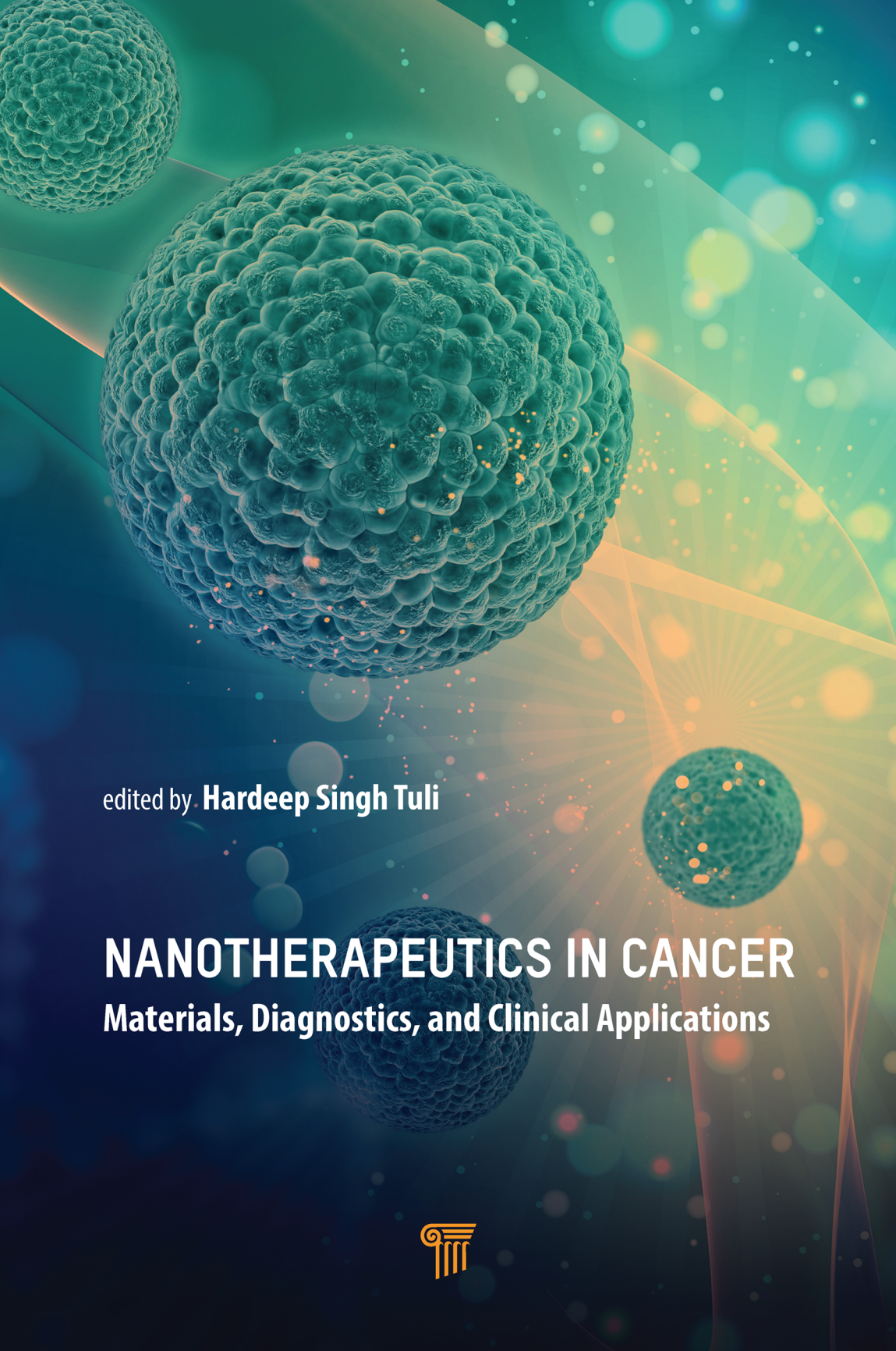
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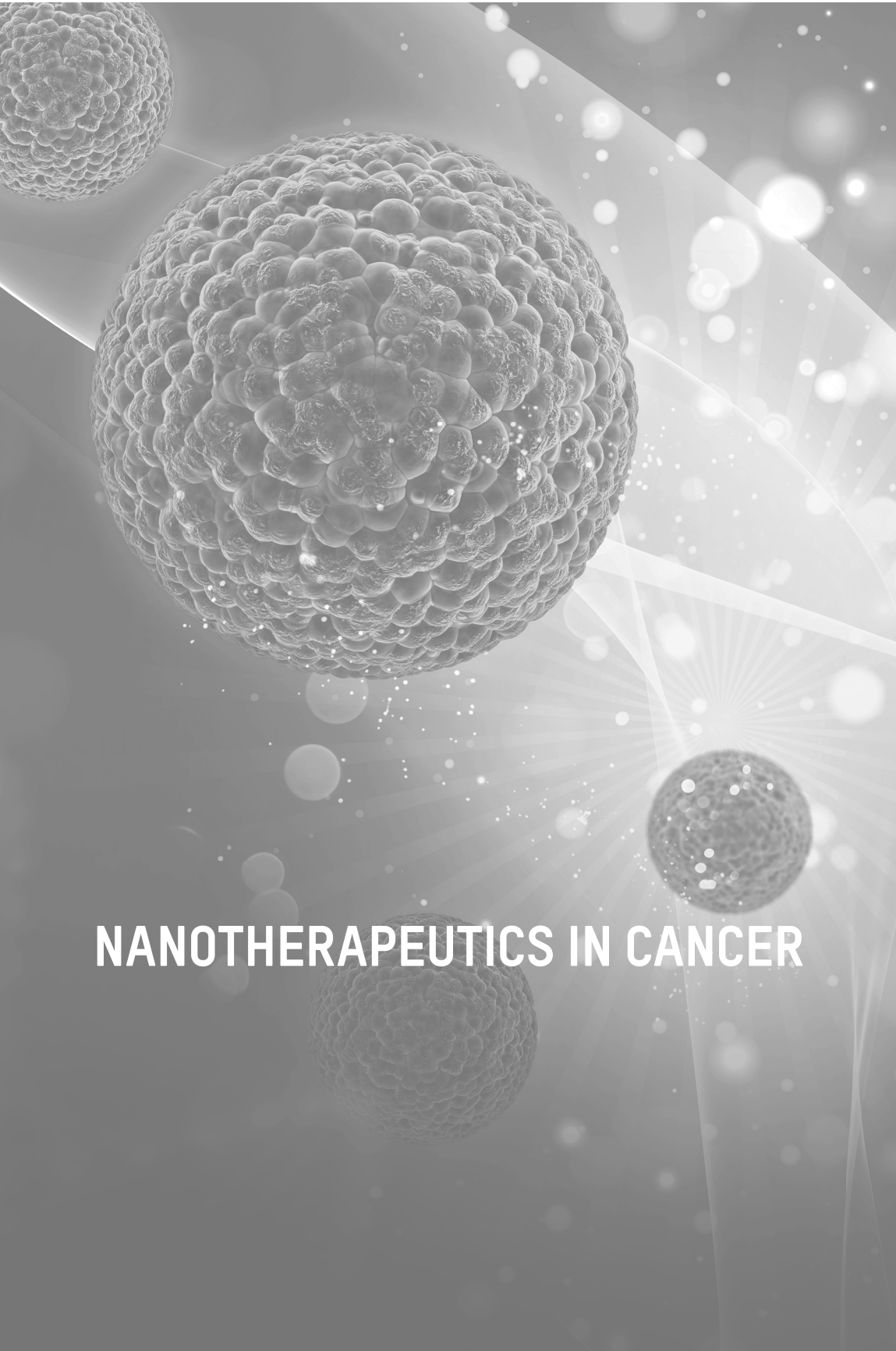
Materials, Diagnostics, and Clinical Applications



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Materials, Diagnostics, and Clinical Applications

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Hardeep Singh Tuli

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Preface

The incidence of cancer diseases is continually increasing worldwide, causing millions of deaths each year. As it is one of the main causes of disease-related death, tremendous academic effort, with substantial research budgets, is being devoted to developing and designing new anticancer drugs and treatment strategies. However, considering the hallmarks of cancer seems to be a more rational strategy for designing and developing efficient anticancer drugs and treatment approaches. The applications of nanoparticulate drug delivery have received abundant interest in the field of cancer diagnosis and treatment. By virtue of their unique features and design, nanomedicines have made remarkable progress in eliminating dreadful tumors. Research in cancer nanomedicine has spanned multitudes of drug delivery systems that possess high tumor-targeting ability, sensitivity toward tumor microenvironments, and improved efficacy. Various nanocarriers have been developed and approved for anti-tumor drug targeting. These nanocarriers, *i.e.*, liposomes, micelles, nanotubes, dendrimers, and peptides, offer a wide range of advantages such as high selectivity, multi-functionality, specificity, biocompatibility, and precise control of drug release. Nanotherapeutics is offering new opportunities for improving the safety and effectiveness of regular therapy. This book provides an overview on the unique features of nanoparticles that are suitable for biological systems, emphasizing on the type of clinically used nanoparticles and their specificity for therapeutic applications, as well as on their current delivery strategies for specific diseases. In this book, the authors have documented the current contexts, including designing nanoparticles for therapeutics, types of therapeutic nanoparticles, and their applications in targeted delivery, along with limitations and disadvantages of therapeutic nanoparticles.

Hardeep Singh Tuli

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Chapter 1

Introduction to Nanotherapeutics: A Synthetic Preview

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1.1 Introduction

Nanotechnology comprises a multitude of generally different areas such as nanoelectronics, information technology, cellular and molecular biology, and biotechnology. Nanotechnology has revolutionized health strategies in recent years to offer improved health facilities with tremendous impact. Nanotherapeutics has been recently originated from nanotechnology-based applications and a wide range of medical services (Noh *et al.*, 2012; Mahato *et al.*, 2016). Nanotherapeutics is the application of nanotechnology to medicine and drug development. For bio-interactions and subsequent easing

*Nahid Rehman and Anjana Pandey contributed equally to this work.

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effects, the design of nanotherapeutics (size, shape, and surface properties) is vital. Nanotechnology-based formulas contain new physical and chemical attributes for a broad range of applications in various diseases (Prasad *et al.*, 2018; Jo *et al.*, 2015).

Nanotherapeutics offers new opportunities for improving the safety and effectiveness of regular therapy. Stable interactions with ligands, size and shape variability, high carrier capacity, and ease of binding of hydrophilic and hydrophobic materials are known to make NPs favorable for the targeted and controlled supply of micro- and macromolecules at targeted site.

NPs combined with the therapeutic agents are used to solve conventional therapy issues, but certain problems such as adverse reactions and toxicity are still discussed. The specific nature of therapeutic NPs and their delivery strategies must therefore be understood. This contributes to the production of new drug delivery systems by different national, international, and pharmaceutical organizations (Prasad *et al.*, 2018).

Over the past two decades, a number of nanotherapeutic products, that have been certified by the Food and Drug Administration (FDA) are for the treatment of hepatitis, cancer, cardiovascular disease apart from autoimmune, diabetes, high cholesterol, Parkinson's disease, and certain infectious diseases.

Over the last several decades, nanotechnology contributed significantly to oncology. The primary class of therapeutic NPs that received clinical approval for cancer treatment was liposomal doxorubicin (LD) including Doxil and Myocet. In addition to other lipid-based NPs, this still accounts for a large proportion of nanotherapeutics at the clinical level.

Recent biomedical exploration has led to a successful improvement in the designing of therapeutic agents in the treatment of diseases. However, the delivery of therapeutic agents in the target area constitutes a major obstacle before the treatment efficiency of various diseases. The use of standard treatment agents is limited by non-selectivity, undesirable adverse effects, and low efficiency including poor biodistribution. Current research activities, therefore, focus on the development of well-controlled and multifunctional delivery systems.

The combination of NPs with therapeutic agents with unique physicochemical and biological characteristics, which develop

their pathway for appropriate targeting, is a promising approach to supply a wide range of molecules to certain body locations. This strategy increases the therapeutic concentration in cells/tissues and thus allows for the use of low doses, especially if the toxic effects of the agent are contradictory. Increasing therapeutic agents' concentration is also enhancing its therapeutic index by increasing the efficacy and/or tolerability in biological systems.

Water-insoluble therapeutic agents can also be combined with NPs to protect and improve their bioavailability against physiological barriers. The association of therapeutic NPs with contrasting agents, on the other hand, is an opportunity to monitor their pathway and to imagine their place of delivery *in vivo* systems.

1.2 Designing Nanoparticles for Therapeutics

The development of safe and effective therapeutic NPs is crucial and one of nanomedicine's ultimate objectives. They are likely to aggregate and to opsonize proteins (protein binding to NP surface as a tag for immune system recognition) once NPs enter the bloodstream. By means of phagocytosis or liver, spleen, and kidney filtration, opsonized NPs could be removed from the bloodstream. This quick and non-specific immune clearance leads to a decreased retention time and therefore limits bioavailability (Yetisgin *et al.*, 2020).

The time of retention can be altered by decorating the surface of a NP with polyethylene glycol (PEG), carbohydrates, and grouping of acetyl or protein moieties (peptide, albumin). Such surface changes may also, however, alter the ability to recognize targeted delivery (Shreffler *et al.*, 2019). Therefore, therapeutic NPs must be cleaned and biodistributed during their design process. Size also plays an important role in controlling the circulation and biodistribution of NPs for therapeutics.

Physiological systems (filtration through the kidney) can readily remove NPs of size less than 10 nm, while the phagocytic cells in the reticuloendothelial system (RES) can remove particles by larger than 200 nm. Therefore, the blood system has more circulation time for therapeutic NPs < 100 nm in size. Several studies have shown a higher accumulation of therapeutic NPs in a size of 20–200 nm in

tumors because they cannot be identified and filtered by the RES (Ernsting *et al.*, 2013). In addition, in the areas of tumors, blood vessels are higher and larger than normal tissues. Thus, NPs of appropriate sizes can relatively easily access the tumor area and accumulate for a longer time, known as the enhanced permeability and retention (EPR) effect (Nakamura *et al.*, 2013).

Passive targeting is in fact used to collect NPs in the tumor site, which is done without working with targeted moiety. The area of the NPs, however, is combined with at least one sort of target moiety in active targeting, such as proteins, peptides, nucleic acids, antibodies, or small molecules (Yu *et al.*, 2012).

Most NPs in the cells are absorbed by endocytosis using mechanisms dependent on clathrin or caveolae (Zhang *et al.*, 2009). Due to its internalization of the targeted cells, the form of NPs is also critical to biodistribution. For instance, cationic NPs in rods are easier targets than cationic NPs of other forms for endosomal uptake, suggesting that these NPs are considered as rod-shaped bacteria by immune system cells.

In their clearance and targeted delivery, the surface load of therapeutic NPs is important. Positively charged NPs provide a higher immune response than neutral or negative NPs. In addition, phagocytose and non-specific interactions of NPs with a surface potential between −10 and +10 mV have been shown to be lowered (Bhatia, 2016). The ideal range however could depend on the material for NPs. Surface load also bears a close connection to NPs' pH sensitivity. These types of NPs can be identified and located in specific cell compartments. Acidic NPs, for example, may have a pH of pH < 6.0 for endosomes or lysosomes for releases of cargo (Bhatia, 2016).

Surface alterations were shown to reduce non-specific protein absorption on the surface of NPs with long-chain polymers, such as PEG. PEG is a favorite polymer for therapeutic NP because its physicochemical properties reduce their phagocytic absorption and accumulation in non-targeted organs (Walkey *et al.*, 2012). Before PEGylation, therapeutic NPs should be considered for factors such as the length, shape, and density of PEG chains affecting surface hydrophilicity and phagocytosis. The combination of target ligands on the surface of PEGylated NPs can improve the target-specific supply of the NPs.

1.3 Types of Nanoformulations

Around 40% of medicines have few restrictions such as low solubility, inferior stability, and highly poor performance with minute biological equivalents (Baumgartner *et al.*, 2014). These issues have led researchers to investigate nanotechnology advances in dome nanoformulation (Fig. 1.1). Two phases of cell targeting approaches have traditionally been used, such as passive targeting and active targeting, which have demonstrated a substantial variation in the behavior of cells between normal tissue and affected tissue. The use of nanoformulation for cancer therapy is, unquestionably, a huge step as compared with traditional processes. Nanoformulation is certainly a major stride. Marketed nanoformulated capsules show high chemical stability with broad reproductivity and biocompatibility characteristics. These formulations are attracted by researchers because of their protective coverings for medicines and other compounds. Nanoformulations for improved medication delivery systems have been created for several procedures.

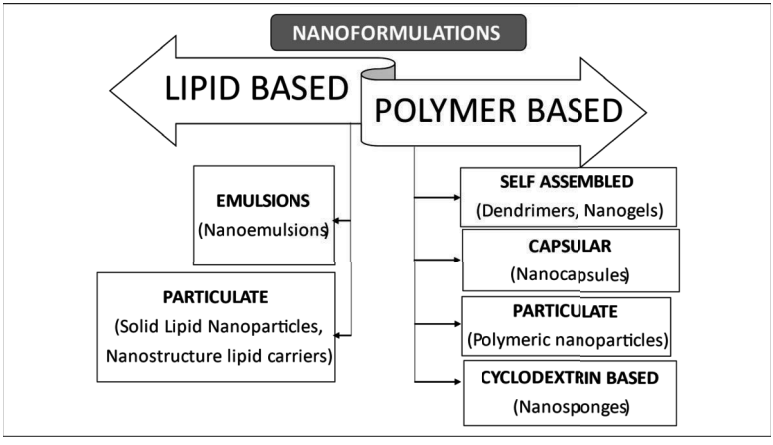

Figure 1.1 Nanoformulations for a better drug delivery system.

1.3.1 Polymeric Nanoparticles

Natural polymers result in poor repeatability and controlled release behavior for the confined drug due to variance in the purity and

consistency between batches. Synthetic polymers are the most widely utilized polymer NPs. In contrast, synthetic polymers with excellent repeatability and purity for batch use facilitating the adjustment of the pattern of drug release of polymer NPs are available. NPs made using synthetic polymers are widely investigated for medicinal products.

Polymeric NPs can shield unstable drug moieties against degradation, avoiding the hazardous medication's adverse effects. Polymers such as alginate, chitosan, albumin, and jelly, are made up of natural polymer NPs. Polymeric NPs with dexamethasone or α-tocopheryl succinate alleviate cisplatin ototoxicity as a result of chemotherapy treatment. NPs that capture, transport and eventually distribute dexamethasone, or α-tocopheryl succinate, can partially reduce ototoxicity from the highest dose of CDDP (generic name of chemotherapy drug of cisplatin) (Saldana *et al.*, 2017). Otherwise, when systemically supplied for prolonged periods, these poorly soluble medicines exhibit serious adverse effects. The integration into the hydrophobic cavity of these therapeutic compounds produces *in vitro* and *in vivo* effects. Decapeptyl®, Gonapeptyl Depot®, Enantone Depot®, and Abraxane are commonly commercialized as polymerized NPs. These polymers can be synthesized in many forms.

1.3.1.1 Nanosponges

Nanosponges are a small, non-toxic, colloidal spongy architecture, with different voids, with the possibility to insert pharmacological active moieties. β-cyclodextrins are commonly used for nanosponges preparation. Ability to integrate both hydrophilous and lipophilic moieties within the nanosponges to load the medication and dispensing systems are the main reason of interest for the researchers. For their preparation and synthesis certain cross-linking components, such as diisocyanate of hexamethylene, carbonyldiimidazole and diphenyl carbonate are used. Also, in any type of carbon-based solvent, they are not soluble in water. These nanosponges have a pH range of 2–11 and are stable up to 300°C, therefore, possess sterilizing characteristics by their nature; (Selvamuthukumar *et al.*, 2012; Ahmed *et al.*, 2013).

1.3.1.2 Dendrimers

Dendrimers are 3D nanostructures, strongly ramified, globular, and polymeric. Water solutions, a low index of polydispersity, customizable structures and vacuum indoor presence are important properties of these dendrimers and are distinct from other nanoformulating systems by a number of functional groups situated in the periphery. Terminal functional groups often provide targeting drug medication and conjugation platforms. Functional groupings on the periphery likewise offer these perfect features and flexibility.

Research on dendrimers has shown that this material is utilized to supply medicinal compounds such as polyamidoamine (PAMAM). Their synthesis is carried out by reacting with methyl acrylate by reacting to two new branches with an esterified end dendrimer. PAMAM dendrimer does not exhibit an immunogenic property that is water-soluble and possesses a terminal amine function group, which may be altered to effectively target medication use. Additionally, dendrimers' solubility nature is studied through the eye, mouth, and transdermal and respiratory systems for their administration. Altering the structure can alleviate toxicity problems (Kumar *et al.*, 2020).

Recent findings reveal that the transdermal permeation of 2-(3-benzoylphenyl) propanoic acid of peptide-containing essential amino acids at the dendrimer terminal was significantly increased (Patri *et al.*, 2002). The results of another investigation indicated that the combination of dendrimeric peptides contributed to ketoprofen skin permeation in just 30 minutes of ultrasonic exposure. This work for the first time has shown that the synthesized peptide dendrimer had improved the transdermal permeation of ketoprofen and enhanced the displayed synthesized peptides ratio up to 3.25 times when compared with passive diffusion (Manikkath *et al.*, 2017).

1.3.1.3 Nanocapsules

Nanocapsules consist of the liquid, semisolid or solid core where the medication can be encased by the naturally-produced (synthetic) polymer membrane. Nanocapsules have been shown to fall between 10 nm and 100 nm in size. Nanocapsules have a lipid core typically produced by the precipitation process. Nanocapsules are now in

demand for biosensing research for the reason that their protective characteristics help easily oxidize electrochemical tests. In addition, these NPs are mostly used as carriers of medications.

The majority of these are utilized in various cancers, HIV, Alzheimer's and other important disorders (Peters *et al.*, 2018). Such characteristics can be evaluated by X-ray diffraction, photoelectron spectroscopy, microscopic electron systems, and electronic microscope transmission methods (Kothamasu *et al.*, 2012). Nanocapsules are suitable for a wide range of medical applications, *e.g.*, agrochemicals, biomedical systems, sanitizers, cosmetics, and sewers (Patel *et al.*, 2018). These nanocapsules can also be studied as strong management medications, radiation, self-medial, pollution treatment, and beneficial for agricultural research (Peters *et al.*, 2018). In future, these technologies for nanocapsulation will open the door to a new age of effective biologically active medicines and other tissue-related chemicals.

1.3.1.4 Nanogels

Nanogels, consisting of hydrophilic flexible polymers, may be prepared as plain gels. While swelling in water, the medication can be spontaneously absorbed into the nanogel. As a result of this gel, solid, dense NPs are formed and the solvent volume decreases. Nanogels provide unique applications for the use of polymer-based drug carrier systems due to their biocompatibility, high humidity content, and favorable mechanical characteristics. The increased surface area of the gels has been used for multipurpose bioconjugation as well as internal network for biomolecular entrapment (Prasad *et al.*, 2018).

The micro-molding and photo-lithographical techniques, biopolymers modification, continuous micro-fluidics heterogeneous, living/controlled radical and free-radical polymerization comprise several synthetic ways for the creation of nanogels. Several requirements for the *in vivo* therapeutic use are necessary to develop and manufacture an efficient nanogel based drug carriers' system. The stability of nanogels for long term blood circulation is an essential requirement. Another unique new feature that can detect receptors on infected cells is the bioconjugation of nanogel surfaces with special ligands (Prasad *et al.*, 2018).

Finally, nanogels' biodegradability should not only change the release of the drug at a specified time but should also enable the empty

device to be removed upon dropping. The importance of nanogel as topical applications of Clobetasol for psoriasis was demonstrated *in vivo* in an anti-psoriasis conducted on an imiquimod model. Zyflex nanogel eyes, sane care nanogel, skin perfect brightening nanogel, Eye-care gel and oxalgin are some of the chosen and marketed nanogel formulations (Sharma *et al.*, 2016).

1.3.2 Lipid-Based Nanoparticles

These systems are one of the most promising bioactive organic molecular colloidal carriers. Present oncology application has changed cancer treatment by enabling numerous chemotherapeutic drugs to improve their anti-tumor action. The advantages of lipid-based nanoparticles (LBNPs) include high time and temperature stability, high loading capacity, easy preparedness, low cost of manufacturing, and big industrial production since they can be made from natural resources. Moreover, the association in lipid NPs of chemical therapeutic agents minimizes the therapeutic active dosage and toxicity, reduces drug resistance, and raises tumor tissue drug levels while reducing them in healthy tissue. Not just in *in vitro* cancer treatment but *in vivo*, LBNPs have been widely tested and certain clinical studies have shown encouraging outcomes (Prasad *et al.*, 2018).

Lipid-based NPs comprise several subsets but are the most common spherical platforms consisting of at least an interior aqueous compartment of at least one lipid bilayer. Lipid-based NPs offer numerous benefits, including simplicity of formulation, self-assembly, biocompatibility, high bioavailability, capacity to carry a wide range of payloads and physicochemical properties, which can be regulated to model their biological properties. For all these reasons, lipid-based NPs constitute the most frequent type of FDA-approved nanomedicines (Mitchell *et al.*, 2021).

The NPs are often made up of phospholipids, which can form unilamellar and multilamellar vesicular structures, with liposomes being one subset of lipid-based NPs with most members. This enables the liposome to bear, transport, and even trap hydrophilic, hydrophobic and lipophilic drugs in the same system, increasing their utilization. *In vitro* and *in vivo* stability of liposomes is affected by the size of the NP, surface load, lipid content, number of lamellas,

and changes in surface (ligands or polymers) that may be modified by synthesis (Mitchell *et al.*, 2021).

As in reticuloendothelial systems, they can be quickly absorbed, liposomes frequently encompass surface changes that increase circulation and improve therapeutic usage. Another important subgroup of lipid NPs (LNPs), which are liposome structures frequently employed in the supply of nucleic acids, is usually known as lipid NPs. They vary largely from typical liposomes since they produce micellar structures inside the particle core that may be modified depending on factors of formulation and synthesis.

Typically, LNPs consist of four major components: (i) cationic and ionizable lipids complex with negatively charged genetics and aid in endosomal escape, (ii) phospholipids structure of particles, (iii) cholesterol for stability and membrane fusion and (iv) PEGylated lipids to improve the stability and circulation. LNPs have been especially useful in customized genetic treatment applications due to the effectiveness of their nucleic acid delivery together with their easy production, modest-sized and serum stability (Mitchell *et al.*, 2021).

Ionizable LNPs are a suitable platform to administer these nucleic acid treatments because they have an almost neutral load at physiological pH but are chargeable in acidic endosomal compartments and foster intracellular escape. Despite these benefits, however, the low loading and biodistribution of drugs that result in high liver and spleen absorption may still be restricted in LNP systems. These LBNPs can be further modified as:

1.3.2.1 Nanoemulsions

Nanoemulsions are an intriguing, thermodynamically stable, and filtrated method of colloidal drug delivery**.** A heterogeneous mix of aqueous oil droplets is responsible for the small dispersion of nanodroplets. The resulting nanoemulsions are assessed by the use of the appropriate surfactant as translucent or transparent, isotropic. There may be three forms of nanoemulsions: (a) water-in-oil nanoemulsion; (b) oil-in-oil nanoemulsion (oil scattered into an aquatic medium; and (c) bicontinuous nanoemulsion. The foul smell of greasy liquids hidden is the most widespread property of nanoemulsions. These also offer extended drug action and hydrolysis and oxidation protection. Consequently, nanoformulations with high

bioavailability can prove to be an effective and impermeable delivery alternative. Nanoemulsions are now widely investigated to target different photosensitizers, anticancer medicines, or therapeutic substances. These nanoformulations provide a number of uses, including medication delivery, biological diagnostics and chemical agents (Prasad *et al.*, 2018). Nanoformulation in *in vitro* and *in vivo* research has been proven to be functional. It specifically reduces endothelial activity and thus monocyte infiltration, which leads to a substantial reduction in lung inflammation in the mouse model. Examples of formulations for nanoemulsion are norvir (ritonavir), restasis, gengraf (cyclosporin A), etomidat-lipuro (etomidate), ropion (flurbiprofenaxtil), diprivan, troypofol (propofol), limethasone and liple (alprostadil palmitate) (Prasad *et al.*, 2018).

1.3.2.2 Solid lipid nanoparticles

Lipid NPs, incorporating solid matrix are known as solid lipid NPs (SLNPs). They are produced by employing a solid lipid using oil-in-water nanoemulsions. The first generation of SLNs was developed at the beginning of 1990. The benefits of SLNs include inexpensive raw ingredients, avoidance of organic solvents, utilization of physiological lipids, easy scale-up, biocompatibility, improved bioavailability, environmental risks, and controlled drug release protection against sensitive moieties.

The polymorphic transition of solid lipids renders them less appropriate for drug delivery systems due to their crystalline structure, drug expulsion, uneven tendencies of gelation and poor drug incorporation. Ciprofloxacin (CIP)-loaded SLNs have recently been developed utilizing ultrasonic melt-emulsification to provide higher antibacterial activity. These have been well constructed with a size of 165 to 320 nm NPs and a poly dispersion index of 0.18 to 0.33 with good trap-induction efficiency. A regulated release pattern with various lipids was presented in the CIP release. Ciprofloxacin SLNs are produced with the greatest bursting impact of stearic acid (CIPSTE). This composition of CIPSTE was established for 120 days at room temperature. SLNs were fully examined based on *in vitro* and *in vivo* assessment on the various delivery routes, such as oral, dermal, pulmonary, ocular and rectal. SLN formulations are available on the market for nano base and nano pearl (Prasad *et al.*, 2018).

1.3.2.3 Nanostructured lipid carriers

The nanosystems consisting of solid lipid integrated into liquid lipids of the second generation form nanostructured lipid carriers (NLCs). These nanocarriers enable the therapeutic medicines to be strongly immobilized and avoid particle coalescence as compared to emulsions. In addition, their potential for drug loading is boosted in comparison with SLNs because of the liquid oil droplets in a solid matrix. Biodegradability, poor toxicity, controlled release, drug protection and the avoidance of organic solvents during manufacturing are among the benefits of NLCs over polymeric NPs. The NLCs for the delivery of hydrophobic and hydrophilic medicines have been widely studied in recent years. The NLCs were created to fulfill industrial validation and qualification criteria, simple technology, size, and cheap costs. NLC formulations, including NLC repair cream and NLC reconstruction cream, are also available commercially. Taking different routes of administration, such as oral, topical, ocular, pulmonary, and parenteral, NLCs have been examined for therapy of various disorders. Fluconazole-loaded NLCs were manufactured using ultrasound probe technique and were studied on a wide variety of Candida species for antifungal activity (Prasad *et al.*, 2018).

1.3.3 Non-polymeric Nanoparticles

Expansion of an active drug carriage method for the treatment of cancer is a worldwide daring task. The predictable drug distribution tactics including above discussed approaches are being used. However, the suppression of cancer is still not conceivable with these methods due to some boundaries of each approach. The development of non-polymeric nanotechnological methods can be resourceful in this path of drug delivery system.

1.3.3.1 Carbon nanotubes

These are carbon-based tubular structures of 1 nm diameter and 1–100 nm length. These structures may be produced by encircling a single graphite layer termed graphene in a continuous cylinder. It includes single-walled nanotubes (SWNTs), multi-walled nanotubes (MWNTs) and C60 fullerenes in the carbon nanotube's structure.

Carbon nanotubes are promising non-polymer carriers for medicinal substances because of their size and stable geometric form. The interior diameters of SWNTs and C60 are particularly between 1 nm and 2 nm, or almost half the typical diameters of DNA helix. By endocytosis or direct insertion into the cell membrane, SWNTs and MWNTs may enter the cell. In the organization of its graphite cylinder, fullerenes differ in their core structure and the existence of a high number of conjugated double bonds. Fullerene experiments suggest that it can be used in medicines such as antibiotics, antiviral, and anticancer drugs. In addition, the damaged mitochondria can be protected by supplying free radicals. This characteristic enables the specific tissue targeting of mitochondria, which can be utilized to provide therapeutic drugs.

1.3.3.2 Nanodiamonds

These are members of carbon-based nanomaterials smaller than 100 nm in diameter and of distinct forms, with two types of distinctive aspects, created by diverse techniques such as detonation, deposition of chemical vapor (CVD) and processes of high pressure/high temperatures. The earliest and most widely used nanodiamond (ND) preparation is the detonation technique by initiating a controlled explosion over a confined chamber of carbon-containing precursors. NDs with their unique characteristic properties, including electrostatic surface properties, low chemical-inert cytotoxicity and low photo-bleaching through the addition of nitrogen deficiencies and functionalized by immobilizing different biomolecules which make them remarkable in biomedical applications, such as magnetic resonance imaging (MRI), contact lens synthesis, and drug delivery for cancer therapy. As a contrast agent for MRI, the NDs can be connected with gadolinium [Gd] (III), and the signal generated from this complex is several times greater than the contrast agent of Gd (III).

1.3.3.3 Metallic nanoparticles

Metallic NPs medical applications employ 1–100 nm size of metal NPs, primarily made up of cobalt, nickel, iron, gold, and their corresponding oxides, such as magnetite, maghemite, chromium dioxide, and cobalt ferrite. They may be manufactured and changed using several functional chemical groups to adorn them with different

molecules such as curative agents, biological molecules like peptides, proteins, and DNA. In addition to stability and biocompatibility, they provide unique features such as magnetic qualities as a carrier. Thus, by employing an external magnetic field, magnetic NPs may be targeted at a particular place in the body. A significant characteristic of its medicinal application is its magnetic susceptibility, defined as the ratio of induced magnetization to the field applied. The super-paramagnetic iron oxide NPs (SPION) are, for example, very vulnerable to MRI, and are thus commonly employed as contrast representatives in clinics. Super-paramagnetic characteristics also allow steady transport into the body/cells of therapeutic substances and appropriate tissue accumulation to ensure reproductive and safe therapy. If metallic NPs are exposed to the magnetic field, heat termed magnetic hyperthermia may be produced that can be used for cancer therapy in the removal of cancer tumors. Metal NPs of gold (AuNP) are utilized frequently in the detection and treatment of cancer due to comparatively low cytotoxicity, unique optical and localized surface plasmon resonance (LSPR) owing to the inertness of gold. In addition, the optical and LSPR characteristics of AuNPs may be adjusted for applications such as imaging, optical and electrochemical detection, diagnostic or photothermal treatments.

1.3.3.4 Quantum dots

These are small, semiconductor material particles or nanocrystals having a diameter of 2–10 nm. These particle components include an inorganic semiconductor core like cadmium selenide (CdSe) and an aqueous organic coated shell-like zinc sulfide (ZnS). Quantum dots (QDs) create unique fluorescence hues, some of which are the consequence of extraordinarily high surface-to-volume ratios of those particles. The core structure of QDs controls the emitted color, while the external aqueous shell can be utilized for the biological conjugation of peptides, proteins, or DNA. Furthermore, QDs may include a cap that enhances solubility in water buffers. Because of their narrow emission, their strong fluorescence, and their excellent photostability, QDs can be utilized to monitor cell/tissue therapeutic drugs. Although the medicinal application of QDs has not been disputed, their surfaces are a superior option for multiplexing bioconjugation, adaptability of photophysical characteristics, and greater stability over lengthy examination durations.

1.3.3.5 Silica-based nanoparticles

These NPs offer significant benefits in nanotechnology since they are applicable for the design and efficiency of complicated systems. Their unique surface properties, porosity, and functionality make them ideal therapeutic tools. Silica NPs possess a large surface area covered by polar silanol groups that support water adsorption and increase therapeutic stability. Furthermore, silica-based NPs can be used as a vehicle for delivery in order to interact with nucleic acids. The size and density of their nanopores may be adjusted to a constant delivery rate. In addition, it provides a solider medium for supplying ingredients for encapsulation of medicinal products in silica-based NPs. Pores of the NPs of silica can be coated with different pacing molecules to enhance the drug release rate of the targeted tissue. In order to release the enclosure medication into the acid tumor tissue, for example, mesoporous silica NPs enclosed with β-cyclodextrin when combined with contrasting substances such as gold, silver, iron oxide, organic dyes, and QDs to enable biological tracking. In order to increase the mechanical characteristics and biocompatibility of the product, these NPs are also employed as an additive in pharmaceutical manufacturing.

1.4 Targeted Delivery Applications of Therapeutic Nanoparticles

Targeted delivery refers to the effective directing and dominating accumulation of the therapeutic substance at the desired location. The agent loading system should be kept for the preferred duration in the physiological system, evading the immune system, targeting certain cells/tissues, and releasing the loaded therapeutic agent (Yetisgin *et al.*, 2020). Targeted NPs delivery in cancer therapy is now widely researched (Fig. 1.2). In anticancer applications, more than 20% of the treatment NPs were previously created in clinics or under clinical assessment. Furthermore, a related study focuses on NP-mediated treatment for certain additional conditions such as neurological disorders, infectious diseases, autoimmune diseases, etc.

