

The new era of nanotechnology, an alternative to change cancer treatment

Ancuta Jurj¹
Cornelia Braicu¹
Laura-Ancuta Pop¹
Ciprian Tomuleasa^{1,2}
Claudia Diana Gherman^{3,4}
Ioana Berindan-Neagoe^{1,5,6}

¹Research Center for Functional Genomics, Biomedicine and Translational Medicine, University of Medicine and Pharmacy "Iuliu-Hatieganu", Cluj-Napoca, Romania;

²Department of Hematology, The Oncology Institute "Prof Dr Ion Chiricuta", Cluj-Napoca, Romania;

³Practical Abilities, Department of Medical Education, University of Medicine and Pharmacy "Iuliu-Hatieganu", Cluj-Napoca, Romania;

⁴Department of Medical Education, University of Medicine and Pharmacy "Iuliu-Hatieganu", Cluj-Napoca, Romania;

⁵Department of Functional Genomics and Experimental Pathology, The Oncology Institute "Prof Dr Ion Chiricuta", Cluj-Napoca, Romania;

⁶MedFuture Research Center for Advanced Medicine, University of Medicine and Pharmacy "Iuliu-Hatieganu", Cluj-Napoca, Romania

Abstract: In the last few years, nanostructures have gained considerable interest for the safe delivery of therapeutic agents. Several therapeutic approaches have been reported, such as molecular diagnosis, disease detection, nanoscale immunotherapy and anticancer drug delivery that could be integrated into clinical use. The current paper aims to highlight the background that supports the use of nanoparticles conjugated with different types of therapeutic agents, applicable in targeted therapy and cancer research, with a special emphasis on hematological malignancies. A particular key point is the functional characterization of nonviral delivery systems, such as gold nanoparticles, liposomes and dendrimers. The paper also presents relevant published data related to microRNA and RNA interference delivery using nanoparticles in cancer therapy.

Keywords: nanotechnology, gold nanoparticles, liposomes, dendrimers, microRNA, RNA interference

Introduction

Malignant cells are resistant to the anticancer action of chemotherapeutic agents and cell division inhibitors, which reflects their ability to change at molecular level and develop tumor survival strategies that activate the angiogenic mechanism, in order to prevent hypoxic conditions and to support nutrient intake. Therefore, the main therapeutic approaches are targeting the hallmarks of cancer, particularly aiming to inhibit tumor angiogenesis. Multiple cellular processes related to apoptosis or cell proliferation lead to alterations in signaling pathways that are responsible for resistance to chemotherapy and drug tolerance in a cancer cell.¹⁻³

The progress of nanotechnology-based screening techniques has led to target-based drug development regimens which increase the survival rate of cancer patients.⁴ Therapeutic agents are becoming highly specific and have a high affinity for various molecular targets, depending on the malignancy's genotype and phenotype.⁵ Cancer treatment strategies include a wide range of combination chemotherapy drugs, in addition to radiotherapy and adjuvant/neo-adjuvant surgery. The main drawbacks related to chemotherapy are the unwanted side effects. Therefore, intensive research is carried out to develop novel therapeutic formulation using specific nanoparticles for targeted delivery in order to avoid the cytotoxic effects on healthy cells.⁶

Nanoparticle-based drug delivery systems show remarkable progress due to their ability to have a "controlled-release reservoir", which can safely deliver therapeutic agents to injury sites or specific cells.^{7,8} For safe use in medicine, nanoparticles must be biocompatible, that is, able to integrate within a biological system without causing immune response or negative side effects when the construct is directly released either into the tumor or into the bloodstream.⁹ Nanoparticles must also provide controlled

Correspondence: Claudia Diana Gherman
Department of Surgery, "Iuliu Hatieganu"
University of Medicine and Pharmacy,
23 Marinescu Street, 400337
Cluj-Napoca, Romania
Email gherman.claudia@umfcluj.ro

drug release, increasing the therapeutic agent's protection and circulation time and thus decreasing toxicity to healthy cells.^{8,10} This can lead to enhanced permeability and retention (EPR) effect.¹¹

