorporation of mesoporous silica nanoparticles into random electrospun PLGA and PLGA/gelatin nanofibrous scaffolds enhances mechanical and cell proliferation properties. Mater Sci Eng C 66:25–32

Meng F, Cheng R, Deng C, Zhong Z (2012) Intracellular drug release nanosystems Materials today 10:436–442

Mo J, He L, Ma B, Chen T (2016) Tailoring particle size of mesoporous silica nanosystem to antagonize glioblastoma and overcome blood–brain barrier. ACS Appl Mater Interfaces 11: 6811–6825

Mo R, Jiang T, DiSanto R, Tai W, Gu Z (2014) ATP-triggered anticancer drug delivery Nature communications 1:1–10

Mozafarinia M, Karimi S, Farrokhnia M, Esfandiari J (2021) In vitro breast cancer targeting using Trastuzumab-conjugated mesoporous silica nanoparticles: towards the new strategy for decreasing size and high drug loading capacity for drug delivery purposes in MSN synthesis. Microporous Mesoporous Mater 316:110950

Narayan R, Gadag S, Cheruku SP, Raichur AM, Day CM, Garg S, Manandhar S, Pai KS, Suresh A, Mehta CH, Nayak Y (2021) Chitosan-glucuronic acid conjugate coated mesoporous silica nanoparticles: a smart pH-responsive and receptor-targeted system for colorectal cancer therapy. Carbohydr Polym 261:117893

Narayan R, Nayak UY, Raichur AM, Garg S (2018) Mesoporous silica nanoparticles: a comprehensive review on synthesis and recent advances. Pharmaceutics 3:118

Natarajan SK, Selvaraj S (2014) Mesoporous silica nanoparticles: importance of surface modifications and its role in drug delivery. RSC Adv 28:14328–14334

Newalkar BL, Komarneni S, Katsuki H (2000) Rapid synthesis of mesoporous SBA-15 molecular sieve by a microwave–hydrothermal process. Chem Commun 23:2389–2390

Niesz K, Yang P, Somorjai GA (2005) Sol-gel synthesis of ordered mesoporous alumina. Chem Commun 15:1986–1987

Nozawa K, Gailhanou H, Raison L, Panizza P, Ushiki H, Sellier E, Delville JP, Delville MH (2005) Smart control of monodisperse Stöber silica particles: effect of reactant addition rate on growth process. Langmuir 4:1516–1523

Otsuka M, Tokumitsu K, Matsuda Y (2000) Solid dosage form preparations from oily medicines and their drug release. Effect of degree of surface-modification of silica gel on the drug release from phytonadione-loaded silica gels. J Control Release 2-3:369–384

Pal N, Bhaumik A (2013) Soft templating strategies for the synthesis of mesoporous materials: inorganic, organic–inorganic hybrid and purely organic solids. Adv Colloid Interf Sci 189:21–41

Pan G, Jia TT, Huang QX, Qiu YY, Xu J, Yin PH, Liu T (2017) Mesoporous silica nanoparticles (MSNs)-based organic/inorganic hybrid nanocarriers loading 5-fluorouracil for the treatment of colon cancer with improved anticancer efficacy. Colloids Surf B: Biointerfaces 159:375–385

Qiu K, He C, Feng W, Wang W, Zhou X, Yin Z, Chen L, Wang H, Mo X (2013) Doxorubicin-loaded electrospun poly (L-lactic acid)/mesoporous silica nanoparticles composite nanofibers for potential postsurgical cancer treatment. J Mater Chem B 36:4601–4611

Roy I, Kumar P, Kumar R, Ohulchanskyy TY, Yong KT, Prasad PN (2014) Ormosil nanoparticles as a sustained-release drug delivery vehicle. RSC Adv 96:53498–53504

Roy I, Ohulchanskyy TY, Pudavar HE, Bergey EJ, Oseroff AR, Morgan J, Dougherty TJ, Prasad PN (2003) Ceramic-based nanoparticles entrapping water-insoluble photosensitizing anticancer drugs: a novel drug− carrier system for photodynamic therapy. J Am Chem Soc 26:7860–7865

Roy I, Stachowiak MK, Bergey EJ (2008) Nonviral gene transfection nanoparticles: function and applications in the brain. Nanomedicine: nanotechnology. Biol Med 2:89–97