- Nanomaterials for implantation
- In nanoelectromechanical system
- In nonviral gene and protein delivery in cancer therapy
- In the photodynamic therapy
- In medical imaging for diagnostic approach
- In biosensors
- For BLOOD PURIFICATION
- In tissue engineering

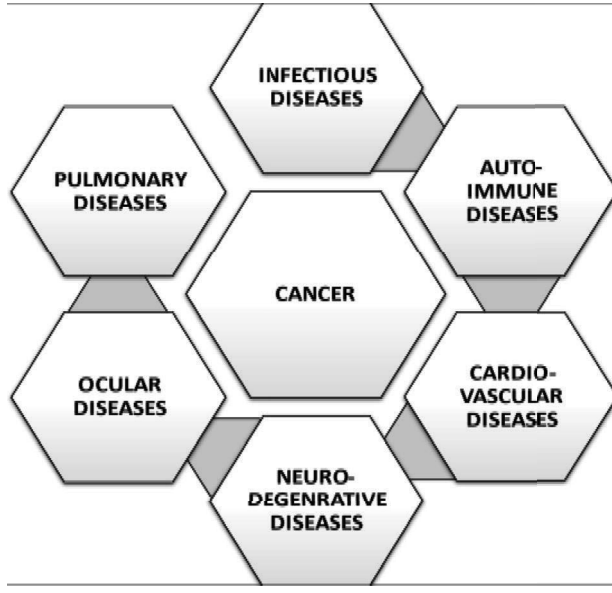

Figure 1.2 Current uses of therapeutic NPs in a number of illnesses for a targeted delivery method.

1.5 Limitations and Disadvantages of Therapeutic Nanoparticles

NPs show promising outcomes in the treatment of a wide range of cancer and glaucoma disorders. Toxicity and safety concerns of nanotherapeutics are a major challenge. Unfortunately, the approach of nanomedical applications on NPs-based methods has

been found to show some limits and drawbacks. Toxicity to NPs, the evasion from the phagocytic system, absence of physiological barriers and the generation of an immunological response are some of the problems that need to be carefully considered while utilizing in living organisms.

Studies *in vitro* and *in vivo* revealed that the size and toxicity of therapeutic NPs are co-related. As the size of NPs decreases, dispersal in the nucleus rises gradually and in return, both at the cellular and systemic levels can induce intrinsic toxicity. Their aggregation trend is another barrier with smaller NPs. For example, smaller micelles, dendrimers, and QDs, which result in low biodiversity, are inclined to aggregate. While functionalizing the surface with PEG is a highly efficient way to reduce accumulation in non-target organs, NPs are frequently called 'stealth NP' as they may evade the phagocytic system and cause cell toxicity.

Therapeutic NPs when united with drugs that are currently being used in medical applications provide various features to the drug and increase the efficiency of the treatment. But it was observed that NP-based drug delivery systems may have limited concentration and penetration into tumor regions due to the heterogeneity of the permeability of the vascular system. To address this, a proposal with a regulated supply with drug-loaded liposomes, which are generated *in vitro* by local heat for the release of medicinal products, was offered (Manzoor *et al.*, 2012). QDs are another form of opposing medicinal NPs. As already noted, QDs exhibit distinctive fluorescence emission characteristics; they are therefore frequently employed in imaging applications. In addition to their advantages during illness detection, QDs have non-inclusive drawbacks such as high intrinsic cytotoxicity. Research on quantitative cadmium dots reveals that metal ions leaking in QDs result in hepatocyte crops being very toxic. The effects of QDs might be different based on the type of coating they have from the liposome or polymer. Another research has shown a low impact on normal human hepatic cells in QDot-lipid complex (QD-LC) and preferentially destroy cancer cells *in vivo* in a dose and duration-dependent manner. In addition, QD-LC NPs induce apoptotic c-Jun N-terminal kinase (JNK) pathway in human liver cancer cells through reactive oxygen species (ROS).

Medication delivery based on nanotechnology has been a major success as seen in the market in some of the nanodrug items.

Nanodrug delivery, however, presents several problems. These include blood circulation, increased surface area, protection of the loaded medication against degradation, biological barriers, and site-specificity. Academic investigators carry out most of the nanodrug delivery experiments. In nanodrug delivery technology, there are also a number of regulatory difficulties. It takes an hour to have various physicochemical and pharmacokinetic rules for nanodrug compounds different from traditional medicines. The Food and Drugs Administration of the United States (FDA) and the European Medicines Evaluation Agency (EMEA) have proactively started action to identify prospective scientific and regulatory challenges. The environmental impacts of regulators and the scientific community have been examined during the previous two decades. The focus was on engineered NPs such as carbon 60 and QDs.

Another key problem for the industry is the large-scale manufacturing of nanomaterials. Because of the technique, processes, and changeable prices of the materials employed, several nanodrug delivery methods are not scalable. Scaling up involves low levels of nanomaterials, agglomeration, and processing. The different obstacles include nanomaterials can more easily be modified in the laboratory than on a large scale or production scale unimpeded by their size and composition. A series of concerted initiatives to tackle the difficulties of this extremely promising platform for medicinal products are therefore necessary.

1.6 Conclusion

Nanotherapeutics is a hefty and developing area with countless positive qualities in the world of pharmaceuticals. Self-assembly nanostructures such as liposomes and polymer micelles can be a potential instrument for effective medication administration and targeting. A range of applications is being investigated from targeted drugs to enhance cancer therapy of nanostructures like SLNs, gold NPs, super-paramagnetic NPs, and aptamers. QDs are used in cancer and imaging diagnostics. Nanotherapeutics provides adaptability and significant commercial potential in combination with all these applications.

Over the last 10 years, the creation of therapeutic NPs has been explored intensively and nanodelivery systems are the main area to be targeted particularly in the treatment of a number of illnesses. At the moment, most NPs are composed of polymers or lipids utilized in the targeted delivery procedure. Although polymeric NPs show tremendous benefit in disease therapy, they also have drawbacks including difficulty in the scaling up of organic solvents, biocompatibility, cytotoxicity, and immunogenicity.

On the other hand, lipid NPs show that they are comparable to cell membranes and of crossing difficult locations even without surface functionalization. Thus, nanodelivery systems based on lipids are regarded as the next generation. Today, only one illness is mostly treated or prevented by therapeutic NPs. Researchers have, however, started combining several medicinal molecules with different types of NPs, thereby guiding the future of therapeutic NPs toward multi-therapeutic NPs for the treatment of more than one illness. While the nanoparticle supply systems substantially contribute to focused therapy with enhanced efficiency, decreased side-effects, and increased bioavailability, the metabolism, clearance, and toxicity of therapeutic NPs remain unknown.

This implies that further investigations on the composition, manufacture, and toxicity of NPs are necessary. In addition, the costs of nanomedicine and bigger production are other key issues to be addressed.

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Chapter 2

Synthesis, Characterization, and Application of Metal Oxide Nanoparticles

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2.1 Introduction

This chapter presents the techniques used to characterize and investigate the inner construction and possessions of nanoparticles by the craved synthesis techniques [1]. The characterized NPs can be transferred out each of two by top-down talk to or by the extremity up approach. The act characterized implement is very climacteric in managing the possessions of evolved nanoparticles [2]. The most extensively used technique for characterizing the NPs is to cut a long story short reported in this chapter. The characterized technique used in our instance is the chemical coagulation process,

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which has been reported in brief. Synthesis of the characterized NPs concerning the resolution of elemental configuration, approximation of track down adulteration, construction analysis, morphologic analysis, recognization of crystalline stages and details on crystal imperfection takes part in a significant function in the quality control and growth of the state of art materials and their use in the implementation needs near quality contemplation [3]. Safekeeping this is mind, synthesis techniques that are used in our instance are described in different characteristics.

2.2 Techniques for Synthesis of Nanoparticles

Characterization of nanoparticles is not directly the mind permission of materials but often needs very distinct manufacturing approaches. There are many methods to manufacture NPs. The maximum techniques used in the nanoparticles characterized can be widely divided into two approaches, *i.e.*, bottom-up approaches and stick approach (Fig. 2.1).

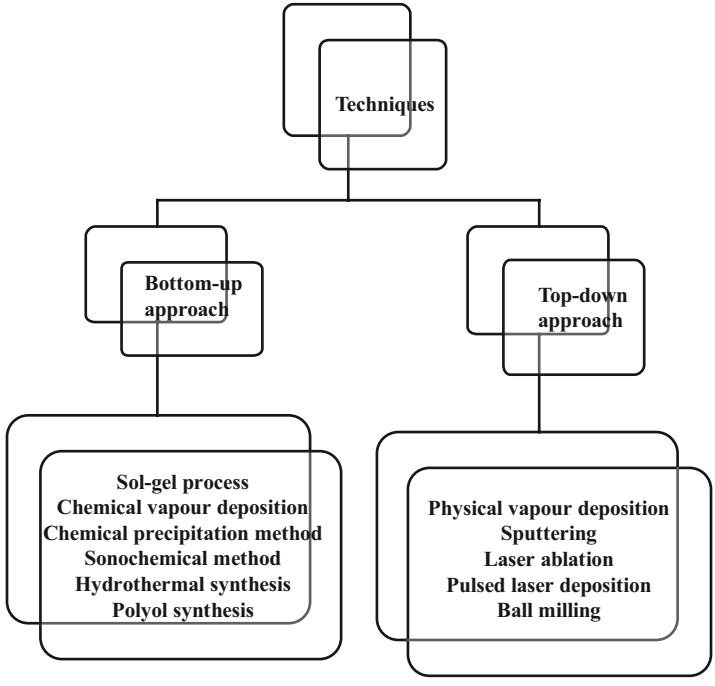

Figure 2.1 Schematic representation of nanoparticles synthesis by using top-down and bottom-up techniques.

2.2.1 Top-Down Approach

The top-down approach uses the conventional factory or little bitty production processes, where apparently managed implements are used to gash, a mile and appearance the materials onto the craved dimensions and organization an uncomplicated to demonstrated a bottom-up process is to believe of hankering a figure out of a huge block of dapple. Reprint processes also belong to this classification. The broadly use bottom-up speak to characterize the NPs incorporate a flat surface is treated to absorb or repel in the desired pattern and ball granulating.

2.2.1.1 Physical method

Physical techniques generate nanoparticles by causing bulk material abrasion, condensation, evaporation, and melting using mechanical pressure, electrical energy, thermal energy, and high-energy radiation. Physical methods are mainly adopted on top-down synthesis which is advantageous as they produce uniform monodisperse NPs and are free from solvent contamination. The major disadvantage of using these synthesis methods is the enormous waste produced, which makes the processes less economical. Some of the physical methods commonly used are high-energy ball milling, physical vapor deposition, electrospraying, sonochemical synthesis, inert gas condensation, etc.

2.2.1.2 Physical vapor deposition

Physical vapor deposition (PVD) is the process of depositing thin materials that have been investigated ranging in size from a few nanometers to a few micrometers.
PVD steps consist of:

1. Vaporization from a solid source,
2. Transportation, and
3. Nucleation and growth.

The most common PVD methods are:

1. Sputtering
2. Laser ablation (LA)
3. Pulsed laser deposition (PLD)

2.2.1.3 Sputtering

Sputtering relies on the vacuum-based process to deposit films and nanoparticles. The basic principle of sputtering is momentum transfer as the ions are bombarded with the target atoms. Then the material deposition can be done by DC, pulsed DC, and radiofrequency powers.

2.2.1.4 Laser ablation

The LA method uses a high-power laser beam to evaporate the materials in the solid source. In this, the laser may be pulsed or continuous. This method was widely used for the production of polymeric microstructures and nanostructures.

2.2.1.5 Pulsed laser deposition

PLD is also a vacuum-based method that uses a high-power laser in which hitting these laser energy pulses to the target surface results in melting, evaporation, and ionization. Later materials are deposited over the substrate. This method is used for the synthesis of polymers, oxides, metallic systems, carbides, fullerenes, etc.

2.2.1.6 Ball milling

A ball mill is a kind of bomber in which around implement is used to crush (or mix) ores, ceramic raw materials, chemicals and dyes are examples of such materials. Ball mills revolve throughout a parallel axis to a certain extent meadow with material to be floor along with the crushing medium. Distinct materials are used as crushing medium counting boulder stone, and unsullied steel balls. An inner pouring result short the material to an excellent powder industrial ball mills can utilize perennial fed at one end and exit at the other. The ball milling process has also been fortunately making use of gash carbon nanotubes from lengthy nanotubes by a crash between milling ball and nano manufactured powders.

2.2.2 Bottom-Up Approach

The backward or self convention approaches to nanofabrication use chemical or physical forces working at the little bitty together

fundamental units into big structures. As a part size declined in nanofabrication backward up approaches supplied more and more significant accompaniment of pragmatic techniques. Several backward-up approaches have been growing for manufacturing nanoparticles varying from the coagulation of atomic vapors on top to the amalgamate of atoms in fluids. The broadly used backward-up approaches encompass the sol–gel method, chemical vapor overthrow, laser deletion, and chemical coagulation processes.

2.2.2.1 Chemical methods

The chemical methods used for nanoparticle synthesis are the sol–gel method, chemical vapor deposition technique, chemical precipitation method, sonochemical method, hydrothermal synthesis, and polyol synthesis.

2.2.2.2 Sol–gel process

Sol–gel methods are a moisture chemical characterized approach that can be used to cause nanoparticles by freezing, coagulation, and hydrogenated treatment. The sol–gel technique is a widely used commercial approach for separating colloidal NPs from fluids, which was improved for the production of advanced nanomaterials. For oxide NPs and nanopowder characterization, sol–gel techniques have been extensively improved. The major advantages of sol–gel techniques for nanoparticle production are the short development achieved and simple shaping and blasting. The sol–gel technique allowed the system to shift from the liquid to the solid state [4].

2.2.2.3 Chemical vapor deposition

Chemical vapor deposition (CVD) is an adaptable technique often used in the semiconductor industry for overturning material on various substrates. Vapor or gas shut down is changed into solids such as narrow films, dust, or several arranged materials inner an atomic furnace. It has also been used to manufacture carbon fibers, string, and tube-shaped carbon materials for several years. Lately, CVD has been used to characterize the calm of several materials. It is an idiomatic upgradable process with many merits over taking part in man-made processes.

2.2.2.4 Chemical precipitation method

The chemical coagulation technique was hand-covered by Michael Faraday in 1857, by step up monodisperse gold colloids. In common chemical, coagulation means the association of a distinct solid material from a solution, either by changing the material procedure into an insoluble form or by exchanging the constitution of the solvent to decline the solubility of the substance in it. The main difference between coagulation and morphology is that the focus seems to be on the method of lowering solubility or on the way of bringing order to the solid substance construction. This technique is used industrially to pull-out metal ions from aqueous solutions, for instance, silver ions in attendance in a solution of explicable salt like silver nitrate are coagulated by the inclusion of chloride ion (sodium chloride). So that the silver ions and chloride ions amalgamate to form silver chloride, a compound that is not solvable in water. For II–VI compound semiconductors, the mixture of chemical reactants containing the group II and VI kinds is the solution and homogeneously stirred. Accordingly, a big number of becoming turn centers are set up. The growing of big particles at the cost of little particles to keep down the higher top free energy-related with a particle of compact dimensions is due to the Ostwald maturing. Which can be restricted by using the chemical/capping agents? These organic and inorganic surface-active/crowning agents from complexing ligands the debt-free. Moreover, temperature, pH, application of the surface-active agent, thrilling rate, and full length are Cineplex the parameters that are in charge of the resulting size dispersal of the nanoparticles. Several characterize techniques have possessed drawbacks and demerits but the chemical coagulation process has been classified as a superior technique for manufacturing competently bright nano phosphors in the expression of method clarity, the success of impurities, lofty yield and has an extremely uncomplicated course of action with minimum spirit equipment [5].

2.2.2.5 Sonochemical method

A sonochemical method is done by applying ultrasound frequency waves to lead to the formation, development, and collapse of microcavities. For example, CuO NPs showed various morphology by the use of precursor (cupric acetate) and reducing agent (polyvinylpyrrolidone) by sonochemical method.

2.2.2.6 Hydrothermal synthesis

Hydrothermal synthesis is used to synthesise nanoparticles of metal oxides by changing their properties under conditions of different temperatures and pressure. The nanoparticles are synthesized from a colloidal suspension consisting of two or more phases (solid, liquid, or gas states) of matter mixed with (e.g. gels and foams) at controlled temperature and pressure. This method has the advantage of synthesizing nanoparticles of the bulk amount with desired size, shape, and surface characteristics.

2.2.2.7 Polyol synthesis

Polyol synthesis involves the use of polyethylene glycol which acts as a reducing, complexing agent for the synthesis of numerous metal-based nanoparticles (Au, Ag, Cu, Pd, Pt), metal oxide nanoparticles (CuO, ZnO, TiO_2), magnetic nanoparticles, and metal hybrid nanoparticles.

2.3 Characterization Techniques

The article has been produced for their constructional morphological, compositional, and optical properties. The several synthesis techniques used in the attending study incorporate X-ray diffraction, Fourier transform infrared spectroscopy, transmission electron microscopy, room temperature photoluminescence, and ultraviolet–visible (UV–Vis) absorption spectroscopy studies.

2.3.1 X-ray Diffraction

X-ray diffraction (XRD) is an unfavorable and basic technique for the constructional synthesis in the precipitate affair, which supply concealed bulk constructions in several proportions [6]. By logical dispersion, the translational regularity of a lattice is shown in a diffraction design, and the atomic kind with their mean site profession is thrown back in potency. In powder diffraction, a filled construction analysis has become practicable as the out-turn of proceeding in casting strategies. On condition that the proportions of deserting lattice grassland area close to the wavelength of X-rays (*i.e.*, they are nano-sorted), or on condition that the lattice the flat

gap is not continual but reported by a dispersal function (developing from pressure and tightness or atomic disarray) after the diffraction side view is regulated and needs side view examination to abstract these parameters [7]. The logical scattering of X-ray by the straight forward yields the comprehensive details about the atomic construction without relying on conversion regularity ability XRD is not touching and the unfavorable technique that blends it perfectly for in-site studies. The qualification of strength gives correct and quantitative details on the atomic positioning at the port [8].

When X-ray radiation passes through the incident, it interacts with the electrons in the atoms, resulting in radiation dispersion. If the atoms are arranged in planes, *i.e.*, the incident is crystalline, and the distance between the atoms is the same as the distance between X-rays, destructive and constructive intrusion will take place. This consequence in deflection where X-rays are emitted at attribute gradient based on the separation between the atoms arranged in crystalline construction called grasslands. In an X-ray diffractometer, X-rays are caused within a shift tube and way out through a casement collected of a light element normally beryllium. Inner the tube, a current is proceeding through a strand (normally tungsten) to give rise to electrons. These electrons are next hastened through a potential difference about a metal target like copper. When the arriving electrons have enough energy to emit electrons from the Mobil-shell (K-shell) of copper, an attribute frequency band is calmed of careful energies, which happen due to X-rays ejected by the lineup of electrons to put back the emitted electrons. Electrons line up from the L-shell to the K-shell to allow stand up to copper K_α peaks, electrons from M-shell allow K_β peaks, and electrons from N-shell allow K_γ peaks. K_β and k_α peaks are the most important peaks in the attributes spectrum and after all the k_γ peaks have feeble constituents so these can be neglected. The k_β and k_α peaks are duplicates owing to the distinct energies of electron line-up from distinct L and M sub orbitals approximately. Electrons will not line up from the L1 sub-orbitals but allow from the L2 and L3 sub orbitals, which allow to $k_{\alpha1}$ and $k_{\alpha2}$ peaks approximately. Due to the little energy distinct between $k_{\alpha1}$ and $k_{\alpha2}$ peaks, the K_α peaks are an intimate position couplet. A close method happens for the electrons' line-up from two of the five M sub orbitals to allow stand up to a K_β couplet. For investigations where monochromatic radiation is

needed, K_α radiation is of attentiveness and a strainer requires to be used to detach the K_β radiation. XRD utilizes the wave Mother Nature of electromagnetic radiation and ass X-ray interrelates with a sample, these impede with each other. It illustrates the reflection of X-rays from the side by side of the flat in a solid. Most of the X-rays go through ruinous interference, which consequences in a signal at the self-same specific angle. The Circumstances for the helpful interference between the dispersed X-ray is specified by Bragg's order. The helpful interference of X-ray from sequential planes happens when the bit distinct is an essential multiple of distance according to Braggs equation:

$$n\lambda = 2d\sin\theta$$

where d is the bury-planer separating and θ is the gradient of occurrence also known as Bragg's gradient. The consequence spectrum schemed between the strength and the 2θ. The details on the construction and crystal dimensions of the nanocrystals were acquired by using Bruker AXS, D8 proceed X-ray diffractometer with CuK_α radiation ($\lambda = 1.54$ Å) as occurrence radiation and provided with the secondary transformation Graphite monochromatic. The study offset the V-shaped scope of 200 to 1000 in the stage size of 0.02. The dimensions of nanocrystals were set from the field thickness at half major (FWHM) of XRD peaks using the Scherer blueprint. The mean crystallite dimensions (D, in nm) were approximately using the Debye–Scherer equation.

2.3.2 Transmission Electron Microscopy

Transmission electron microscopy (TEM) is a tomography technique that is worn to acquire details from the selected that are sufficient to transfer electrons [9]. In this technique joist of electrons of enough strength is concentrated on the sample. The transmitted electrons are normally used to form as soon as the other a likeness or a diffraction design of the specimen and extend kind materialize on a fluorescence screen or to be existence by a CCD camera. TEM is competent of imagery at a notably higher intention due to the little de-Broglie wavelengths of electrons. This warrants the equipment to be skillful to survey the excellent characteristics, even as little as a single column of the atoms which is tens of thousands rhythm trivial

than the smallest reconcilable object in a brightness microscope [10]. TEM forms a critical study process in a compass of researchers' fields, in either biological or physical science when a gem of lattice separating *d* is lit up with electrons of wavelength. The separate split part waves will be manufactured at particular angles for $n = 1$, fulfilling Bragg's circumstances. The separate split part waves form diffraction marks on the stern focal plane. In an electron microscope, the utilisation of electron glass permits the systematic positioning of diffraction pots to be forecast on a partition and the electron diffraction design can be noticed. On the condition that the transfer end of the diffracted joists interferes on the likeness plane, enlarged portrayal can be discerned. The association of likeness and electron diffraction in TEM can be appreciated from the simplified ray [11]. The structured are made up of an electron origin [Filament: LaB_6]. The increased voltage is changeable between 20–200 kV. Present-day TEMs have two of a kind condenser glasses. The first condenser glasses are powerful lenses, to decrease the gun. Distinct currents allow distinct spot sizes. The second condenser (frail lens) is now second-hand to conduct the first condenser glasses intersection downstairs to the instance. The top dimensions of the subsidiary condenser glasses aperture are set on by the globe-shaped irregularity of the subsidiary glasses. One of these days little apertures are picked to acquire better coherent glitter. In operation, mark dimensions downwards to just about 100 nm can be acquired by using this dual condenser complex. The condenser glasses are shaped with apertures, which are normally little platinum circles with interstitials of numerous sizes [12].

2.3.3 Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectroscopy is a strong instrument for recognizing the variety of chemical bonds attending in a molecule by manufacturing an infrared immersion continuum of a sample with immersion peaks keep in touch with the periodicity of shaking between the bonds of atoms manufacturing the substance [13, 14]. Each matter is an individual amalgamation of distinct atoms, *i.e.*, no two of a kind compounds manufactures the entirely self-same infrared spectrum. Consequently infrared spectroscopy outcomes

in recognition (denary analysis) of distinct co-operative of matters [15]. It can be put into the examination of solids, gases, and liquids. In inclusion, the dimensions of peaks in the continuum are the shortest indicator of the quantity of matter present. The expression "infrared" offsets the compass of the electromagnetic spectrum in the middle of 0.78 μm and 1000 μm [16]. In the circumstances of infrared spectroscopy, distance is regular in the spatial frequency of a wave (cm^{-1}) (Table 2.1).

Table 2.1 Range values of FTIR w.r.t. zones

Zone	Wavelength	Wavenumber compass
Nearby	0.78–2.5	12800–4000
Central	2.5–5.0	4000–200
Distant	5.0–10.0	200–10

The functional infrared zone reclines in the middle of 4000–670 cm^{-1}. The foundational immersion frequencies also known as classification frequencies of the molecules are essential for unpicking. The construction-shadowy connections of the connected molecular quivering. The quivering spectrum of a molecule is thought to be an individual physical possession and is an attribute of the molecule.

Basic Principle

The infrared spectrum is set up as an outcome of the immersion of electromagnetic radiations at commonness that matches up the shaking of a particular place of chemical bonds inside a molecule. It is significant to throw back the dispersal energy owned by a molecule at some specified moment, explained as the ad of the donating energy terms [17, 18]:

$$E_{\text{TOTAL}} = E_{\text{VIBRATIONAL}} + E_{\text{ROTATIONAL}} + E_{\text{TRANSLATIONAL}} + E_{\text{ELECTRONIC}}$$

2.3.4 UV–Visible Absorption Spectroscopy

UV–Vis emission spectroscopy is the quantification of the intensity and wavelength of immersion of close by UV and visible light by a representative [19]. Visible and UV lights are active sufficient to encourage outer electrons to lofty energy levels. It is normally

put in molecules and inorganic ions or composite in solution. This manner is supportive to fluorescence spectroscopy [20], it calculates transition from the earth state to the animated state while immersion distributes transition from the animated state to the ground state. The involvement of light by the mixture is one of the elderly and motionless ones of the lion's shares functional nanotechnology for design processes [21]. The wavelength of light involvement by a combination is predictable of its chemical construction. A particular zone of the electromagnetic spectrum is soaked up by thrilling particular kinds of molecular and atomic translating to lofty energy levels. Immersion of microwave radiation is normally due to the excitation of molecular spinning motion. Infrared immersion is correlated with the quivering motions of molecules [22]. The involvement of UV and visible radiation is connected with the excitation of electrons, in both together molecules and atoms, to elevated energy states. The molecules go through electronic excitation patronage the involvement of lights, yet most of the molecules need sky-scraping energy radiation. The molecules accommodating coupled electron systems have sufficient light in the UV–Vis region, as the stage of junction inclines the spectrum to carry about lower energy. The UV–Vis spectra are widely acknowledged as a powerful process that can provide huge intuition in the direction of gathering decoration [23]. Thus examination involvement spectra allow the precious details regarding the emergence of molecule composite for the examination of collection, kasha, and comrade put forward a representation named as exciton integrate model. According to imitation, for attribute collection, immersion band would break interested two bonds, single is a small bathochromic transferred band and additional is expanded hypsochromic transfer band, considering that for the cluster, expanded or blue transport sort immersion band could be noticed [24].

2.3.5 NMR Spectroscopy

The ability of nuclear magnetic resonance analysis of molecular structure is to investigate the environment of an independent atomic nucleus [25]. This method can provide acceptable details about the constituent's properties, and the unique area participate in the intermolecular, which is important for the molecule's hardness

[26, 27]. Nuclear magnetic resonance is a powerful tool to explain functional implement for indicating the incident of halogen bonding interaction in molecule draw pull force [28, 29].

2.3.6 Thermal Analysis

For a lengthy time, the solidity of molecule complex has been deliberate by warm examination process counting thermogravimetry distinctive inspect calorimeter and other processes [30]. They allow the valuable particulars regarding compounds in several methods for instance because of substantiating the compound emergence transform in the middle of the compound and straightforward solution, resolution of stoichiometry proportion examination of guest-host compounds, an inspection of warm firmness [31], and explanation of decay methods especially, differential scanning calorimetry (DSC) is qualified to compute the vitality exchange with phase exchange which is an extraordinary ingredient to report the intermolecular forces of appeal in attendance in the compounds, while on the contrary, TGA control mass exchanges as a purpose of reversal which is due to evaporation of vaporous substance and decomposition and sublimation of complexes. These exchanges can also designate the attendance of a compound [32].

2.3.7 Scanning Electron Microscope

The scanning electron microscope (SEM) is a microscope that employs electrons instead of light to create an image [33]. There are a lot of advantages in utilizing the SEM rather than a light microscope. With the invention and development of scanning the electron microscope, new areas of study in physical, chemical, bioengineering, and medical science have evolved. Numerous specimens of bigger sizes have become possible to investigate with the SEM. A well-focused beam of high-energy electrons is being used to generate different signals at the surface of a solid specimen by the SEM [34]. The output signal produced from the electron specimen interactions reveals information containing the surface topology, its chemical composition, and other physical properties. In SEM, a beam of high-energy electrons is generated by an electron gun placed at the top of

a microscope. The electron beam travels through the microscope in a vertical path that is contained within a vacuum. The beam is focused downward toward the sample with the help of electromagnetic fields and lenses. The primary electron is scattered by atoms in the sample when it strikes the surface of the sample. The primary beam mostly spreads and occupies a teardrop-shaped volume, known as the interaction volume through the scattering phenomena. The accelerating voltage of the beam, the atomic number of the sample, and the density of the samples are the factors that influence the size of the interaction volume [35]. Secondary electrons are ejected from interactions in this zone, they are then detected, converted to a voltage, and amplified to develop an image. In SEM, various kinds of signals can be generated such as secondary electrons, backscattered electrons, specimen current and specimen current. For detection of all these signals, specific detectors are needed and in a single instrument are equipped with all the necessary detectors [36].

2.3.8 Energy-Dispersive X-ray Analysis

The elemental and chemical composition of a specimen is done by using the energy-dispersive analysis of X-rays (EDAX) method. The analysis of a material may be felt through interaction between electromagnetic radiation and the matter by X-ray fluorescence spectroscopy since each element has a different atomic structure that permits a special combination of peaks on its electromagnetic emission spectrum [37]. To restore the discharge of characteristics X-ray from a specimen so that an atom within the specimen consists of ground state electrons in discrete energy level or electron shell bound to the nucleus. A charged-particle beam (such as of electron) of high energy or an X-ray beam is spotted on the sample being investigated. An electron residing in the inner shell may get excited when the incident beam falls on the sample and finally eliminates it from the shell. The created hole is being filled up by an electron from a high-energy shell. The energy difference between the high-energy shell and lower-energy shell gets eliminated as an X-ray. The number and the energy of the X-rays that are eliminated from a sample can be determined with the help of an energy-dispersive spectrometer

[38]. The energies of the generated X-rays are determined by the energy difference between the two shells and the atomic structure of the eliminating sample. Hence the elemental composition of the sample under investigation can be evaluated by using EDS.

2.3.9 Selected Area Electron Diffraction

The crystal structures of individual nanomaterials and the crystal structure of a different region of the specimen can be found with the help of the selected area electron diffraction (SAED) pattern. Estimation of Bravais lattice and lattice parameters of the crystalline specimen can be done by utilizing the pattern. The development of diffraction patterns in TEM is demonstrated by a ray diagram. The back focal plane of the objective lens gets the focus from the intermediate lens so the image from the transmitted beam and the entire diffracted beam is shaped [39]. The intermediate aperture, is a second aperture, this allocates in the image of the objective lens & restricts the diffraction pattern to a selected area of the specimen, that's why this approach is termed as "selected area electron diffraction." To select the region of interest, the specimen is inspected in image mode initially. The intermediate aperture is embedded and placed over the region of interest. The activation of the diffraction mode of the microscope is done after that. The selected region in the image mode produces the SAED pattern. The lattice spacing can be estimated by utilizing the formula given below

$$RL = \lambda d,$$

where R is the radius, L is the camera length, and λ is the wavelength of light. The plane can be determined from the d value [40].

2.3.10 Energy-Dispersive X-ray Spectroscopy

The microanalytic method of energy-dispersive X-ray (EDX) spectroscopy is employed in combination with scanning electron microscopy. To describe the elemental composition of the examined volume, the EDX method detects X-rays produced from the sample during bombardment by an electron beam. Electrons from a higher state fill the resultant electron vacancies, and an X-ray is produced to balance the energy difference between the two electrons' states. The

X-ray energy is unique to the element that it was emitted from. The relative quantity of emitted X-rays against their energy is measured by the X-ray detector in EDX. In a solid-state device, the detector is usually lithium drifted silicon. When an incident X-ray collides with the detector, it causes a charge pulse proportionate to the X-energy. The charge sensitive preamplifier converts the charge pulse to a voltage pulse. After that, the signal is transmitted to a multichannel analyzer, which sorts the pulse by voltage. Each incident X-ray is delivered to a computer for display and additional data assessment, and the energy was calculated from the voltage measurement [41]. The elemental composition of the measured volume is determined by analyzing the X-ray energy versus counts spectrum. The EDX data for green produced nanoparticles was collected using a HITACHI Model S-3000H with a 15 kV accelerating voltage.

2.3.11 X-ray Photoelectron Spectroscopy

X-ray the most frequently used surface examination technique is photoelectron spectroscopy which is also known as electron spectroscopy for chemical analysis. It may be used on a wide variety of materials and gives useful quantitative and chemical status information from the material's surface. For an XPS measurement, the typical depth of analysis is about 5 nm. XPS devices may produce bands with a lateral spatial resolution of 7.5 μm. the spatial distribution information may be acquired by scanning the sample surface with a micro-focused X-ray beam. Combining XPS measurements with ion milling to describe thin-film material can provide depth distribution information [42]. Many industrial and research applications where surface or thin-film composition plays a critical role in performance such as catalysis, nanomaterials, electronic device, corrosion and photovoltaics. XPS is generally performed by bombarding a sample surface with monoenergetic rays, which cause photoelectrons to be released. The energy of the released photoelectrons is measured using an electron energy analyzer. The elemental identity, chemical state and amount of detected element may be calculated using the binding energy and intensity of a photoelectron peak. Physical electronic XPS equipment work similarly to SEM/EDS instrument, which generates SEM picture for sample viewing and point spectra or images for compositional

analysis using a highly focused electron beam. A tightly focused X-ray beam is scanned with the XPS equipment to generate secondary electron pictures for sample viewing and point spectra or images for compositional analysis [43]. To assist the efficient examination of bigger samples with homogeneous composition, the size of the X-ray beam can be increased. XPS is a surface analysis method with a typical analysis depth of less than 5 nm, which makes it more suitable for the compositional study of thin-layer and thin microscale sample features than SEM/EDS, which has a normal analysis depth of 1–3 m.

2.3.12 Differential Scanning Calorimetry

DSC is a type of thermal study that examines how temperature affects a material heat capacity. The heat capacity of a sample of known mass is measured as the variation in the heat flow. Different techniques may be detected such as phase shift, melts and glass transitions. DSC is utilized in numerous sectors including food, printing, medicines, paper electronics, polymers and semiconductors, because of its versatility and the fact that most materials display some type of transition. Energy is equally injected into a sample cell and a reference cell in a simple DSC experiment and both cells' temperatures rise at the same rate. The quantity of excess heat absorbed or emitted by the molecule in the sample corresponds to the difference in input energy necessary to match the sample temperature to that of the reference (during an endothermic and exothermic process, respectively).

2.3.13 Photoluminescence Spectroscopy

Photoluminescence (PL) spectroscopy is a touching smaller, counter damaging process for exploring the electronic construction of substance. Photoluminescence spectroscopy is a strong technique for exploring the electronic construction either extrinsic or intrinsic of semiconducting and semi-insulating substances. It can be nearly newed to decide the band gap of semiconductors, doping level and imperfection observation, substance standard, and to appreciate the physics supporting the reconnect methods. On light up the semiconducting substances with a light origin results in elevation of electron to the conduction band that leads to supporting holes in the valence band. The connection of these electron level combinations

may cause the emission of gamma quantum energy along with wavelength property of the material.

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Chapter 3

Current Scenario of Nanomaterials in Cancer Diagnostics

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3.1 Introduction

Cancer is one of the leading causes of death worldwide. It causes the patients to live a poor-quality life and reduces their survival expectations (Siegel *et al.*, 2016). There are several anticancer drugs available in the market, however, most of them tend to be cytotoxic due to their high pharmacokinetic distribution volume and low molecular weights. In addition, they are easily secreted out of the body due to their low molecular weight, which means they need to be administered in higher concentrations in the body. Further, these drugs cause side effects like slouching of the epithelial cells in the gut, alopecia (hair loss), and suppression of bone marrow because of their non-specific binding to the non-cancerous cells

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(Luo and Prestwich, 2002). Most of the anticancer drugs lack bioavailability and solubility making it necessary to formulate these agents in toxic solvents before administration (Kwon, 2003). Further, various treatment approaches such as radiotherapy, chemotherapy, and surgery are available for the treatment of cancer, however, the mortality rates remain high. In addition, radiotherapy and cancer therapy cause unwanted side effects on the normal cells due to the poor specificities of these technologies. For example, a chemotherapeutic agent, doxorubicin (DOX) induces apoptosis of normal cells in addition to normal cells. The surgery does not always have a one hundred percent success rate in removing the cancerous cells (Zhou *et al.*, 2017b). Therefore, nanomaterials such as liposomes, carbon nanotubes, polymeric micelles, etc. (Misra *et al.*, 2010; Tiwari, 2012) have been investigated and used for the treatment of cancerous cells. Nanomaterials are used for the development of nano delivery systems for different drugs especially drugs for cancer as they improve the drug efficiency with minimal side effects by specifically targeting the reaction affected tissues, use of biodegradable drug delivery system, and controlled release of the drug (Bharali *et al.*, 2009). The nanomaterials increase the efficiency of treatment and reduce the side effects by accumulating at the tumor sites *via* enhanced permeability and retention (EPR). The nanomaterials could be inorganic like gold nanoparticles (AuNPs), quantum dots (QDs), magnetic nanoparticles, carbon nanotubes, or organic molecules such as liposomes, dendrimers, polymeric micelles, etc. (Fig. 3.1).