Nanoparticles are used in medicine to improve bioavailability,^{12,13} to enhance the delivery of therapeutic agents¹⁴ or to develop novel imaging techniques,^{15–17} in order to assure the control of biological systems for single molecules or groups of molecules.¹⁸ A wide range of nanostructures such as liposomes, nano-diamonds, quantum dots, peptides, cyclodextrin, carbon nanotubes (CNTs), graphene and metal-based nanoparticles are used for diagnostic or therapeutic purposes (Figure 1).¹³ Nanoparticles are able to enhance the accumulation and release of pharmacologically active agents at the tumor site, improve therapeutic efficacy and decrease the intensity of side effects on the healthy tissues. Due to the intrinsic features of nanoparticles, it is possible to integrate both diagnostic and therapeutic agents into a single nanoparticle. These features allow monitoring the biodistribution and accumulation of agents at the target site, and thus, the release of drugs can be visualized and quantified, which may lead to proper assessment of their therapeutic efficiency.^{19,20}

Low size of nanoparticles allows them to cross cellular membranes and avoid detection by the reticuloendothelial

system, thus preventing their degradation. Their high surface area enhances the loading of therapeutic agents, making them ideal for medical purposes.²¹ A wide range of anti-cancer drugs such as rituximab, lestauritinib, carboplatin, paclitaxel, doxorubicin and tyrosine kinase inhibitors have been loaded onto nanoparticles with a potential effect against various cancers, and a superior therapeutic efficacy than free chemotherapeutics.^{22–26}

Due to the high incidence of death among cancer patients, there is an urgent demand for the development of novel and innovative delivery systems for therapeutic agents. For most cases, a single targeted therapeutic agent may not be sufficient,²⁷ and therefore, nanoparticles are designed to assure an efficient delivery.

Our review paper summarizes the most recent data in the field of nanoparticle design and functionalization in the context of targeted and efficient therapy. The manuscript presents in a well-structured manner the newest developments in the field of nanotechnology, one of the most rising research niches from our times. Besides the basic strategies, we also discuss the latest forms of therapy involving nucleic acid delivery at targeted sites in the form of microRNA (miRNA) and small interfering RNA (siRNA). The present paper is also complemented by the comprehensive presentation of the chimeric antigen receptor (CAR) T cells therapy,

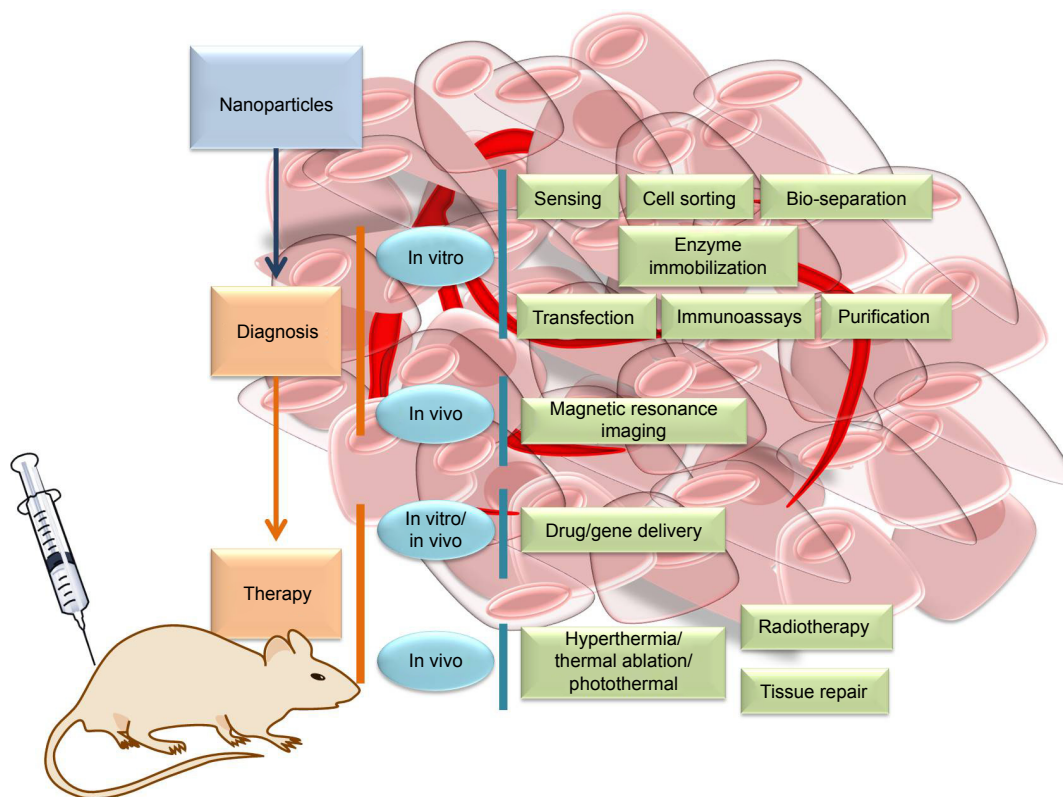


Figure 1 Different applications of nanoparticles involved in therapy and diagnosis.

