

Advances in Biological Science Research

A Practical Approach

Edited by
Surya Nandan Meena and Milind Mohan Naik



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21.1 Introduction

Nanotechnology refers to the branch of science and engineering dedicated to materials with dimensions in the range 1–100 nm. Nanoparticles (NPs) are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. Morphological and topographical features of NPs play important roles in their potential applications in various fields like biomedical, optical, storage system, magnetic separation, targeted drug delivery, electronics, etc. Consequently, with a wide range of applications available, NPs have the potential to make a significant impact on society. The characteristic feature of NPs is that the physical and chemical properties are significantly different from bulk counterparts [1]. In the case of magnetic nanomaterials, the quantum size effects and large surface area dramatically changes their magnetic behavior and they exhibit superparamagnetic phenomena with quantum tunneling of magnetization because at nano-dimensions below a critical size each particle behaves as a single magnetic domain [2]. The research associated with nanomaterials in the biological field is mostly directed toward their use in medical diagnosis and treatment of cancer-related ailments. The potential for drug delivery systems involving NPs offers several advantages such as (1) the ability to target specific locations in the body; (2) reduction of the drug quantity needed to attain a particular concentration in the vicinity of the target cells; and (3) reduction of the concentration of the drug in the normal cells to minimize the severity of size effects [3]. This chapter gives an overview of NPs in advanced biomedical research. The biomedical roles of metallic, nonmetallic (metal oxide NPs), and carbon-based nanomaterials with special reference to cancer diagnosis and treatment have been discussed.

21.2 Cancer therapy

Cancer refers to a group of diseases characterized by abnormal cell growth with the potential to invade or spread to other parts of the body with the exception of benign tumors which do not spread to other parts of the body. With severe health consequences, cancers are a major cause of death worldwide [4,5]. According to the World Health Organization, worldwide deaths due to cancer are estimated to be 8.2 million, which is about 13% of the total deaths, and this is expected to rise to 22 million by 2030 [6,7]. In the United States alone, 1,735,350 new cancer cases and 609,640 deaths due to different types of cancers are projected to occur in 2018. In India, the estimated number of people living with cancer is around 2.5 million, and every year over seven lakhs are diagnosed for cancer out of which 5,56,400 deaths occur due to cancer-related diseases [8]. The most common types of cancer include breast cancer affecting females, which is the leading cause of cancer mortality next to lung cancer [9]. There is no single cause for cancer; it is caused due to the interaction of many factors together, which may be genetic, environmental, or constitutional characteristics of individuals. The treatments for cancer depend on its type, advancement in the body, all of which have some limitations and side effects [10]. The commonly used cancer treatments include: surgery, radiation therapy, chemotherapy, immunotherapy, hormone therapy, stem cell transplant, and precision medicine. The major disadvantage of the existing cancer therapies is their inability to deliver specific drugs to the target, causing drugs to act on both cancerous and healthy cells, leading to systemic toxicity, which also prevents sufficient drug concentration to be delivered to tumor sites, thus making the cancer treatment deemed to be ineffective. Most of anticancer agents used in conventional methods for cancer treatment are hydrophobic with low solubility and high metabolism, consequently, the bioavailability of drug decreases [11]. Furthermore, the available conventional chemotherapeutic treatments are limited in their solubility, selectivity toward tumor cells and are increasingly multidrug resistant (MDR), and hence, the resistance of tumors against anticancer drug increases [12]. This has generated the necessity for alternative methods for cancer treatment, and one approach that has evolved in the recent past is targeted drug delivery. It works selectively on cancer cells with minimum side effects on normal cells, tissues, and the body as a whole. For an effective cancer treatment, it is desirable to increase the efficacy of anticancer drugs, which can be achieved by specific targeted drug delivery, thereby minimizing the side effects. The recent development of nanomedicines offers tremendous opportunities in specific targeted drug delivery for anticancer therapy. The binding ability and specificity of NPs to bind malignant tumor cells can be enhanced by conjugation with suitable biomolecular ligands and high surface area that may be utilized as carriers for therapeutic and diagnostic agents [13]. Among metallic NPs, colloidal silver, in addition to its antitumor activity exhibits excellent *in vivo*