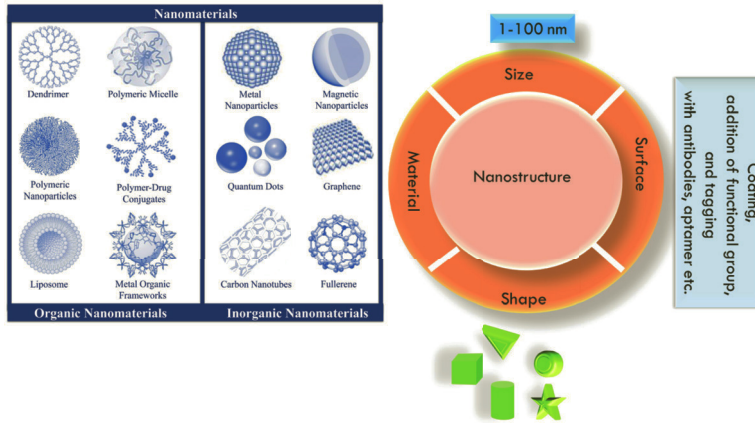

Figure 3.1 Schematic representation of nanomaterials along with their properties such as shapes, sizes, and surface changes.

3.2 Advantages of Using Nanomaterials in Cancer Therapy

The advancement of nanotechnology has paved the path for the development of various nanostructures such as liposomes, inorganic nanoparticles, and polymeric micelles that can be used as diagnostic and therapeutic agents (Couvreur and Vauthier, 2006) in cancer therapies (Torchilin, 2005; Wagner *et al.*, 2006). The nanomaterials serve a number of advantages in cancer therapies such as their small size which is far smaller than the biological molecules like cancer cells. Their smaller size increases their chances of intracellular uptake which make them excellent choice material for drug delivery (Goldberg *et al.*, 2007). They can be used for the manipulation of target signal pathways involved in the proliferation and survival of cancer cells due to their interactions at specific intracellular compartments (Tang *et al.*, 2003). Further, the nanomaterials can overcome the biological defense system and vascular barriers of the body, *i.e.*, they are not cleared easily out of the body due to their extremely smaller sizes (Peer *et al.*, 2007; Mok *et al.*, 2009). Another major advantage of using nanomaterials is their high surface area which enables labeling with numerous therapeutic and imaging agents. For instance, around 2,000 drug molecules can be loaded onto nanomaterials of an average diameter of 70 nm (Bartlett and Davis, 2007). Further, their surfaces can be easily modified using antibodies, peptides, or small molecules to enhance their imaging and therapeutic potential (Montet *et al.*, 2006; Hong *et al.*, 2007). Most of the chemotherapeutic agents like paclitaxel (PTX) are insoluble in water which hinders their anti-cancerous effect in the body. However, loading these chemotherapeutic agents in nanomaterials like liposomes and polymeric micelles enhances their water solubility making them readily available in the body (Hubbell, 2003). The encapsulation of chemotherapeutic drugs in nanomaterials reduces their cytotoxicity as the drug is released in a controlled manner at specific tumor cells without causing adverse effects on the healthy cells.

3.3 Nanomaterials Used for Cancer Diagnostics

3.3.1 Liposomes

The small lipid vesicles with closed membrane structure and size ranging from 50 nm to 1000 nm are known as liposomes (Fahmy *et al.*, 2007), and are promising drug carriers due to their reduced cytotoxicity, targeted drug delivery, and protection of degradation of drugs (Torchilin, 2007b). Liposomes are formed by phospholipid dispersion and contain hydrophilic heads and hydrophobic cationic/anionic long chain tails. Due to their structural properties, they can be used to load both water-soluble drugs (in aqueous core) and lipophilic drugs (in lipid bilayer) (Huang, 2008; Cai *et al.*, 2014). They are classified as small uni-lamellar vesicles (SUV), large uni-lamellar vesicles (LUV), and multi-lamellar vesicles (MLV) depending on their number of bilayers and their size. Based on their intracellular delivery mechanism and composition, they can be classified as pH-sensitive liposomes, immunoliposomes, conventional liposomes (CL), long-circulating liposomes (LCL), and cationic liposomes. The liposomes are synthesized from natural non-toxic phospholipids and cholesterol by hydration, vortexing, and extraction under high pressure using ultracentrifugation or column chromatography. The pharmaceutical molecules can be entrapped in the liposomes using a membrane contactor module and ethanol-injection method for liposome synthesis (Jaafar-Maalej *et al.*, 2011). Vast research is being conducted on the use of liposomes to be used as drug carriers, the only known effective commercialized formulation is Doxil (DOX). Anticancer drugs such as DOX encapsulated into PEGylated liposomes has been approved by the US Food and Drug Administration (FDA) to treat cancer due to improved tissue distribution and plasma pharmacokinetics (Marcato *et al.*, 2008; Chang and Yeh, 2012). In another study, DOX was encapsulated into pH-responsive liposomes modified with $H_7K(R_2)_2$ and used for targeting specific delivery in response to the mildly acidic pH in glioma cells (Zhao *et al.*, 2016). The anticancer drugs encapsulated in pH-responsive liposomes exhibited excellent anticancer properties with minimal renal and hepatic toxicity (Chiang and Lo, 2014). In addition to drugs, the liposomes are also used to load nucleic acids and enzymes (Pakunlu *et al.*, 2006; Zhang *et al.*, 2014) and *in vivo* distribution of the agents

to the targeted sites (Peer *et al.*, 2007; Takara *et al.*, 2012; Rengan *et al.*, 2014). Although providing drug delivery in the cancerous cell, the liposomes suffer from various disadvantages such as small capacity to load drugs in them, inter-batch variation, sterilization, and stability.

3.3.2 Dendrimers

Dendrimers are three-dimensional geometric patterns that are achieved by the arrangement of repeated arms of polymeric macromolecules through convergent and/or divergent methods of synthesis. The convergent method was first introduced by Hawker and Frechet (Derfus *et al.*, 2004), in which the monomer units are linked together to form the surface wedges which are then attached to a central unit giving it the shape of a dendrimer, for example, polyaryl ethers and polypropylenemine (PPI). On the other hand, in the divergent method the branches of the repeating units are originated from the core unit, for example, polyamidoamine (PAMAM) dendrimers (Tomalia *et al.*, 1985). A typical dendrimer structure is composed of three parts: terminal functional groups, branches, and an initiator core. The initiator core (G0) is the center of the dendrimer, and the monomers attached to G0 are called first-generation monomers (G1). Second-generation monomers (G2) are formed by attaching to G1 *via* functional groups and so on, doubling the molecular weight of the dendrimer after every generation (Tomalia, 2005). Depending on the desired application of the dendrimer, its terminal groups can be modified using different functional groups to obtain lipophilic, hydrophilic, or charged dendrimer (Bai *et al.*, 2006). They serve as ideal carriers for drug delivery due to their size comparable to biological molecules such as proteins, and DNA, and they possess feasible functionality, dimension, and topology (Gillies and Frechet, 2005) which enables the drug conjugation on the surface or encapsulation in the core. These structures can be used to load genes and drugs *via* covalent conjugation, encapsulation, and electrostatic interactions. They possess a high density of surface functional groups like -COOH and $-NH_2$ and empty internal cavities. DOX was covalently bound to the 3-arm polyethylene oxide *via* hydrazone linkage. It was observed that when this drug formulation was used in murine melanoma cells

(cell line B16F10), breast cancer cells (cell lines MDA-MB231 and MDA-MB-435), and monkey kidney fibroblast CV-1 cells, the cells exhibited limited *in vitro* cytotoxicity, and the *in vivo* half-life of DOX was increased with minor accumulation of the formulation in the organs like heart and liver (Padilla De Jesús *et al.*, 2002).

In another study, DOX was conjugated to PAMAM dendrimers *via* PAMAM-hyd-DOX bonds and targeted to the Ca9-22 gingival carcinoma cells through the photochemical internalization (PCI) technique, which significantly improved the accumulation of DOX in the nucleus of cancerous cells, thus, destroying them efficiently (Lai *et al.*, 2007). Time sequenced propagation technique was used to synthesize a poly (amide-amine)-based dendrimer with four direction branches and a cyclic core, which was used to conjugate 1-bromoacetyl-5-fluorouracil to form dendrimer-5FU. It was observed from the pharmacokinetics of the dendrimer-5FU that could serve as an excellent carrier for anti-cancerous drugs and their controlled release in the targeted cells (Zhuo *et al.*, 1999). Another excellent example of dendrimers for drug delivery is the use of PEGylated dendrimers, synthesized by conjugation of polyethylene oxide (PEO) or polyethylene glycol (PEG) to dendrimers, as they have reduced accumulation in cells, reduced levels of cytotoxicity, and prolonged circulation time in the blood. The effectiveness of these dendrimers was studied by using polyester-based dendrimer-PEO-DOX to successfully inhibit the progression of C-26 tumor implanted in BALB/c mice (Lee *et al.*, 2006). Further, prolonged drug delivery with minimal hematologic disturbances was achieved in albino rats *in vitro* using the PEGylated PAMAM dendrimers-5FU (Bhadra *et al.*, 2003). In addition, PEGylation reduced the hemolytic toxicity and leakage of the drug, thus, leading to the increased stability and drug-loading efficiency of the drug. Their smaller size, *i.e.*, 1-15 nm, enable their clearance from the body through renal extraction, thus, reducing their cytotoxicity.

3.3.3 Polymeric Nanoparticles

Polymeric nanoparticles can be used for drug encapsulation, dissolution, absorption, and entrapment (Devulapally *et al.*, 2014; Masood and C, 2016) and have increased drug stability and drug-loading efficacy. The polymeric nanoparticles can be synthesized

using methods like nanoprecipitation (Govender *et al.*, 1999; Wang *et al.*, 2016a), salting out (Liu *et al.*, 2011; Owen *et al.*, 2013), supercritical antisolvent method (Tam *et al.*, 2016), solvent extraction or evaporation and emulsification (Patil *et al.*, 2009), and electrospray nanoprecipitation (Luo *et al.*, 2015).

The polymeric nanoparticles can be used to deliver various anticancer drugs, proteins, and genes as they have prolonged circulation, biocompatibility, biodegradability, and reduced side effects. Paclitaxel (PTX) was loaded into PEGylated PLGA NPs for inhibition of tumors in HeLa cells and it was found that these polymeric nanoparticles explicated three times higher cytotoxicity toward the HeLa cells when compared to Taxol (Danhier *et al.*, 2009). The gene therapy for cancerous cells is usually performed using viral carriers, however, it has been observed that the viral carriers pose safety issues like tumorigenicity, immunogenicity, and inherent toxicity. Therefore, polymeric nanoparticles can be used to perform gene therapies for cancer treatment. In a study, brain tumor survival was extended *in vivo* in F98 and 9L rat glioma cell lines using the polymeric NPs when compared to Lipofectamine 2000, a commercially available anticancer reagent (Mangraviti *et al.*, 2015). DOX, immobilized in 2-nitroimidazole derivative conjugated carboxymethyl dextran polymeric nanoparticles possessed higher cytotoxicity against hypoxic cells and thus significant anti-cancerous efficacy (Thambi *et al.*, 2014). Further, polymeric nanoparticles loaded with magnetic nanoparticles and gadolinium complexes are used in magnetic resonance imaging (MRI) to image cancer. In another study, liver cancer cells were imaged using magnetic nanoparticles and sorafenib co-encapsulated in folate-conjugated PEGylated PLGA-based nanoparticles (Li *et al.*, 2015). Apart from imaging, these conjugates also posed inhibited the tumor growth in the liver cells. Similarly, gemcitabine-52-monophosphate was immobilized in polymeric nanoparticles and used to image and inhibit MDA-MB-231 tumor cells *in vivo* simultaneously (Li *et al.*, 2016a).

3.3.4 Polymeric Micelles

The nano-sized colloidal and spherical particles containing hydrophobic core and hydrophilic shell are called as polymeric

micelles (PMs) and are prepared from the amphiphilic co-polymers (Torchilin, 2007a; Zhang and Ma, 2009). The hydrophilic shell is constructed using a material like polyethylene glycol (PEG) which stabilizes and protects the *in vivo* degradation of the carriers (Kataoka *et al.*, 2012; Zhou *et al.*, 2017a). The hydrophobic core is constructed using the materials like poly(lactic-co-glycolic acid) (PLGA), poly(lactide) (PLA), poly(ε-caprolactone) (PCL), and polysaccharides which enhances the water solubility of drugs by entrapping the drug into the hydrophobic core. The PMs are prepared by oil-in-water emulsion method, solid dispersion method, nanoprecipitation method, and dialysis method (Kwon and Okano, 1996; Jones *et al.*, 1999). The PMs serve as ideal drug delivery systems as they reduce the non-specific toxicity, improve the pharmacokinetics of the loaded drug, and deliver the drug to the specific site. In addition, its narrow distribution and small size increases the circulation time and prevents the rapid renal excretion of the anti-cancerous drug (Fonseca *et al.*, 2015; Biswas *et al.*, 2016). In a study, lymph node metastasis was inhibited by using an anticancer drug, (1,2-diaminocyclohexane) platinum (II) entrapped in cRGD-conjugated PMs (Makino *et al.*, 2015). The tumor cells have a natural pH gradient therefore, pH-sensitive degradable micelles can be used to deliver anticancer drugs (Helmlinger *et al.*, 2002; Vander Heiden *et al.*, 2009). For instance, intracellularly acid-switchable micelles composed of a pH-sensitive diblock polymer (DOX and photosensitizer) were used in the therapy against drug-resistant tumors (Wang *et al.*, 2016b). The activated micelles generated reactive oxygen species (ROS) when exposed to near-infrared (NIR) laser irradiation which could be imaged using photoacoustic (PA) imaging. In another work, DOX was delivered in cytosol using pH-sensitive mixed micelles prepared using poly(histidine)-PEG and DSPE-PEG (Wu *et al.*, 2013) where these nanomaterials explicated enhanced anti-cancerous efficacy.

3.3.5 Polymer Drug Conjugates

Water-soluble polymers conjugated to agents using biodegradable linkage leads to the formation of polymer-drug conjugates (Duncan *et al.*, 2005; Duncan, 2006; Greco and Vicent, 2009). The drugs are delivered through endocytosis and the EPR effect is used to target

the tumor cells. To date, polymer-drug conjugates have proved to be the most successful delivery vehicles for cancer treatment (Duncan, 2006; Bonomi, 2007; Li and Wallace, 2008). The drugs are loaded onto the polymer-drug conjugates through the covalent bonding of the functional groups of the drug to the polymer with or without a spacer. DOX was loaded onto the polymer-drug conjugate made up of a matrix metalloproteinase 2 (MMP2)-sensitive peptide linker, PEG, and a TAT, which provided an improved anti-cancerous efficacy by P-gp inhibition and specific tumor targeting (Tu and Zhu, 2015). Similarly, increased anti-cancerous efficacy was achieved with DOX covalently bound to polyethylenimine (PEI), 2,3-dimethylmaleic anhydride, and fragment antibody (HAb18 $F(ab2)_2$) (Zhou *et al.*, 2015).

The major advantage of using polymer-drug conjugates for cancer therapy is that they could be used to conjugate and deliver multiple drugs to provide the synergetic effect of drugs (Greco and Vicent, 2009), which is very well needed in cancer treatment (Broxterman and Georgopapadakou, 2005). For instance, mitomycin C (Mit-C) and DOX conjugated to HPMA through pH-sensitive hydrazone bonds were used to prepare polymer-drug conjugate for the treatment of EL-4 cancer cells (Kostková *et al.*, 2013). Similarly, demethylcantharidin (DMC) and oxaliplatin were conjugated on the polymer-drug conjugates for the treatment of SKOV-3 cancer cells. It was found that the co-polymerized drugs showed higher cytotoxic effects against the cancer cells in comparison to the free drugs.

3.3.6 Gold Nanoparticles

Clustered or colloidal particles made up of an Au core and a surface coating with few to several hundred nanometer diameters are known as gold nanoparticles (AuNPs). Depending on the synthesizing agents and conditions used, different types and sizes of AuNPs can be prepared. AuNPs can be synthesized using salt reduction (Majdalawieh *et al.*, 2014) or seed-mediated growth method (Millstone *et al.*, 2008; Sánchez *et al.*, 2013). In the salt reduction method Au salt is reduced in organic or water solvent leading to the formation of AuNPs. In seed-mediated growth method, small seed particles of Au are used to prepare the AuNPs. Due to their unique optical, electronic, and chemical properties, synthetic versatility,

and high absorption of X-rays, they have been used as novel radiosensitizers. In addition, AuNPs can be used in labeling, sensing, and delivering drugs and thus find potential in cancer therapies (Cristina Popescu and Mihai Grumezescu, 2015; Kodiha *et al.*, 2015; Singh *et al.*, 2015). A radiotherapeutic and chemotherapeutic effect was observed by using core/shell NPs containing DOX and AuNPs in tumor cells resulting in the highest anti-cancerous effect (Kim *et al.*, 2016). The particle size of AuNPs influences the radiosensitization and contrast of CT images. For instance, AuNPs with 13 nm diameter could possess significant radioactive disruption and excellent CT contrast ability simultaneously, allowing real-time radiotherapeutic inhibition and CT imaging of tumors in mice (Dou *et al.*, 2016). The high contrast in imaging is due to the higher scattering intensity and absorption of AuNPs in comparison to most organic dyes (Link and El-Sayed, 1999). Folic acid and cisplatin prodrug were conjugated to fluorescent gold nanoclusters (FA-GNC-Pt) for targeting and imaging breast cancer cells in 4T1 tumor-bearing nude mice (Zhou *et al.*, 2016). It was observed that the FA-GNC-Pt was accumulated at the tumor sites leading to a strong fluorescent signal and inhibiting the growth of tumors in the mice. In another study, four times higher CT intensity images were observed in prostate cancer cells using aptamer-AuNP bioconjugate (Kim *et al.*, 2010). In another study, platinum (IV) was delivered to prostate cancer cells by conjugating it to AuNPs causing specific and efficient anti-tumor effects on the cells (Kumar *et al.*, 2014).

AuNPs can be functionalized easily and thus can be used to deliver drugs to the targeted cancer cells (Fernandes *et al.*, 2017). In a work conducted by Farooq *et al*. (2018) two drugs: DOX and bleomycin (BLM) were delivered simultaneously to the cervical cancer cells (HeLa cells). This approach served different advantages such as high stability, enhanced cellular uptake by HeLa cells, high loading capacity, specific cancer cell environment mediated release of the drug, and can overcome of drug resistance. Colorimetric detection of analytes has gained interest as it has easy preparation steps and has high stability at low-cost input. AuNPs can be conjugated to enzymes and measure the activity in the reaction. Cellular glutathione (GSH) was detected using the colorimetric response of peroxidase by GSH stabilized gold nanoclusters (AuNCs) (Feng *et al.*, 2017). The cancerous cells contain higher levels of GSH which is essential in

cellular metabolic and protective functions of the cells. The higher concentration of GSH in cancerous cells help in the detection of tumors at an early stage (Xianyu *et al.*, 2015). The catalytic activity of AuNCs is inhibited and free radicals like ·OH are scavenged by GSH causing an anti-cancerous effect in the cells. The nanoprobes prepared using AuNPs can specifically distinguish the cancerous cells from the normal cells.

3.3.7 Magnetic Nanoparticles

Magnetic nanoparticles (MNPs) are used for hyperthermia treatment, drug delivery, and imaging in cancer cells due to their MRI and magnetic targeting properties (Sun *et al.*, 2008; Singh and Sahoo, 2014; Gobbo *et al.*, 2015; Hajba and Guttman, 2016). MNPs can be synthesized using methods like co-precipitation, hydrothermal synthesis, micelle synthesis, and thermal decomposition (Lu *et al.*, 2007; Laurent *et al.*, 2008). The iron salts like iron acetylacetonate and iron pentacarbonyl are reduced at high temperatures in aqueous solutions. The size of MNPs can be reduced using the surfactants during the synthesis processes. MNPs are mostly used to image the cancer cells at earlier stages using MRI (Song *et al.*, 2005; Lee *et al.*, 2007; Hayashi *et al.*, 2013). The MRI images are generated by microscopic field gradient generated by MNPs in the presence of a strong magnetic field. This gradient causes diphase or shortening of longitudinal (T1) or transverse relaxation times (T2) of proton nuclei which are detected on the MRI map as hyper-intensities for T1 and hypo-intensities for T2. A polymeric nanocapsule was prepared using super magnetic hydrophobic MNPs for magnetic targeting and imaging of tumor cells (Bai *et al.*, 2016). Early-stage imaging of pancreatic ductal adenocarcinoma (PDAC) cells was achieved by using iron oxide (maghemite, ɣ-Fe_2O_3) NPs and recombinant human serum albumin (rHSA) targeting the galectin-1 receptor (only present in pancreatic cancer cells). The images were recorded using single-photon emission computed tomography-computer tomography (SPECT-CT), MRI, and a handheld ɣ camera (Rosenberger *et al.*, 2015).

MNPs can be modified using the aptamers to prepare multifunctional target specific NPs (Xiao *et al.*, 2012; Zhang *et al.*, 2018) as they can easily penetrate the cells and can be retained by

the cells for longer times (Xiang *et al.*, 2015). *In vivo* and *in vitro* synergistic chemo-PTT effect in cancer cells was observed using aptamer functionalized MNPs@carbon@DOX (Apt-Fe_3O_4@C@DOX) (Zhao *et al.*, 2019). It was observed that when the nanocomplex, Apt-Fe_3O_4@C@DOX was irradiated with laser (808 nm) it converts the radiations into heat energy leading to the complete removal of the tumor A549 cells. This approach could simultaneously detect and remove the cancer cells without causing any adverse side effects. The results were backed up by the fact that hematoxylin and eosin (H&E) staining after treatment with the prepared nanocomplex only the normal cells were observed, and the cancer cells were absent.

3.3.8 Silica Nanoparticles

In biological systems, silica-based nanostructures have gained recent interest (Vivero-Escoto *et al.*, 2012) as their surface chemistry, shape, porosity, and size can be controlled during their synthesis. several anti-cancerous agents have been successfully encapsulated in the silica-based nanoparticles (SiNPs) (Couleaud *et al.*, 2010; Hao *et al.*, 2015; Paris *et al.*, 2015; Liu *et al.*, 2016; Yang *et al.*, 2016). SiNPs are used as optical imaging agents and drug delivery carriers due to their favorable colloidal properties, photophysical stability, biocompatibility, and ease of modification using antibodies and aptamers (Wang *et al.*, 2008; Li *et al.*, 2016b). SiNPs are synthesized using the reverse microemulsion (Arriagada *et al.*, 1992) and Stöber method (Stöber *et al.*, 1968). In the reverse microemulsion method, tetraethoxysilane (TEOS) is catalyzed in the presence of ammonium salts in water-in-oil microemulsion where the size of the SiNPs is controlled by water-to-organic solvent ratio. In the Stöber method, controlled condensation, and hydrolysis of TEOS is carried out in water and ethanol. SiNPs as drug delivery carriers serves the advantages such as they can provide a stimulus-controlled release and high payload of the drug (Mekaru *et al.*, 2015; Hakeem *et al.*, 2016). In a study, SiNPs were used to deliver siRNA and DOX simultaneously which was used to overcome drug resistance in breast cancer by exhibiting synergetic inhibition of tumors *in vivo* (Meng *et al.*, 2013). In another study, SiNPs coupled with an anti-HER2 monoclonal antibody, trastuzumab, and PEI-PEG were used to deliver siRNA to breast cancer cells. It was found that the SiNPs

specifically targeted the HER2-positive breast cancer cells without causing any toxic effects on the normal cells (Ngamcherdtrakul *et al.*, 2015).

3.3.9 Quantum Dots

QDs, sized nearly 10 nm in diameter, are multimodal, photostable, and optically tunable particles used as luminescent probes for various biomedical and biological applications (Chan and Nie, 1998; Alivisatos, 2004). The small size of the QDs facilitates their "honing in" at the targeted sites by allowing unimpeded systemic circulation (Gao *et al.*, 2004; Michalet *et al.*, 2005). In addition, they can be simultaneously used in tissue engineering (Goldberg *et al.*, 2007), *in vivo* imaging and drug delivery as different therapeutic agents can be attached to their surfaces (Howarth *et al.*, 2005). For target-specific drug delivery, the QDs can be modified using different surface molecules (Hoshino *et al.*, 2004; Alivisatos *et al.*, 2005). The QDs were first used by Gao *et al*. (Gao *et al.*, 2004) in living animals for *in vivo* imaging and cancer targeting by administering QD probes *via* systemic injection and QD-tagged prostate cancer cells *via* subcutaneous injection to attain multicolored fluorescence and sensitive imaging of the cancer cells. A similar study was conducted by Bagalkot *et al*. (Bagalkot *et al.*, 2007) in which the prostate cancer cells were targeted and imaged using the QDs-based fluorescence using QD-aptamer-DOX conjugates. Various coating materials have been used to reduce the cytotoxicity of the QDs, however, in some cases, the QDs may prove to be toxic probes (Derfus *et al.*, 2004). For example, long term circulation of CdTe particles causes the loss of the protective cover and production of ROS, and CdSe particles produce cadmium ions when termed to a prolonged ultraviolet (UV) light exposure, causing cell death (Green and Howman, 2005; Lovrić *et al.*, 2005; Cho *et al.*, 2007).

The QDs with desirable optical properties, sizes, shapes, and compositions can be generated by optimizing the precursors, surfactants, and reaction temperature during the synthesis process. During the synthesis of QDs, the addition of solvents like trioctylphosphine (TOP) and trioctylphosphine oxide (TOPO) can stabilize the particles and prevent them from agglomeration. Compared to the organic dyes, the QDs possess narrow emission

and broad absorption spectra which give them better optical properties (Chan and Nie, 1998; Chen *et al.*, 2012). They provide a stable photostable signal even at lower concentrations which enables the acquisition of images long after their emission. Unlike the dyes they can emit in the near-infrared region, considerably reducing the autofluorescence. Their optical advantages make them a material of choice for cancer detection and treatment (Qin *et al.*, 2015; Bwatanglang *et al.*, 2016; Michalska *et al.*, 2016). HER2 biomarker in lung and breast cancer cells was detected using anti-HER2-antibody-QD conjugate (Rakovich *et al.*, 2014). In another study, tumor-bearing mice cells were detected and treated using the ZnO-Gd-DOX QDs without potential toxicity to the normal cells (Ye *et al.*, 2016). The PbS QDs were used to encapsulate ribonuclease-A (RNase-A) and it was found that they could penetrate deep into the muscle tissues and achieve excellent fluorescent images even at the ultra-low concentrations (Kong *et al.*, 2016).

The QDs possess the properties like biocompatibility, facile production, strong photoluminescence, high drug-loading capacity, and excellent physiological capacity which make them an excellent choice for drug delivery (Chen *et al.*, 2017). Graphene QDs functionalized with aptamer and PEG were prepared for the delivery of porphyrin (GQDs-PEG-P) (Cao *et al.*, 2017) which possess low toxicity and the ability to generate singlet oxygen (Kou *et al.*, 2017). Porphyrin is used as a second-generation photosensitizer in photodynamic therapy (PDT) for cancer treatment, where it transfers the photon energy to its surrounding oxygen molecules to generate ROS like singlet oxygen. These ROS then kill the cancerous cells upon irradiation with light of desired wavelength (Tian *et al.*, 2013; Cheng *et al.*, 2015). The GQDs-PEG-P nanosystem serves the advantages of better physiological stability, low toxicity, and good biocompatibility. The GQDs in GQDs-PEG-P nanosystem has a larger surface area to provide the microRNA (miRNA) delivery to cancer cells by distinguishing them from somatic cells. The developed nanosystem had a high quantum yield of singlet oxygen (1.08) and photothermal conversion efficiency of 28.58%, making it an excellent candidate for PDT. The temperature of the GQDs-PEG-P rises to 53.6°C upon irradiation with a laser of wavelength 980 nm for 10 min which leads to the ablation of A549 cancer cells. The exposure to laser also causes early apoptosis and destruction of the

cell membrane in A549 cells with good stability and reproducibility after each on/off laser light cycle. One of the prominent reasons for cancer propagation in humans is the mutation caused by the oxidative damage by 8-oxoguanine (8-oxoG) leading to G:C to T:A transversions (Viel *et al.*, 2017) which can be removed using human 8-oxoG DNA glycosylase I (hOGG1) (Hu *et al.*, 2018). Using this principle, cadmium tellurium (CdTe) QDs-labeled multifunctional DNA nanocages were developed and loaded with DOX for the fluorometric detection of hOGG1 and treatment of MCF-7 cancer cells (Jie *et al.*, 2019). To prepare the nanosystem, 8-oxoG containing DNA template (DNA HP1) was cleaved specifically by hOGG1 enzyme forming DNA2. Next, 3'-recessed DNA duplex was prepared by the hybridization of DNA2 and DNA HP2. This duplex was digested with ExoIII and cycling amplification was initiated (cycle I) to generate unholder DNA, *i.e.*, HP2 DNA fragments containing Cover and Pedestal DNA and nucleolin aptamer. The Cover and Pedestal DNA and unholder DNA are combined to form hexahedral DNA nanocages which were then labeled with CdTe QDs and loaded with DOX. The nanosystem binds specifically to the nucleolin of MCF-7 cancerous cells to enter the cells where it disperses releasing the DOX. The fluorescence turns ON upon the release of DOX which detects the presence of tumor and treat it. Similarly, QDs have been used to diagnose and treat cancerous cells with great specificity and efficiency.

3.3.10 Carbon Nanotubes

The seamless tubular structures made up of thin sheets of benzene ring carbons were first reported in 1991 by Iijima as carbon nanotubes (CNTs) (Iijima, 1991). There are two types of CNTs: single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) (Liang and Chen, 2010). CNTs have been used as gene and drug delivery vehicles in thermal and PDTs for cancer treatment. CNTs can be synthesized from a carbon source and energy using chemical vapor deposition, laser ablation, and arc discharge method (Brownson and Banks, 2012; Park *et al.*, 2013; Gougis *et al.*, 2014). In chemical vapor deposition method, the carbon source is carbon monoxide, acetylene, or methane and energy to decompose these hydrocarbons is provided using high temperatures. This

method is used for the large-scale manufacturing of CNTs. In the laser ablation method, laser pulses are used to decompose the carbon electrodes synthesizing the CNTs. The most-reported method for CNTs synthesis is arc discharge where a potential difference of approximately 20 V decomposes the carbon electrodes. This is the easiest method reported for the synthesis of CNTs.

CNTs possess excellent physiochemical properties and tunable surface modifications which make them a choice for drug delivery in cancer cells. In a study, PTX was conjugated to the PEG chains on SWCNTs and delivered to tumor cells. It was found that PTX-PEG-SWCNTs was accumulated at the tumor site *in vivo* and circulated in the blood for a longer time, thus, providing better anti-tumor efficiency when compared to free Taxol (Liu *et al.*, 2008). The SWCNTs have also been used for multi-drug delivery. For instance, salinomycin and PTX were conjugated to PEG chains on SWCNTs through hydrazine bond and delivered to cancer stem cells (CSCs) and breast cancer cells. The drugs were released in a pH-dependent manner providing enhanced therapeutic efficacy which was confirmed using imaging techniques like MRI and bioluminescence (Al Faraj *et al.*, 2016). Another successful treatment using CNTs was observed by Zhang *et al*. (Zhang *et al.*, 2009), where DOX was immobilized on SWCNTs and delivered to cancerous cells. It was found that the acidic conditions and pH of the tumor cells enable the quick release of DOX into the nucleus of cells resulting in cell apoptosis. The DOX-SWCNTs system could detect and kill cancerous cells far more effectively and selectively in comparison to free DOX. Not only do the CNTs provide anti-tumor properties but can also be used as imaging agents as their dark color absorbs excellently in the NIR region. The MWCNTs were modified with RGD-conjugated silica-coated gold nanorods for the photoacoustic imaging of the gastric cancer cells *in vivo* with a very low non-specific toxicity (Wang *et al.*, 2014). The prostate stem cell antigen (PSCA) was imaged using PSCA antibody-conjugated MWCNTs (CNT-PEI(FITC)-mAb) *in vivo* and *in vitro* with great specificity (Wu *et al.*, 2014).

3.3.11 Nanographene

First separated from graphite in 2004, graphene is an atom-thick single layer of carbon atoms forming a two-dimensional

(2D) honeycomb structure and is used to prepare other graphitic materials (Kopelevich and Esquinazi, 2007; Rao *et al.*, 2009; Geim and Novoselov, 2010). Graphene along with its derivatives like reduced graphene (rGO) and graphene oxide (GO) have special optical, chemical, and physical properties (Gurunathan *et al.*, 2015; Luo *et al.*, 2016; Wei *et al.*, 2016). These properties make them useful candidates in various biomedicine applications such as disease treatment and cancer therapies. GO is prepared by oxidation of graphene and can be functionalized using various functional groups such as hydroxyl, carboxylic acid, and epoxide groups. The functionalization of GO attributes to its excellent biocompatibility and dispersity in the biological medium. The 2D structure of nanographene allows hydrophobic interactions, non-covalent π-π stacking, and provides a high surface area which makes them suitable for drug delivery in cancer cells. The functional groups on the surface of GO allow binding of the molecules through electrostatic interaction, hydrogen bonding, and covalent conjugation (You *et al.*, 2015; Zhao *et al.*, 2015; Bikhof Torbati *et al.*, 2017). A labeled fluorescein probe was immobilized on a GO-based nanocarrier was used to image the tumors in mice *in vivo* and *in vitro*. In addition, to confocal fluorescence imaging, the nanocarrier also induced apoptosis in the cancerous cells (Tian *et al.*, 2016). When GO is reduced thermally, chemically or with UV radiation, it leads to the formation of rGO (Park *et al.*, 2009). Similar to GO, rGO is also used to image and treat cancer cells. For example, anti-HER2 antibody-conjugated poly-L-lysine functionalized rGO was used to deliver DOX to the nucleus of MCF7/HER2 cells where they caused the apoptosis of the cancerous cells (Zheng *et al.*, 2016).

3.4 Cytotoxicity Caused by Nanoparticles

A vast amount of research has been carried out recently regarding the use of nanotechnology and nanoparticles for humans, though there is limited information available on their toxic effects. Therefore, it is important to study the effect of nanoparticles *in vivo* and their intracellular response. It has been found that the titanium dioxide/zinc oxide nanoparticles present in the sunscreens can enhance the *in vitro* oxidative damage to cultured human fibroblasts

and DNA (Dunford *et al.*, 1997). In another report, it has been found that gold nanoparticles (AuNPs) when injected in pregnant rats, can cross the placenta, and reach the fetus from the mother (Wootliff, 2004). In addition, the toxicity related to the oxidation of single-walled carbon nanotubes (SWCNT) has also been found to cause morphological changes and probable dermal toxicity (Shvedova *et al.*, 2003) and pulmonary toxicity (Shvedova *et al.*, 2004). Further, the silver nanoparticles can also cause toxicity in keratinocytes, growing human fibroblasts, and lesioned skin upon crystallization of the nanoparticles (Poon and Burd, 2004).

3.5 Conclusion

This chapter discusses nanotherapeutics offering new opportunities for improving the safety and effectiveness of regular therapy. Stable interactions with ligands, size and shape variability, high carrier capacity, and ease of binding of hydrophilic and hydrophobic materials makes nanoparticle favorable for therapeutic as well diagnostic purpose. It provides an overview of the unique features of nanoparticles in the biological systems, emphasizing the type of clinically used nanoparticles and their specificity for therapeutic applications.

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Chapter 4

Emerging Antineoplastic Potential of Nanoparticles Against Different Types of Cancer

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4.1 Introduction

In 2020, an estimated 19.3 million new cancer cases with almost 10.0 million cancer-related deaths were reported worldwide. Moreover, during the next two decades, the cancer burden is expected to

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further increase by about 47%, meaning that in 2040, 28.4 million new cancer cases will be diagnosed [1]. Such a continuous rise in global cancer incidence presents a great challenge for the scientific community to develop new and more effective strategies for fighting against this dreadful and often incurable disease. One promising approach along this road involves the improvement of the drug delivery process by using different types of nanosized carriers.

Although the age of nanoparticles started already in the 1950s with the design of a polymer (polyvinylpyrrolidone)-drug (mescaline) conjugate [2], the first nanosized anticancer drug, *i.e.*, albumin-bound paclitaxel (Abraxane) was approved by the US Food and Drug Administration (FDA) for the treatment of breast cancer still in 2005 [3]. Since then, different types of nanocarriers have been synthesized and tested with the aim to increase the tumor tissue concentrations and therapeutic efficacies of anticancer agents, by improving pharmacokinetic parameters, bloodstream circulation times, and cellular uptake of various chemotherapeutics [4]. Today, it is well elucidated that nanoparticles can target tumor tissue in either a passive or active manner. The passive targeting is based on the altered architecture of tumor vasculature with the so-called enhanced permeability and retention (EPR) effect leading to increased accumulation of nanoparticles in the tumor site [4]. The optimal size of nanoparticles (100–200 nm) makes it possible to take advantage of the EPR effect and extravasate via vascular fenestrations of tumors; while, at the same time, escaping hepatic and splenic filtration [5]. The active targeting, on the other hand, involves conjugation of nanoparticles with certain receptor ligands (such as vitamins, peptides, and antibodies), allowing the binding of nanoproducts to specific cellular targets through recognizing particular receptors at the surface of tumor cells, thereby essentially improving the selective delivery of nanodrugs [6]. Following their systemic administration, nanotherapeutics are commonly removed by the reticuloendothelial system (RES) [4].