a novel domain with high-ranking chances of success in the clinical scenario. Therefore, we hope that this review will feed the need of both researchers and clinicians in the attempt to develop and implement superior forms of therapies for the benefit of patients. Moreover, the comprehensive presentation of the current achievements will stimulate the idea of hybrid nano-strategies that will combine their advantages of different therapies, minimizing at the same time the downsides associated with each type of therapy.

Kinetics, biodistribution and release profile of nanoparticles

The kinetics, biodistribution and release profile of the active compounds are modified by the nanodrug formulation, thus improving cancer treatment while enhancing EPR. To be more effective, the nanodrugs must be accumulated at the target tissues, for which the nanoparticles must be smaller than the mean pore size of the vasculature of the target tissues.

Nanodrugs are complex, and scientists must understand their structure and physical and chemical characteristics, as well as the biological principles employed to attach or encapsulate the therapeutic agents.²⁸ Drugs could be selectively targeted to tumors through “active targeting” by using a peptide or an antibody that specifically binds to a molecule that is selectively expressed on targeted cancer cells. Drugs should “passively target” cell-specific functions or local environments in order to facilitate the uptake and accumulation in tumor tissues and inflammatory sites (Figure 2).²⁹ In addition, there are some nanoparticle properties that determine their *in vivo* distribution, such as particle size, charge, core, surface properties, shape, flexibility, multivalence and controlled synthesis.

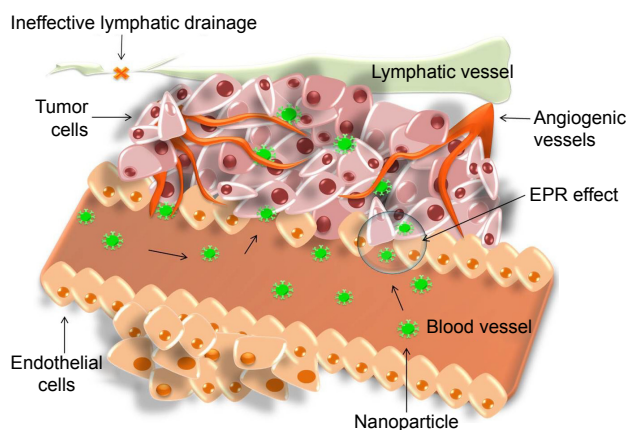


Figure 2 Passive targeting relies on cell-specific functions or local environments specific to target the tissue to facilitate uptake and accumulation in tumor tissues and inflammatory sites.

Abbreviation: EPR, enhanced permeability and retention.

Key areas of investigation in nanoparticle research are the pharmacokinetics and biodistribution, related to the nanoparticle size and its behavior. It was shown that the optimal size for the drug delivery systems is between 1 and 100 nm. Small nanoparticles increasingly accumulate and penetrate into tumor tissues through the EPR effect, with elimination in the spleen being avoided. Multivalence is characterized by a high surface area-to-volume ratio, which offers high loading capacity for different imaging agents, targeting ligands and therapeutic agents. The shape of nanoparticles influences their internalization into cells, determining the *in vivo* behavior and biological function (Figure 3). To improve or reduce circulation time, the surface and charge of the nanoparticles can be changed; positive charge is correlated with a higher rate of nonspecific internalization and a shorter blood circulation time, when compared to negative and neutral charges.¹⁹

An important mechanism by which therapeutic agents are uptaken by the cells is the endocytic pathway. After passing through the cellular membrane, these agents are entrapped in endosomes and are degraded in lysosomes by specific enzymes. The main advantage of this mechanism is that it facilitates the endosomal escape and ensures cytosolic delivery of the agents, which helps achieving an effective biological-based therapy.