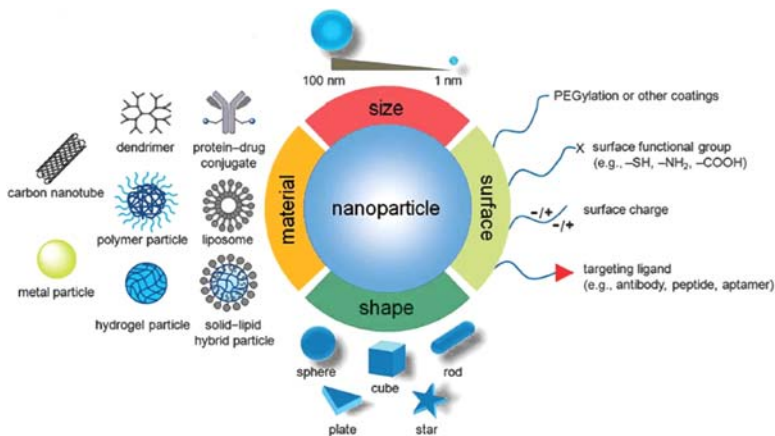


FIGURE 21.1 Physicochemical properties of nanomaterials.

distribution and very low toxicity [14,15]. The *in vitro* antiproliferative activity of silver NPs (AgNPs) is reported on breast cancer cell lines (MCF-7, MDA-MB-231) [16]. Gold NPs (AuNPs) display unique physicochemical properties that can be utilized in building multifunctional platforms for transport of low solubility and poor pharmacokinetic profile therapeutics to the tumor target as well as to sensitize cells and tissues to the treatment [17]. Superparamagnetic iron oxide nanoparticles (SPIONs), due to their low *in vivo* toxicity and high tolerability, offer great advantage in cancer diagnosis as well as anticancer therapy by hyperthermia [18]. Carbon-based nanomaterials such as graphene oxide (GO), reduced graphene oxide (rGO), and carbon nanotubes (CNTs) have been explored as promising drug carriers for targeted drug delivery systems [19]. GO has attracted more attention due to its effective endocytosis, biocompatibility, and large surface area for drug loading. Furthermore, GO and rGO display good dispersing capability in water and physiological environments because of the surface functionalities that may help in forming hydrogen bonds with the associated drug molecules [20,21]. This hydrogen bonding, in addition to the π - π stacking and hydrophobic interaction, can assist drug loading on the nanocarriers [22]. Physicochemical properties of various nanomaterials are illustrated in Fig. 21.1.

21.3 Metal nanoparticles as drug delivery and anticancer agents

Metal nanoparticles (MNPs) have enthralled researchers for over a century and are now profoundly utilized in material science, catalysis, fuel cells, biomedical sciences, electrochemical sensors, and biosensors. MNPs' surface can be suitably modified with different functional groups that help them to

conjugate with antibodies, ligands, and drugs of interest and thus open a wide range of potential biomedical applications. The size- and shape-controlled synthesis of MNPs with high surface area is important in present-day cutting-edge materials. NPs of noble metals such as Pt, Au, Ag, and Cu have potential applications in catalysis and other fields [23–27]. Biomedical functions and applications of noble metals like Au and Ag are presented in the following section.

21.3.1 Gold nanoparticles

Gold nanoparticles are being looked upon as an ideal candidate for various biomedical applications because of their characteristic properties such as small size, unique photophysical features, easy surface functionalization, and biocompatibility (Fig. 21.2). These properties render AuNPs as a versatile nanoplatform for emerging biomedical applications like cell imaging, ultra-sensitive detection, transfection, drug transport and delivery system, antiviral agent, efficient material for photothermal ablation, etc. [28,29]. X. Zheng et al. [30] have obtained the renal clearable ~ 2 nm glutathione-coated AuNPs. The zwitterionic coating was found to minimize nonspecific MPS uptake in balb/c mice. Further, the pharmacokinetic measurements in animal model have indicated rapid distribution and circulation with a blood-elimination half-life