Sakamoto Y, Kaneda M, Terasaki O, Zhao DY, Kim JM, Stucky G, Shin HJ, Ryoo R (2000) Direct imaging of the pores and cages of three-dimensional mesoporous materials. Nature 6811:449–453

Sánchez T, Salagre P, Cesteros Y (2013) Ultrasounds and microwave-assisted synthesis of mesoporous hectorites. Microporous Mesoporous Mater 171:24–34

Schmidt HK, Geiter E, Mennig M, Krug H, Becker C, Winkler RP (1998) The sol-gel process for nano-technologies: new nanocomposites with interesting optical and mechanical properties. J Sol-Gel Sci Technol 1:397–404

Sharma KK, Asefa T (2007) Efficient bifunctional nanocatalysts by simple postgrafting of spatially isolated catalytic groups on mesoporous materials. Angewandte Chemie International EditionApr 16:2879–2882

Slowing I, Trewyn BG, Lin VS (2006) Effect of surface functionalization of MCM-41-type mesoporous silica nanoparticles on the endocytosis by human cancer cells. J Am Chem Soc 46:14792–14793

Slowing II, Vivero-Escoto JL, Wu CW, Lin VS (2008) Mesoporous silica nanoparticles as controlled release drug delivery and gene transfection carriers. Adv Drug Deliv Rev 11:1278–1288

Song JT, Yang XQ, Zhang XS, Yan DM, Wang ZY, Zhao YD (2015) Facile synthesis of gold nanospheres modified by positively charged mesoporous silica, loaded with near-infrared fluorescent dye, for in vivo X-ray computed tomography and fluorescence dual mode imaging. ACS Appl Mater Interfaces 31:17287–17297

Song SW, Hidajat K, Kawi S (2005) Functionalized SBA-15 materials as carriers for controlled drug delivery: influence of surface properties on matrix− drug interactions. Langmuir 21:9568–9575

Soni N, Jain K, Gupta U, Jain NK (2015) Controlled delivery of gemcitabine hydrochloride using mannosylated poly(propyleneimine) dendrimers. J Nanopart Res 17:458. https://doi.org/10.1007/s11051-015-3265-1

Soto RJ, Yang L, Schoenfisch MH (2016) Functionalized mesoporous silica via an aminosilane surfactant ion exchange reaction: controlled scaffold design and nitric oxide release. ACS Appl Mater Interfaces 3:2220–2231

Suthar T, Navneet, Jain K (2021) Nutraceuticals against neurodegeneration: understanding the mechanistic pathways. In: Lokhande JN, Pathak YV (eds) Nutraceuticals for aging and anti-aging: basic understanding and clinical evidence. CRC Press, ISBN: 9781003110866; pp 133–168

Suthar T, Jain VK, Popli H, Jain K (2022) 12 - Nanoemulsions as effective carriers for targeting brain tumors. In: Kumar L, Pathak Y (eds) Nanocarriers for drug-targeting brain tumors. Elsevier, pp 347–363 https://doi.org/10.1016/B978-0-323-90773-6.00008-7

Suthar T, Patel P, Singh P, Datusalia AK, Yadav AK, Jain K (2023) Hesperidin microemulsion: Formulation optimization, characterization, and in vitro evaluation. J Drug Del Sci Tech 80 (104166):1–12

Taratula O, Garbuzenko OB, Chen AM, Minko T (2011) Innovative strategy for treatment of lung cancer: targeted nanotechnology-based inhalation co-delivery of anticancer drugs and siRNA. J Drug Target 10:900–914

Trachootham D, Alexandre J, Huang P (2009) Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? Nat Rev Drug Discov 7:579–591

Vivero-Escoto JL, Slowing II, Trewyn BG, Lin VS (2010) Mesoporous silica nanoparticles for intracellular controlled drug delivery. Small 18:1952–1967

Wang G, Otuonye AN, Blair EA, Denton K, Tao Z, Asefa T (2009) Functionalized mesoporous materials for adsorption and release of different drug molecules: a comparative study. Journal of Solid-State Chemistry 7:1649–1660

Wang Q, Hu W, Cai L, Huang Y, Qian Z (2017) Nanomedicines in bone cancer—from diagnostics to therapies. J Biomed Nanotechnol 8:911–930

Wang Y, Han N, Zhao Q, Bai L, Li J, Jiang T, Wang S (2015) Redox-responsive mesoporous silica as carriers for controlled drug delivery: a comparative study based on silica and PEG gatekeepers. Eur J Pharm Sci 72:12–20