There are various mechanisms for endosomal escape that facilitate the release of the therapeutic agents into the cytosol. Pore formation occurs through the interactions between the membrane tension that extends the pores and the line tension that closes the pores. This causes the forming of pores

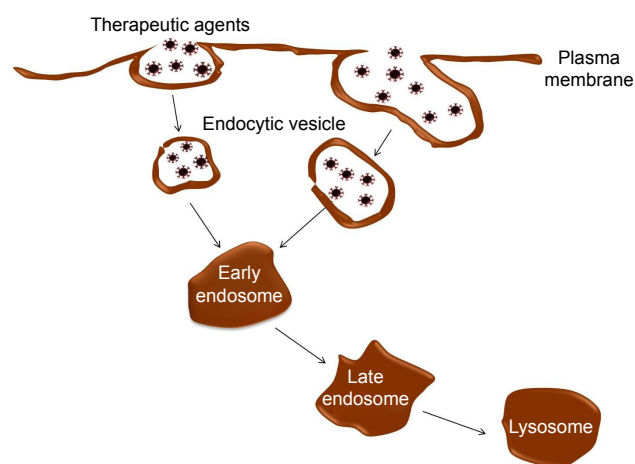


Figure 3 Nanoparticles internalization. Nanoparticles enter the cell via endocytosis, which is the main pathway for crossing the cellular membrane. Also, nanoparticles are internalized into the cells, and the cargo is released inside. Nanoparticles administered are cleared in the liver and spleen, which remain in these organs for a long time and are then uptaken by macrophages. Then, the nanoparticles exit the cell via exocytosis.

in the lipid bilayer mediated by aggregates of peptides that enter into the membrane in a perpendicular orientation, thus leading to an inward curving of the membrane. The proton sponge effect (pH-buffering effect) is a mechanism caused by the ability of agents to inflate when protonated. Through this step, called “protonation”, the entrapped components are released due to the inflow of ions and water into the endosomal environment, leading to endosomal membrane ruptures. Another mechanism is represented by the fusogenic peptides that have the ability to destabilize the endosomal membrane, which plays an important role in cellular trafficking and endocytosis. Endosomal membrane can be destroyed photochemically, by exposure to light. This method induces the formation of reactive singlet oxygen with a short lifetime which destroys the endosomal membrane, allowing the therapeutic agent to be delivered to the cytosol.³⁰

A nanoparticle's intracellular fate is based on the selected endocytic pathway. However, many researches showed that the transport pathway can be affected by the physicochemical characteristics of nanoparticles, such as size, charge, shape, different endocytic machineries in various cell types,³¹ aggregation state and surface chemistry.³² Peñaloza et al showed that nanoparticles can be taken by recycling endosomes back to the extracellular environment, or may be degraded in lysosomes or trapped in an organelle, without releasing their content at the desired site.^{13,33}

Delivery systems used in cancer research

This section highlights the ideal delivery systems used in cancer therapy, such as vectors, gold nanoparticles, liposomes, hybrid systems, dendrimers and CNTs. The main purpose of this section is to describe delivery systems' designs, and to present their benefits and effects in the medical field. Novel nanoparticles that can target multiple altered mechanisms represent an important tool in cancer treatment and can be employed in viral and nonviral delivery systems,³⁴ each of which has its specific advantages and disadvantages (Table 1).

The optimal way to deliver gene therapy is by direct administration of the therapeutic gene to the target site. However, this is extremely inefficient, unreliable and feasible only in tumors. Generally, in gene therapy approaches, the genetic material is delivered via the intravenous route; as nucleic acids are susceptible to degradation by nucleases and rapid clearance in systemic circulation,²⁸ a vector is required to pack, protect and transport the genetic material to its site of action.

Viral delivery systems

Viral vectors are viruses capable of delivering genetic material into specific cells with the purpose of increasing gene expression or inhibiting the production of a target protein.^{35,36} Among the viral vectors used for gene delivery are adenoviruses, retroviruses and lentiviruses.³⁴ Viral vectors are efficient in gene delivery and expression, but their drawbacks, such as low transgenic size, high cost,³⁴ immunogenicity, oncogenicity³⁵ and toxicity,³⁶ limit their use. Retroviruses can be used for miRNA delivery inside somatic and germline cells. These types of viral vectors belong to RNA virus family, and their size is between 7 and 11 kb. The cargo is delivered and integrated inside the target cell's genomic DNA during the mitotic phase of the cell cycle, infecting just the dividing cell. Lentiviruses belong to the retrovirus family and incorporate the foreign genetic material inside the host genome. These viruses are able to affect both dividing and nondividing cells through infecting postmitotic and terminal differentiated cells. Lentiviruses exhibit a high transfection efficiency and long-term stable expression. Adenoviruses contain double-stranded DNA (dsDNA) and are specific for miRNA gene delivery. Similar to lentiviruses, adenoviruses infect both dividing and nondividing cells.³⁷ In addition, several tests employ adeno-associated virus-mediated gene delivery, which has the advantage to overcome resistance to conventional anticancer therapies^{38,40} and lead to a cell differentiation inhibition.³⁹