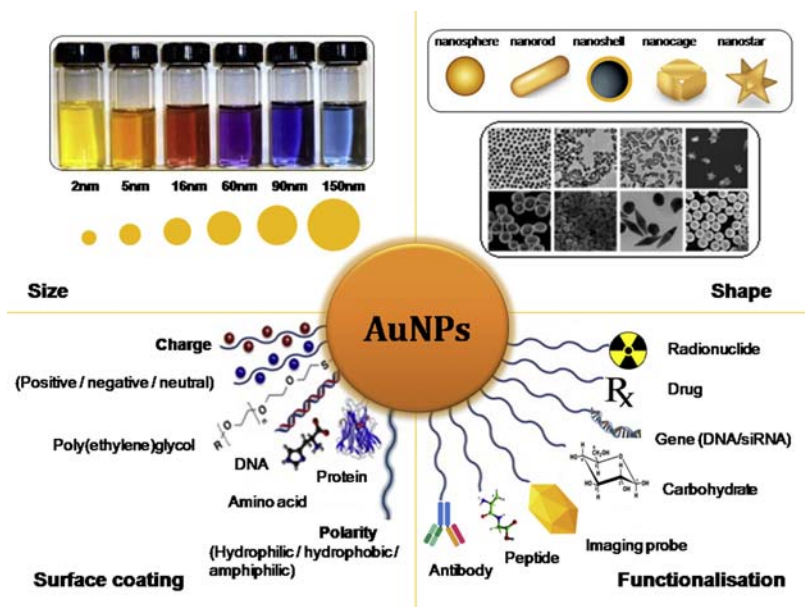


FIGURE 21.2 Schematic representation of AuNP characteristics in biomedical research.

of 12.7 hr. The long blood retention time signifies the ability of these coated AuNPs to passively target tumor-bearing nude mice [31]. K. Huang et al. [32] observed superior cancer cell penetration and in vivo tumor accumulation for N-(2-mercatopropionyl)glycine-coated ultrasmall AuNPs (2 nm) as compared with 6 and 15 nm particles. P. Xu et al. [33] have studied remote control drug release employing supramolecular assembly system and synergistic chemophotothermal therapy for cancer treatment. They have used cucurbit (CB) [7] uril-stabilized gold nanostar (GNS) to encapsulate anticancer drug camptothecin (CPT) via host-guest chemistry. Importantly, the drug release was triggered using near infrared (NIR) light and CB [7] performs a dual role, that is, acting as surfactant to improve stability of GNS in aqueous solution and as cage for intermolecular assembly of CPT molecules. S. Gulla et al. [34] have evaluated the bioactivity of novel tumor vasculature targeting noncytotoxic Au-CGKRRK nanoconjugates. They observed >70% enhancement in overall survivability in melanoma-bearing mice by intraperitoneal administration of the Au-CGKRRK NPs complexed with both PD-L1siRNA and STAT3siRNA; while, the biodistribution study using NIR dye-loaded Au-CGKRRK nanoconjugates has revealed the accumulation of dye in tumor site in the mice. S. Ke et al. [35] have observed higher potency in promoting apoptosis for AuNPs combined with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in nonsmall-cell lung cancer cells. The AuNPs-TRAIL combination causes excessive mitochondrial fragmentation in cancer cells, which is accompanied by dramatic increase in mitochondrial recruitment of dynamin-related protein 1 (Drp1), mitochondrial dysfunction, and enhancement of autophagy induction resulting in increased apoptosis in exposed cells.

21.3.2 Silver nanoparticles

Silver NPs are one of the most vital and fascinating nanomaterials extensively explored in biological and medical research. AgNPs are being employed in various fields such as medical, food, health care, consumer, as well as industrial purposes due to their unique optical, electrical, thermal, and biological properties [36]. Commercially, AgNPs find use as antibacterial agents in industrial, household, health care-related products, in consumer products, medical device coatings, optical sensors, and cosmetics. As well, AgNPs also find biomedical applications such as drug delivery and anticancer agents in targeted drug therapy [37,38]. P. Roychoudhary et al. [39] investigated the antiproliferative activity of AgNPs against leukemic cell lines (K562, MOLT-3, REH) through MTT assay. AgNPs synthesized by *Lyngbya majuscula* displayed dose- and time-dependent activity in REH cells and 4',6-diamidino-2-phenylindole (DAPI) staining clearly revealed the fragmentation of cancer cells due to treatment of AgNPs. J. Blanco et al. [40] have investigated effects of AgNPs on a human lung carcinoma cell line (A549). They have observed decrease in p53, p21, MDM2, and caspase-3 expression after