In this book chapter, a thorough overview of the synthesis and characteristics of nanoparticles-conjugated chemotherapeutics is presented, describing their anticancer activity in different tumor types. Also, the benefits of these nanotherapeutics over their parent intact drugs are highlighted, as well as the possible bottlenecks impeding their clinical application are analyzed.

4.2 Nanotherapeutics in Diverse Range of Cancer

Cancer is a major concern in health all over the world. Approximately 19.3 million new cases of cancers were reported globally in 2020. There are many drugs available in the market which is used to treat cancer but the patients experience severe kind of side effects. Currently, Nanoparticles (NPs) are employed as therapeutic agents to deliver drugs, antibodies, and ligands. Various NPs are used in the treatment of cancers like silver, gold, magnetic particles, miRNAs, etc. which are conjugated to various drugs that help in increasing the efficacy of these drugs in the patients. In this section, we are summarizing the role of various NPs in different types of human cancers.

4.2.1 Role of Nanoparticles in Brain Cancer

Brain cancer is one of the most prevalent malignancies whole over the globe, which accounts for a noticeable percentage of all cancer types [7, 8]. Conventional chemotherapy is certainly effective against many cancer types [9–12]. Such effectiveness is partially attributed to diagnostic approaches and improved screening. However, the fact that conventional chemotherapy is not devoid of certain side effects and drug resistance, cannot be neglected [13–16]. Further, the lack of bioavailable serum or plasma concentrations of many chemotherapeutics is also a major hurdle in the present-day treatment of cancer [9, 10]. Brain cancer is also not an exception when accounting for these aspects. To counter such hurdles, the use of nano-therapy is being regarded as a major life savior approach [17, 18]. Apart from improving the bioavailability of chemotherapeutic agents, the use of nanoparticles is also beneficial in terms of the slow release of the required drug to sustain the available drug for the optimal therapeutic effects against brain cancer. This not only counters the side effects of brain cancer chemotherapeutics but also improves the efficacy of the treatment to counter the carcinogenic events linked with the onset and progression of brain cancer. A variety of nanotherapeutic approaches such as nano titanium wires [19] for local drug delivery, drug-loaded nanofiber disks [20], magnetic and PEGylated nanoparticles [21, 22] with enhanced brain

tissue penetration are being investigated and considered in the treatment of brain cancer.

4.2.2 Role of Nanoparticles in Head and Neck Cancer

The porphysomes have been shown to enable fluorescence and photoacoustic imaging of buccal and tongue carcinomas with complete removal of primary tumors and metastatic regional lymph nodes, while sparing the nearby critical structures/functions [23]. Gold is a promising radiosensitizer and gold nanoparticles have shown therapeutic applications along with photothermal therapy, intravascular drug/gene delivery, and ionizing radiation augmentation [24]. They are reported to have beneficial clinical applications toward head and neck cancers and have led to the initiation of two, phase 1 human trials investigating gold conjugated tumor necrosis factor (TNF) treatment of solid tumors and photothermal therapy of refractory head and neck cancer [25]. Passivated gold nanoparticles with polymers such as polyethylene glycol (PEG) are reported to accumulate in solid tumors [24]. The PEG coating buffers the complex from the immune responses and is vital for successful drug delivery. *In vivo* studies have confirmed that PEG-nanorods accumulate about one-third of the gold in the tumor supporting their potential applications in head and neck cancers [26]. An array of targeting ligands has already been explored to improve tumor uptakes such as folate, transferrin, arginine–glycine–aspartic acid peptide, antibodies, or antibody fragments to cell surface receptors, etc. In another report, a combination of radiotherapy and 24 h pre-treatment with antisense Epidermal growth factor receptor polymeric nanoparticle showed a synergistic antitumor effect on the squamous cell carcinomas of the head and neck (HNSCC) SCCVII cell line. In phase III randomized trial, Epidermal growth factor receptor inhibitors as radiosensitizers were shown to significantly increase survival in regionally localized advanced head and neck cancer [27]. In another study gold nanoparticles have been reported to enhance X-ray irradiation-induced apoptosis in head and neck squamous cell carcinoma *in vitro*. A combination of 4 Gy X-ray irradiation and 1.0 nm gold nanoparticles was shown to significantly reduce the number of cells by enhancing the cytotoxic effects on human head and neck cancer cells *in vitro*, through the induction of apoptosis [28].

4.2.3 Role of Nanoparticles in Breast Cancer

A range of nanomaterials is under investigation against breast cancer which includes liposomes, micelles, dendrimers, protein, microneedle, polymer-based conjugates, etc. Goldman *et al.*, 2017 demonstrated that 100 nm liposomes effectively target triple-negative murine breast cancer metastasis [29]. In a study, genetically engineered synthetic multivalent antibodies retargeted exosomes (SMART-Exo) genetically displaying both anti-human CD3 and anti-human HER2 antibodies, dually targeting T cell CD3 and breast cancer-associated HER2 receptors, exhibited highly potent and specific antitumor activity both *in vitro* and *in vivo* by redirecting and activating cytotoxic T cells toward attacking HER2-expressing breast cancer cells [30]. Liu *et al.*, 2020, demonstrated a combination of curcumin-loaded polymeric nanoparticles, and a nano-vaccine containing CpG and antigenic peptides injected into the 4T1 breast cancer model, efficiently triggered immunogenic cell death of cancer cells and activated dendritic cells (DC). This combination resulted in a significant improvement in tumor-specific $CD8^+$ T cell response causing inhibition of tumor growth [31]. In another study, injection of anti-PD-1 peptide-loaded gold nanoparticles along with subsequent irradiation at the tumor site have shown excellent antitumor effects in breast cancer [32]. In another report, *Cuminum cyminum* L. (cumin) seed extract, chemically synthesized silver nanoparticles (AgNPs) and biosynthesized silver nanoparticles (bio-AgNPs) from cumin seeds were shown to exhibit toxicity-free features and have profound antitumor effects on human breast adenocarcinoma cell line (MCF-7) and human breast adenocarcinoma metastatic cell line (AU565) [33]. More recently a new category of nanoparticles has shown beneficial effects on TNBC treatment in *in vitro* and *in vivo* studies and are soon expected to deliver promising results in clinical trials. These are the bio-inspired and smart nanoparticles; also known as smart drug delivery systems (SDDSs). SDDSs are stimuli-responsive nanocarriers that are developing as a great replacement for conventional drug delivery systems in TNBC treatment, due to their tumor site-specific distribution, controlled drug release, prolonged drug retention, and minimal off-target drug release in response to various physiological stimuli such as pH, hypoxia, oxidative stress, and enzyme expression [34].

4.2.4 Role of Nanoparticles in Gastric Cancer

Gastric cancer is one of the most diagnosed and aggressive cancers that remains the second most common cause of cancer-related mortalities word wide despite considerable advancements in diagnostic and prognostic approaches [35, 36]. The use of nanoparticles in treating most complex malignancies has been suggested in several studies including gastric cancer [9–11, 35, 37]. Nanoparticles govern a variety of pathological events while curbing tumorigenesis. These include apoptosis, autophagy, drug resistance, and cell cycle arrest among several others. Preliminary studies considering the use of nanoparticles are encouraging and the mounting evidence suggests that the use of nanoparticles can indeed open novel therapeutic avenues while curbing cancer development and progression including gastric cancer [35]. In view of this, a previous study demonstrated the curative effects of target-activated nanosizer comprising epigallocatechin-3-gallate-loaded fucose-chitosan/polyethylene glycol-chitosan/gelatin nanoparticles on gastric cancer cells. Curative mechanisms of the treatment were found to be dependent on reduction in gastric acidosis, induction of apoptosis, and reduction in vascular endothelial growth factor expression [36]. Moreover, an *in vivo* study with an orthotropic gastric cancer model by the same group of authors revealed a significant lowering in gastric and liver inflammatory responses. Similarly, another recent study with miR-200C nanoparticles demonstrated the decrement in PD-L1 expression in gastric cancer cells. Interestingly, a combination of miR-200C nanoparticles with radiotherapy revealed synergistic therapeutic effects against gastric cancer which was supposed to rely on the reversal of epithelial-mesenchymal transition and inhibition of immune escape events dependent on PD-L1 expression and abrogation of cancer-stem cells-associated characteristics as compared to naked mIR-200C [38]. Similarly, Zinc-Oxide nanoparticles (ZnO-NP) were found to restrain cell proliferation, cell migration, and invasion, and induce apoptosis in gastric cancer cells *in vitro*. Moreover, ZnO-NP also reduced the IC_{50} concentration of cisplatin while confining cell proliferation of cisplatin-resistant SGC7901 cells. However, the authors suggested further investigation in the context of gastric cancer treatment considering the use of ZnO-NPs [39]. Furthermore, Yu and

colleagues (2020) also demonstrated a significant enhancement of gastric cancer therapeutic potential of gold nanoparticle-delivered mIR-26a to gastric cancer cells as compared to mIR-26a alone. The enhancement in therapeutic potential due to the formation of gold nanoparticles was demonstrated by suppression of cellular growth and proliferation [40]. Therefore, the use of nanoparticles in the treatment of some of the most complex gastric cancers seems to have enormous therapeutic potential.

4.2.5 Role of Nanoparticles in Lung Cancer

Liu *et al.* in 2014 studied on effects of internalized gold nanoparticles with respect to cytotoxicity and invasion activity in lung cancer cells. This study concluded that the size of nanoparticles is an important concern for affecting cell proliferation, apoptosis, cell cycle, and cell invasion. The great cytotoxicity is caused when small particles are endocytosed into the cells, whereas large particles have no significant cytotoxicity. Second, in addition to particle size, cell type is also an important factor and third is small Au-NPs upregulate the expression of MMP9 [41]. Stocke *et al.* used the spray drying technique to compose D-mannitol and iron oxide magnetic NPs which leads to hyperthermia in lung cancer cells under alternating magnetic fields [42]. Tseng *et al.* used Pt–Fe-HAP (platinum-iron-hydroxyapatite) NPs having lesser side effects, acting as chemo-hyperthermia to treat lung cancer [43]. Ma *et al.* in 2015 worked on magnetic nanoparticle clusters (MNCs) and found that the rate of tumor inhibition was high in the NCI-H460 mouse xenograft model when treated with these NPS as there is an increased level of apoptosis in these cells [44].

4.2.6 Role of Nanoparticles in Pancreatic Cancer

Yanyan Huai *et al.* 2019 revealed that treatment of pancreatic cancer cells with AuNPs, which is pre-treated with gemcitabine, repressed colony-forming ability and migration of these cells. These NPs suppressed gemcitabine-induced stemness, EMT, and MAPK activation. So their results showed that these NPs could be used as a potent agent against pancreatic cancer cells [45]. Lei Wang *et al.* 2018 worked on the synthesis of gold nanoparticles from

leaf *Panax notoginseng* and its anticancer activity in pancreatic cancer PANC-1 cell lines. These NPs successfully induce cytotoxicity, producing reactive oxygen species (ROS), and causes apoptosis in PANC-1 cells by accenting intrinsic apoptotic gene expressions. The final result of this paper is that the synthesis of AuNPs from *Panax notoginseng* shows antimicrobial and anticancer effects [46]. Ristorcelli *et al.* in 2008 showed the antitumor role of exosome NPs reducing the proliferation of tumor cells. The counteraction of NPs against the PI3K/Akt pathway leads the cancerous cells toward apoptosis. This was the first study that depicted the role of tumor cells-derived NPs against tumor cells [47]. According to Li *et al.* in 2019, Zn-CuO NPs can decrease the tumor growth in pancreatic cancer cells when treated *in vitro* as well as *in vivo* by ROS-mediated pathway [48]. Many researchers used chitosan and its combination with various drugs as NPs to treat pancreatic cancer. One of the research groups showed the role of chitosan- gemcitabine in PC cells [49], other group used folate- chitosan- gemcitabine combination and found that these combinations not only decreased the side effects but also increased apoptosis and cell toxicity in cancerous cells [50]. Taniuchi *et al.* also showed that delivery of siRNA-FA-PEG-COL NPs in pancreatic cells can inhibit metastasis and invasiveness of pancreatic tumors [51]. Arya *et al.* in 2011 used HER2-Gem-CS-NPs for pancreatic cancerous cells and revealed that efficient delivery of this combination showed anti-proliferative activity and increased S-phase arrest which leads to cell death [52].

4.2.7 Role of Nanoparticles in Ovarian Cancer

Arvizo *et al.* in 2013 found that MICU1 increases the Ca^{2+} levels in malignant cells but the silencing of MICU1 reduces the expression of Bcl-2, enhances both the activity of caspase-3 and levels of cytochrome c which initiates apoptosis in ovarian cancer cells by mitochondrial pathway, further elevated by $^{+}$AuNPs [53]. Recently, Hanna and Saad in 2021 demonstrated that when there is elevated internalization of cellular folic acid-coated SnO2 NPs in SKOV3 cancer cells, it will induce cytotoxicity in these cells. These NPs induced cytotoxicity through ROS-mediated apoptosis via the mitochondrial pathway. Moreover, these NPs are safe for living systems. The authors suggested the use of these NPs as a potential

therapeutic target for ovarian cancer in humans [54]. Liu *et al.* in 2019 conducted a study on SKOV2 ovarian cancer cells which underwent photothermal effect of NPs Ag@ Fe_3O_4 at different concentrations. These cells were treated with different concentrations of 808 nm near-infrared laser rays. Cell counting kit-8 assay was used for measuring cell proliferation. The results were very promising and the cell proliferation was inhibited and cell morphology was also devastated in these cells which underwent photothermal effect only but did not depend on concentration–time manner. Hence this therapy can help in treating ovarian cancer [55]. Aboutalebi *et al.* in 2021 showed that lipid NP containing the essence of Artemisia extraction initiated the apoptotic pathway in the SKOV-3 cell line than the pure essence. This anti-apoptotic effect was determined by flow cytometry and MTT assay [56]. The authors revealed that magnetic liposomal paclitaxel NPs (PTX-PEG-ML) acted as a more potent therapeutic target and showed more cytotoxicity than PTX-PEG-L in A2780CP ovarian cancer cells [57]. The researchers found that inorganic SeNPs (selenium nanoparticles) showed significant cytotoxicity and inhibits ovarian cancer cell lines SKOV-3 and OVCAR-3 by elevating histone methylation [58]. A study conducted by Haber *et al.* in 2020 showed that large anionic NPs help in treating ovarian cancer cells by accumulating in tumor-associated macrophages when intraperitoneally administered in the mouse model. These NPs showed significant results when treated with human samples too [59]. The authors introduced albendazole and bovine serum albumin into NPs in both *in vivo* and *in vitro* models of ovarian cancer cells with ascites and found that the number and volume of ascites cells, as well as expression of VEGF and SPARC, were also decreased [60].

4.2.8 Role of Nanoparticles in Prostate Cancer

Autio *et al.* concluded that progression-free survival was better in patients with metastatic castration-resistant prostate cancer (mCRPC) in which they are treated with BIND-014 NPs containing docetaxel drug which is a well-known drug used for the treatment of various cancer [61]. Chandratre and Dash in 2015 conducted a study by using paclitaxel and cyclopamine, two anticancer drugs, in DU145 TXR, DU145, and Wi26 A4 cells coated inside solid lipid

poly(glycolic-lactic) acid (PLGA) as well as glyceryl monooleate (GMO)-chitosan NPs. The latter NPs showed considerable cytotoxicity than the former NPs [62]. The authors delivered GA/albumin NPs in DU-145 prostate cancer cells, after internalization, these NPs induced necrosis, impaired the Ca^{2+} homeostasis, and causes stress and bursting of lysosomes. These NPs also lead to light-induced cytotoxicity inside the cancerous cells by generating ROS [63]. The researchers injected direct magnetic NPs in humans in two, separate phase I studies. They employed magnetic NP thermotherapy with permanent seed brachytherapy and also magnetic NP thermotherapy alone. The possibility and good tolerability were found in both trials. Although the patients felt discomfort under a strong magnetic field and uneven distribution of heat inside the tumor cells yet this therapy can be evaluated in conjunction with radiation therapy in patients with prostate cancer [64]. Xiang *et al.* in 2013 administered dual-modified liposomes in 22Rv1 cells which enhances cellular uptake and downregulated the expression of polo-like kinases and initiates apoptosis in cancerous cells [65]. Gold NPs loaded with doxorubicin when conjugated with PSMA aptamer acted more potent against LNCaP cells than PC3 cells [66]. Wolfe *et al.* revealed that the antigens specific to the tumor cells cause NPs to internalize within the cells when these NPs conjugated to these antigens and lead to radiosensitization. These authors used goserelin-conjugated AuNRs to radiosensitize prostate cancer cells by using megavoltage radiation [67].

4.3 Conclusion and Future Perspectives

Nanotechnology has shown a theranostic role in delivering small molecules like microRNAs, DNA, siRNA, drugs, polymers, etc. which helps in detecting, diagnosing cancer, biomedical imaging, and drug delivery. With the help of this technology, small and large molecules to the targeted or localized manner. On the other hand, scientists also faced many challenges with toxicity, stability, and clinical development associated with nanoparticles. Improvement in technology will surely help in targeting multiple molecules of cancerous cells simultaneously and adopting appropriate therapeutic approaches. From the future point of view, nanotechnology will

make an immense revolution not only against cancer but also in medical fields like imaging and drug delivery.

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Chapter 5

Nanomaterials-Mediated Oxidative Stress in Cancer: Recent Trends and Future Perspectives

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5.1 Introduction

Cancer is emerging as the second leading cause of death worldwide after cardiovascular disease; it is defined as the disruption of the normal functioning of intracellular signaling mechanisms and the formation of uncontrolled growing cell populations due to genetic and environmental factors (Aggarwal *et al*., 2019; Dancey *et al*., 2012; Rumgay *et al*., 2021; Varol, 2020b; Wu *et al*., 2018b). Every

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year, tens of millions of people worldwide are diagnosed with cancer, and more than half of the patients die due to this phenomenon (Heron and Anderson, 2016; Ma and Yu, 2006). Published cancer statistics and the future perspectives show an increase in the incidence of cancer cases and death rates and indicate that in 2020, approximately 19.3 million new cancer cases were diagnosed worldwide and approximately 10 million people died due to cancer, and predict that in 2030, approximately 21.7 million people will be affected by cancer each year (Bray *et al*., 2018; Cabral *et al*., 2018; Sung *et al*., 2021; Vinay *et al*., 2015).

The malignant tissue that is formed as a result of carcinogenesis, which causes the cells to continuously increase their dividing capacity by exhibiting abnormal behavior, may damage the surrounding tissues and prevent vital organs from performing their normal functions, and thus the homeostatic balance of the organism is adversely affected and the patients are also suffered from the damaged surrounding tissues (Chen and Zhang, 2017; Lobo *et al*., 2007). When the carcinogenesis process is examined, it is seen that the proteins involved in intracellular signaling pathways undergo structural changes as a result of mutations and some epigenetic mechanisms, or they undergo changes in the form of a decrease or increase in their intracellular amounts (Mittal and Rajala, 2020; Noorolyai *et al*., 2019; Sarasin, 2003; You and Henneberg, 2018). On the other hand, targeting these intracellular signaling proteins regardless of the nature of the pathway in which they act is not considered a rational strategy to treat cancers due to the prolonged and complex multistage nature of carcinogenesis (Varol, 2020a). Conventional methods such as chemotherapy, surgical approaches, and radiotherapy are still widely used in cancer treatment; however, cases where such treatment approaches cause systemic toxic effects, blood clots, damage to nearby tissues, development of drug resistance, and evolution to more aggressive cancer types are frequently encountered (Demir *et al*., 2019; Greenwell and Rahman, 2015; Sultana *et al*., 2014; Varol, 2016a). Additionally, the conventional treatment modalities can cause different side effects depending on the type of cancer (Gegechkori *et al*., 2017). It is currently known that there are more than 200 cancer types, and it is seen that the tumor tissue formed as a result of carcinogenesis consists of cells with

different cytological and histological features, molecular profiles, and mutations and has a heterogeneous and dynamic structure that can lead to different clinical outcomes (Dagogo-Jack and Shaw, 2018; Kuijjer *et al*., 2018; Meacham and Morrison, 2013; Moses *et al*., 2018; Varol and Varol, 2020). Therefore, it is widely considered as a more rational strategy to focus on the hallmarks of cancer in researching drugs and strategies for cancer treatment (Arya and Bhansali, 2011; Varol, 2020a, b; Varol and Varol, 2020). Hanahan and Weinberg (2000, 2011) described the common mechanisms of carcinogenesis as maintenance of proliferation signals, avoidance of growth suppressors, resistance to apoptosis, the establishment of replicative immortality, induction and activation of angiogenesis, and acquisition of metastatic ability (Hanahan and Weinberg, 2000, 2011). Moreover, they reported that the irregularities in the cellular energetics and the escape from the immune system can be observed in the progression of carcinogenesis (Hanahan and Weinberg, 2011).

Designing and discovering new cancer treatment strategies and drug components to improve patients' quality of life and life expectancy by focusing on common mechanisms of carcinogenesis is of paramount importance, and substantial research budgets are spent by governments and research authorities around the world (Hainaut and Plymoth, 2013; Varol, 2016a). As a result of microenvironmental stress, malignant transformation of cells in carcinogenesis begins with hyperproliferation, in the ensuing process, cells become insensitive to growth suppressing factors (evasion), develop resistance to programmed cell death (apoptosis), exhibit invasive nature (invasiveness), produce angiogenic factors to induce the formation of new capillaries from existing ones (angiogenesis), and acquire metastatic ability to settle down in distant parts of the organism through the blood vessels (Hanahan and Weinberg, 2000, 2011). It is well known that all the hallmarks of cancer are tightly associated and regulated by the production of intracellular reactive oxygen species (ROS) (Varol, 2020b). Therefore, ROS generation–mediated strategies are considered as the convenient treatment modalities for a wide range of cancers, and currently designed many nanomaterials target pathways underpinning the common mechanisms of carcinogenesis thanks to their antioxidant or pro-oxidant features (Cairns *et al*., 2011; Varol, 2020b).

5.2 Molecular Mechanisms of Oxidative Stress in Carcinogenesis

The processes of carcinogenesis are characterized by excessive production of ROS both in the cancer cell microenvironment and in various cellular compartments, and this excessive accumulation of ROS affects multiple cellular signaling cascades and progressively impairs the genetic stability of cells, which cause the formation of more aggressive cancer cells (Galadari *et al*., 2017; Kumari *et al*., 2018; Storz, 2005). The balance between ROS and antioxidant factors, which enable healthy cells to cope with oxidative stress, cannot be achieved in cancer cells because endogenous and exogenous ROS generators cause excessive ROS production that cannot be tolerated by the antioxidant defense (Dickinson and Chang, 2011; Droge, 2002). Intracellular ROS can exist as non-reactive radical species with no unpaired electron or as radical species with at least one unpaired electron (Hecht *et al*., 2016). The radical species such as superoxide ($O_2^{\bullet-}$), peroxyl ($RO_2^{\bullet}$) and hydroxyl ($OH^{\bullet}$) are considered as highly electrophilic and the short-lived molecules that cause significant cytotoxicity by oxidizing nucleic acids, proteins, lipids, and the other key components of cells, and these cytotoxic radical species can be formed by the non-reactive radical species including hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3) and singlet oxygen ($^1O_2$) (Chio and Tuveson, 2017; Jones, 2008; Zhang and Huang, 2017). Exogenous ROS producers for cells are seen as inflammatory cytokines, chemotherapeutics, environmental toxins, ultraviolet rays, and ionizing radiation, while endogenous ROS sources are transition metal ions, NADPH oxidases, cytochrome P450, lipoxygenases, mitochondria, endoplasmic reticulum, and peroxisomes (Krumova and Cosa, 2016; Snezhkina *et al*., 2019). There is a general belief that the mitochondrion is the primary endogenous source of ROS. This is so because the amount of ROS produced by mitochondrion is detected to be much higher than normal during mitochondrial isolation procedures using outdated techniques, resulting in functional damage; however, with the application of new mitochondrial isolation procedures, it was determined that the amount of ROS originating from mitochondria was much less than the amount of ROS previously determined (Hecht *et al*., 2016; Nohl *et al*., 2003).

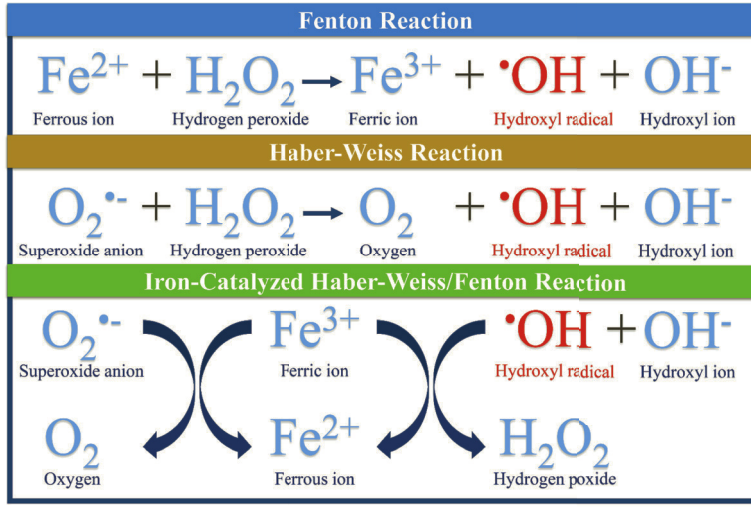

Figure 5.1 Fenton and Haber–Weiss reactions.

The intracellular ROS production can be accomplished through enzymatic and non-enzymatic reactions, and arachidonic acid, cyclooxygenase (COX), cytochrome P450 enzymes, lipoxygenase (LOX), NADPH oxidases (NOXs), uncoupled endothelial nitric oxide synthase (eNOS) and xanthine oxidase (XO) play substantial roles in the enzymatic ROS generation (Aggarwal *et al*., 2019; Gorrini *et al*., 2013; Sosa *et al*., 2013; Varol, 2020b; Zhang and Huang, 2017). On the other hand, the mitochondrial respiratory chain can cause non-enzymatic ROS generation during aerobic ATP production because of the electron leakage from the electron transport system (ETS) that cause the formation of superoxide ($O_2^{\bullet-}$) by reduction of approximately 1-2% of oxygen molecules, which are reduced to water molecules by cytochrome c oxidase in ETS (Brown *et al*., 2010; Murphy, 2009; Snezhkina *et al*., 2019). In the ensuing process, the formed superoxide molecules form hydrogen peroxides by spontaneously or the activity of the superoxide dismutase (SOD) enzyme, and the Fe^{2+} or Cu^{2+} ions-catalyzed Fenton reactions cause further conversion of hydrogen peroxide molecules to the hydroxyl radicals ($OH^{\bullet}$) (Fig. 5.1) (Handy and Loscalzo, 2012; Hecht *et al*., 2016; Kumari *et al*., 2018; Varol, 2020b). Moreover, the highly toxic hydroxyl radicals that are formed by the Fenton reactions can cause genomic instability and various cellular damages because of the

formation of oxidized nucleic acids, proteins, and lipids (Aggarwal *et al*., 2019; Chio and Tuveson, 2017; Dizdaroglu and Jaruga, 2012; Varol, 2020b).

5.3 Mechanism of Nanomaterials-Mediated ROS Generation

Nanotechnology has gained an important place in medicine due to the excellent properties of nanomaterials such as suitable pharmacological parameters, good biocompatibility, optimal physical and chemical properties and intrinsic targeting properties, and the rapid development in the use of nanotechnology in different treatment strategies over the last decade has had a major impact on medicine, which opened up a new field of medical application named nanomedicine (He *et al*., 2019; Huyan *et al*., 2020; Wu and Yang, 2017; Zamani Kouhpanji and Stadler, 2020). Nanotechnology contributes to the development of different nanomedicine strategies by enabling the production and processing of materials at the nanometer scale, enabling the development of new tools for the diagnosis, treatment, monitoring, and control of various diseases (Bansal *et al*., 2020; Lemmerman *et al*., 2020). The most widely used nanotechnology platforms in nanomedicine are nanoparticles defined by the American Society for Testing and Materials (ASTM) as having two or more dimensions on the nanometer scale (Limongi *et al*., 2019).

Nanoparticles have especially improved physical and chemical properties over their corresponding bulk materials, such as high surface-area-to-volume ratio and a unique quantum size effect due to certain electronic structures (Mauricio *et al*., 2018; Singh, 2016). Additionally, the physical and chemical properties of nanoparticles can be manipulated depending on their size and shape (Dienerowitz *et al*., 2008; Tian *et al*., 2019). To facilitate the cellular uptake of nanoparticles and to obtain monodisperse nanoparticles, it is necessary to manipulate their shape and size, thus minimizing aggregation (Behzadi *et al*., 2017; Chithrani and Chan, 2007). Thanks to these manipulative features, nanoparticles are frequently used as diagnostic, therapeutic and carrier agents in various biomedical applications (Blanco *et al*., 2015; Overchuk and Zheng, 2018; Wang

et al., 2012). Nanoparticles can be also employed to redound the improved stability and extended shelf life of drugs (Dang and Guan, 2020; Santamaría-Aguirre *et al*., 2018; Shahbazi and Shavisi, 2018).

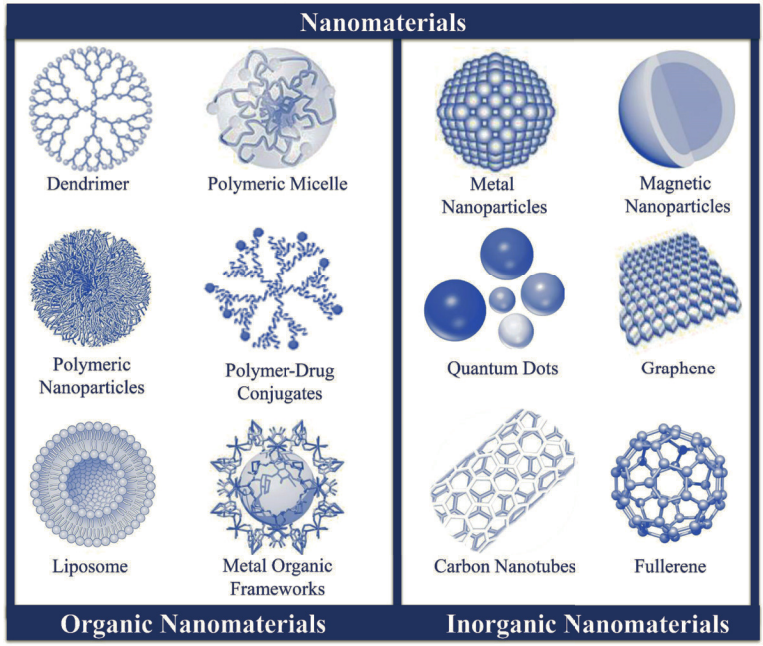

Figure 5.2 Organic and inorganic examples of nanomaterials.

Nanomaterials allow for a nanoscale control mechanism for the gradual delivery of drug particles and delivery tools to various parts of the body while maintaining the pharmacological properties (Ma *et al*., 2013; Vieira and Gamarra, 2016). During the last decades, tremendous progress has been made in nanomaterials such as micelles, liposomes, niosomes, carbon dots, carbon nanotubes, artificial polymers, and drug-polymer polysaccharide linkages (Fig. 5.2) (Crommelin *et al*., 2020; d'Amora and Giordani, 2018; Desbrieres *et al*., 2018; Peng *et al*., 2017; Tang *et al*., 2019; Wadhwa *et al*., 2019). Among nanomaterials with different types, chemical and physical properties, metal-based nanoparticles and carbon nanotubes have aroused considerable commercial interest due to their very important intrinsic properties such as electrical responsivity, conductivity, and high tensile strength, which can meet

the needs of specific applications of nanomedicine (Manke *et al*., 2013; Raval *et al*., 2018; Tangboriboon, 2019).

Despite the high commercial interest in nanomaterials, it is striking that there are not many studies on the negative effects of these materials. It is thought that this deficiency in the literature is due to the complexity and difficulties caused by the fact that nanomaterials have a large number of physicochemical parameters such as size, shape, structure, and basic components in the investigation of harmful effects such as toxic effects (Aillon *et al*., 2009; Gatoo *et al*., 2014; Manke *et al*., 2013; Podila and Brown, 2013; Poljak-Blaži *et al*., 2010).

Nanomaterial-induced toxicity is generally thought to arise from paradigms such as inflammation, oxidative stress, disruption of cell signaling pathways, genetic damage, suppression of cell division, and cell death (Ganguly *et al*., 2018; Naqvi *et al*., 2018; Yuan *et al*., 2019). Most of the data in the literature suggest that nanomaterials especially trigger the formation of intracellular ROS and as a result cause cell damage by creating oxidative stress (Mendoza and Brown, 2019; Peng *et al*., 2020). For example, it is known that cell signaling pathways that result in increased expression of pro-inflammatory and fibrotic cytokines are mediated by carbon nanotube-induced oxidative stress (Li *et al*., 2010). Moreover, it has been reported that inflammatory cells such as macrophages and neutrophils are activated by some nanoparticles, thereby increasing ROS production and oxidative stress (Kennedy *et al*., 2009; Lee *et al*., 2009; Manke *et al*., 2013). On the other hand, it should be noted that nanomaterials are considered to be very promising materials in antioxidant activity, especially in the scavenging of ROS, and they are employed both as antioxidant agents and in the transport of antioxidants to improve their activity (Akhtar *et al*., 2017; Cheng *et al*., 2018; Valgimigli *et al*., 2018).

Due to the pro-oxidant feature of nanomaterials, they target ROS-related metabolic processes by inducing intracellular ROS generation, triggering disruption of antioxidant mechanisms, and ultimately causing tumor cell death (Ruan *et al*., 2021; Yang *et al*., 2018a; Zhang *et al*., 2021). The ROS generation mechanisms of nanoparticles differ according to their physicochemical features, and the basic cellular mechanisms that affect ROS production are not yet fully understood for nanomaterials. However, nanomaterial-

mediated ROS generation can be caused by pro-oxidant functional groups on the reactive surface of nanoparticles and interactions of nanoparticles with cells, and it is well known that the reactive surface of nanoparticles plays an important role in the formation of oxidative stress (Knaapen *et al*., 2004; Qiu *et al*., 2016). For example, free radicals on the surface of nanoparticles such as $SiO^{\bullet}$ and $SiO_2^{\bullet}$ trigger the formation of ROS such as $OH^{\bullet}$ and $O_2^{\bullet-}$ inside the cells (Fubini and Hubbard, 2003; Lehman *et al*., 2016; Rubio *et al*., 2019). The surface of nanoparticles can absorb oxidative molecules such as ozone and nitrogen dioxide, and the nanoparticle size decreasing along with changes in electronic properties cause the structural defects that lead to reactive groups on the particle surface (Donaldson and Tran, 2002; Puckett *et al*., 2005). Furthermore, the defected nanoparticles react with molecular oxygen (O_2) to form $O_2^{\bullet-}$, which causes further ROS generation via the Fenton reaction (Fig. 5.1) (Oberdörster *et al*., 2005; Zhou *et al*., 2019). On the other hand, it is well known that ROS generation also increased with the dissolution of some nanoparticles and subsequent release of metal ions (Eixenberger *et al*., 2017; Song *et al*., 2010). Most of the metal-based nanoparticles display a toxic effect because of the formation of free radicals via Fenton-type reactions, whereas the mitochondrial damage is usually caused by ROS generation triggered by carbon nanotubes (Kim *et al*., 2017; Qian *et al*., 2019; Ranji-Burachaloo *et al*., 2018; Visalli *et al*., 2019). Fenton reactions typically occur as a result of the interaction of a transition metal ion with hydrogen peroxide forming a hydroxyl radical and the oxidized form of the metal ion (Canaparo *et al*., 2021). The formation of the hydroxyl radical, which is highly toxic to biomolecules, can occur as a result of the reduction of hydrogen peroxide in the presence of iron ions (Fe^{2+}) in the cells. Although it is well known that iron and copper-based nanoparticles cause oxidative stress through the Fenton reaction, oxidized metal ions can also react with hydrogen peroxide to form hydroxyl radicals in the Haber–Weiss reaction (Dayem *et al*., 2017). Nanoparticles with transition metals such as chromium, cobalt, silica, and vanadium can catalyze both Fenton and Haber–Weiss type reactions (Canaparo *et al*., 2021). On the other hand, intercellular free radical generating signaling pathways involving MAPK and NF-κB can be triggered by the nanoparticles containing some metals such as arsenic, beryllium, cobalt, and nickel (Ahamed *et al*., 2019; Ganguly *et al*.,

2018; Smith *et al*., 2001; Wu and Kong, 2020). Mitochondria that are considered an important source of endogenous ROS generation, also play an important role in the formation of nanoparticle-mediated oxidative stress (Pathak *et al*., 2015; Sharma *et al*., 2012; Yu *et al*., 2020). Smaller particle size nanomaterials are known to generate higher ROS, and the nanoparticles that have the ability to easily access the mitochondria, can trigger the excessive ROS formation by causing structural damage and depolarization of the mitochondrial membrane, disrupting the electron transport chain, and activating NADPH-like enzymes (Huerta-García *et al*., 2014; Manke *et al*., 2013; Sioutas *et al*., 2005; Tee *et al*., 2016; Xia *et al*., 2018).