Nonviral delivery systems

The alternative vectors available for drug delivery are nonviral vectors. Due to their cationic charge, these nanostructures interact with negatively charged DNA or RNA structures through electrostatic interactions obtaining cationic polymers (polyplexes) and cationic lipids (lipoplexes) (Figure 4).³⁵ Cationic polymers are completely soluble in water and do not contain a hydrophobic moiety. They can be synthesized with different functional groups that are attached by substitution or addition, in different lengths and with different geometry. Cationic lipids are amphiphilic molecules that contain positive charges. Through the positive charge, lipoplexes are bound to a hydrophobic domain including two alkyl chains. This charge is associated with an amine group with different degrees of substitution, that is, amidine, guanidium and pyridinium. Cationic polymers differ from cationic lipids in some properties such as chemical structures, nucleic acid interactions and their behavior inside the cell.³⁹ Nonviral vectors can be delivered through physical as well as chemical methods. When delivered through physical methods, the

Table 1 Examples of therapeutic nanoparticles conjugated with different types of molecules

Nanoparticle type	Size	Molecules used for nanoparticles conjugation	Advantages	Disadvantages
Polymer gold nanoparticles	2–10 nm	Small molecule (curcumin)	Effective delivery and extended release; increased the binding capacity for small molecule; safe and nontoxic in in vitro test	Insolubility of small molecule under aqueous condition and low internalization rate ^{80,81}
Gold nanoparticles	4 nm	WGA-HRP	Lower dose delivered at the target organ; this type of conjugated nanoparticles has the potential to eliminate unwanted side effects by requiring a smaller effective dose	Side effects such as nausea and seizures, nonspecific biodistribution and neuronal hyperactivity ⁸²
	11.2 nm	miR-21	Targeted BCL-2 genes and c-Met protein levels, increased apoptosis and diminished proliferation of glioma cells	Absence of significant adverse side effects and safe as delivery system ⁸³
	12 nm	Tyrosine-kinase (lestauritinib, sorafenib and quizartinib) and FLT3 inhibitors (lestauritinib, midostaurin, sorafenib and quizartinib)	Inhibition of BCR-ABL and FLT3 genes	Increased resistance to chemotherapy and higher risk for disease relapse ²²
	41.16±14.30 nm	cmHsp70.1 monoclonal antibody	Improved detection of tumors; in perinuclear area, the aggregates with diameter between 1 and 2 µm are detected; effective accumulation at the tumor site; enhanced radiation therapy by increasing the rate of tumor cell death	No toxic effects at concentrations lower than 1–10 µg/mL ⁸⁴
Cationic arginine-functionalized gold nanoparticles	50 nm	Doxorubicin	Increased cellular uptake and cytotoxicity towards the MDR and transported the drug across the blood–brain barrier	Cardiotoxicity and myelosuppression ⁸⁵
		Oxaliplatin	Increased in vitro cytotoxicity after conjugation, and conjugated particles were efficiently uptaken	No selective toxicity
		Platinum (IV) (cisplatin, the active analog of Pt[IV] prodrugs)	Increased cytotoxic effects of the Pt(IV)-AuNP complex and free active form of the drug (cisplatin)	Kidney toxicity and irreversible nerve damage
Liposomes	179±8 nm	siRNA	High effective siRNA transfection strategy, and cytosolic delivery of siRNA avoiding endosomal sequestration; effective for in vitro applications	No side effects ⁸⁶
Liposomes	80–100 nm	EGFR, doxorubicin, epirubicin, vinorelbine	Increased antitumor effect (nude mice with MDA-MB-468 breast cancer cell line overexpressing EGFR)	Low tumor internalization rate for intravenous treatment, retrieved in interstitial space being degraded and unspecific drug release ⁸⁷
	90–100 nm	Anti-HER2 monoclonal antibody fragments, doxorubicin	Enhanced antitumor effect and increased drug accumulation into the cancer cell line (nude mouse with HER-2 overexpressing Bt-474 breast cancer cell line) ⁸⁸	
	~100 nm	Folate, doxorubicin	Increased antitumor effect (murine lung cancer)	Targeting efficiency of folate-linked vesicles was affected by the amount of folate-PEG-lipid; a higher molar fraction of folate-PEG-lipid in folate-linked liposomes decreased liposome uptake into cells ⁸⁹
	100 nm	Nucleosome-specific monoclonal antibody 2C5, doxorubicin	Enhanced cytotoxicity and increased drug accumulation into the cancer cell line in vitro and in vivo (in murine breast or lung cancer)	Introduction of new side effects such as skin toxicity manifested as hand–foot syndrome and mucositis ^{90,91}