low dosage daily and high single dosage exposure to AgNPs; the opposite effect was noticed with changed frequency of doses/administration, which clearly indicate time- and dose-dependent antiproliferative activity. M. Khan et al. [41] observed smallest IC_{50} values for A549 with higher cytotoxic activity than the reference tamoxifen for AgNPs decorated with highly reduced graphene oxide. Further, the cytotoxic effect was found to be proportional to the concentration of AgNPs and cell death mechanism due to cell cycle arrest at G0/G1 phase and apoptosis induction. P. Yuan et al. [42] reported the synthesis and cytotoxic studies of GO-AgNPs nanocomposite against human neuroblastoma cancer cells (SH-SY5Y). The GO-AgNPs nanocomposite displayed significant cytotoxicity at lower concentrations. Further, they have established the molecular mechanism of cytotoxicity. P. Netchareonsirisuk et al. [43] have reported the antiproliferative activity of AgNPs capped with sodium alginate and poly(4-styrenesulfonic acid-comaleic acid) sodium salt (PSSMA) against human normal skin fibroblast (CCD-986SK) and malignant melanoma (A375). The sodium alginate-capped AgNPs displayed high selectivity against A375 cell line through apoptosis and necrosis, while PSSMA-capped AgNPs exhibited nonselective toxicity. R. Bhanumathi et al. [44] investigated drug discharge capacity and anticancer effect of folic acid (FA) and berberine (BBR), an isoquinoline alkaloid-loaded AgNPs. They have used BBR encapsulated on citrate-capped AgNPs in conjugation with polyethylene glycol (PEG)-functionalized FA. The in vivo antitumor efficiency of NP-encapsulated drug showed significant restraint of tumor progression. The toxicities behavior of FA-PEG@BBR-AgNPs against different organs was established by histopathological observations.

21.4 Metal oxide nanoparticles as drug delivery and anticancer agent

Metal oxide nanoparticles (MONPs) are important compounds in the materials chemistry field, attracting considerable interest due to the potential technological applications of these compounds. The importance of these materials in different areas such as medicine, information technology, catalysis, energy storage, piezoelectric devices, corrosion protection coatings, and sensing, etc. has driven much research in developing synthetic pathways to such nanostructures. MONPs with different morphological features such as nanorods, nanotubes, nanospheres, nano-hollow spheres, and nanofibrous materials can be conveniently synthesized using different techniques such as hydrothermal, precursor, sol-gel, etc. MONPs have a unique structure, high surface area, unusual redox properties, good mechanical stability, and biocompatibility. For these reasons, MONPs have attracted considerable interest in the fields of biomedical therapeutics, bioimaging, and biosensing and are important components in medical implants, cancer diagnosis and therapy, and in neurochemical monitoring [45–50].

21.4.1 Iron oxide nanoparticles

Iron oxide NPs (IONPs) have been investigated for magnetic properties, which find several important applications such as magnetically mediated hyperthermia for cancer treatment [51], contrast agent MRI [52], treatment of anemia [53], etc. However, the recent studies have indicated that the ability of NPs to generate reactive oxygen species (ROS) can be used in cancer therapy [54,55]. Among iron-based NPs, SPIONs are being explored extensively, especially for their therapeutic and diagnostic applications. SPIONs, in particular magnetite (Fe_3O_4) and maghemite ($\gamma\text{-Fe}_2\text{O}_3$), are considered as means of transport for targeted drug delivery as they can be guided to the tumor site through an external magnetic field and this prevents drug diffusion to the rest of the body. Due to their superparamagnetic nature, SPIONs lose their magnetism and enter the blood circulation when the external magnetic field ceases [56,57]. The effectiveness of NPs in diagnostic or therapeutic applications can be increased by surface coating, which enhances the physicochemical and biological properties of nanomaterials by providing protection against corrosion and environmental degradation. Also, the biocompatibility and colloidal stability of NPs increases, thereby enhancing the drug release at therapeutic site [58]. N. Mallick et al. [59] have investigated the chondroitin-4-sulphate (CS)-capped SPIONs for loading of the anticancer agent doxorubicin hydrochloride (DOX). The in vitro drug release profile indicated 96.77% of DOX release within 24 hr and MTT assay in MCF7 cells has revealed significantly higher toxicity for CS-SPIONs-DOX with IC_{50} value 6.294 ± 0.4169 . S. Mondal et al. [60] have investigated hydroxyapatite (HAp)-coated IONPs synthesized using solvothermal and chemical precipitation for magnetic hyperthermia-mediated cancer therapy. The nontoxic nature of IONPs-HAp was established by trypan blue and MTT assay. The hyperthermia study performed on osteosarcoma cells (MG-63) displayed excellent hyperthermia effect with specific absorbance rate value 85 W/g. They have achieved hyperthermia temperature of about 45°C within 3 min, which could kill nearly all the studied cancer cells within 30 min. C. Saikia et al. [61] have studied FA-tagged aminated starch/ZnO-coated SPIONs for targeted delivery of anticancer drug, curcumin. The cytotoxicity study of the drug-loaded NPs was analyzed by MTT assay in human lymphocytes, liver cancer cells (HepG2), and MCF7, wherein NPs were found to be compatible with human lymphocyte cells and reduce the cell viability up to 61% with 0.5% ZnO concentration in HepG2 and MCF7 cells. The cell uptake efficiency and ROS generation was studied using HepG2 cell lines. The ROS generation was found to enhance with increasing ZnO concentration in the system. N. Moghadam et al. [62] reported improved antiproliferative effect of the nevirapine (Nev) on cancer cell line (Hela) by loading onto chitosan-coated magnetic iron oxide nanoparticles (MIONPs). The in vitro ct-DNA-binding study has revealed DNA aggregation on Nev-loaded MIONPs through groove-binding mode.