Apart from the direct intracellular ROS production of nanomaterials, they can be activated to produce ROS by employing different methods for cancer treatment such as sonodynamic therapy (SDT), photodynamic therapy (PDT), and chemodynamic therapy (CDT) (Chen *et al*., 2020; Li *et al*., 2021a; Liang *et al*., 2020; Um *et al*., 2021; Yan *et al*., 2020; Yang *et al*., 2019; Zhong *et al*., 2020). Nanomaterial-mediated chemodynamic cancer therapy is accomplished by the Fenton reaction mostly using cellular hydrogen peroxide molecules to convert them into ROS and hydroxyl radicals that are lethal to cells, and CDT offers many advantages such as being highly selective, tumor-specific, less systemic side effects, and no need for external stimulation (Li *et al*., 2021a). Photodynamic therapy (PDT) is applied by generating singlet oxygen and ROS in target cells based on synergistic interactions of a non-toxic photosensitizer; low-energy, non-thermal visible light; and tissue oxygen, and nanomaterials are employed in PDT as the photosensitizer (Chen *et al*., 2020; Varol, 2019). SDT and PDT applications are very similar; however, SDT is applied with acoustic waves that penetrate deeply into the tissue and a sound-sensitive molecule named "sonosensitizer" that can be triggered to generate ROS by these waves (Liang *et al*., 2020; Varol, 2016b). Regardless of the ROS generation mechanism and the applied treatment modality, nanomaterials are evaluated as substantial toxic agents, especially for cancerous cells and tissues thanks to their ROS production abilities, and are used to damage and destroy biomolecules and organelles that are vital for the cancerous cells.

5.4 Metal-Based Nanoparticles-Mediated ROS Generation

Metal-based nanomaterials have been extensively investigated in terms of biomedical applications and therapeutic aspects since these nanomaterials tend to modify with numerous functional groups according to the interested target. Apart from tailoring the characteristics in nanodimensions by using a myriad of moieties ranging from basic chemical functional groups to complex biological molecules, these nanostructures can be also synthesized at a variable shape and size including nanoparticles, nanowires, nanorods, nanosheets, and nanocages exhibiting shape and size-dependent features (Chen *et al*., 2021; Gurunathan *et al*., 2018; Mauricio *et al*., 2018; Mody *et al*., 2010). The majority of the studies in the literature covers the synthesis of metallic nanoparticles including gold, platinum, and silver nanoparticles and metal oxide nanoparticles such as iron oxide, copper oxide, cerium oxide, and so on owing to their relatively simple synthetic procedures and superior electronic, magnetic and optical properties along with their higher biocompatibility and lower toxicity (Andreescu *et al*., 2012; Patra *et al*., 2010; Porcel *et al*., 2010).

Among the metallic nanoparticles, gold nanoparticles (Au NPs) have become particularly significant due to the conjugation ability with biological molecules containing thiol, mercaptan, and amine functionalities for the preparation of highly effective novel conjugates (Alivisatos *et al*., 1996; Calavia *et al*., 2018; He *et al*., 2021). Although they are not classified as redox-active agents, it is reported that Au NPs can induce the related interactions in biological systems leading to biological redox responses (Sims *et al*., 2017). Furthermore, Au NPs' effect on ROS generation and oxidative stress-mediated DNA damage has been increasingly studied in recent years, even though their utilization as photosensitizers in PDT and PTT still requires intense examination because of the insufficient therapeutic effect (He *et al*., 2021; Yang *et al*., 2018b). As an elucidatory example, Au NPs were conjugated with biotin, and the light-induced ROS generation ability of the presented photoactive conjugate was examined to improve the photothermal therapy efficacy for brain cancer cells (He *et al*., 2021).

Similarly, apart from the well-known antimicrobial activity of silver nanoparticles (AgNPs), it is reported that AgNPs can induce ROS production and oxidative stress leading to DNA damage and apoptosis (Ahamed *et al*., 2010a). Besides, AgNPs are promising candidates as cytotoxic agents for cancer treatment owing to their remarkable antitumor potential (Sriram *et al*., 2010). Thus, the researchers have examined these properties *in vitro* for breast and lung cancer and cervical carcinoma (Chugh *et al*., 2018; Foldbjerg *et al*., 2011; Gurunathan *et al*., 2013; Vasanth *et al*., 2014).

Inspired by the conventional use of cisplatin in cancer therapy, researchers studied the use of platinum nanoparticles (PtNPs) as an anticancer agent and it was concluded that PtNPs can reduce oxygen to hydrogen peroxide, whereas converting hydrogen peroxide to water and oxygen. The mentioned property of PtNPs can be adapted to mimic SOD and catalase enzymes for cancer treatment (Kajita *et al*., 2007). As innovative research in this field, Hao and coworkers (2020) synthesized PtNPs loaded ROS-responsive prodrug to utilize as a chemophotodynamic therapeutic agent for colon cancer (Hao *et al*., 2020). This ROS-responsive prodrug was synthesized through a thioketal bonding which was linked with camptothecin and a photosensitizer, namely, 2-(1-hexyloxyethyl)-2-devinyl pyropheophorbide-a. It was concluded that the produced PtNPs containing prodrug showed a catalytic effect on the hydrogen peroxide decomposition to yield oxygen which enhanced the photosensitizer consumption under 660 nm laser irradiation leading to ROS generation improvement. These promising results paved the way in the field of combined therapeutic strategies for future developments in colon cancer treatment (Hao *et al*., 2020).

Metal oxide nanoparticles and metal oxide–containing nanostructures in biomedical applications have comprised a fascinating research area owing to their unique redox and optical properties, enhanced chemical stability, biocompatibility, and antioxidant activities as well as comparably reduced production costs (Kwon *et al*., 2018; Mauricio *et al*., 2018; Nikolova and Chavali, 2020; Pandey *et al*., 2016).

Iron oxide nanoparticles (IONPs) and iron-containing nanomaterials have been the most widely examined nanomaterials for ROS generation due to their biocompatible nature in recent years. However, IONPs have come into prominence because of the

superparamagnetic features and ROS generating capabilities as a result of Fenton and Haber–Weiss reactions. It should be also noted that the magnetic or superparamagnetic effect of IONPs provides to improve targeted cancer treatment strategies in case of an external magnetic field is applied which leads to heat generation or direction of related particles to the specific tissues (Guardia *et al*., 2012; Singh *et al*., 2010). As the products of Fenton and Haber–Weiss reactions, iron ions yield extremely reactive hydroxyl and hydroperoxy radicals which induce oxidative stress leading to lysosomal or mitochondria malfunctions and DNA damage. It has been also reported that IONPs exhibit an enzyme-like activity under acidic conditions by catalyzing the oxidation reactions to produce hydroxyl radicals from H_2O_2 which can be utilized in cancer therapy (Ghosh *et al*., 2020; Li *et al*., 2021b; Mansur *et al*., 2020; Wu *et al*., 2014). Yu and coworkers (2019) examined *in vitro* and *in vivo* interactions between IONPs and H_2O_2 to elevate ROS therapeutic efficacy (Yu *et al*., 2019). For this purpose, a core-shell iron carbide nanostructure denoted as $Fe_5C_2@Fe_3O_4$ was synthesized and tested for utilization in cancer therapy. The researchers confirmed that $Fe_5C_2@Fe_3O_4$ core-shell nanostructure enabled an effective discharge of Fe^{2+} ions in acidic media for H_2O_2 production and promoted ROS generation with improved safety (Yu *et al*., 2019). In another study conducted by Ma and coworkers (2019), Pd nanosheets and Fe_3O_4 nanoparticles were combined to synthesize Janus nanoparticles (Fe_3O_4-PdJNPs) to enhance ROS generation for breast cancer treatment (Ma *et al*., 2019b). It was reported that the obtained nanoparticles showed a synergistic effect on ROS generation due to the catalytic and hyperthermia effects of Fe_3O_4-PdJNPs. On the other hand, minimal side effects were observed and tumor growth in orthotropic 4T1 tumor-bearing mice was completely inhibited in case of Fe_3O_4-PdJNPs were administered (Ma *et al*., 2019b).

Despite the incontrovertible role of IONPs in terms of ROS generation based on Fenton and Haber–Weiss reactions, copper oxide nanoparticles have a great potential thanks to their ability of Cu-based Fenton reactions in which hydrogen peroxide is converted into hydroxyl radicals in a wider pH range. Furthermore, kinetic studies demonstrated that the reduction rate of Cu^{2+} ions is remarkably higher than Fe^{3+} ions in the presence of H_2O_2, while the hydroxyl radical production rate of Cu^{+} ions is approximately

100-fold higher than Fe^{2+} ions. Therefore, there is increasing attention to copper oxide nanoparticles and copper-including nanomaterials to develop novel oxidative cancer treatment agents (Hu *et al*., 2019; Li *et al*., 2021a). In a comprehensive study by Xiong *et al*. (2020), dose and time-dependent inhibitory effects of cuprous oxide nanoparticles were examined for different bladder cancer cell lines and the results indicated that these nanoparticles induced cell cycle arrest and apoptosis through ROS generation activated ERK signaling pathway and autophagy (Xiong *et al*., 2020). ROS-dependent activities of copper oxide nanoparticles were also examined for human lung epithelial cells, A549 cell line, and HEp-2 cell line by other researchers (Ahamed *et al*., 2010b; Fahmy and Cormier, 2009; Karlsson *et al*., 2009).

ROS-induced inhibitory effect on tumor cell growth and antioxidant properties have made cerium oxide nanoparticles (CeO_2NPs) and cerium-based nanomaterials significant for the therapeutic aspects of cancer (Datta *et al*., 2020; Li *et al*., 2021b; Mauricio *et al*., 2018). Additionally, the co-existence of Ce^{3+} and Ce^{4+} ions in cerium-based nanostructures enables not only anti-oxidative activities but also pro-oxidative ones through a pH-dependent phenomenon providing a large-scale utilization of these nanomaterials (Sims *et al*., 2017; Walkey *et al*., 2015). In a recent study by Datta *et al*. (2020), CeO_2NPs were examined as the pro-oxidant agent for colorectal carcinoma cell line and the obtained results showed that the enhancement of ROS generation by CeO_2NPs led to DNA fragmentation and consequently to cellular apoptosis via p53-related mitochondrial signaling pathway (Datta *et al*., 2020). As reported by Yao *et al*. (2018), the enzyme-like activity of upconversion mesoporous CeO_2NPs in neutral and acidic microenvironments provided the decomposition of endogenous H_2O_2 in a tumor and ultimately enabled PDT to cause cellular apoptosis (Yao *et al*., 2018).

It is worth giving special attention to metal-organic frameworks (MOFs), which are classified as a brand new type of highly organized porous materials since they provide flexible pore size and composition with enhanced biodegradability. Depending on the types of metal centers and the ligands that form the framework, morphological and structural properties of MOFs can be tuned while tailoring also their functions (Liu *et al*., 2020; Wu and Yang, 2017; Zhang *et al*., 2019). Recent studies about iron-based MOFs

revealed that they can be utilized as peroxidase mimicking agents to produce hydroxyl radicals in cancer cells (Tang *et al*., 2019). Apart from the enzyme mimicking nature, based on the principle of Fenton reactions catalyzed by ferrous ions, iron-based MOFs were successfully employed for tumor-targeted CDT. MOFs can be also utilized as the PDT agents by combining metal centers with photosensitizer molecules such as porphyrins and phthalocyanines to elevate the efficacy of the cancer treatment strategy. Outstanding examples of iron, copper, cobalt, titanium, and manganese centered MOF-based cancer treatment strategies have been reported more recently in the literature (Falsafi *et al*., 2021; Han *et al*., 2021; Liang *et al*., 2021; Tian *et al*., 2021; Yin *et al*., 2021).

5.5 Carbon-Based Nanomaterials-Mediated ROS Generation

Since metal-based nanomaterials have been predominantly employed as ROS generative agents in cancer treatment strategies, carbon-based nanomaterials have been utilized as well, individually or in combination with other carbon-based or metal-based nanomaterials. Unique properties of carbon-based nanomaterials such as larger surface area, the capability of surface modification, and superior biocompatibility have drawn the attention of researchers in the last years (Gurunathan *et al*., 2018; Krasteva *et al*., 2019; Kumawat *et al*., 2019; Mariadoss *et al*., 2020; Tabish *et al*., 2018). Carbon nanotubes (CNT), graphene and its derivatives, fullerene, carbon nanoclusters, and graphene quantum dots are widely used carbon-based nanomaterials in biomedical applications, however, a literature survey reveals the extensive utilization of CNT, graphene and its derivatives and their roles in ROS generation induced cancer treatment.

Since the discovery by Iijima in 1991, CNTs have been used in many applications ranging from industry to medicine thanks to their excellent chemical and physical properties based on sp^2-hybridized carbons in their rolled hexagonal graphene-like structures (Gurunathan *et al*., 2018; Iijima, 1991). CNTs can be formed as single-walled, double-walled, or multi-walled layers, and depending on the number of concentric rolls, chemical and physical properties may

vary. Furthermore, tailoring the physicochemical properties of CNTs including size, surface reactivity, charge, and the presence of metals or functional groups directly influences their pro-oxidant effects to be utilized for cancer treatment (Lam *et al*., 2004; Manke *et al*., 2013). CNT-mediated ROS generation results from mitochondrial damage, unlike metal-based nanomaterials in which Fenton reactions play an important role in ROS generation (He *et al*., 2011). Wang *et al*. (2020) reported a targeted photothermal-delivery nanoplatform based on the polyethylene glycol decorated chitosan nanoparticles and single-walled carbon nanotubes (Wang *et al*., 2020). It was observed that the pH-sensitive surface of the nanoplatform enabled the mitochondrial targeting with facilitated tumor cell uptake and ultimately induced ROS generation as a result of mitochondrial damage. Apart from their utilization in PDT, CNTs have displayed a prominent role in SDT. Behzadpour *et al*. (2020) prepared polypyrrole-coated multi-walled carbon nanotubes for the absorption of ultrasound irradiation to be examined as sonosensitizer (Behzadpour *et al*., 2020). *In vitro* investigations on the melanoma tumor model and C540 (B16/F10) cell line confirmed that improved thermal activity and ROS generation caused detrimental SDT effects.

Graphene was discovered and isolated through the exfoliation of graphite in 2004. Owing to its two-dimensional structure composed of sp^2-hybridized carbon atoms containing monolayer sheets, graphene exhibits enhanced electronic properties, higher thermal conductivity, and mechanical strength with a direct interaction ability with biomolecules (Du *et al*., 2008; Novoselov *et al*., 2004; Pop *et al*., 2012; Sims *et al*., 2017). In terms of theranostic applications, graphene and its derivatives such as graphene oxide (GO), reduced GO (rGO), graphene foam and graphene quantum dots displayed selective tumor uptake and improved ROS production with minimized side effects posing an enormous potential for cancer treatment (Cho and Choi, 2012; Hu *et al*., 2015; Tabish *et al*., 2018). Kumawat *et al*. (2019) presented the synthesis of a hybrid nanomaterial consisting of two-dimensional GO and zero-dimensional graphene quantum dots (GQDs) which was formed via polyethylene imine bridge (GO-PEI-GQDs) to assess its photothermal activity and oxidative stress response for MDA-MB-231 breast cancer cells (Kumawat *et al*., 2019). It was reported that the combination of different nanomaterials in the single nanostructure showed a synergistic

effect on photothermal and cytotoxic activities along with stable fluorescence imaging even at lower doses. In a recent study by Ma *et al*. (2021), negatively charged bovine serum albumin modified manganese (IV) oxide nanoparticles (MnO_2NPs) were attached to PEI modified rGO nanosheets to form rGO@MnO_2 nanocomposite to be tested as CDT and PDT combined therapeutic agent (Ma *et al*., 2021). The authors concluded that owing to MnO_2NPs based Fenton reactions, intracellular glutathione was oxidized, while Mn^{2+} ions converted H_2O_2 to hydroxyl radicals leading to intracellular ROS level increment. In addition to the effect of MnO_2NPs based Fenton reactions, temperature increment via the rGO photothermal effect increased the hydroxyl radical formation rate by Fenton reactions and improved CDT efficiency to kill HeLa cells (Ma *et al*., 2021).

5.6 Nanovehicles in ROS-Mediated Cancer Therapy

Instead of acting directly on the target cancer cells, the nano-sized drug delivery systems target tumor regions spatiotemporally and transport drugs that will show activity into the cells, and nanomaterials with different properties have been developed for this purpose (Fig. 5.3) (Elmowafy *et al*., 2019; Zeinali *et al*., 2020). Since nanocarriers interact with drugs physically and chemically, they effectively deliver these drugs to the cells in the target tumor tissues by passing through the vascular system without the effects of increased permeability, retention, extravasation, and passive targeting (Del Burgo *et al*., 2014; Kaasgaard and Andresen, 2010; Shin *et al*., 2021). Moreover, the development of innovative nanovehicles that ensure the delivery of anticancer drugs to target cells, maximizing their activities, and minimizing side effects has been possible thanks to the superior physicochemical properties of nanomaterials (Mughees *et al*., 2021; Naqvi *et al*., 2020; Yuan *et al*., 2020). During the last decades, targeted drug delivery using nanovehicles along with intracellular ROS production has been shown to improve the efficacy of chemotherapeutic agents that are frequently used as amplification agents of oxidative stress to generate ROS such as cisplatin, camptothecin, docetaxel, paclitaxel, cinnamaldehyde, β-lapachone, *etc*., and thus preferential killing of

targeted cancer cells has been shown to be an enhanceable feature for amplification agents of oxidative stress (Feng *et al*., 2020; Lu *et al*., 2021; Noh *et al*., 2015; Qiao *et al*., 2018). On the other hand, it is possible to encounter problems arising from nanocarriers during the transport of drugs to target cells, some of these problems can be observed in the form of nanocarriers that remain trapped in endosomes, do not degrade inside the cell and trap the drug, and do not release the drug effectively (Chou *et al*., 2011; Shin *et al*., 2021). Therefore, the design of nanocarriers that provide controlled release of their therapeutic load in response to a specific stimulus emerges as a more rational strategy to ensure precise delivery of drugs and improve the antitumor activity (Wang *et al*., 2013).

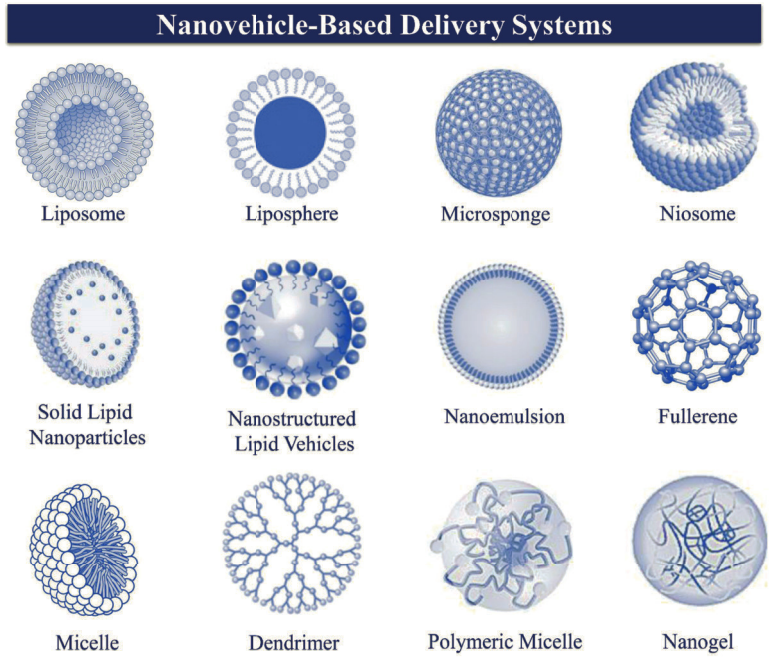

Figure 5.3 Different types of nanovehicles.

The specific stimulus can be classified as endogenous (pH, enzymes, redox reactions, *etc*.) and exogenous stimuli (temperature, light, ultrasound, *etc*.), and thanks to the operation of endogenous and exogenous stimuli, it is possible for nanocarriers to release their therapeutic load into the target cells in a controlled manner, and

selective and highly efficient drug therapy can be applied (Karimi *et al*., 2016; Karimi *et al*., 2015; Ma *et al*., 2019a; Qin *et al*., 2015; Zhang *et al*., 2017). It is well known that PDT, SDT, and CDT strategies in ROS-based cancer treatments use external stimuli such as light, ultrasound, and chemicals, respectively. These cancer treatment modalities are also used in the controlled release of drug loads to target cells by nanocarriers, and among the external stimulation methods, the use of light, which provides a non-invasive application, easy intensity adjustment, and excellent temporal and spatial control, is emerging as the preferred strategy (Anderski *et al*., 2019; Bechet *et al*., 2008; Calixto *et al*., 2016).

Nanocarriers capable of absorbing light, an electromagnetic wave type, use photon energy to trigger changes in chemical bonds, polarity, and chemical groups and induce the formation of heat and ROS (Croissant *et al*., 2018; Wu *et al*., 2018a). pH-sensitive nanocarriers take advantage of the fact that tumor tissues often have a lower pH than healthy tissues (Thews and Riemann, 2019). Tissue perfusion and removal of metabolic wastes are insufficient because tumor tissues consist of cells that proliferate uncontrollably, and so the pH in tumor tissue is around 6.8, which is more acidic than in healthy tissues (Shin *et al*., 2021; Thews and Riemann, 2019). Additionally, intracellular endosomes and lysosomes have a highly acidic pH level, and pH-sensitive nanocarriers can release the loaded drugs with the help of degradation of chemical bonds and the changes in chemical structure and hydrophilicity through protonation and deprotonation by acid-catalyzed cleavage, thus preventing the nanocarriers from being trapped in endosomes (Bae *et al*., 2012; Iversen *et al*., 2011; Sim *et al*., 2017). Consequently, nanovehicles are considered as a rational strategy to improve the efficiency and activity in cancer treatment approaches by advancing pharmacological and pharmacokinetic features of the loaded drugs.

5.7 Concluding Remarks and Future Prospects

Although the therapeutic strategies developed for the use of nanotechnology in cancer treatment have provided encouraging results in preclinical and clinical trials, it seems that there are some handicaps in the successful introduction of ROS-based

nanomaterials into clinical applications. The ongoing concern about the potential adverse effects of nanomaterials on human health and safety remains the common thread of nanomedicine applications. Therefore, characterization, evaluation, and explanation of the molecular mechanisms underlying the short and long-term toxicological effects of nanomaterials have great importance in nanomedicine applications. Commercial drugs are of course not completely free of toxicity and potential side effects, but designing safe, precisely targeted, functionally optimized, and non-invasive nanomaterials is of great importance for both ethics and human health. It may not be possible to design the biological reactivity of nanomaterials with ROS-mediated activity; however, obtaining more complete information on how nanomaterials induce off-target effects on the immune, nervous and other systems should be considered as the most logical approach. Predictive toxicology approaches may be more useful than descriptive toxicology methods in determining off-target effects on organ systems and healthy cells. Nanomaterials can generate oxidative stress due to their physicochemical properties, including chemical composition, particle size, surface reactivity, surface charge, and presence of transition metals, and as a result of interactions with cellular mechanisms such as immune cell activation cascades, mitochondrial respiration pathways, and the NADPH oxidase system. Thus, a comprehensive characterization of physicochemical properties of nanomaterials should be performed to design and produce safer nanomaterials. Redox imbalance created by nanomaterials may cause undesirable pathophysiological phenomena such as inflammation, genotoxicity, fibrosis, and carcinogenesis. Therefore, it is of great importance to understand the cellular and molecular mechanisms of nanomaterials-mediated oxidative stress and to develop strategies to reduce the off-target side effects of nanomaterials. Photo-sensitive, sono-sensitive, and pH-sensitive smart nanocarriers seem to be promising in the transport and deposition of anticancer drugs into tumor cells, thereby increasing the effectiveness of anticancer drugs and minimizing systemic side effects. Recently, nanocarriers that can respond to two or more stimuli have been developed and it is aimed to develop a more effective cancer treatment strategy by using these multifunctional nanocarriers. Intravenous administration of nanocarriers may result in the lack of desired activity due to the

untargeted uptake and elimination from the body before effective accumulation at target sites. Therefore, it may be useful to focus on alternative delivery systems and potential new strategies such as subcutaneous injection, oral administration, intratracheal administration. Consequently, nanomaterial-mediated ROS generation seems to have a special place in nanotechnology-based cancer treatments and other nanomedicine applications. Questions about where and how nanomaterials cause the production of ROS and the mechanisms of enhancing oxidative stress seem to have preoccupied the scientific community for a long time. However, ROS-mediated apoptosis induction of target cells by rational transport and deposition of oxidative stress amplification agents with the multifunctional nanovehicles seems to be quite promising as an important treatment strategy.

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Chapter 6

Role of Nanotherapeutics in Inhibiting Cancer Angiogenesis: A Novel Perspective

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6.1 Introduction

Cancer is a broad-spectrum disease that leads to indiscriminate cell proliferation and their dislocation. It has a very high mortality rate compared to many other common diseases like diabetes and cardiac malfunctions. The proliferation of cancer cells can be slowed down

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through different means such as chemotherapy, radiation therapy, immune and hormone therapy. While these techniques have helped improve survivability in patients and target the cancer cells to stop them from proliferating, they also can affect the functioning of normal body cells leading to serious side effects. Drug targeting has today become the biggest challenge in cancer therapy [1], including various barriers to drug delivery such as enzyme activity, cell membrane barrier, and immune response. Moreover, due to the rapid and indiscriminate proliferation of cancer cells, they undergo mutations at higher rates and could become resistant to the drugs [2] such as patients that showed initial partial recovery through chemotherapies could relapse into various side effects. Therefore, new ways of delivering drugs to specific cell types have become imperative. Tumor targeting strategies have been classified into (i) angiogenesis-associated targeting via vascular endothelial growth factor receptors [3], αvβ3 integrins [4], matrix metalloproteinase receptors, and vascular cell adhesion molecule-1 and (ii) targeting uncontrolled cell proliferation markers via human endothelial receptors, transferrin receptors, and folate receptors [5].

Owing to the serious effect that drugs can have on normal cells, especially in the case of drugs that directly target cellular signaling, a novel approach was used to instead inhibit cancer cell proliferation [6]. These tumor cells can create new blood vessels around them from existing capillaries and thus create a source of oxygen and nutrition for their proliferation, called angiogenesis [7]. This process also allows the cancer cells to go into the bloodstream and metastasize to move to different organs and create malignant tumors. Drug resistance is a major challenge in the field of angiogenic therapies. Very limited therapies against the antiangiogenesis mechanism have been notified through genetic variability of cancer cells during targeting the protumorigenic pathway [8]. Antiangiogenic therapy also fails in cases where tumor cells grow close to existing vessels, thus not requiring extension or creation of new vasculature and hence avoiding angiogenesis [9]. Hence, it is crucial to understand the failure of antiangiogenic drugs and how such flaws can be fixed.

There are also challenges in targeting specific growth factors such as VEGF as they can conflict with the activity of other angiogenic factors like FGF-2 which may be inversely up-regulated and create

resistance against the anti-VEGF treatment [10, 11]. This has led to drugs that inhibit both VEGF and FGF at the same time such as VF-trap fusion protein which inhibited both these growth factors and suppressed tumor growth [12].

Antiangiogenesis methods target areas around the newly proliferating tumor cells to prevent the formation of these blood vessels and inhibit the growth and movement of the cancer cells. While it has less severe side effects, there is still a need to improve drug targeting to increase the effectiveness of the antiangiogenesis process. Figure 6.1 shows the inhibition of angiogenesis by drug delivery using NPs (NPs). NP delivery systems and nanotherapies may be used for targeted delivery and diagnostics toward cancer cells.

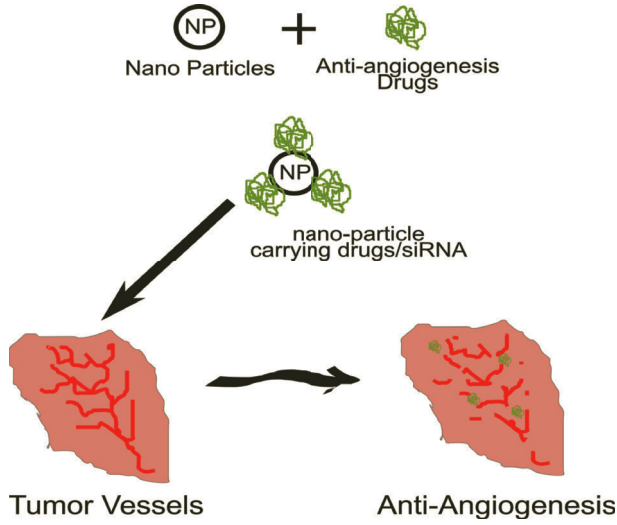

Figure 6.1 Role of NPs in drug delivery against cancer angiogenesis.

Progress in the synthesis of NPs can also overcome multi-drug resistance (MDR) [13] as small-sized NPs can avoid anti-drug targets and reach tumors owing to their better permeability [14]. In this chapter, we discuss the role of angiogenesis in cancer, their prevention through traditional drug therapies, and the novel effective NP-based methods for angiogenesis prevention in cancer.

6.2 Angiogenesis: A Critical Hallmark in Cancer

All cells whether a tumor or non-malignant normal cells need oxygen and nutrients to survive. These resources are provided to these cells through blood vessels—especially capillaries that form the exchange region of nutrients from the blood to the cells. These capillaries are formed by endothelial cells and smooth muscle cells. The formation of new blood vessels occurs in the healthy body through angiogenesis. It involves many stages driven by pro-angiogenic factors that activate the endothelial cells to divide while breaking down the capillary walls to make new vascular branches [15]. One of the main steps in this process is the migration of endothelial cells into the extracellular matrix (ECM). In a healthy body, angiogenesis plays an important role during embryo growth. It can get triggered in adults due to pathological disorders that could potentiate the factors and cell death where the disease could lead to the start of tissue healing processes even without any injuries or stimuli.

The angiogenesis signaling involves various molecules and elements that regulate the growth of vessels, breakdown of the cellular matrix, *etc*. These constitute growth factors such as VEGFs, FGFs, and matrix metalloproteinases (MMPs) [16]. They also involve receptors on cell surfaces that combine with the growth factors to activate the process of angiogenesis such as IGF with IGF receptor to increase angiogenesis [17]. There are two ways the drugs target angiogenesis: either by targeting these signaling pathways to stop the cancer cells from stimulating endothelial cells for and initiating the process of angiogenesis or by targeting the endothelial cells near the tumor cells directly to stop them from creating new vasculature. Many inhibitors have been found as possible drug therapy options to reduce cancer cell proliferation and metastasis by directly inhibiting the growth factors, but these need to be injected in the right dosage to keep the homeostasis. Many clinical trials have been conducted to test their efficacy in cancer treatment.

Angiogenesis inhibition is considered a viable and effective option for cancer treatment [7]. The most targeted processes in angiogenesis include the growth factor receptors, such as VEGFR,

PDGFR-b, and angiotensins (ANGs). Table 6.1 shows the drugs currently used in antiangiogenic therapies. Bevacizumab is a monoclonal antibody that inhibits the activity of VEGF. It has been found to delay the progression of renal cell carcinoma. Sunitinib, pazopanib, sorafenib, and vandetanib block tyrosine kinase (TK) actions and indirectly inhibit VEGFR and PDGF and have been found effective in renal, thyroid, and hepatocellular cancers. Everolimus is found to inhibit the mammalian target of rapamycin (mTOR), the multifunctional signal-transducing proteins, and ultimately reduce tumor growth.

Table 6.1 Antiangiogenic drugs against various cancers

Drugs	Target Factors	Treatment
Bevacizumab	VEGF-A	Advanced stage lung, breast, and renal cancer [18]
Sunitinib	Small molecule tyrosine kinase	Advanced pancreatic cancer [19]
Pazopanib	Small molecule tyrosine kinase	Renal cell cancer [20]
Sorafenib	Small molecule tyrosine kinase	Renal and hepatocellular carcinoma [21]
Axitinib	Multikinases	Lung, thyroid, breast, pancreatic cancers [22]
Cediranib	Broad-spectrum agent—inhibits multiple targets	Non-small cell lung cancer and rectal cancer [23]
Vatalanib	Small molecule tyrosine kinase	Tumors of the central nervous system, brain tumors, and colorectal cancer [24]
Brivanib	VEGFR, FGFR	Tumors of colorectal and hepatocellular carcinoma [25]
Vandetanib	All VEGF and TK-receptors	Thyroid cancer [26]
Regorafenib	Multikinases	Colorectal cancer [27]
Ranibizumab	VEGFR	Preclinical trials for macular degeneration [28]

These drugs however can become ineffective due to the high number of mutations found in cancer cells. Some of these alterations can be mediated by the use of combinations of different therapies but these can still lead to side effects. Tyrosine kinase inhibitors (TKI) are also antiangiogenic molecules that block ATP sites in pro-angiogenic receptors in tumor cell environments, further inhibiting tyrosine kinase receptors and stopping further signaling pathways. TKI is found to also inhibit pathways other than VEGFRs such as PDGFR and tyrosine kinase with immunoglobulin-like and EGF-like domain (TIE-2). Tumor sites can also have adverse environments such as extreme pH levels and lack of oxygen. These can also lead to poor drug effectiveness and allow for faster tumor progression. Aflibercept and bevacizumab in combination with cytotoxic agents showed the highest activity.

6.3 Antiangiogenic Nanotherapy

Nanotechnology has been developed significantly over the last decade as a stable commercially available medical technique. The biggest hurdle in targeting angiogenesis in tumor cells using the above-mentioned drugs is their inability to effectively reach the binding sites of the growth factor receptors. It is well known that the endothelial cell networks in cancer cells differ considerably from the normal vasculature. Thus, delivery based on these differences could allow for improved efficacy of the drugs and further reduce any side effects. NPs can be potential agents that can combine with these antibodies to bind with the corresponding receptors. Low molecular weight heparin and ursolic acid have been successfully used to inhibit MMPs [29, 30]. These particles show antiangiogenic and anticoagulant activity. These showed significantly better stability and distribution properties.

NP-coated polyethylene glycol (PEG) has been shown to circumvent abnormal blood barrier resistance and allow for longer circulation time [31]. HER2 (human epidermal growth factor receptor 2) inhibitor allowed intracellular delivery in breast cancer sites [32]. Taxane with NPs has also been able to surmount poor perfusion rates in tumor sites [33]. Some NP-based approaches can allow

overcoming adverse tumor microenvironments. Doxorubicin when combined with hypoxia-sensitive micelles, allows it to be delivered successfully in hypoxic tumor sites [34]. Similarly, polyHIS-PEG and gelatin NPs have been found to allow drug delivery in varying pH values [35, 36].

Antiangiogenic therapy can also improve through the use of NPs by the application of new methods that are not possible through traditional mechanisms. These may involve controlled delivery of drug dosage at the cancer cell site over time, which may not be possible through oral or intravenous administration. Several cases have already been in use—nanopolymeric Lodamin was shown to provide longer circulation time, controlled drug release, and much narrow targeting at tumor sites. Cyclosporin A [37], Mesoporous silica NPs [38], and Doxorubicin in NPs [39] have been synthesized to avoid drug resistance. The NP-based drug delivery mechanisms have successfully shown improved cellular uptake of drugs, reduced side effects, and efficient delivery to targeted cells.

Additionally, bevacizumab has also been found to have higher survivability in patients with colorectal cancer when used in combination with 5-fluorouracil (5-FU) and irinotecan-based drugs as compared to being used on its own. It was also better in advanced colorectal cancer as a secondary treatment strategy when used with oxaliplatin-containing therapy [40]. DOX and mitomycin C (MMC) in conjunction with polymer–lipid hybrid NPs have been used in drug-resistant human breast tumors as they show better targeting and improved survivability. Curcumin, a natural antiangiogenic molecule also had improved performance when coloaded with DOX into poly-(beta-amino ester) copolymer NPs [41].

VEGF has been marked as one of the most influential targets for antiangiogenic effects. Numerous NP-based therapies, therefore, target and bind to the VEGF receptor, inhibiting its activity and reducing the formation of new blood vessels. The NP-based antiangiogenesis approaches can be categorized into their material types—metal, non-metallic, and metallic oxide and polymer-based nanomaterials, including the others such as tetrac, peptide, and carbon-based nanomaterials. Figure 6.2 indicates the role of different NPs against angiogenesis.