Wanka G, Hoffmann H, Ulbricht W (1994) Phase diagrams and aggregation behavior of poly (oxyethylene)-poly (oxypropylene)-poly (oxyethylene) triblock copolymers in aqueous solutions. Macromolecules 15:4145–4159

Watermann A, Brieger J (2017) Mesoporous silica nanoparticles as drug delivery vehicles in cancer. Nano 7:189

Xie X, Li F, Zhang H, Lu Y, Lian S, Lin H, Gao Y, Jia L (2016) EpCAM aptamer-functionalized mesoporous silica nanoparticles for efficient colon cancer cell-targeted drug delivery. Eur J Pharm Sci 83:28–35

Xiong L, Yang T, Yang Y, Xu C, Li F (2010) Long-term in vivo biodistribution imaging and toxicity of polyacrylic acid-coated upconversion nanophosphors. Biomaterials 27:7078–7085

Yamada H, Urata C, Higashitamori S, Aoyama Y, Yamauchi Y, Kuroda K (2014) Critical roles of cationic surfactants in the preparation of colloidal mesostructured silica nanoparticles: control of mesostructure, particle size, and dispersion. ACS Appl Mater Interfaces 5:3491–3500

Yang KN, Zhang CQ, Wang W, Wang PC, Zhou JP, Liang XJ (2014) pH-responsive mesoporous silica nanoparticles employed in controlled drug delivery systems for cancer treatment. Cancer Biol Med 1:34–43

Yang L, Yin T, Liu Y, Sun J, Zhou Y, Liu J (2016b) Gold nanoparticle-capped mesoporous silica-based H2O2-responsive controlled release system for Alzheimer's disease treatment. Acta Biomater 46:177–190

Yang P, Zhao D, Margolese DI, Chmelka BF, Stucky GD (1999) Block copolymer templating syntheses of mesoporous metal oxides with large ordering lengths and semicrystalline framework. Chem Mater 10:2813–2826

Yang Y, Achazi K, Jia Y, Wei Q, Haag R, Li J (2016a) Complex assembly of polymer conjugated mesoporous silica nanoparticles for intracellular pH-responsive drug delivery. Langmuirm 47: 12453–12460

You Y, Yang L, He L, Chen T (2016) Tailored mesoporous silica nanosystem with enhanced permeability of the blood–brain barrier to antagonize glioblastoma. J Mater Chem B 36:5980–5990

You YZ, Kalebaila KK, Brock SL, Oupicky D (2008) Temperature-controlled uptake and release in PNIPAM-modified porous silica nanoparticles. Chem Mater 10:3354–3359

Zhang B, Luo Z, Liu J, Ding X, Li J, Cai K (2014b) Cytochrome c end-capped mesoporous silica nanoparticles as redox-responsive drug delivery vehicles for liver tumor-targeted triplex therapy in vitro and in vivo. J Control Release 192:192–201

Zhang L, Li Y, Jimmy CY, Chen YY, Chan KM (2014a) Assembly of polyethylenimine-functionalized iron oxide nanoparticles as agents for DNA transfection with magnetofection technique. J Mater Chem B 45:7936–7944

Zhao D, Feng J, Huo Q, Melosh N, Fredrickson GH, Chmelka BF, Stucky GD (1998a) Triblock copolymer syntheses of mesoporous silica with periodic 50 to 300 angstrom pores. Science 5350:548–552

Zhao D, Huo Q, Feng J, Chmelka BF, Stucky GD (1998b) Nonionic triblock and star diblock copolymer and oligomeric surfactant syntheses of highly ordered, hydrothermally stable, mesoporous silica structures. J Am Chem Soc 24:6024–6036

Zhao P, Li L, Zhou S, Qiu L, Qian Z, Liu X, Cao X, Zhang H (2018) TPGS functionalized mesoporous silica nanoparticles for anticancer drug delivery to overcome multidrug resistance. Mater Sci Eng C 84:108–117

Zhuang J, Zhou L, Tang W, Ma T, Li H, Wang X, Chen C, Wang P (2021) Tumor targeting antibody-conjugated nanocarrier with pH/thermo dual-responsive macromolecular film layer for enhanced cancer chemotherapy. Mater Sci Eng C 118:111361