21.4.2 Miscellaneous

Photocatalyzed titanium dioxide NPs (TiO_2 NPs) have been shown to eradicate cancer cells. However, the required *in situ* introduction of UV light limits the use of such therapy in humans. Hydrophilized TiO_2 NPs (HTiO_2 NPs) produce ROS *in vivo* when activated by ultrasound to eradicate tumor. HTiO_2 NPs-based sonodynamic therapy generates a high level of ROS both *in vitro* and *in vivo* that can cause destruction of the tumor [63]. Cerium oxide NPs (CONPs) are a novel and very interesting material for radiation therapy, possessing the “smart” capacity to selectively induce the death of irradiated cancer cells by increasing oxidative stress and apoptosis, while protecting the surrounding tissue from radiation-induced damage and oxidative stress. Therefore, CONPs have the unique feature of acting as radio-protecting, as well as radio-sensitizing agents simultaneously [64]. Application of zinc oxide NPs (ZnONPs) has shown that they are most efficacious on cancer cells (T98G), moderately effective on tumor cell line (KB), and least toxic on normal human (HEK) cells. These results demonstrated that treatment with ZnONPs sensitizes T98G cells by increasing both mitotic (linked to cytogenetic damage) and interphase (apoptosis) death [65]. Copper oxide NPs can be used to kill human liver cancer cells. The small spherical NPs generate ROS (such as superoxide anions, hydroxyl radical, and hydrogen peroxide) that damages the membranes and the DNA of the cancer cells, eventually inducing their death. ROS also turned on several death-triggering genes, driving the cancer cells to commit mass suicide. Also, these NPs have shown cytotoxic effects on A549 cells [66]. A. Wani et al. [67] reported that PEGylation of mesoporous silica nanorods (MSNRs) prevented dose-dependent hemolysis in concentration range 0–10 mg/L, improved colloidal stability of MSNR, and increased mitoxantrone (MTX) release. Also, decrease in the IC_{50} of MTX and MTX-loaded MSNR was observed under hypoxic conditions.

21.5 Carbon-based nanoparticles as drug delivery and anticancer agents

Graphene, carbon nanotubes, and fullerenes are important classes of carbon-based nanoparticles. They have a unique pore structure, adsorptive capacity, electronic properties, and acidity. This has generated tremendous interest in these nanomaterials for applications in fields such as physics, chemistry, biology, and medicine. The basic building blocks of all the carbon nanostructures are a single layer of graphite that consists of hexagonally aligned sp^2 hybridized carbon atoms forming a hexagonal honeycomb-like lattice. Graphene is the thinnest two-dimensional single layer of hexagonal packed carbon atoms that has attracted researchers all over the world [68–71]. Graphene oxide is a product of chemical exfoliation of graphite. It is typically synthesized by reacting graphite powders with strong oxidizing agents such as

KMnO_4 in concentrated sulfuric acid. GO is described as a graphene sheet modified with different types of organic functionalities such as carboxyl (-COOH), carbonyl (-CO), hydroxyl (-OH), and epoxy (C-O-C). The presence of these functionalities renders GO hydrophilicity and the potential to improve the solubility of some water-insoluble drugs. Hence, GO is preferred over pristine graphene for drug delivery application [72]. Reduced GO is another most commonly explored graphene material for biomedical applications. It is generally obtained by chemical, thermal, and electrochemical reduction of GO [73]. Both GO and rGO hold great potential in biomedicine such as polymer composites, biological sensors, bioimaging, targeted drug delivery, and photothermal therapy (PTT) [74]. CNTs are allotropes of carbon, which consist of hexagonally aligned sp^2 hybridized carbon atoms interlinked with each other to form a tubular shape with an outer diameter ranging from 4 to 30 nm. Structurally, they are similar to graphite sheets that are rolling upon themselves. The rolled sheets can be single, double, or many walls, and therefore they are named as single-walled, double-walled, or multiwalled carbon nanotubes (MWNTs), respectively. Due to their unique physical, chemical, and mechanical characteristics, these materials have wide applications in various areas including polymer science, biomedical research, energy storage, electrodes, gas sensors, catalyst support, etc. [75,76].