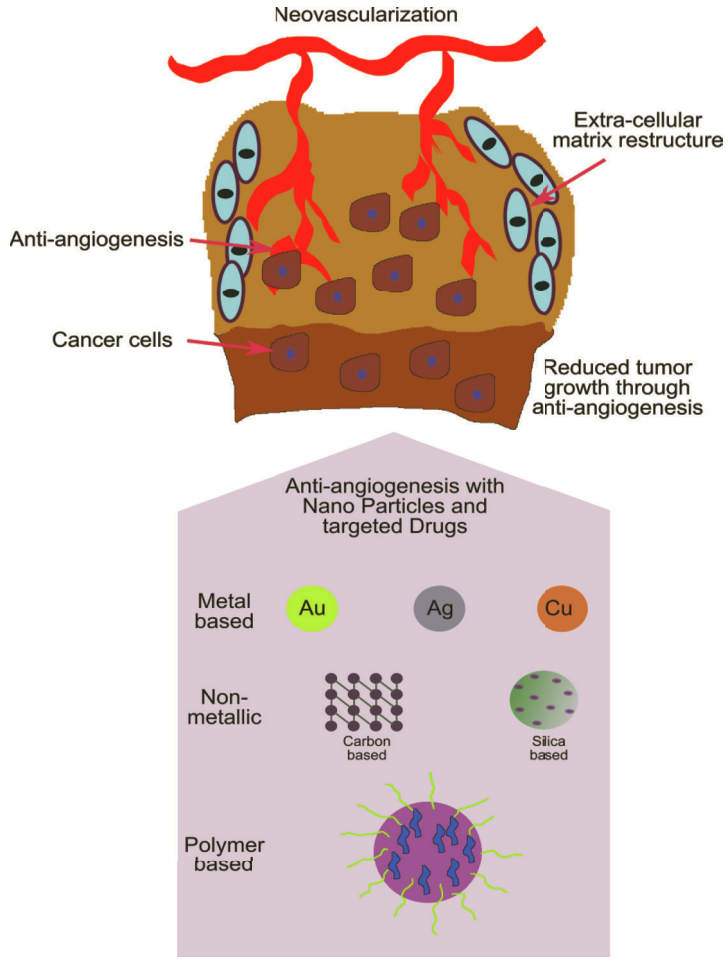

Figure 6.2 Schematic representation of different ways to target the tumor microenvironment (TME) through various NPs, which target the ECM and cancer cells, showing antiangiogenesis.

6.3.1 Metal and Metallic Oxide NPs

Many metals show natural binding toward various drugs, while selective attraction toward biomolecules. Heavy metals such as gold, silver, and copper have been found to target VEGF and are therefore used in antiangiogenic therapies. Table 6.2 indicates the use of different NPs against angiogenesis and cancer.

Table 6.2 Different major NPs against angiogenesis

Nanotherapy type	NPs used	Mechanism
Metal-based NPs	Gold NPs	Inhibits VEGFR2, Akt phosphorylation [42]
Metallic oxide NPs	Copper Oxide	Inhibits HUVEC proliferation, migration, and cell cycle [43]
	Cerium Oxide	Inhibits VEGF165 based proliferation of VEGFR2 [44]
Non-metallic NPs	Carbon-based NPs	Accumulated in cancer cell microenvironment [45]
	Silicon-based NPs	Antiangiogenesis in the retinal vasculature and ovarian cancers [46]
Lipid-based NPs	Nanopolymeric micelles	Inhibit tumor progression and angiogenesis. Can accumulate only in tumors.
	Nanoliposomes	Can target somatostatin receptors. Increase the antiangiogenic ability of PTX [47]
Polymeric nanotherapeutic drugs	PLGA PEG-PLA	Decreased metastasis by inhibiting necrosis factors [48]
	Chitosan	Found to inhibit angiogenesis in breast cancer [49]
	Aptamer-based poly NPs	Significant vascular regeneration in ischemic tissues [50]

6.3.1.1 Gold NPs

Gold NPs (Au NPs) were found to have the antiangiogenic ability in human umbilical vein endothelial cells (HUVECs). Au NPs are negatively-charged molecules, bound to the positively charged heparin site of the VEGF165 and inhibit the cell surface kinase receptor. Au NP also binds with FGF receptors and is found to inhibit VEGFR2 and threonine kinase phosphorylation [42]. Pan *et al*. examined the impact of Au NPs on the interaction of VEGF and VEGFR2, the VEGF165-induced VEGFR2, and Akt phosphorylation. The authors also revealed the anticancer activity of Au NPs in xenograft as well as ascites models [39].

6.3.1.2 Silver NPs

Silver NPs (Ag NPs) show both chemical and biological antiangiogenic properties. Ag NPs have also been found to inhibit VEGF-induced proliferation of endothelial cells. Ag NPs were found to block the PI3K/Akt-dependent pathways [51]. Recently, several research groups demonstrated the antiangiogenic therapeutic ability of biologically and chemically synthesized AgNPs. Baharara *et al.* designed AgNPs using *Salva officinalis* extracts [52]. Their analysis shows the antiangiogenic effects of NPs. A recent study has also shown that AgNPs also show antibacterial activity and act as efficient drug delivery transport [51]. The previous study also stated that the inhibition of VEGF- and IL-1 beta (IL-1β)-induced vascular absorptivity by an Src-dependent pathway in porcine retinal endothelial cells (PRECs). AgNPs inhibit the VEGF- and IL-1β-induced Src phosphorylation at Y419 [52]. Authors revealed that AgNPs prevent VEGF stimulated cell proliferation, and survival in bovine retinal endothelial cells (BRECs) by regulating PI3K/Akt pathway [53]. The inhibitory effect of AgNPs was demonstrated by the progression of apoptosis and augmentation in caspase-3 activity, which lead to show high antiangiogenic activities.

6.3.1.3 Copper NPs

Copper oxides have been known to be an essential vehicle for drug delivery. Cuprous oxide and copper NPs (Cu NPs) have also been found to show antiangiogenic properties in HUVEC proliferation and migration [54]. The antiangiogenic effects of Cu NPs have also been found to suppress VEGFR2 expression at protein and mRNA levels. However, since copper is known to be a vital element in vessel growth, certain dosage levels of Cu NPs have also been shown to generate new blood vessels. By various assays, such as Matrigel plug assay, prevention of *in vivo* blood vessel formation observed by CD31 staining, supporting the role of Cu NPs in antiangiogenesis. Furthermore, the antiangiogenesis activity of Cu NPs was accompanied by the inhibition of VEGFR2 expression at both the protein and mRNA levels in a dose and time-dependent manner. In a recent study, Zhang *et al*. demonstrated that αvβ3 conjugated soft copper oleate NPs (αvβ3-Cu NPs) were efficiently delivered as a potent antiangiogenic pro-drug, fumagillin [55].

6.3.2 Non-metallic NPs

Non-metallic NPs include carbon and silicon-based organic agents. Silica-based NPs can be used as either antiangiogenic themselves or deliver antiangiogenic drugs to tumor sites. In retinal models, silica-based NPs inhibited VEGFR-2 phosphorylation. They have also been developed recently to deliver small interfering RNA (siRNA) in ovarian tumors in mice [56] using a magnetic mesoporous silica core with a polyethylenimine (PEI) cap. The system was found to have highly efficient siRNA delivery and inhibition of angiogenesis without any toxic side effects. The magnetic core also allows it to be used as a biomarker in cancer diagnosis. These nanotherapy show reduction in VEGF expressions in immune-histological analysis. A recently developed silica NP (NAMI-A@MSN-RGD) using NAMI-A (a well-known antiangiogenic agent) has been used as an antiangiogenic agent in HUVEC models [57]. The conjugate uses ROS triggered apoptosis that leads to 'Sub-G1'-phase arrest in HUVEC. Silica NPs have also been found to initiate autophagy in endothelial cells and suppress angiogenesis [58].

Similarly, carbon-based nanomaterials have strong antiangiogenic characteristics for the treatment of cancer. These include graphene, carbon nanofibers, nanodiamonds, carbon nanotubes, nanodots, and fullerenes. Studies have observed antiangiogenic activities of carbon NPs in a chorioallantoic membrane (CAM) model [59] where these significantly suppressed tumor area, volume, and weight by inhibition of VEGF and b-FGF2-induced angiogenesis. Diamond NPs also inhibit VEGF-R to suppress the proliferation and migration of endothelial cells [60]. These materials have been found to provide antiangiogenic effects even in hypoxic microenvironments. A broad-spectrum study on different carbon nanomaterials found that while many of these materials had antiangiogenic properties, fullerene had pro-angiogenic characteristics, and graphene had no significant effect on angiogenesis.

6.3.3 Polymer-Based NPs

Polymer-based drug delivery approaches have been used in various diseases for many years. Owing to their biocompatible and biodegradable properties the polymers such as PEG and polylactide

(PLA) can be used as transport for drugs. Specifically, in the case of cancer, antiangiogenic drugs like paclitaxel (PTX) have been used in conjunction with extra domain B (EDB) to modify PEG-PLA NPs to target cancer cells. Such modified PTX delivery mechanisms have been found to be significantly effective compared to the non-targeted approach. TNP-470 is another angiogenesis inhibitor that has been shown to significantly inhibit metastasis in HUVECs by encapsulating them in polymer-based NPs modified with specific (APRPG) peptides [61]. These have successfully suppressed tumor proliferation in mice models of ovarian cancer [61]. Carbon as the second most abundant element in the human body attracted a lot of attention in nanomedicine.

Chitosan and cellulose are naturally occurring polymers that are potent candidates for NP coupling for targeted delivery. They are biocompatible having low immune response and toxicity. Early studies in carcinoma xenografts have provided evidence of their antiangiogenic properties of chitosan [62]. It has been found to block the VEGF receptors and suppress the endothelial cell proliferation pathways. Like non-metallic NPs, the chitosan-like polymers can be used to encapsulate and deliver anti-RhoA siRNA to cancer cell microenvironments and have been found to effectively reduce tumor growth in breast cancer mice models [63]. An interesting study by Kohane *et al.* showed successful use of a polymer NP-based approach to allow binding of VEGF165a isoform but not to VEGF165b, which is known to be antiangiogenic. This allows for a strongly targeted inhibition of VEGF-controlled signaling.

6.3.4 Tertac NPs

Tetrac is derived from L-thyroxine and inhibits the antiangiogenic activity of different cell surface growth factors involving the thyroid hormone. Tetrac NPs were found to suppress tumor growth in renal cell carcinoma xenografts [64]. Specific doses of these NPs showed strong anti-tumor efficacy and were found to inhibit tumor angiogenesis in the chick CAM model. Tetrac, also a blocking agent of L-thyroxine and integrin αvβ3, shows excellent anti-tumor efficacy in tumor xenograft. Their study revealed the antiangiogenic and anticancer activities of Tetrac and Tetrac NP for the prevention of cancer.

6.3.5 Peptide NPs

Various peptides conjugated with metal NPs have been observed to improve the antiangiogenesis effects of these NPs. Peptide-coated gold NPs (Au NP) have been shown to inhibit angiogenesis in *in vitro* models. Similarly, silver NPs (Ag NP) in conjugation with P3-peptide lead to an increase in ROS concentrations and inhibit angiogenesis by blocking blood vessel formation. Some researchers [65] used PEG-PLA NPs with APTEDB peptides to demonstrate antiangiogenic properties.

The oligoethylene glycol capped gold nanospheres were incubated with a peptide that selectively interacts with receptors of cells, leading to the inhibition of angiogenesis without causing toxicity. The antiangiogenic activity was investigated by various assays.

6.3.6 Carbon-Based Nanomaterials

Carbon-based nanomaterials are the most auspicious materials including nanofibers, carbon nanotubes, nanodiamonds, fullerenes, graphene, and nanofibers. They have various properties such as high mechanical strength and large surface area, and hence show numerous sites for physical as well as chemical conjugation. Single-wall carbon nanotubes (SWCNTs) could be important vehicles of antiangiogenic agents. Also, Masotti *et al.* reported that polyamidoamine dendrimer (PAMAM)-coated carbon nanotubes (CNTs) and polyethyleneimine (PEI) were the proper delivery systems for microRNAs (miR-503 oligonucleotides) for regulation of angiogenesis [66]. More recently, Su *et al*. designed a dual-targeted delivery system based on iRGD-modified MWCNTs for usage in antiangiogenic therapy [67]. Different carbon-based NPs along with their allotropes showing intense antiangiogenic activities, as indicated in several assays. Grodzik *et al*. revealed the antiangiogenic properties of ultra-dispersed detonation diamond (UDD) NPs against a glioblastoma multiforme (GBM) tumor model developed on a CAM model [68]. Among those NPs, diamond NPs and multi-walled nanotubes showed the highest antiangiogenic properties by reducing the expression of VEGF. However, fullerene exhibited pro-angiogenic activity. Also, graphite NPs had no effect on the regulation of angiogenesis.

6.4 Nanotechnology and Gene Therapy in Cancer

A new and rather interesting approach in antiangiogenesis and cancer treatment in general is the combination of gene therapy and nanotechnology. The idea behind combining these two techniques is to be able to deliver specific genes—that can alter the cellular behavior of tumor cells—using NPs. A common technique for gene delivery today is through the use of virus vectors. However, the viruses can have toxic side effects whereas NPs in general are much safer than viruses [69, 70]. A recent study used NPs to deliver a mutant Rag gene to tumor sites leading to inhibition of bFGF and VEGF pathways [71]. NPs, called CPPC [72], have also been used to encapsulate with PEDF blocking genes and effectively suppress C26 tumor growth. Small interfering RNA (siRNA) can be used to knockout genes responsible for tumor growth. Silica-based NPs have been used to encapsulate siRNAs and deliver in adverse tumor microenvironments [73].

6.5 Current Approved Nanotherapies for Cancer Treatment

Several approved drugs using nanotherapy have been approved by FDA for cancer treatment.

Doxil [74]: Doxil was the first-ever NP-based drug approved by FDA in 1995. It has been used in the suppression of metastasis in ovarian cancer and breast cancer. Doxil has also been used in several solid tumors such as ovarian cancer.

DaunoXome [75]: This drug has been approved by FDA since 1996 for Kaposi's sarcoma.

Abraxane [76]: Approved by FDA in 2005, it has been successfully used in breast cancer. Recent studies have found it to be potential in the treatment of pancreatic cancer when used in combination with gemcitabine in xenograft mouse models.

Myocet [77]: Used in Europe since 2000 to prevent metastasis in breast cancer.

Depocyt [78]: Used in the treatment of Lymphomatous Meningitis.

Genexol [79]: Approved in South Korea for treatment of breast cancer. It has also been trialed as a potential treatment for pancreatic cancer by FDA.

Oncaspar (PEG-L-asparaginase) [80]: FDA-approved treatment of lymphoblastic leukemia.

Endoderm (Iron oxide–based NPs) [81]: Used as a carrier for detection of lesions associated with metastasis in liver cancer and various benign tumors.

6.6 Conclusion and Future Perspectives

Nanomaterials were comprehensively used in various medicinal applications (targeted drug/siRNA/shRNA delivery, immunoassays, biosensorics, *etc*. due to their functional properties. However, they also can exhibit long-term toxicity in the human body. This necessitates the systematic study of NPs and substances during animal model studies to understand various facets of their interaction with the body—such as their effect on immune cells, pharmacokinetic effects on normal cells, their metabolic results, toxicity, and efficacy. During clinical trials, the NPs need to be studied for their biocompatibility, biodegradability, dosage, and ways of administration and type of drugs they can best be conjugated with. Safe and effective NPs can largely improve the success rates of already FDA-approved antiangiogenic drugs. The advancement in chemical and molecular techniques in recent decades has given way to the development of stable NPs that can be tailored for specific requirements. Such advancements have allowed the NPs to bind with antiangiogenic drugs to be delivered to tumor sites and inhibit the neovascularization initiators. The NPs can be designed with different characteristics based on the parameters of the tumor microenvironment. The size, shape, and chemical and biomolecular binding properties need to be considered during the development of NPs. Big NPs may not be able to get to the target sites if they get filtered out by the dense cellular matrix of the vessels at the tumor site. Similarly, the shape of the conjugates plays an important role in their distribution within the cellular matrix. While the ideal NP

design could allow their use in broader cases such designs are yet under preclinical developmental stages. At present, the design of NP-based approaches is limited to very specific use case scenarios that target tumor environments of well-defined properties such as pH and oxygen levels, vascular density, and cellular matrix structures. Newer drugs and their NP counterparts also need to be designed to target not only VEGF receptors but other receptors such as FGFRs, PDGFRs, and other factors to allow for more effective suppression of angiogenesis.

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Chapter 7

Inhibition of Cancer Cell Metastasis by Nanotherapeutics: Current Achievements and Future Trends

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7.1 Introduction

Cancer is one of the world's most dreaded diseases, causing nearly 10 million deaths by 2020 [1]. The early stage of cancer is treated with various therapeutic strategies and metastasis is prevented by such therapies as surgery, radiation therapy, chemotherapy, immunotherapy, radiofrequency ablation, hormone therapy, and cryo-ablation. But, in later stages, tumor metastatic spread occurs.

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Tumor metastasis results from cancer cells losing their attachment to the primary tumor site and traveling to distant locations. It has been reported that approximately 90% of cancer-related deaths result from tumor metastasis [2]. Consequently, there is an urgent need to prevent malignant cells from acquiring a metastasizing quality. Tumor metastasis is the result of a series of events in which cancer cells become resistant to programmed cell death (anoikis), invade the extracellular matrix and enter circulation through blood vessels and the lymphatic system, and eventually disperse to distant organs and tissues. Upon extravasation, cancer cells utilize self-induced angiogenesis to build massive metastatic masses then use proliferative signaling molecules to sustain their growth [3–6]. Even though researchers have gained valuable insight into tumor metastasis mechanisms, some challenges still exist that must be overcome to develop effective treatment strategies for cancer metastasis prevention and cure. The first challenge would be the identification and detection of metastasis-related biomarkers that aid in identifying the stages of the tumor, preferred sites of metastasis, and the chances of tumor recurrence. For example, alpha-fetoprotein (AFP) is used to diagnose hepatocellular carcinoma [7], carbohydrate antigen (CA19-9) for pancreatic cancer [8], a combination of carcinoembryonic antigen (CEA), neuron-specific enolase (NSE), and carbohydrate antigen (CA-125) used for small cell carcinoma [9], cytokeratin-19 (CYFRA-21-1) + CEA+ NSE+ CA-125 used for detection of squamous cell carcinoma [9]. Additionally, estrogen receptors, progesterone receptors, and human epidermal growth factor receptors have all been identified as biomarkers for breast cancer detection [10].

Anti-metastatic therapeutics commonly use those compounds that recognize the biomarkers involved in different signaling pathways of cancer cell metastasis, including tumor proliferation and survival, tumor invasion, tumor extravasation, angiogenesis, immune checkpoint, and tumor growth. For example, cancer cell invasion targeting matrix-metalloprotease inhibitors, angiogenesis inhibitors, and immune-checkpoint inhibitors against programmed cell death (PD-L1) and cytotoxic T lymphocyte antigen (CTLA 4), *etc*. [11].

The second challenge is antigenic heterogeneity on the surface of metastatic cancer cells that creates a major roadblock for the effective

inhibition of cancer cell metastasis. For example, the disappearance of three surface antigens (*i.e*., CD9, CD29, and CD49c) and β-integrin when the breast cancer cell undergoes epithelial to mesenchymal cell transition (EMT) [12, 13]. The third challenge is that most metastasized tumors are resistant to chemotherapy. As an example, Blagoev *et al*. reported that clinical samples of metastatic prostate cancer treated with different combinations of prednisone, docetaxel, mitoxantrone, and abiraterone showed strong drug resistance [14]. Fourth, it will be necessary to effectively kill the tumor cells without damaging normal cells. It can be concluded from these findings that conventional cancer therapy does not eliminate metastasis and relapse effectively. Although chemotherapy, photodynamic therapy (PDT), gene therapy, and photothermal therapy (PTT) have been developed to overcome tumor metastasis problems they have some limitations such as less cancer cell killing efficiency of chemotherapy and PDT due to low oxygen environment in the tumor, high non-specific targeting in gene therapy, and restricted light penetrating ability in PTT.

Nanotechnology provides a new avenue for preventing tumor metastasis and recurrence by overcoming all the challenges discussed above. Nanotechnologies facilitate drug transport across biological barriers, target tumor cells specifically, and provide long-term drug release approaches. Specifically, the use of nanomaterials as nanocarriers for gene delivery in gene therapy and drug delivery to treat tumor metastasis by improving the solubility and stability of antimetastatic drugs prevent the drug from enzymatic degradation and premature clearance, sustains drug release, increases the half-life to prolong their circulation time in blood, improves the absorption, distribution, metabolism, excretion, transportation (ADMET) profile of the drug, helps in the delivery of multiple drugs at tumor sites. Nanomaterials-based nanomedicine could potentially improve host immune responses to fight cancer, and functionalized nanoparticles have been used to disrupt epithelial–mesenchymal transitions, reducing tumor metastatic potential and invasion. Thereby, nanoparticle-based nanomedicines provide a ray of hope by modernizing drug delivery systems and thus improving the treatment of tumor spread and relapse, which is difficult to do with molecular or chemical medications.

Here in this chapter, the significance of various nanoparticles is described that facilitate the inhibition of invasion and tumor metastasis. Furthermore, different nanomedicine-based strategies and therapeutic approaches for inhibiting the growth and dissemination of metastatic cancer are discussed, along with a stance toward future clinical trials with different functional nanoparticles for prevention of cancer metastasis and relapse to improve the treatment of metastatic tumors.

7.2 Impact of Nanocarriers Physicochemical Properties in Tumor Inhibition

7.2.1 Nanoparticles Size and Morphology

Nanoparticles may affect absorption, metabolism, excretion, transport, biodistribution, and bioavailability in specific organs depending on their size. These aspects directly affect the therapeutic efficacy of nanoparticles [15]. The kidneys tend to remove nanoparticles with sizes smaller than 10 nm, while particles larger than 200 nm, which are larger than the splenic fenestrations, are removed faster by the kidneys and liver [16]. However, nanoparticles, which are typically 10 nm to 100 nm in size, remain in blood circulation for longer and can reach lymph nodes by way of lymphatic vessels [16]. With the use of labeled fluorescent dyes conjugated to nanoparticles, it is easy to quantify and trace these nanoparticles as they are transported to the target sites [17].

Furthermore, a previous study reported the biodistribution studies of drug-silica nanoconjugates with different sizes, including 20 nm, 50 nm, and 200 nm out of which 20 nm-sized nanoparticles are much better than their counterparts because they experienced better tumor internalization, lower systemic clearance, and the highest accumulation at target sites [18]. As the morphology of nanoparticles influences the effectiveness of nanoparticles, they have a crucial role to play in controlling the therapeutic efficacy. As of today, nanoparticles can take different morphologies, such as rods, stars, cuboids, triangles, plates, and spheres [3]. The longer blood circulation times, the greater margination effects, and the stronger penetration of non-spherical particles into solid

tissues and malignancies are thought to be benefits of non-spherical particles [15].

7.2.2 Nanoparticle Surface Charge

In addition to surface charge, another vital factor affecting nanoparticle uptake by cancerous cells is the charge on the nanoparticle's surface. The surface charge is important both for cancer cell uptake and host immunity. Nanoparticles with neutral or negatively charged charges are less likely to be absorbed by local dendritic cells than those with positively charged charges. In a recent study, it was found that nanoparticles with a negative charge elicit a weaker immune response compared to their positively charged counterparts [19, 20]. Despite this, negative-charged nanoparticles demonstrate greater permeability to the tissue since their positively charged counterparts are trapped by the extracellular matrix that has a negative charge [21]. Considering the observations we discussed above, positively charged nanoparticles are likely to be useful as nanocarriers, but they may result in platelet aggregation and hemolysis, which inconvenience lymphatic trafficking in vessels [22], resulting in a later release of antigen.

7.2.3 Nanoparticle Surface Chemistry

An important factor affecting the absorption and dispersion of nanomaterials is their surface chemistry. To fabricate nanomaterials for biological and medical applications, surface chemistry needs to be designed. The ability to target nanoparticles at tumor sites has been improved by using a variety of biological ligands. Specific receptors located in tumor tissues are often bound to these surface ligands. By modifying nanoparticle surfaces with biological ligands, therapeutically active nanoparticles are better able to penetrate tumor cells and increase treatment efficacy [23]. In many studies, polymer-based nanomaterials have been shown to have an important impact on biological interactions because of their surface chemistry. For example, nanoparticles coated with drug were more effective at preventing lung tumors in mice than free drugs which led to an increase in survival and a reduction of detrimental effects [23, 24]. To investigate cellular absorption, PLGA [poly-(lactic-co-glycolic acid)]

nanoparticles were coated with polyvinyl alcohol (PVA) or vitamin E TPGS (tocopherol polyethylene glycol succinate). In contrast to PVA-coated PLGA and bare PLGA nanoparticles, Vitamin E TPGS-coated PLGA nanoparticles had a significantly higher capacity to internalize cells [24]. Due to the considerable differences between the surface coatings, chemical modification of nanomaterials could be one of the most efficient strategies for controlling and limiting nanomaterial cellular interactions, and hence the biological effects of nanomaterials. Carbon nanotubes have also been extensively studied with regard to surface chemistry and their use in cancer therapy as the vehicle of choice for small interfering RNA (siRNA), paclitaxel, and doxorubicin (DOX) delivery [25–28]. Through the surface chemistry of nanostructures, therapeutic benefits can be augmented by reducing side effects. In contrast, surface functionalization requires further research before it is applied in the clinic. The above observations suggest that ligand surface conjugation may alter the nanoparticle's fate; therefore, it is essential to carefully investigate nanomaterials after surface decoration to avoid undesirable toxic effects and fully assess any enhanced specificity and sensitivity that may be induced by surface modification.

7.3 Nanomedicine-Based Strategies for Inhibition of Tumor Metastasis

There is a general understanding that anticancer medications are significantly less effective once they reach their target, resulting in therapy being futile and potentially having unintended consequences [38]. It is only possible to achieve successful anticancer drug therapy when every dose is provided at the right time and the drug is designed especially to target tumor cells. Therefore, nanomedicine designed to target tumor cells should have the capability to accumulate drugs within and around tumor cells, limiting the chances of toxicity to healthy cells. The ideal nanomedicine should have a prolonged half-life in the bloodstream, excessive drug accumulation at the target site, an improved pharmacokinetic profile, capability of crossing the blood-brain barrier and tumor stroma, and increased biocompatibility [39]. The efficient distribution of nanomedicines to target tissues has been studied in detail as discussed below as well

as different nanomaterials-based treatment strategies are listed to inhibit tumor metastasis (Table 7.1).

Table 7.1 List of different strategies that have been reported using nanomedicines to target tumor metastasis

S. No.	Design	Strategy	Target	Refs
1.	Polymer nanoparticle loaded with paclitaxel and dual functionalized with K237 peptide and Ep23 aptamer	CTCs must be eradicated from the neovasculature	Onset and spread of metastatic disease	[30]
2.	DOX-loaded PBA-LMWH-TOS micellar nanoparticle	Inhibit MMP-9 expression in tumor cells Interactions between tumor cells and platelets should be cut off	Initiation and spread of metastatic disease	[31]
3.	Self-assembling tumor-associated tissue factor (TF) siRNA delivery system based on peptides	Both TME and CTCs have TF expression knocked down Interactions between tumor cells and platelets should be cut off TME's hypercoagulable state must be reversed	Initiation and spread of metastatic disease	[32]
4.	Carfilzomib-loaded PLGA nanoparticle coated on the membrane of neutrophils	Reduce the number of CTCs in circulation and prevent PMN formation	Dissemination and colonization of metastatic lesions	[33]
5.	Dextran-octadecanoic acid sialic acid Micelles loaded with DOX to target E-selectin	Stop cells from migrating CTCs that are spread through the blood must be eliminated Reduce the size of lesions that have already formed	Initiation and spread of metastatic disease	[34]

(*Continued*)

Table 7.1 (*Continued*)

S. No.	Design	Strategy	Target	Refs
6.	DOX-loaded mesoporous silica nanoparticle for targeting EpCAM and CD44	CTCs must be eliminated CTC extravasation should be prevented	Dissemination and colonization of metastatic lesions	[35]
7.	DOX and indocyanine green loaded platelet and neutrophil hybrid cell membrane coated nanocage	CTCs and tumor-derived exosomes are captured and cleared TME's immunosuppressive effects must be reversed	Initiation, dissemination, and colonization of metastatic disease	[36]
8.	An immunopotentiator and DOX are loaded into a PBA-LMWH-TOS nanoparticle	Inhibit the implantation of CTCs Interfere with granulocytic myeloid-derived suppressor cells' recruitment to PMNs and vascular destruction (G-MDSCs) G-MDSCs' MMP-9 expression should be reduced	Dissemination and colonization of metastatic lesions	[37]

Source: Reproduced from Ref. [29] with permission from Elsevier.

7.3.1 Active Targeting

Active targeting is a ligand-based targeted method based on enhancing uptake by target cells through enhanced recognition, retention, and uptake by target cells [40]. Molecular interactions between the ligands and their targets are responsible for the binding affinity, which comprises receptor-ligand interactions, charge-based interactions, and motif-based interactions with substrates [41, 42]. In addition to antibodies, proteins, nucleic acids, peptides, carbohydrates, and ligands include small molecules such as vitamins [43–45]. Surface molecules expressed by diseased cells, including lipids, carbohydrates, and proteins in organs and

molecules belonging to and emanating from tumor cells in their microenvironment or even the physicochemical environment in the proximity can be target substrates. Tumor microenvironments are known to be complex, with extensive distribution of vessels, restricted tissue structures, low permeability, and an acidic pH [46, 47]. Due to low permeability, nanoparticles in this milieu are unlikely to preferentially find their way into malignant and metastatic tissue. Researchers commonly modify nanoparticle surfaces with ligands, antibodies, and targeting probes to enhance drug accumulation at tumor sites and bestow upon them the ability to control cancer cells specifically [48–50].

An active targeting strategy has been developed to improve drug delivery performance by increasing nanoparticle uptake by target cells (Fig. 7.1). Recently, trastuzumab, an antibody that binds to the human epidermal growth factor receptor II (HER-2), was used for the study. Researchers have demonstrated that trastuzumab can effectively attack HER-2-positive breast cancer by utilizing nanoparticles [51]. The researchers developed nanoparticles that mimic red blood cells (RBC-iRGD-NPs) combined with arginine-glycine-aspartic acid peptides to improve biocompatibility and blood circulation time (iRGD). The team designed a model of metastatic 4T1 breast cancer to conduct antitumor studies [52]. Research findings have demonstrated that RBC-iRGD-NPs can suppress primary tumor growth and lung metastasis by more than 90% and 95%, respectively, whereas iRGD-NPs without red blood cell membrane could prevent lung metastasis only by 70% [52]. In this study, researchers determined that nanoparticles coated with cell-like membranes can trick the immunity of the host, evade macrophages, persist in the bloodstream for extended periods, as well as transport drugs directly to the tumor site to suppress metastatic spread [52]. Nevertheless, numerous variables, such as ligand conjugation, ligand density, and hydrophobicity on the surface of nanoparticles, must be tuned for optimal usage of active-targeted cancer treatments. The crucial aspect of this conjugation is maintaining the conjugated ligands in the unfavorable physiological environment, and several methods have been attempted to accomplish this [40]. There was a surprising correlation between ligand density and overall affinity along with the target site. Molecular saturation, incorrect ligand orientation, bond restrictions, and steric constraints from neighboring

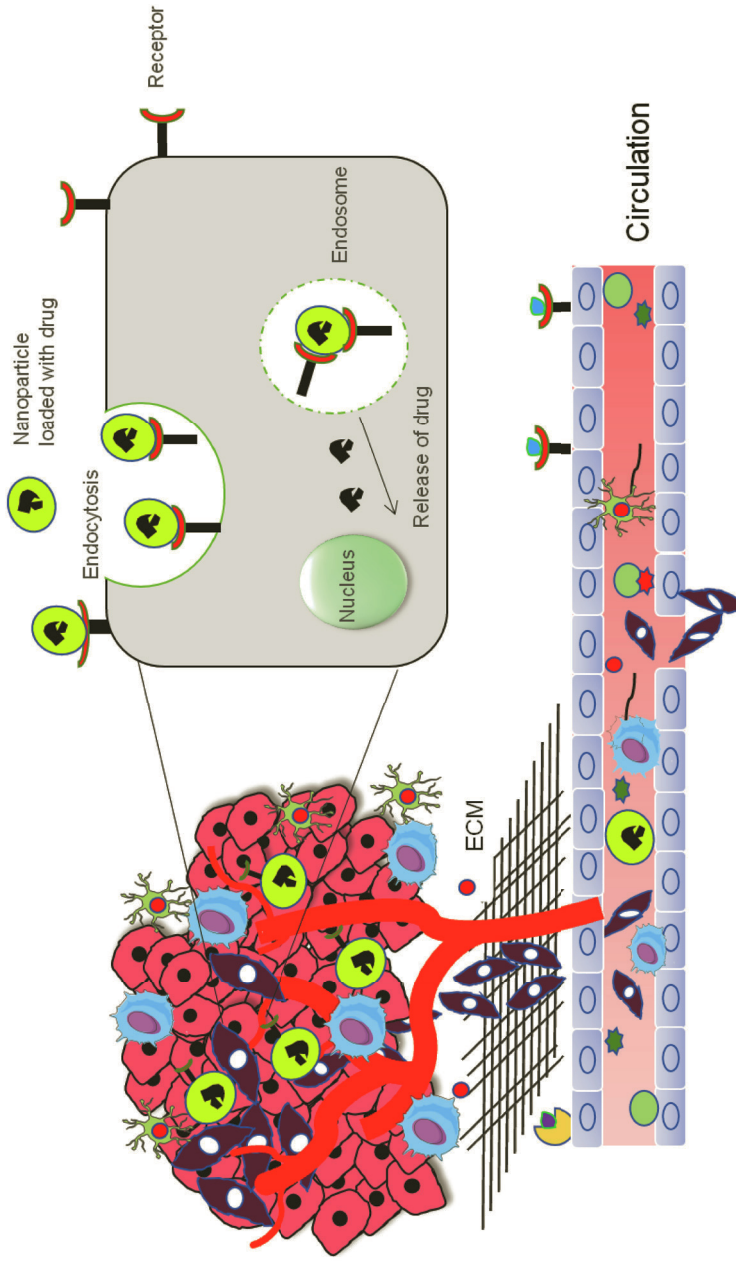

Figure 7.1 Schematic representation of targeting strategies of nanomedicine for the prevention of tumor metastasis.

molecules on the nanoparticles have been used as explanations for this phenomenon [53]. As well, increasing the hydrophobicity of the nanoparticles made them more prone to macrophage ingestion without enhancing target cell uptake significantly [54]. This result highlights how the choice of a targeting moiety, conjugation method, and density of therapeutic nanosystems plays a significant role in achieving the desired outcomes.

7.3.2 Passive Targeting

Tumor cells have a compromised vascular barrier when compared to healthy cells, allowing nanomedicine to accumulate in tumor tissue [55]. Tumor vasculature is disrupted by vascular bed perturbation when endothelial gaps are formed between cells. These gaps may be as large as 200 to 2000 nm based on the type, site, and microenvironment of the tumor. Furthermore, because lymphatics are inefficient, nanoparticles do not clear quickly and concentrate within the tumor interstitial space [56]. As such, it is called the enhanced permeability and retention (EPR) effect, and it lies at the foundation of passive targeting [57]. For effective drug distribution in passive targeting, the drug has to circulate at target sites for a considerable amount of time (Fig. 7.1). The subsequent decades have seen researchers develop a variety of strategies using nanomedicines, drug carriers, and nanoprobes to target cancer and monitor its spread, which is essential for early detection and successful treatment of tumors and metastasis [58–60]. Furthermore, increased nanoparticle accumulation at tumor sites necessitates a greater level of targeting ability on the part of the nanoparticles themselves (Fig. 7.1). Nanoparticle size and shape, nanoparticle retention duration, lymphatic function, tumor perfusion, nanoparticle surface charge, interstitial penetrations, vascular permeability, blood pressure, extracellular matrix (ECM), and tumor microenvironment all influence nanocarriers build-up at tumor sites [11]. First, a crucial part of the EPR function is the relative size ratio of NPs to perforations in blood vessels. A lower ratio means that nanoparticles will be more likely to cross tumor vascular walls. The number of tumor cells accumulated in liposomes with particles between 100 and 200 nm is fourfold higher than that for particle sizes less than 50 nm or exceeding 300 nm [61,

62]. Second, the nanoparticle's shape affects how long it takes to circulate in the blood, how many cells it captures, and how much tumor it accumulates. There has been an increase in drug dosage, a longer time for blood to circulate, and an improvement in tumor penetration with non-spherical nanoparticles [40, 62, 63]. Third, tumor tissues have poor lymphatic outflow, which can be used to transport theranostic drugs via nanocarriers. Nanoparticles can typically remain at tumor locations for an extended time. There was an accumulation of liposome-drug formulation in regional lymph nodes and non-regional lymph nodes of 6.6 and 5.35 times more than free drugs [64]. Fourth, positive surface-charged nanoparticles accumulated and exhibited greater cytotoxicity at tumor sites than neutral or negatively charged nanoparticles. In addition, positive nanoparticles exhibit shorter blood circulation times due to their tighter interactions with cell membranes and demonstrate higher cytotoxic effects than negative and neutrally charged nanoparticles [65, 66]. Fifth, nanoparticles can enter cancerous tissues through blood vessels because they occupy the larger spaces between endothelial cells, which are reliant on interstitial fluid flow (IFF) instead of tumor interstitial fluid pressure (TIFP). Nanoparticles with high TIFP can be transported to tumor areas even though the IFF rate is slow [67–69]. Sixth, a rise in vascular permeability leads to increased TIFP, which poses a barrier to therapeutic agent transport. A high expression of vascular endothelial growth factors (VEGF) leads to increased blood vessels permeability, and anti-VEGF medication can be administered to reduce TIFP, which can increase the effectiveness of chemotherapy [70, 71]. Seventh, for effective tumor therapy, the carrier therapeutic molecules must remain in tumor cells for an extended time before they are excreted. The increased retention duration of therapeutic compounds in cancer cells is caused by impaired lymphatic function. Anti-angiogenesis therapy improves therapeutic molecule retention in malignant tissues [72, 73]. The eighth problem is that high blood pressure makes it easier for drugs and vaccines to invade cancerous tissues. Because tumor blood vessels lack smooth muscle layers, increasing blood pressure linearly increases blood volume. As a result of high blood pressure, angiotensin-II-induced hypertension increases blood volume, resulting in an increase in permeability of diagnostic and therapeutic agents into tumor tissue

[74, 75]. Ninth, the presence of ECM, on the other hand, impedes the transport of targeted therapeutic medications by interfering with the formation or dissolution of therapeutic molecules within tumor tissues via the diffusion of molecules across the vascular architecture. ECM can capture nanoparticles and prevent them from entering a specified tumor site. To improve drug delivery, the ECM must be modified to reduce the distance between the blood vessel and target tissues [70, 76]. Many factors influence the EPR phenomenon, including tumor necrotic factor, bradykinin, carbon monoxide, matrix metalloproteinase (MMPs), heme oxygenase, peroxynitrite, nitric oxide, VEGF, and others. Peroxynitrite boosts the EPR effect by activating matrix metalloproteinase, a protein that is highly expressed in cancer cells and is associated with invasion, angiogenesis, and metastasis. Tumor treatment can be substantially boosted by integrating the EPR effect with PEG (polyethylene glycol)-hemin [76, 77]. However, the lack of adequately recapitulated solid tumor models in individuals limits our knowledge of EPR effects. In essence, the majority of our present understanding has been derived from subcutaneous tumor xenograft models that grow rapidly, characterized by extremely significant EPR effects. As a result, the experimental findings utilizing these models may give a deceptive indication of the effectiveness of passively targeting nanomaterials [78]. Furthermore, it is critical to recognize a scarcity of clinical evidence regarding EPR effects. As a result, future advances in tumor biology, including a better understanding of EPR effects in various tumor types, are critical. Such in-depth understanding will be important in the pragmatic customization of nanoparticles, which can then be employed for customized tumor treatment to achieve even greater therapeutic advantages.

7.3.3 Cancer Stem Cells Targeting

A cancer stem cell (CSC) is a type of tumor cell that has the ability to self-renew, differentiate, and establish tumor growth; they are thought to be one of the chief drivers of cancer heterogeneity between and within tumors [79]. The bulk of non-CSC tumor cells can be eliminated with standard chemotherapy, but CSCs are often drug-resistant, resulting in tumor recurrence and metastasis. Furthermore, CSCs can evade strict regulation through the use of a

few crucial dysregulated self-renewal signaling pathways (SRSPs), such as proto-oncogene tyrosine-protein kinase Src (SRC) signaling, signal transducer and activator of transcription (STAT) signaling, and Wnt/β-catenin signaling, all of which lead to cell proliferation [80, 81]. Since CSCs differ in their ability to regulate their activity, we have focused on developing therapies that combine chemotherapy with inhibitors of CSC-regulating pathways as a fundamental issue in developing CSC-targeting therapies. Functionalized nanoparticles can target CSCs in a variety of ways, including targeting ligands, surface biomarkers, inhibitors of SRSPs, miRNA (micro-RNA), shRNA (small hairpin RNA), and therapeutic compounds [11]. The tumor microenvironment's thick matrix and stromal barriers, however, frequently hinder traditional synthetic nanocarriers, including liposomes, micelles, and inorganic nanoparticles. Recent developments have led to the development of biomimetic delivery vehicles with inherent biological functions that play crucial roles in tumor progression and metastasis, thereby successfully targeting and delivering chemotherapeutic drugs or siRNAs to CSCs. In colon and pancreatic cancer, for example, inhibiting STAT3 transcription factor with napabucasin inhibitor results in suppression of *c-myc*, *nanog*, and *sox2* genes, which reduces metastasis and tumor relapse in mice models [82]. In a recent study, gemcitabine-loaded autologous exosomes generated from pancreatic ductal adenocarcinoma effectively inhibited tumor development and metastasis in mice when compared to free gemcitabine therapy at a high dose [83]. Because of the tumor-accumulating capabilities and cloaking properties of cell membranes, cell membrane-camouflaging nanoparticles are also appealing delivery platforms for targeting CSC. Using this method, researchers may load chemotherapeutic agents to satisfy a variety of pharmacological needs in synthetic nanoparticles with improved targeting ability and biocompatibility. Zhang *et al*. produced nanoparticles coated with cancer cell membranes [84]. They made human pancreatic cancer and pancreatic stellate cell hybrid tumor-bearing animals to better model the tumor microenvironment of pancreatic cancer in mice. When compared to free DOX and doxil drugs, the nanoparticles demonstrated immune escape, rapid tumor invasion of the stroma, and increased tumor accumulation, all of which contributed to the improved antitumor activity.

7.3.4 Epithelial–Mesenchymal Transition Targeting

In epithelial–mesenchymal transition (EMT), epithelial cells lose their polarity and adherent properties and acquire mesenchymal appearance, leading to migratory behavior. A study has shown that EMT is necessary for the generation of CSCs and stem-like cells, which in turn contribute to drug resistance and cancer relapse [85]. By preventing the spread of tumor cells by suppressing EMT before they have metastasized, cancer cells may be prevented from causing systemic spread. In addition, in cases when metastases are already established, such an approach may improve sensitivity to chemotherapy. Cadherin switch is a defining characteristic of EMT, and E-cadherin is down-regulated [86], resulting in loss of epithelial cell adhesiveness, leading to both dissociation from the tumor cluster and induction of intracellular transformation. In some instances, transcription factors such as Snail/Slug, SIP1/ZEB2, and TWIST decrease E-cadherin expression [87, 88]. Thus, blocking the transcription factors responsible for re-establishing E-cadherin activity is likely to be beneficial to maintaining tumor cells. Several targets have been identified for their role in commencing EMT: platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), VEGF, transforming growth factor (TGF), fibroblast growth factor (FGF), Notch pathway, and epidermal growth factor (EGF) [89]. Tumor tissues in epithelial ovarian cancer express EMT markers to a high degree, the tumor is highly drug-resistant, and it recurs frequently. Inhibiting EMT biomarkers (e.g., TWIST) is essential for preventing tumor spread [90, 91]. Using mesoporous silica nanoparticles, Roberts *et al*. delivered siRNA to ovarian cancer tissues to target TWIST [92]. The researchers found that functional nanoparticles can specifically target ovarian cancer cells, modulate the expression of TWIST, and limit the EMT process as well as the growth of ovarian cancer tissue [92]. Another study found Au NPs cured ovarian cancer *in vitro* by Arvizo *et al*. have demonstrated that Au NPs reduce cancer cell invasion and spread by decreasing nearly all cytokines responsible for tumor growth and metastasis [93–95]. This therapeutic approach could be employed in clinics to treat tumor metastasis and drug resistance.

7.3.5 Remodeling Tumor Microenvironment

The tumor microenvironment (TME) has impacts tumor cell metastasis through complicated and evolving intercellular interactions in the form of secreted factors and cell-to-cell contacts between CSCs, non-CSCs, and stromal cells [96]. Metastatic tumor cells are thought to dwell in a specific compartment within the TME, which governs metastatic tumor cell's destiny via cell-to-cell interactions or secreted milieu cues. TME differs from healthy cells in physiological conditions in several ways, including low oxygen levels, high temperatures, low pH values, high enzyme expression, and high concentrations of reduced glutathione (GSH), among others [97]. As a corollary, using these TME characteristics, it is possible to dramatically boost the therapeutic efficacy of antitumor medications by exploiting these TME features. Furthermore, several kinds of research have concentrated on reprogramming the CAFs to provoke quiescence, which resulted in the suppression of stromal cell regeneration, metastasis, and drug resistance, or remodeling of the TME while preserving the external layer of stromal cells to facilitate drug absorption to the internal tumor and target CSCs [98]. To overcome protein denaturation during the loading process and preserve therapeutic release at metastatic sites, researchers recently overexpressed ECM, which inhibits drug accumulation at tumor metastasis. In one recent study, a collagenase nanoparticle (*i.e.*, collagozome) was designed to target collagen and disrupt the ECM in the pancreatic cancer tumor microenvironment. The collagenase was placed into the collagozome, which protected the enzyme from early deactivation in the plasma and prolonging its activity. As a result, pre-treatment with collagozome allows for improved drug permeation into pancreatic primary and metastatic TMEs [99]. Xu *et al*. reported an injectable nanoparticle generator (iNPG) that could bypass several biological barriers and transport anticancer medicines to treat primary and metastatic malignancies in another investigation [100]. To begin, polymerized DOX was chemically bonded to L-glutamic acid to create pH-responsive pDOX, which would then be loaded into the nanopores of disk-shaped silicon-based carrier particles to generate the iNPG-pDOX nanocomposite. Because of the TME's low pH, the pDOX nanocomposite can achieve TME-stimulated drug release to metastatic tumors [100]. The

researchers applied this nanocomposite to a 4T1 lung metastasis model using BALB/C mice and discovered that the iNPG-pDOX nanocomposite has a superior ability to release the medication at metastasis with effectiveness that surpasses free DOX and free pDOX. This method demonstrated that iNPG-pDOX nanocomposite could accumulate and stay at lung tumors in mice, significantly improving drug delivery to metastatic tumors [100]. Similarly, another study was undertaken to improve the anticancer effect of the chemotherapeutic agent paclitaxel (PTX) on drug-resistant and metastatic breast cancer by co-delivering PTX with a siRNA to reduce the expression of the *Akt* gene [101]. Poly [(1,4-butanediol)-diacrylate-β-N,N-diisopropylethylenediamine]-polyethyleneimine (BDP), a pH-sensitive amphiphilic polymer was manufactured to produce PTX-loaded BDP micelle/siRNA nanocomposite (PMA). It was shown that PMA was sustainable including both neutral and tumor extracellular pH, and it could release cargo drugs in the endosome and lysosome acidic environment led to the downregulation of *the Akt* gene and P-glycoproteins, as well as the upregulation of *Caspase-3* gene in 4T1 cancer cell line [101]. Down-regulated P-glycoprotein gene expression inhibits PTX efflux, raising intracellular concentration, enhancing cytotoxicity, and inhibiting 4T1 cancer cell migration and invasion to other regions. As a result, the PTX-loaded BDP micelle/siRNA pH-sensitive nanocomposite was demonstrated to be an excellent drug delivery method for the therapy of suppressing breast cancer metastasis by circumventing drug resistance while also limiting lung metastasis [101]. These nanomedicines, when combined, disrupted the core of the tumor through improved internalization and anticancer activity, as well as successfully preventing tumor cell dissemination and metastasis.

7.3.6 Circulating Tumor Cell Targeting

Circulating tumor cells (CTCs) circulate in the bloodstream during the early stages of tumor metastasis, overcoming several obstacles on their way to spread and settle in multiple organs for the development of metastatic sites on various host organs [102, 103]. As a result, targeting CTCs in the blood is an important step in the diagnosis and prevention of tumor metastasis. Because nanoparticles may be easily changed and functionalized to target

CTCs, they can be utilized as an armory to capture and target CTCs from the bloodstream. These advantages enable successful CTC capture from the bloodstream in the vicinity of other host blood cells and biological components. Several ways for particular targeting and removal of CTCs from blood are now being developed, including the creation of immune cells membrane mimicking nanomedicine [104], CTCs specific immunomagnetic nanosystems [105], and microfluidic technology [11]. Neutrophils are immune cells that can target CTCs and can scavenge pre-metastatic surroundings prior to the entrance of CTCs. Kang *et al*., for example, created a nanostructure that mimics neutrophil membranes by covering neutrophil membranes on PLGA-NPs loaded with carfilzomib drug. This disguised technique selectively decreased CTCs in circulation and prevented early metastasis as well as niche progression when compared to free carfilzomib and uncoated nanoparticles containing carfilzomib [106]. An additional method for removing CTCs from the bloodstream is to create immune-magnetosomes, which resemble the membrane of leukocytes and reduce the absorption of blood leukocytes on the surface of nanoparticles [107]. These immune-magnetosomes detect CTCs with high sensitivity and successfully eradicate CTCs from the bloodstream. Microfluidic technology, in conjunction with functionalized nanoparticles, can also be used to create microfluidic chips that recognize and particularly eradicate CTCs from the bloodstream. Park *et al*. created a microfluidic device containing pentanethiol functionalized gold nanoparticles to successfully eradicate CTCs [108]. This microfluidic device can be utilized to distinguish CTCs from metastatic breast cancer and epithelial cancer cells utilizing the thiol exchange process while causing minimal damage to normal blood cells [108]. According to the data shown above, these nanomedicines are effective at capturing CTCs from blood and preventing tumor metastasis.

7.3.7 Gene Editing

A potent CRISPR/Cas9 gene-editing technique has been discovered lately through the study of bacterial defense mechanisms [109]. With this technology, scientists can modify the genomes of eukaryotes with greater precision as well as efficiency relative to prior techniques. A CRISPR/Cas9 system consists of three parts: a single guide RNA

(sgRNA) that is unique to the target DNA; the Cas9 protein, which acts as a DNA endonuclease; as well as tracrRNA, which binds to Cas9 [110, 111]. Cas-9-mediated targeted editing of the human genome was reported by Cong *et al*. Throughout the past decade, CRISPR-Cas9 gene editing has been gaining traction offers an alternative method of treating tumors [112]. Cancer cells produce receptors on their surface through the expression of specific genes. By mutating them, they could reduce the adhesion capability of cells and enhance tumor cell proliferation. Inhibiting primary tumor metastasis by targeting and knocking down mutant genes in the human body is possible with Cas-9 [113]. In general, the Cas-9 mechanism has mostly been passed through plasmids and viruses. Unfortunately, a slew of problems has been linked to these two delivery systems. It is well known that the Cas-9 and SgRNA components of plasmids possess strongly negative charges and are rather large sized. In this way, it is difficult to carry big RNA into tumors [114, 115]. Despite the high efficiency of viral vectors for transferring genes, these vectors are inadequately safe since they can lead to mutagenesis and other harmful effects [116]. Within the last few years [117–119], it has been proven that nanoparticles can be used for delivering Cas-9 to cells and facilitate the editing of genes. When focal adhesion kinase (FAK) is disrupted, DNA is damaged, leading to increased sensitivity to radiation exposure and resistance to chemotherapy. As well, the presence of FAK in tumor xenografts using CRISPR/Cas9 methods has been shown to contribute to the tumorigenic potential of the KRAS mutation [120, 121]. Furthermore, FAK overexpression correlates with poorer clinical performance among individuals afflicted by non-small cell lung cancer (NSCLC) [122–124], making it an extremely appealing target for treating NSCLC and preventing metastases. It has been shown that miRNA disrupts cancerous cells and is essential to cellular function. In addition, higher levels of expression of mir-487a, mir-539, along with mir-323b, have been linked to metastasis and worse clinical outcomes in individuals with lung adenocarcinomas, predominantly non-smokers [125]. Due to this, treatment with these miRNAs could prevent lung adenocarcinoma patients from acquiring distant metastasis and dispersion of tumor cells. There was a marked decrease in cell motility *in vitro* and a decreased risk of lung metastases with CRISPR-mediated deletion in the CXCR2 (IL-8 receptor) in breast

cancer cells according to another study [126]. Researchers have identified MARK4 and FERMT2 as potential targets of CRISPR/Cas9 for regulating breast cancer cell motility and metastasis [127–129]. CRISPR-based deletion of Nogo-B caused a dramatic reduction in tumor development as well as distant metastasis *in vivo* and *in vitro*, as well as impressive decreases in cell growth and apoptosis *in vitro* [130]. According to the preceding investigations, nanoparticles can be used to transport Cas-9, which has been demonstrated to be effective in overcoming delivery barriers, reducing off-target rates, and achieving gene editing of targeted genes. Further clinical trials and *in vivo* investigations for these altered genes could be conducted in the future as inhibitors are developed.

7.4 Experiences from Clinical Trials

We addressed recent underway or successfully completed clinical trials of nanomedicines that efficiently target tumor metastatic sites for tumor metastasis inhibition in this section (Table 7.2) [11]. In addition, various genes have been reported that play a significant part in the inhibition of invasion and tumor metastasis. A plethora of constraints exist that hinder effective therapy for metastatic tumors, such as drug extravasation restrictions in endothelial cells, inadequate drug penetration into tumor tissues, drug allocation in normal tissues, and significant drug resistance. During metastasis, the majority of metastatic cancer cells usually disseminate to certain organs, like metastases in the lungs or liver in breast cancer. Conventional anticancer medications are incapable of accumulating selectively at tumor locations due to these challenges. As a result, effective nanocarriers capable of delivering antitumor or antimetastatic medications to specific metastatic tumor sites are badly needed [166]. Nevertheless, as previously said, designing effective tumor nanomedicines remains a significant difficulty and nearly a few of these formulations have reached clinical trials. First, functional characterization of nanomaterials is crucial for drug administration, and it must be done carefully to limit the risk of nanoparticles causing unintended toxicity to normal cells, as well as to increase nanomaterial biocompatibility [3]. Second, there has been a considerable delay in the commercialization of

Table 7.2 Various clinical trials of nanomedicines for inhibition of tumor proliferation and metastasis

NCT no.	Metastasis type	Phase	Status	Nanomedicine	Summary	Refs
01861496	Metastatic breast cancer, skin cancer, and prostate cancer	Phase I/II	Ongoing	LiPlaC (liposomal formulation of Cisplatinum)	A single-arm study of LiPlaC is being conducted on patients with advanced or refractory solid tumors, including metastatic breast cancer, prostate cancer, and skin cancer.	[131]
02340117	Metastatic pancreatic cancer	Phase II	Ongoing	SGT-53 (Liposome encapsulated with the plasmid containing human p53 DNA)	A single-group, open-label study assessed the efficacy of intravenously administered SGT-53 with gemcitabine and nab-paclitaxel in patients with metastatic pancreatic cancer.	[132]
02596373	Metastatic breast cancer	Phase II	Ongoing	Mitoxantrone HCl (hydrochloride) liposome injection	A study examining the effects of injecting mitoxantrone HCL liposomes on the growth and recurrence of advanced recurrent or metastatic breast cancer; open label, randomized.	[133]
02833766	Metastatic triple-negative breast cancer, EGFR positive	Phase II	Ongoing	Anti-EGFR-IL-dox (anti-EGFR immunoliposomes loaded with DOX)	In the case of advanced triple-negative, EGFR positive breast cancer, anti-EGFR-IL-dox as first-line therapy; open-label, single-group study.	[134]

(*Continued*)

Table 7.2 (*Continued*)

NCT no.	Metastasis type	Phase	Status	Nanomedicine	Summary	Refs
02739633	Metastatic pancreas adenocarcinoma	Phase II	Ongoing	Genexol®-PM (CrEL-free polymeric micelle formulated paclitaxel)	In a single-group study, we evaluated the effect of combining Genexol®-PM and gemcitabine in patients with recurrent and metastatic pancreatic adenocarcinoma.	[135]
01784120	Metastatic breast cancer	Phase II	Ongoing	Genexol®-PM	DOX/ Genexol®-PM efficacy and toxicity in metastatic breast cancer; open-label, single-group assignment.	[136]
01770795	Metastatic NSCLC	Phase II	Ongoing	Genexol®-PM	A single-group, open-label, randomized trial evaluating Genexol®-PM and gemcitabine in patients with metastatic NSCLC who have not been treated.	[137]
02551991	Metastatic pancreatic adenocarcinoma but previously untreated	Phase I/II	Ongoing	MM-398 (nanoliposomal Irinotecan)	Analyzing the safety, tolerability, and preliminary efficacy of nanoliposomal Irinotecan when used alone or in combination with other anticancer therapies.	[138]
03823989	Metastatic solid tumors	Phase I	Ongoing	Promitil® (mitomycin-C PEGylated liposome)	An open-label, multicenter, single-arm, prospective study administered liposomal Promitil to cancer patients while they received external radiotherapy.	[139]

NCT no.	Metastasis type	Phase	Status	Nanomedicine	Summary	Refs
02138955	Different metastatic cancers	Phase I/II	Ongoing	LipoCURC (liposomal curcumin)	Evaluating the safety, tolerability, and pharmacokinetic profiles of body surface area with adjusted doses of LipoCURC in an open-label, single-group study.	[140]
00504998	Metastatic pancreatic cancer	Phase I/II	Ongoing	Rexin-G (retrovector harboring a construct of cytocidal cyclin G1)	Identifying the optimal dose of Rexin-G and determining its overall safety; open-label, parallel assignment, an uncontrolled study.	[141]
00572130	Metastatic osteosarcoma	Phase I/II	Ongoing	Rexin-G	Researchers are evaluating the clinical effectiveness and safety of Rexin-G in a non-randomized, open-label, parallel assignment study.	[142]
00951054	Triple-negative breast cancer, metastatic disease	Phase II	Ongoing	NK-102 (SN-38-releasing polymeric micelle)	NK012 is being tested in a multicenter, single-arm, open-label study to determine whether it is safe and effective for advanced and metastatic triple-negative breast cancer patients.	[143]

(*Continued*)

Table 7.2 (*Continued*)

NCT no.	Metastasis type	Phase	Status	Nanomedicine	Summary	Refs
01644890	Metastatic breast cancer	Phase III	Approved	NK-105 (paclitaxel-incorporating micelle nanoparticle)	An open-label, randomized, multinational study concluded that NK105 did not exhibit a statistically significant difference from paclitaxel in terms of progression-free survival in patients with metastatic or recurrent breast cancer.	[144]
03771820	Head and neck squamous cell carcinoma, metastatic disease	Phase I/II	Ongoing	NC-6004 (polymeric micelle-containing cisplatin)	Assessing the optimal tolerable and recommend phase IIb dose in recurrent or metastatic squamous cell carcinomas of the head and neck; open-label, parallel assignment, randomized.	[145]
03109158	Head and neck squamous cell carcinoma, metastatic disease	Phase I/II	Ongoing	NC-6004	Initiating a trial for NC-6004, 5-FU, and Cetuximab, establishing a recommended dose for Phase II, providing an efficacy signal, and establishing open-label, single-group assignment.	[146]
00826085	Relapse of breast cancer at the chest wall	Phase I/II	Ongoing	ThermoDox (thermally sensitive liposomal DOX)	Open-label, single-group research is being conducted to determine if ThermoDox combines well with therapeutic chest wall heating to treat recurrent regional breast cancer.	[147]

NCT no.	Metastasis type	Phase	Status	Nanomedicine	Summary	Refs
00441376	Liver metastatic tumor	Phase I	Ongoing	ThermoDox	Using open-label, single-group studies of ThermoDox in combination with radiotherapy ablation to determine its maximum tolerable dose in the treatment of primary and metastatic liver tumors.	[148]
01612546	Stomach, esophageal, or gastroesophageal, cancers, metastatic disease	Phase I/II	Ongoing	CRLX101 (cyclodextrin-based nanoparticles camptothecin)	This study will test CRLX101 for the advanced or metastatic stomach, gastroesophageal, or esophageal tumor that cannot be surgically removed and has progressed through at least one chemotherapy regimen.	[149]
01380769	Metastatic NSCLC	Phase II	Ongoing	CRLX101	A randomized controlled study that compares median overall survival between patients with advanced NSCLC treated with CRLX101 and best supportive care.	[150]
02187302	Metastatic renal cell carcinoma (RCC)	Phase II	Ongoing	CRLX101	CRLX101 in combination with bevacizumab is compared with standard care in patients with metastatic disease (RCC) in an open-label, randomized study.	[151]

(*Continued*)

Table 7.2 (*Continued*)

NCT no.	Metastasis type	Phase	Status	Nanomedicine	Summary	Refs
02707159	Metastatic pancreatic cancer	Phase II	Ongoing	Nab-paclitaxel (nanoparticle albumin-paclitaxel)	Study investigating the potential of CTCs in metastatic pancreatic cancer patients given gemcitabine and nab-paclitaxel. Open-label; patients receive a mixture of gemcitabine and nab-paclitaxel.	[152]
03401827	Metastatic pancreatic ductal adenocarcinoma	Phase IV	Approved	Nab-paclitaxel	GnP (gemcitabine and nab-paclitaxel) will be studied as a second-line treatment for locally advanced or metastatic pancreatic ductal adenocarcinoma following FOLFIRINOX failure.	[153]
03410030	Metastatic pancreatic cancer, untreated	Phase IB/II	Ongoing	NABPLAGEM (nanoparticle paclitaxel protein bound + cisplatin + gemcitabine)	In patients with untreated metastatic pancreatic cancer, open-label ascorbic acid plus NABPLAGEM was used.	[154]
01463072	Metastatic breast cancer	Phase I/II	Ongoing	Abraxane (albumin-bound nanoparticle paclitaxel)	Abraxane is being studied in an open-label, single-group study (metastatic) for older patients with breast cancer that has spread to nearby tissues or lymph nodes (locally advanced) or other parts of the body.	[155]

NCT no.	Metastasis type	Phase	Status	Nanomedicine	Summary	Refs
03304210	Stomach, breast, ovarian, & pancreatic cancer	Phase I	Ongoing	Abraxane	The study looked at the maximum tolerated dose of Abraxane given with repeated pressurized intraperitoneal aerosol chemotherapy. It was a double-blind, multicenter, multinational study (PIPAC).	[156]
00821964	Skin metastases in breast cancer	Phase II	Ongoing	Abraxane	An open-label trial to examine how well topical imiquimod may help treat patients who have skin metastases from breast cancer by using it in conjunction with Abraxane.	[157]
01437007	Hepatic tumor metastases, Inoperable	Phase I	Ongoing	TKM-080301 (lipid nanoparticle formulation containing siRNA against PLK1)	TKM-080301 is being tested in humans for use in a wide variety of cancers with liver metastasis, including colorectal, pancreatic, gastric, breast, and ovarian cancers that do not respond to conventional treatments.	[158]

(*Continued*)

Table 7.2 (*Continued*)

NCT no.	Metastasis type	Phase	Status	Nanomedicine	Summary	Refs
00046527	Metastatic breast cancer	Phase III	Approved	ABI-007 (nanoparticle colloidal composition of protein-stabilized paclitaxel)	Comparison of ABI-007 to Taxol-treated patients with metastatic breast cancer, open label, to see if it is as effective.	[159]
00046514	Metastatic breast cancer	Phase II	Ongoing	ABI-007	ABI-007 monotherapy for metastatic breast cancer patients who have previously received Taxol will be studied open label to determine safety, tolerability, and antitumor activity.	[160]
01792479	Metastatic NSCLC	Phase II	Ongoing	BIND-014 (docetaxel nanoparticles for injectable suspension)	This multicenter, open-label clinical trial is testing BIND-014 on patients with advanced NSCLC.	[161]
01812746	Metastatic castration-resistant prostate cancer	Phase II	Ongoing	BIND-014	The goal of this open-label, multicenter study is to evaluate the efficacy and safety of BIND-014 in patients with metastatic castration-resistant prostate cancer (mCRPC). BIND-014 was also tested in patients with chemotherapy-naive mCRPC.	[162]

NCT no.	Metastasis type	Phase	Status	Nanomedicine	Summary	Refs
01300533	Metastatic solid tumors	Phase I	Ongoing	BIND-014	Phase II open-label study; ascertaining BIND-014's safety, tolerability, pharmacokinetics, and pharmacodynamics; defining BIND-014's recommended dosage; researching all of these aspects.	[163]
02442531	Metastatic solid tumors	Phase I	Ongoing	Docetaxel CriPec® (docetaxel containing nanoparticle)	An open-label, multicenter study that investigates CriPec® safety, docetaxel's tolerance, pharmacokinetics, and pharmacodynamics; and determines the maximum docetaxel dosage that is safe and effective for patients with solid tumors.	[164]
03101358	Non-melanoma cancer cutaneous metastases	Phase I/II	Ongoing	SOR007 (nanoparticle -paclitaxel, uncoated) ointment	Efficacy, tolerability, and preliminary safety of SOR007 Ointment in non-melanoma cutaneous metastases are investigated.	[165]

Source: Reproduced from Ref. [11] with permission from Elsevier.

nanomedicines to limit cancer cell metastasis due to a paucity of a thorough understanding of nano-bio interfacial interactions. Third, nanoparticles' storage and half-life in biological systems may have an impact on their pharmacokinetics and pharmacodynamics profiles [167, 168]. Fourth, toxicological studies of nanomaterials should be conducted to minimize any negative consequences that may arise as a result of unintended interactions between nanoparticles and biological systems. Fifth, a key continuing challenge is the lack of appropriate biological models that connect *in vitro* and *in vivo* drug release profiles. Sixth, because the physicochemical features of nanoformulations vary from batch to batch, large-scale manufacture of commercial nanomedicine products is technically problematic.

However, for preclinical and clinical trial research, only modest amounts of nanomedicine are used. Seventh, regulatory approval of nanomedicines is a major worry because the Food and Drug Administration (FDA) is yet to publish particular criteria for nanotherapeutic goods. Furthermore, the high cost of raw materials and the numerous procedures involved in the creation of nanotherapeutics make the commercialization of nanomedicines a costly proposition. As a result, it is critical to use well-planned and engineered manufacturing procedures, as well as clinical outcomes significant enough to justify the production expenditures.

7.5 Conclusion and Future Perspectives

The invention of new therapeutics for tumor metastasis is becoming increasingly popular and necessary. The goal of preventing tumor cells from spreading throughout the body is a consistent one, with the event directing the case's clinical therapy. The early steps of the cascade are the most appealing target due to the rational possibilities of targeting specific biomarkers or cellular signaling pathways associated with cancer cell survival, invasion, tumor proliferation, extravasation, angiogenesis, and tumor growth and re-establishing normal homeostasis [169–171]. Nanomaterials-based drugs have increased the attention of diagnostic research as promising methods for tumor treatment and prevention of metastasis, due to the advancements in materials science and nanotechnology. The well-designed nanomaterials allow for perfect

control of their physicochemical properties as well as their surface functionalization [172, 173], which improves the target specificity required for effective tumor growth and metastasis treatment. For tumor diagnosis and treatment, a thorough understanding of nano-bio interfacial interactions and nanoparticles specific targeting to tumor cells is required. All of these tactics can help to avoid unwanted systemic toxicity at tumor sites while preserving healthy cells. Furthermore, Kumar and colleagues have recently accredited the development of multi-functional nanoparticles as an impending therapy against tumor metastasis in the future [174, 175]. Nanotherapeutics can overcome the drawbacks of traditional molecular drugs, such as therapeutic efflux out of tumor cells by transmembrane glycoproteins (p-gp) overexpressed on tumor cell membranes, lack of tumor-targeting ability, unwanted side effects from non-selective distribution, and lack of co-delivery of multiple drugs to target primary tumors, CSCs, circulating T cells, and tumor metastasis. Despite the numerous advantages of nanotherapeutics for tumor treatment, the commercial application of nanotherapeutics remains a difficult task [176, 177]. Clinical studies are suffering substantial failures due to a paucity of knowledge of nanotherapeutic toxicity and *in vivo* behavior. As a result, only a few nanomedications for tumor therapy are now available on the market. Further advances in nanotechnology, on the other hand, will result in discoveries that will mark a fundamental change in the treatment of primary and metastatic tumors, and can considerably enhance clinical care. At this point, adaptive designing may be able to strengthen materials for future nanotherapeutics, and innovations may be able to provide improved tactics for tumor growth and metastasis treatment.

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Chapter 8

Nanotherapeutics as Potential Carriers for the Delivery of Anticancer Drugs

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8.1 Introduction

Cancer is a disease that causes harm to any part of the body due to uncontrolled cell growth and spreads to other parts of the body. Changes in DNA sequencing which are caused by mutations lead to cancer. As per WHO, 2021, cancer can be caused by genetic factors or external agents which include physical carcinogens such as ultraviolet and ionizing radiation, chemical carcinogens such as asbestos, components of tobacco smoke, and biological

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carcinogens such as infections from certain viruses and bacteria. Various types of cancer affect the lung, breast, cervical, colorectal, liver, stomach, leukemia, and many more. The most common cases estimated by WHO of cancer were; breast cancer followed by lung, colon and rectum, prostate, skin, and stomach. Cancer can be treated by taking chemotherapeutic drugs, surgery, or systemic therapy and radiotherapy, but these treatments cause toxicity and have many side effects. Therefore, nanotherapeutics which is a recent application of nanotechnology has been developed, which overcomes the limitations caused by the chemotherapy techniques and provides better drug delivery to the exact target part of the body [1, 2]. Nano drug delivery is a very useful system to deliver drugs because of its nanosize. Due to their small size, that is, their dimensions, they can cross the biological and physiological barriers and enter a different types of cells and tissues. For example, cellular uptake increases when colloidal nanoparticles pass through the cell membrane. Tubes, rods, and wires, which are examples of elongated nanostructures, have longer circulation time which results in reduced metabolic clearance [3]. Nano drug delivery can occur in various forms such as quantum dots, carbon nanotubes, polymeric nanoparticles, liposomes, dendrimers, *etc*., which act as carriers.

Dendrimers are highly branched, monodisperse polymeric macromolecules, which are made from starting atoms, such as nitrogen, to which carbon and other elements are added and it forms a series of chains in the form of a sphere. They act as a prodrug and carry antibodies or hormones to the target location. Nanoemulsions are colloidal dispersions of two immiscible liquids where they are composed of oil droplets dispersed in an aqueous medium and are stabilized by surfactants. They have enhanced bioavailability and increased loading of drugs. Examples of nanoemulsions can be in the form of creams, liquids, sprays, *etc*. Carbon nanotubes are a type of carbon nanomaterials that have a large surface area and good electronic and thermal conductivity as they have layers made of graphite. Magnetic nanoparticles are one of the most common forms of the carriers which are involved in drug delivery as they provide adequate dose with active and passive targeting, and are easy to handle [4]. Liposomes are molecules that consist of one or more bilayers of lipid which surround the aqueous medium. They can entrap both the hydrophobic and the hydrophilic, in the lipid membrane and the core, respectively [5]. Encapsulating the

hydrophobic or hydrophilic molecules in their cores or by protecting drug degradation improves drug properties by the nanocarriers. Transport of drugs to the site of the action takes place by carriers that are covered with proteins and by macrophages of the liver and spleen after intravascular administration. Pathologies such as hepatic metastases are improved by controlled biodistribution and therefore, reduce the concentration of drug in the undesired locations, hence decreasing toxicity of the drug [6]. Therefore, this chapter highlights the different classifications of drug carriers that act as nanotherapeutics for the delivery of anticancer drugs. Furthermore, it also includes the benefits and challenges of individual nanocarriers along with their therapeutic applications in the field of cancer.

8.2 Various Nanotherapeutics Used for the Delivery of Anticancer Drugs

The advancements of nanotechnology in chemotherapeutics have mainly been to overcome the drawbacks associated with conventional forms of therapy like radiation, treatment with chemical drugs, and surgery, which have an effect not only on the tumor cells but also surrounding area and tissues, thereby leading to toxic effects [7]. Nanoformulations exhibit properties like the targeted release of the API, enhanced cellular uptake of the released uptake, along with having prolonged residence time in the bloodstream for effective pharmacological action [8–11]. The different nanoformulations for drug delivery in cancer treatment, along with their therapeutic applications, benefits, and challenges summarized in Table 8.1, are discussed as follows.

8.2.1 Delivery of Baicalin and 5-Fluorouracil Using Polyamidoamine Dendrimers

PAMAM dendrimers are able to directly conjugate and physically entrap drug molecules, thus making them an ideal carrier for chemotherapeutic drugs. However, they have been reported to cause toxicity due to the presence of amine groups present freely on the surface of the dendrimer. This toxicity is countered when the free amine groups are made to react with anionic or neutral

moieties [12, 13] like PEG [14] or acetic anhydride [15–17] which also results in an increase in the dendrimer's solubility. For instance, Lv *et al*. developed 5 mg baicalin containing PAMAM dendrimers for anticancer activity [18] on epithelial cell and lung tumors [19].

8.2.2 Delivery of Hydroxycamptothecin and Doxorubicin Using Biodegradable Dendrimers

Mainly consisting of polyester chains, these dendrimers get hydrolyzed, either enzymatically or chemically by esterases [20–23]. Biodegradable dendrimers emerged because there was a dire need for carriers with high molecular weight and a large size which got accumulated and retained in tumors while getting easily eliminated through urine. Studies conducted by Ou *et al*. and Kohman *et al*., have tried to directly address the problem of non-specific degradation by introducing target specific sites into the polyester chains [24, 25]. In the study by Szoka and Frechet, there were reports of "bow-tie" biodegradable dendrimers which exhibited enhanced stability with anticancer molecules and a slow degradation profile. However, this poses a problem of low filtration rate of the dendrimers through urine which may lead to toxicity [26]. In spite of this, they are a popular choice for loading of anticancer molecules [21, 27, 28], agents for gene therapy [25, 29, 30], and boron neutron capture agents [17, 28, 31]. For instance, Morgan *et al*. developed 6 μM hydroxycamptothecin containing biodegradable dendrimers for anticancer activity on breast tumors [32].