21.5.1 Graphene oxide/reduced graphene oxide for drug delivery

Functionalized GO has been extensively investigated for anticancer therapy because of its high water solubility and biocompatibility. Z. Rao et al. [77] investigated complex of amino-modified GO with carboxymethylcellulose as a carrier of DOX. A cumulative drug release of 65.2% was observed at pH 5. The cytotoxicity studies on Hela cell and mouse fibroblasts (NIH-3T3) cells by MTT assay have indicated good biocompatibility with no cytotoxicity. N. Duran et al. [78] have developed hybrids by coupling small interfering RNA (siRNA)-GO-PEG (6ARM-poly(ethylene glycol)amine-PEI (poly-ethylenimine) as a carrier to administer DOX in nonmuscle invasive bladder cancer (NMIBC) treatment. In vivo studies revealed 60% normal bladder diagnosis for the association of GO-COOH-DOX and GO-PEG-PEI/siRNA. A. Deb et al. [79] demonstrated use of the anticancer drug camptothecin (CPT) loaded onto GO nanomaterial with PEG and FA against MCF-7 by MTT assay. They observed higher cytotoxicity to the cancer cell with CPT loaded onto GO-PEG-FA in comparison with free drug. Also, the nontoxic nature of the drug composite was confirmed by cell viability assay wherein 95% of cell count was observed after 24 hr incubation. N. Hussien et al. [80] investigated aptamer-conjugated magnetic graphene oxide (MGO) nanocarrier for targeted drug delivery cancer treatment. They used nanosize magnetite particles on GO layer with aptamer as a targeting moiety and paclitaxel (PAC) as an anticancer agent. In vitro results have indicated 95.75% entrapment efficiency and

pH-sensitive drug release. Cytotoxicity studies have shown biocompatibility of MGO nanocarrier with over 80% cell viability for fibroblast cell line (L-929) with high cytotoxic effect of PAC-loaded MGO on MCF-7. S. Kiew et al. [81] studied dextrin-conjugated GO nanocarrier as drug delivery system to respond to a tumor-associated stimulus, α -amylase. They observed 1.5-fold higher release and 2-fold increase in the cytotoxic effect of DOX in dextrin-conjugated GO. Also, higher permeabilities through fenestrated endothelial barrier were observed for GO-based nanocarrier. R. Sousa et al. [82] developed hyaluronic acid (HA) functionalized-rGO for cancer PTT. The rGO was obtained by greener route employing L-ascorbic acid as reducing agent and functionalized using hyaluronic acid-based amphiphilic polymer. The functionalization with amphiphile improved its thermal stability, cytocompatibility, and internalization by CD44 overexpressing cells, which indicates its potential for targeted cancer therapy.

21.6 Conclusions

Nanotechnology has provided novel and powerful materials that may be used in the treatment and diagnosis of cancer. However, there are still limitations due to the heterogeneity of the cells used for each tumor model in vitro and/or in vivo, which make it difficult to do a comparison between the different studies. Another limitation is the formation of protein corona when NPs reach the blood and interact with the plasma proteins, affecting in vivo distribution and clearance. Nonetheless, the majority of products, reagents, and drugs being used for the development of these nanoscale theranostic agents have still to be approved by the main supervising agencies, such as the FDA and EMA. Research continues in this area, and more information about the distribution, biocompatibility, and low toxicity for normal tissues is necessary prior to clinical trials. Thus far, there are some questions whose answers still provide no clear understanding about the design and application of NPs, such as pharmacokinetics, biodistribution, and side effects of the nanotherapies, and safety profile of NPs before and after conjugation and toxicity. Even though there is no general mechanism for making NPs universally “nontoxic” to all living cells and all organisms, there are important findings that can be applied for increasing nanoparticle biocompatibility and reducing cytotoxic interactions in vivo and in vitro. Although both metallic and nonmetallic NPs have shown potential to be powerful tools against cancer, they still need further optimization and characterization for complete understanding of therapeutic mechanisms. It is now time to start translating these promising nanoplatforms to the clinical settings toward widespread effective therapy strategies in the fight against cancer.

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