8.2.3 Delivery of Trastuzumab and Doxorubicin Using Amino Acid–Based Dendrimers

The properties of amino acids summed up in Table 8.1, help the dendrimers to be more specific, selective, and targeted in nature by offering selective sites for interactions with the drugs non-covalently [17]. Amino acids reported to have been used for making dendrimers include leucine [33, 34], phenylalanine [35], valine [33, 34], tryptophane [36], glutamic acid [35, 37], alanine [34], aspartic acid [37] and glycine [34]. For instance, Miyano *et al*. developed 33 mg trastuzumab containing amino acid dendrimers for anticancer activity on breast tumors [38].

Table 8.1 Chemotherapeutic applications using nano drug delivery

S.No.	Nanoparticle	Advantages	Challenges	Chemotherapeutic Application	Refs
1.	PAMAM dendrimers	They exhibit high solubility in water, possess versatile chemical surface groups, and are small in size.	The amine groups causing toxicity are also responsible for the enhancement of delivery of anticancer drugs into the cytoplasm.	5-Fluorouracil containing PEG-folate-PAMAM dendrimers for the KB cell line which causes epidermoid cancer in humans.	[99, 100]
2.	Biodegradable dendrimers	They are biocompatible, exhibit low toxicity, and have a good degradation profile.	Due to their large size, they undergo hydrolysis non-specifically, leading to premature release and toxicity.	Doxorubicin containing PEO (polyethylene oxide) dendrimers for the B16F10 cell line causing melanoma in murines.	[21, 27–31, 101–103]
3.	Amino acid dendrimers	They possess properties of amino acids like optical activity, chirality, biorecognition, and hydrophobic/philic nature.	It shows significant toxicity to cells and fails to exhibit enhanced efficacy.	10 μg/ml doxorubicin containing polylysine dendrimers for the MC-7 cell line causing breast cancer in humans.	[104, 105]

(*Continued*)

Table 8.1 (*Continued*)

S.No.	Nanoparticle	Advantages	Challenges	Chemotherapeutic Application	Refs
4.	Glycodendrimers	They have increased specificity and targeting properties.	They have shown accumulative toxicity in the brain, possibly due to the autophagy initiated by the dendrimers.	100 µmol/L cytarabine containing maltose polypropylenimine dendrimer for the HL-60 and 1301 cell lines causing myeloid and lymphoblastic leukemia in humans respectively.	[106, 107]
5.	Hydrophobic dendrimers	Allows for better encapsulation of hydrophobic drugs within the voids of the dendrimer.	The inclusion of hydrophobic parts may impact water solubility and formulation aspects of the dendrimer.	30 µL of carboxyl hydrophobic dendrimers for boosting lymphocyte (T cell) response against cancer after intradermal injection into BALB/c female mice.	[17, 101, 108–110]
6.	Asymmetric dendrimers	Have customizable molecular weights and structures.	The asymmetric nature of dendrimers gives rise to higher radial decay.	20 µM biotin-SB-T-1214 taxoid containing asymmetric PAMAM dendrimers for MX-1 and ID-8 cell lines causing human breast and ovarian cancers respectively.	[111, 112]

S.No.	Nanoparticle	Advantages	Challenges	Chemotherapeutic Application	Refs
7.	Antibody liposomes	Have improved targeting and specificity properties.	It does not exhibit an impressive pharmacokinetic profile along with being an idiosyncratic immunogenic agent.	0.02 μM fluorodeoxyuridine containing mAb (CC52) liposome for the CC531 cell line causing colon cancer in rats.	[113–118]
8.	Thermoresponsive liposomes	Improve the fusion of tumor cells to the drug and enhance the release of the drug by making the pH alkaline at the site of action.	It could lead to overheating of the local tissues. Macrophages from the spleen and liver can engulf the liposomes.	10 mg/kg gemcitabine containing thermoresponsive liposomes for the MiaPaCa-2 cell line causing pancreatic cancer in mice.	[119–121]
9.	Enzyme-sensitive liposomes	Overexpression of enzymes by cancer cells can be used as a target for modifying drug release.	The enzyme targeting moieties may be susceptible to inactivation due to exposure to high temperatures involved in the manufacturing of these liposomes.	10 μM siRNA containing PSA (prostate-specific antigen) liposome for the PC-3 cell line causing prostate cancer in humans.	[122–125]

(*Continued*)

Table 8.1 (*Continued*)

S.No.	Nanoparticle	Advantages	Challenges	Chemotherapeutic Application	Refs
10.	Nanoemulsions	Improve a lipophilic drug's bioavailability and induce uniformity in its blood plasma concentrations.	They sometimes show low permeability, biocompatibility, and bioavailability issues.	10 mg/kg piplartine containing nanoemulsion for B16-FO cell line causing melanoma in mice.	[126–128]
11.	Chitosan nanoparticles	Biocompatible, biodegradable, and less expensive with a low toxicity profile.	It might show instability as it is difficult to control its pore size.	24.60 μM raloxifene containing HA chitosan nanoparticles for A549 and Huh-7 and HepG2 cell lines causing non-small cell cancer of the lung and liver cancer in humans respectively.	[129, 130]
12.	Silica nanoparticles	Exhibits high porosity, biocompatibility, and easy modification ability for adding functionalities.	The synthesis proves difficult as there is non-uniform size distribution.	10.1 and 12.4 nmol docetaxel containing lactosamine MSNs for SMMC7721 and HepG2 causing hepatoma in humans.	[131, 132]

S.No.	Nanoparticle	Advantages	Challenges	Chemotherapeutic Application	Refs
13.	PLGA nanoparticles	Has high purity, modifiable molecular weight, biodegradability and is approved by US FDA and EMA (European Medicines Agency) for human use.	It undergoes degradation upon being subject to sterility techniques, the amount of the drug loaded may be low, along with poor reproducibility on a large-scale process.	10 mg docetaxel containing folate PLGA nanoparticles for MCF7 and HeLa cell lines causing breast and cervical cancer respectively in humans.	[82, 133–136]

8.2.4 Delivery of Cytarabine and Fludarabine Using Glycodendrimers

The principle on which these carriers are based is the interaction between ligands and receptors. It is reported that as the number of carbohydrate ligands increases, so does the interaction between a ligand and its receptor [39, 40]. Hence, to achieve multivalent ligand-receptor interactions, macromolecules are being developed having a large number of carbohydrate ligands attached to the dendrimeric chain. Furthermore, dendrons functionalized by sugar ligands have also been developed to stimulate immunity against tumor cells [41]. Thus, glycodendrimers are not only used as site-specific anticancer drug carriers but also as immunity boosters [17]. For instance, Gorzkiewicz *et al*. developed 400 μM fludarabine containing glycodendrimers for anticancer activity on lymph cell tumors [42].

8.2.5 Delivery of Paclitaxel and Doxorubicin Using Hydrophobic Dendrimers

It is known that dendrimers need to be water-soluble in order to be compatible for systemic administration. However, recent studies have reported that the inclusion of a little hydrophobicity allows for better solubilization of drugs hydrophobic in nature. Furthermore, functioning just like amphiphilic micelles, hydrophobic dendrimers lead to longer retention of the hydrophobic drug in its core [43, 44]. Moreover, it overcomes the disadvantages of micelles as it does not need a specific minimum concentration to be functional [17]. For instance, Pourjavadi *et al*. developed 1.2 mg/mL doxorubicin and 2 mg/mL paclitaxel containing hydrophobic dendrimers for anticancer activity on breast and cervical tumors [45].

8.2.6 Delivery of Biotin-SB-T-1214 Taxoid and mAb Using Asymmetric Dendrimers

Symmetric dendrimers exhibit high monodispersity and symmetry. However, the inclusion of asymmetry to the dendrimer's structure gives rise to a range of novel structures with different architectures, which has been shown to affect their pharmacokinetic properties favorably [17]. Furthermore, a study conducted by Lee *et al*. included

reports of synthesis of an asymmetric dendron synthesized by click chemistry. It was found that this synthesis had huge potential for introducing bifunctionalities with a target moiety to improve biocompatibility and cell specificity while another part binds to the anticancer drug [46]. For instance, Shah *et al*. developed 1.87 mg/mL MAC4, RA3-6B2, and 1D3 mAb containing asymmetric dendrimers for immune-boosting activity on T and B cells [47].

8.2.7 Delivery of Hesperidin and Fluorodeoxyuridine Using Targeted Liposomal Approach

Usually, specific antibodies for the tumor cells are added to the surface of the liposome, now called ILP (immunoliposomes) [48]. However, this approach cannot be implemented for all cancers as not all cancer cells contain an antigen. Liposomes can induce an immune response in the body simply by entrapping hydrophobic and hydrophilic antigens in its core and the lipid bilayer respectively [49, 50]. The adjuvants could be directly attached to the liposomal surface either through adsorption or chemical bonds [51]. Other targeting ligands may also be added to the liposome–API conjugate for targeting and specificity properties [52]. For instance, Morsy and Nair developed 10 mg hesperidin containing M6P-bovine serum albumin conjugated liposomes for anticancer activity [53] against liver tumors [54].

8.2.8 Delivery of Silibinin and Gemcitabine Using Thermosensitivity-Based Liposomes

These liposomes basically work in the presence of an externally based trigger system which, in this case, is the temperature [39, 55]. Inducing hyperthermia locally can prove beneficial by increasing blood flow to the site of action, the permeability of the endothelium to the liposomes, and the permeability of the target cells to the drug released from the liposomes [52]. To counter the overheating of local tissues, chemotherapy using magnetically mediated liposomes emerged. It mainly includes using nanoparticles of oxides of iron for inducing the magnetic effect to make magnetic liposomes which are utilized for treating drug-resistant cancer, as it shows no major leaching of liposomes [56]. The recommended size range for

optimum pharmacokinetic profile is 50 nm- 200 nm [57]. Coating of liposomes with PEG [58] or including cholesterol in the liposomes to prevent drug leakage, in turn, improves the stability of the entire system and protects it from engulfment by the comparatively large-sized macrophages (150 nm) [59, 60]. For instance, Han *et al*. developed 8 μg/ml silibinin containing thermoresponsive liposomes for anticancer activity [61] against liver tumors [62].

8.2.9 Delivery of siRNA and Antisense Agent Using Enzyme-Sensitive Liposomes

This approach emerged as it was found that people diagnosed with cancer often have high levels of particular enzymes in their bodies. For example, sPLA2 levels rise in pancreatic, breast, and prostate cancers; elastase levels rise in breast, lung, and skin cancers and cathepsin B levels rise in lung, prostate, breast, and brain cancers. One way this approach can be applied is by coating the liposomes with PEG which works as a targeting moiety and stabilizes the attached API sterically thus increasing the pharmacological activity of the drug and reducing its toxicity [52, 63, 64]. For instance, Ghavami *et al*. developed a 50 mM antisense (r_8) nucleic acid–peptide complex containing enzyme-sensitive liposomes for anticancer activity [61] against cervical tumors [65].

8.2.10 Delivery of Apigenin and Piplartine Using Nanoemulsions

Over 90% of novel molecules discovered are lipophilic in nature [66]. Nanoemulsions work toward enhancing the permeation of poorly soluble API transdermally [67, 68]. Due to this property, they can also function as adjuvants to chemotherapy for tumors present in difficult-to-access locations in the body [69, 70]. Furthermore, recent reports indicate the utilization of nanoemulsion technology in chemotherapeutics for sustained release of the drug after an intramuscular injection by facilitating the transport of the drug through the lymphatic system [71]. The potential of nanoemulsions for delivering oligonucleotides to tumor cells has also been explored as interactions have been reported between cell surfaces with a

negative charge and nanoemulsions with a positive charge [72–75]. For instance, Jangdey *et al*. developed 5% w/w apigenin containing nanoemulsion for anticancer activity [76] against skin tumors [77–79].

8.2.11 Delivery of Quercetin and Raloxifene Using Chitosan Nanoparticles

Chitosan, or deacetylated chitin, includes amine groups present freely in the structure of chitosan which provides cross-linkage and controlled release of entrapped API [80, 81]. It works by passive, active [82], and stimuli-responsive targeting. Passive targeting mainly focuses on improving the permeability of the drug as well as its retention in the target cells. For stimuli-responsive targeting, pH-sensitive chitosan microgels were designed by Zhang *et al*. which exhibited swelling after cellular uptake leading to an increase in retention time and prolonged sustained action of the anticancer drug [83]. For instance, Rashedi *et al*. developed 0.5 g quercetin containing chitosan NPs for anticancer activity [84] against colon tumors [85].

8.2.12 Delivery of Curcumin and Docetaxel Using Silica Nanoparticles

MSNs have gained researchers' attention as they offer a large surface area for loading and adsorption of nano-sized drugs [81, 86] and easy transfer of targeting moieties to increase specificity and reduce overall toxicity of drugs [87, 88]. They distribute the loaded drug homogeneously throughout the structure leading to spike-free plasma concentration profiles of the API [89]. Like nanoemulsions, MSNs can be modified to obtain a positive charge to deliver nucleic acids, which are negatively charged. Furthermore, MSNs have been exploited to deliver zero premature release systems for cancer therapy so as to avoid drug release in non-target sites of action. Different approaches like enzymes [90], pH change [91], magnetic particles [92], and photo-stimulants have been utilized to achieve this type of release with MSNs. For instance, Kuang *et al*. developed 10 mg curcumin containing MSN for use in PDT (photodynamic therapy) in chemotherapeutics [93, 94].

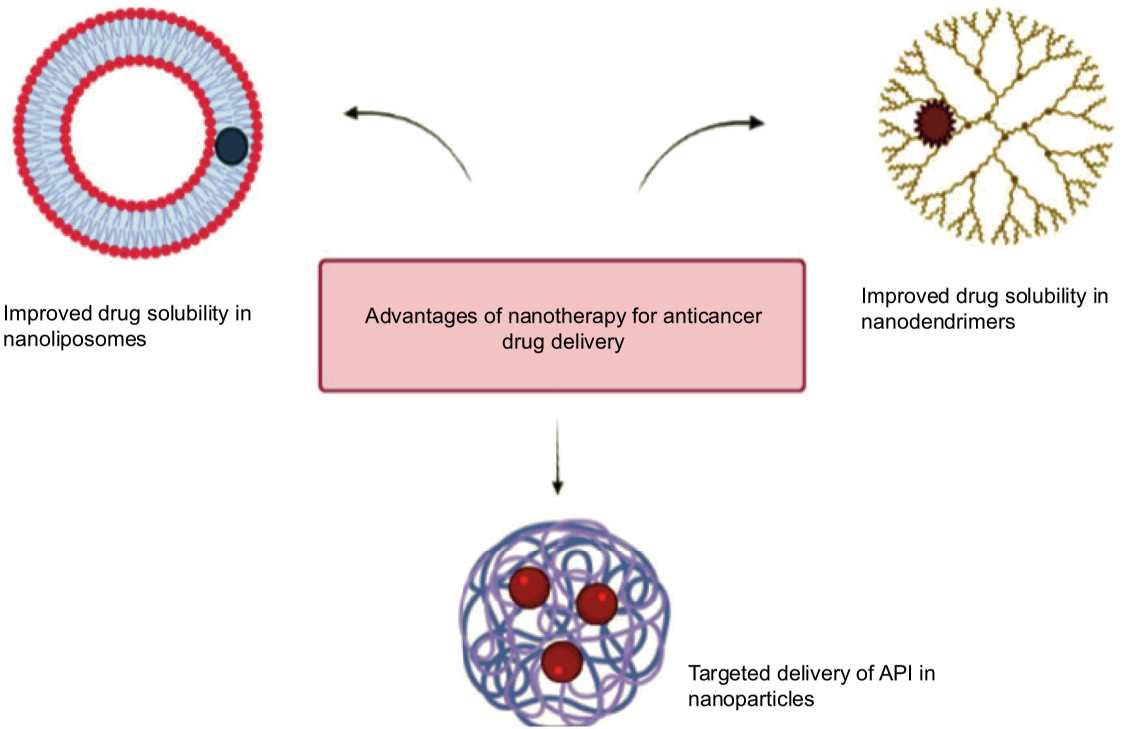

Figure 8.1 Advantages and illustrations of some commonly used types of nanocarriers in anticancer drug delivery.

8.2.13 Delivery of Kaempferol and Docetaxel Using PLGA Nanoparticles

PLGA nanoparticles are usually used for specific and targeted drug delivery as targeting moieties, like hyaluronic acid (HA) for CD44 overexpressed receptors on tumors, can easily be grafted or coated onto the surface of these particles [81]. These nanoparticles can also be used in presence of an external magnetic field used to guide the carrier to the exact site of the tumor [95, 96]. For instance, Luo *et al*. developed 250 μL/ml kaempferol containing PLGA nanoparticles for anticancer activity [97] against ovarian tumors [98].

Nanotherapeutics especially help to reduce the side effects of chemotherapy by targeting and releasing the API, along with prolonging its action, at the exact target site, as illustrated in Fig. 8.1. This helps to achieve optimum therapy and efficacy in the treatment of cancer [137].

8.3 Effect of Nanoformulations to Stabilize Therapeutic Agent

Nanoformulations not only improve the pharmacokinetics and pharmacodynamics of the chemotherapeutics agents but also kill tumor cells without causing damage to the other normal cells, and reduce dose-limiting toxicities [2]. The application of nanotherapeutics is vast and is used in varying fields such as protein and peptide delivery, nano-electrochemical system, implants, cancer therapy, photodynamic therapy, nanotechnology-based nutritive agents, and diagnostic imaging. They surround poorly soluble drugs and protect therapeutic molecules and their blood circulation and tissue distribution are modified.

Nanoparticles get accumulated by active or passive targeting within tumors which are hence involved in vascular targeting, tumor cell targeting, nuclear targeting, and many more [2]. Passively targeted nanoformulations traverse the leaky capillaries surrounding the neoplasm and move into the interstitium. This phenomenon is called EPR (enhanced permeation and retention) [138–140]. This transport mechanism and uptake of the drug by the neoplastic cells mainly depends on the API concentrations in

the environment. However, it becomes very difficult to maintain a concentration gradient as there is constant uptake not only by the cancerous but also mononuclear phagocytic cells [141]. Due to these drawbacks, the concept of active targeting emerged [138]. In active targeting approach, nanoformulations take advantage of certain receptors or antigens characteristic to neoplastic cells. Transfer of a targeting ligand on the nanocarrier enables an interaction between the receptor-ligand complex [142]. It is followed by cellular uptake coupled with minimal side effects to the surrounding environment. Nanotherapeutics can use both these approaches to achieve optimum benefit in the treatment of neoplasms [141–144].

8.4 Conclusion and Future Perspectives

Nanotechnology has wide applications in the field of medicine and is growing to be the most useful and important method in the healthcare system. Nanotherapeutics, which is a part of nanotechnology, plays an important role especially in cancer treatment by proving advantageous over conventional forms of therapy due to its negligible side effects.

There are many studies ongoing currently by scientists to study nanomedicines in detail and overcome their challenges by ensuring they are safe and effective and have prolonged residence time in the bloodstream hence exhibiting efficacious pharmacological action. Research is being conducted regarding the toxicity profile of synthetic nanoparticles and efforts are going on to ensure the nanoformulations are being produced in sufficient quality and quantity to provide fast and effective treatment of cancer. Thus, we can conclude that delivery of anticancer drugs using nanocarriers is a promising approach to nanotherapeutics.

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Chapter 9

Nanoparticle-Associated Toxicity and Concept of Edible Nanoparticles: Promising Therapeutics in Near Future

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9.1 Introduction

Nanotechnology is a useful means for delivering therapeutic biomolecules packaged into nano-sized particles to their specific target [1]. Thus, due to their nano-size, they are able to penetrate through biological and physiological barriers which are normally impermeable for large particulate structures [2]. It was found that

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nanocarriers have been used as drug delivery systems. Examples of nanocarriers include liposomes, solid lipids nanoparticles, dendrimers, polymers, silicon or carbon materials, and magnetic nanoparticles [3]. For example: for the treatment of cancer and to carry anticancer agents such as cytotoxic drugs, chemo modulators, antiangiogenic agents; such nanocarriers can be used [4]. The use of nanoparticles in the field of medicine for the purpose of drug delivery and imaging has been on a rise for the past few years. Recent studies have reported significant advantages of nanotherapeutics for delivering anticancer and other drugs to the target sites of action [5, 6]. The most basic design for targeted drug delivery with or without covalent linkage includes either encapsulation, adsorption, or electronic interaction. The drug to be delivered can be controlled with the use of covalent linkage [3, 7]. Nanoparticles play a crucial role in the direct or indirect production of reactive oxygen species which are taken up by target cells like macrophages or epithelial cells. The interaction of nanoparticles with cell membranes and receptors is associated with the ability to combine nanoparticles with biological molecules which forms protein corona. The level of reduction of superoxide (ROS) production inside or outside the cell regulates the activation of pathways, nuclear factors, and specific genetic programs either directly or indirectly. It has been found that nanoparticles have the ability to interact with membrane receptors leading to possible aggregation of these receptors. These interactions lead to modulation of signaling pathways in target cells and thus could be used for therapeutic purposes [8]. Nanoparticles work by both active and passive targeting, which increases their duration of circulation in the blood and shows prolonged pharmacological effect [9, 10]. Active targeting is obtained through physical stimuli, whereas passive targeting is achieved by using recognition ligands [3]. Nanoparticles provide good pharmacokinetic properties such as enhanced permeability and retention and low toxicity, for clinical treatment for several diseases [7]. They have also proven to increase uptake of drugs by cells and have the potential to be used in MDR (multidrug-resistant) diseases [11, 12].

With the advancement of studies to cure various physiological disorders, it was found that plants are used as nanofactories for the production of nanoparticles, now called edible nanoparticles. Edible nanoparticles are nano-sized extracellular vesicles, having structures

similar to exosomes present in mammalian cells [13]. Plant-derived edible nanoparticles can cross the Blood-Brain Barrier (but not the placenta) [14]. They have been used as natural therapeutics for the treatment of various diseases and hence have great potential for use in targeted therapeutic delivery systems [13, 15], because of their characteristics like morphology, biocompatibility, and biodegradability. They have specific tissue targeting systems and are very much capable to be used for large-scale pharmaceutical production [13]. For example, nanoparticles obtained from gliadin were used as transporters for oral anticancer or lipophilic medications. Plant-derived nanostructures that are obtained from wheat, soybean, corn are used as antitumor medications. Nanoparticles derived from ginger, tomato, lemon, carrots, broccoli have intrinsic therapeutic activity and are used in the treatment of cancer [13, 15]. It was also proved that nanoparticles obtained from grapefruit prevent cancer [16]. Thus, edible nanoparticles provide significant advantages over nanoparticles and hence are explored more in detail in this chapter.

9.2 Nanoparticle-Associated Toxicity

The toxicity of therapeutic nanoparticles is usually attributed to their ability to produce ROS which in turn increases the oxidative stress on the cells. Increased oxidative stress is associated with DNA damage, protein damage, lipid peroxidation, organelle dysfunction, and inflammation [17]. As demonstrated by Hall *et al.*, 2007, nanoparticles when administered intravenously, come into contact with blood cells and may interfere with their function causing aggregation of platelets and hemolysis [18]. They may also interfere with the immune function of the body and lead to toxicity [19]. All these effects disrupt the homeostasis of the cell and may even cause apoptosis. In a study by Huang *et al.*, 2017, nanoparticles were reported to suppress cell proliferation by arresting a phase of the cell cycle, thus leading to cell apoptosis [20]. In a study by Kisin *et al.*, 2007, nanoparticles were found to exhibit genotoxic effects which occurred due to the oxidative damage to genetic material [21]. Furthermore, the biodegradation of nanoparticles upon administration may lead to accumulation and accretion of the components in certain organs like the liver and kidney, giving rise

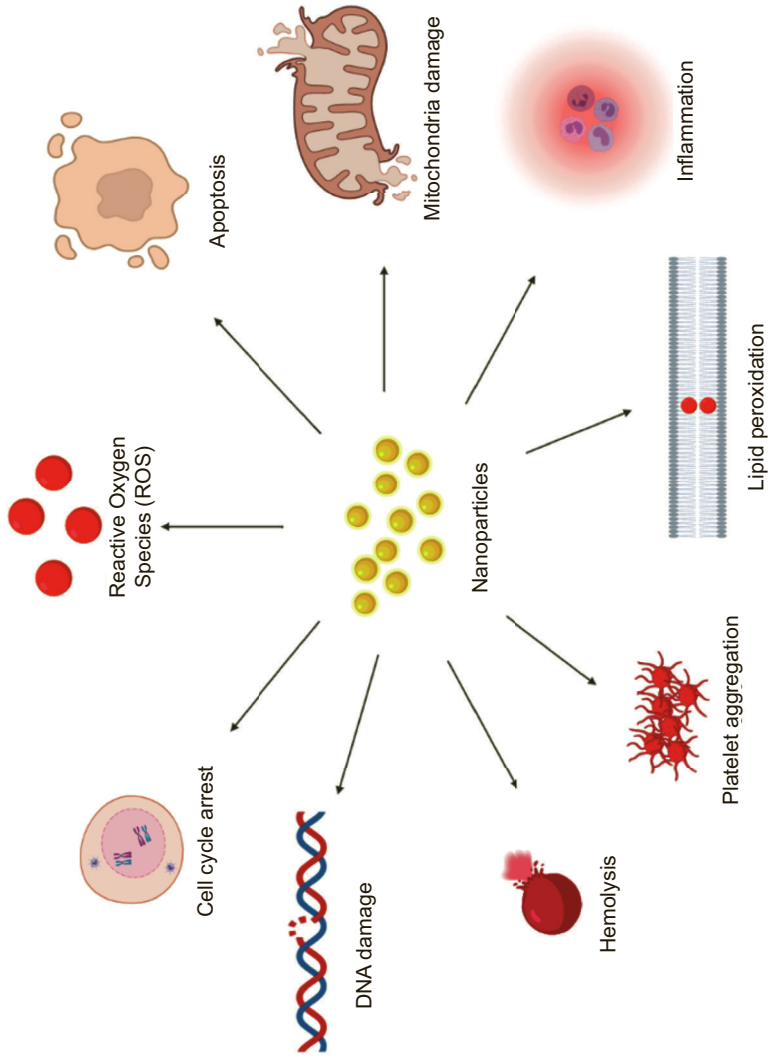

Figure 9.1 Nanoparticle-mediated toxicities.

to toxicity as seen in Fig. 9.1 [19, 22]. There have been reported in many studies summarized in Table 9.1, instances of nanomaterial toxicity which highlights the toxicological profile of nanoparticles.

Table 9.1 Nanoparticle-associated toxicity in *in vitro* cell lines

Organ	Nanoparticle	Cell Line	*In vitro* Effect	Refs
Lungs	CuO	A549 cell line	Oxidative DNA damage, mitochondrial damage, apoptosis	[23]
	Zn Ni Fe Ag	A549 cell line	Mitochondria-dependent apoptosis associated with ROS	[24]
	QD	Human lung adenocarcinoma cells	Mitochondria-dependent cellular apoptosis	[25]
Heart	Carbon nanotubes	Microvascular endothelial cells	Dose-dependent DNA damage	[26]
	Ag	Catla heart cell line (SICH)	Increased lipid peroxidation and decreased levels of GSH, SOD, and CAT	[27]
	Cadmium telluride QD	HepG2 cells	Apoptosis associated with ROS	[28]
Liver	CNT	Human hepatoblastoma C3A cell line	Mitochondria-dependent cellular apoptosis associated with depletion of GSH, ROS dismutase, ROS, IL8, and oxidative damage to DNA	[29]

(*Continued*)

Table 9.1 (*Continued*)

Organ	Nanoparticle	Cell Line	*In vitro* Effect	Refs
	TiO_2	BRL 3A cell line	Mitochondria-dependent cellular apoptosis associated with ROS, reduction of superoxide dismutase, depletion of GSH, and oxidative damage to DNA	[30]
	QD	Primary rat hepatocytes	Increased levels of ROS in the liver, believed to be a consequence of the release of free Cd ions	[31]
Kidney	TiO_2	HEK293 cell line	Genotoxicity	[32]
	CNT	HEK293 cell line	Damage to the cell membrane, lipid peroxidation, reduced GSH levels, and release of LDH	[33]
	CuO	HEK293 cell line	Reduced cell viability	[34]
Brain	CdSe	Primary rat hippocampal neuron cells	Reduced cell viability	[35]
	Au	Zebrafish embryos	Increased permeability and cytotoxicity	[36]
	QD	Neuron like PC12 cells	Axonal degeneration and cell death	[37]

Nanoparticles may be used for targeted drug delivery. However, they have limitations too as they might show *in vivo* toxicity before clinical application and the production scale for synthesizing is also limited. Therefore, to overcome these limitations, nanoparticles are derived from natural sources [13].

9.3 Plant-Derived Extracellular Vesicles as Vehicles for Delivery of Therapeutic Agents

PDEVs act as vehicles for the delivery of therapeutic agents that permit high efficiency of transfection without toxicity or host immune response. It was studied by Rome *et al.*, 2019, that EVs have the ability to bind to hydrophobic agents, increasing their bioavailability and their cellular uptake. For example, lipids derived from plants could bind to hydrophobic agents and deliver siRNA to treat brain tumors.

It was also found that EVs coated with membranes containing specific receptors or drugs are used to increase the specificity of drug delivery. For example, ginger-derived EVs loaded with doxorubicin were used to treat colon cancer and were enhanced when it was conjugated with the targeting ligand folic acid. Therefore, the use of ginger-EV-FA could reduce systemic toxicity of the drug and extend circulation time, and could also cure tumors [38].

9.3.1 Composition of Plant-Derived Extracellular Vesicles and Their Biological Action

EVs, derived from plant extracts, are mainly composed of lipids like phosphatidic acid (PA), phosphatidylethanolamine and phosphatidylcholine; and miRNAs. PA is present most commonly in plant-derived vesicles like grapefruits, sunflower seeds, ginger and helps in cell proliferation and survival signaling whereas phosphatidylethanolamine and phosphatidylcholine, both present mainly in grapefruits, are involved in membrane fusion and regulation of the normal cell cycle. MicroRNAs are small non-coding RNAs that in plants and animals, regulate the level of proteins. It was found in a study conducted by Rome *et al.*, 2019, that these molecules have two important functions similar to plant-derived EVs; namely, to play a role in immune response and cancer-related pathways [38].

9.3.2 Isolation of Plant-Derived Edible Nanoparticles

Based on characteristics such as size, surface-specific proteins, and density; various methods have been used to isolate EVs. Pin *et al.* divided these methods to isolate EVs into five types, which are ultracentrifugation (UC)-based, precipitation-based, immunoaffinity capture-based, microfluidics-based, and size-based techniques (Fig. 9.2). The ultracentrifugation-based technique is the most preferred method for the isolation of PDEVs and its procedure is seen in Fig. 9.3 [39]. Many studies conducted by scientists like Zhuang *et al.*, 2014 and Zhang *et al.*, 2021 [40, 41] and Wang *et al.*, 2014 and Chen *et al.*, 2021 [42, 43] use ultracentrifugation as a method of preparation of ginger, grapefruit, and tea derived nanoparticles respectively.

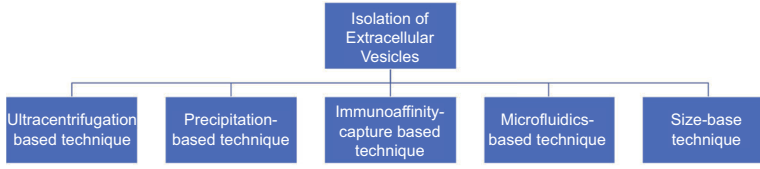

Figure 9.2 Classification of isolation of extracellular vesicles.

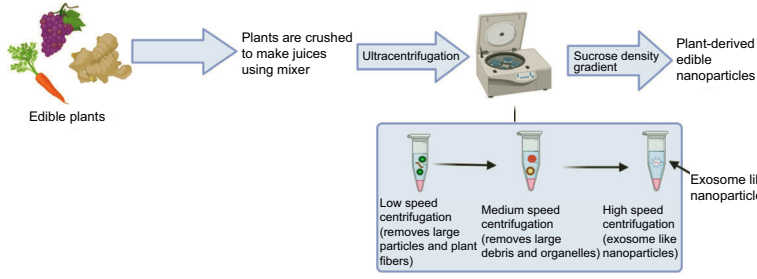

Figure 9.3 Procedure for isolation of plant-derived edible nanoparticles.

9.4 Therapeutic Applications of Plant-Derived Edible Nanoparticles

Edible nanoparticles have been used as natural therapeutics for the treatment of various diseases. Ginger-derived nanoparticles have

been used for the treatment of alcohol-induced liver damage [40]. Lemon-exosome-like nanoparticles are used in the treatment of *Clostridiodes difficile* (C. diff) infection, which causes diarrhea and pseudomembranous colitis in humans [44]. Lemon-exosomes like nanoparticles, by RNase P-mediated specific tRNA decay, enhance lactobacilli toleration to bile which in turn enhances the stress survival of gut bacteria, thus proving helpful to the human body [45, 46]. Mushroom-derived nanoparticles help in the synthesis of metallic nanoparticles and are used in the bio-detection of pathogens, in AIDS, cancer, and as drug and gene delivery systems. These antioxidants are also used as antibacterial, anti-fungal, wound healing agents, and many other pharmacological effects [47]. Many other edible nanoparticles such as ginger-derived nanoparticles, *Lycium barbarum* derived nanoparticles have been highlighted in Table 9.2 given below.

Therefore, from the above table, it was shown that PDEVs are used for the treatment of cancer. In addition to this, multiple scientists are trying to exploit the potential of this novel concept of natural nanomedicines to achieve targeted, non-toxic, and selective drug delivery for the treatment of various diseases [13].

Table 9.2 PDENs and their associated therapeutic use

Plant-derived edible nanoparticles	***In vivo*/*in vitro* cell line**	**Therapeutic use**	**Refs**
Ginger-derived nanoparticles	Colon cancer cells were used for both *in vivo* and *in vitro* study	Used for colorectal cancer therapy	[48]
	RAW 264.7 macrophage and Colon-26 cells were used for *in vitro* study.	Prevents and treat colitis-associated cancer	[41]
	Female C57BL/6 or FVB/NJ mice were used for *in vivo* study		

(*Continued*)

Table 9.2 (*Continued*)

Plant-derived edible nanoparticles	*In vivo*/*in vitro* cell line	Therapeutic use	Refs
Lemon-derived edible nanoparticles	Gastric cancer cells were used for *in vitro* study. The cell lines used are AGS, BGC-823, and SGC-7901	Used as antitumor for gastric cancer	[39]
	For *in vitro* studies, cell lines used were the human lung carcinoma cell line A549 and chronic myeloid leukemia cell line.	*In vitro* used as an antineoplastic activity for different solid and hematologic cancer cell lines	[49]
	LAMA84 cells and cancer cells were used for *in vitro* studies. NOD/SCID mice were used for *in vivo* tumor xenograft model	*In vivo*, suppresses the CML xenograft model	
Exosome-like nanovesicles from edible tea flowers	Breast cancer cells were used for *in vitro* study. Cell lines are MCF-7 cells, 4T1 cells, A549 cells, and HeLa cells	Treats metastatic breast cancer	[43]
Lycium barbarum lipid-derived nanoparticles (LBLNs)	Macrophages were used for *in vitro* study	Protects against ulcerative colitis	[50]

9.5 Discussion

Advances in nanotherapeutics for drug delivery have opened wide avenues for the administration of problematic drugs to the human body for the treatment of various diseases, especially multi-drug resistant [11, 12]. However, there have been multiple reports of toxicity associated with nanoparticles. The most commonly associated toxicity was mediated through oxidative damage and the generation of ROS [17]. Subsequent reactions include an attack on the DNA and RNA causing breakage, generation of protein radicals, impairment of the electron transport system, and ultimate damage to the mitochondrial structure [51, 52]. Multiple efforts have been taken to improve the targeting and specificity properties of nanoparticles by coating liposomes with PEG, thereby reducing the therapeutic dose to reduce the toxic effects of NPs. However, many concerns still remain regarding the adverse effects of synthetic nanoparticles [57]. PDENs are a novel technique of drug delivery that include extracellular vesicles derived from plants being used as carriers for the delivery of problematic drugs. These natural nanocarriers are non-toxic to the human cells, free from idiosyncratic immunogenicity [13, 41], show targeted delivery and biodegradability [13, 15]. Furthermore, PDENs have shown promise in chemotherapeutics by decreasing the cytokine inflammatory mediator levels, inhibiting tumor cell proliferation, and inducing tumor cell apoptosis. The surface of PDENs can also be modified to tune the delivery of PDEN–API complexes directly to different cancer tissues, hence further reducing toxicity [1, 56].

9.6 Conclusion and Future Perspectives

Nanotherapeutics has many uses as drug delivery agents but scientists have discovered some significant cellular toxic effects associated with nanoparticles including activation of inflammasomes and induction of cell stress leading to apoptosis [17–21, 53]. PDENs are the next thing to look out for as they have managed to overcome the limitations of nanoparticles for efficient delivery of drugs *in vivo* [54] as well as show massive applicative potential basis its ability to

be produced on a large scale [13]. These biocompatible nanocarriers do not cross the placenta hence proving beneficial in drug delivery to pregnant women [54]. Multiple scientists are currently adopting studies including PDENs and utilizing them in the delivery of problematic drugs for multi-drug resistant diseases including cancer [21]. PDENs have the potential to act as ideal drug carriers of the future utilizing them in the various fields of drug delivery and therapeutics [55]. Therefore, PDENs are considered safe and biodegradable platforms for curing various diseases.

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