(Continued)

Table 1 (Continued)

Nanoparticle type	Size	Molecules used for nanoparticles conjugation	Advantages	Disadvantages
	<200 nm	Thiolated herceptin, paclitaxel, taxol	Enhanced cellular uptake in vitro and antitumor effects in vivo against BT-474 breast cancer cell line	Side effects given by drugs; due to PEGylation, PILs exhibited a steric effect that proved to be more cytotoxic than taxol, PLs and herceptin ⁹²
Dendrimers	17 nm	Paclitaxel (paclitaxel-conjugated generation 5 PAMAM dendrimers)	Had the ability to stall mitosis in a dividing cell	Cytotoxicity was due to paclitaxel-stabilizing microtubules; necessity for further careful toxicity studies ⁹³
	29.1±3.9 nm	Trastuzumab – monoclonal antibody in combination with docetaxel	Induced apoptosis and enhanced cellular uptake; trastuzumab-conjugated dendrimers caused lower hemolysis than plain dendrimers	Side effects: PAMAM dendrimers caused hemolysis because of their cationic nature ⁹⁴
	50–100 nm	Doxorubicin-conjugated dendrimer nanoparticles using glycyphenylalanyl-leucylglycine tetrapeptide (Gly-Phe-Leu-Gly, GFLG)	Improved anticancer activity, decreased the growth inhibition and increased the accumulation of dendrimers-conjugated doxorubicin at the tumor site	Side effects due to doxorubicin and accumulation of dendrimers in the kidney, liver and brain rather than the solid tumor tissue ⁹⁵
Carbon nanotubes	200 nm	Paclitaxel (multiwalled carbon nanotubes functionalized with hyperbranched poly citric acid and paclitaxel)	Increased cell penetration	Exhibited a higher cytotoxic effect compared with unconjugated paclitaxel; accumulation in internal organs, and not safe ^{77,96}
Single-walled carbon nanotubes	2–3 nm	Doxorubicin	Loading of doxorubicin onto SWNTs reduced the toxicity associated with free drugs; doxorubicin is directly loaded on the carbon nanotubes surface; stable drug loading, significantly prolonged blood circulation, less toxicity and increased tumor uptake by the EPR effect	Side effects due to the free doxorubicin that caused disruption of the intestinal lining consistent with gastrointestinal mucositis, and complete loss of columnar epithelial cells at the tips of villi; accumulation in internal organs, and not safe ^{77,97}

Abbreviations: WGA-HRP, wheat germ agglutinin horse radish peroxidase; MDR, multidrug resistance; AuNP, gold nanoparticle; siRNA, small interfering RNA; PEG, polyethylene glycol; PILs, PEGylated immunoliposomes; PLs, PEGylated liposomes; PAMAM, polyamidoamine; SWNTs, single-walled carbon nanotubes; EPR, enhanced permeability and retention.

vector delivers the gene to the target by applying a physical force that increases the cell membrane permeability. These methods cause cell injuries and increase the apoptotic rate, but are unable to prevent nuclease cleavage.³⁷ The physical methods used are electroporation, ultrasound, microinjection and hydrodynamic applications. Meanwhile, natural and synthetic viral vectors can be delivered through chemical methods to deliver the gene to the target. These delivery systems were developed to improve the ligand's ability to attach on the surface, or to be able to encapsulate and deliver foreign genetic materials into the specific cell type. The main advantages are represented by the low immunogenic response,

capability to carry large inserts, selected modifications,³⁴ easy synthesis and cell-/tissue-specific targeting.³⁵ Viral and nonviral delivery systems have different features, which are detailed further. Due to their ability to transfer their genetic material into host cells, viral vectors present higher transfection efficiency.^{37,40} However, there are difficulties in large-scale production mainly due to the size of the carried DNA, mutagenesis,⁴¹ toxicity and immunogenicity,³⁷ which limit the viral vectors progression. Nonviral vectors have the ability to deliver nucleic acids into cells, with lower transfection efficiency than viral vectors,⁴¹ but are safer,³⁷ protect the cargo from the immune system and can manage

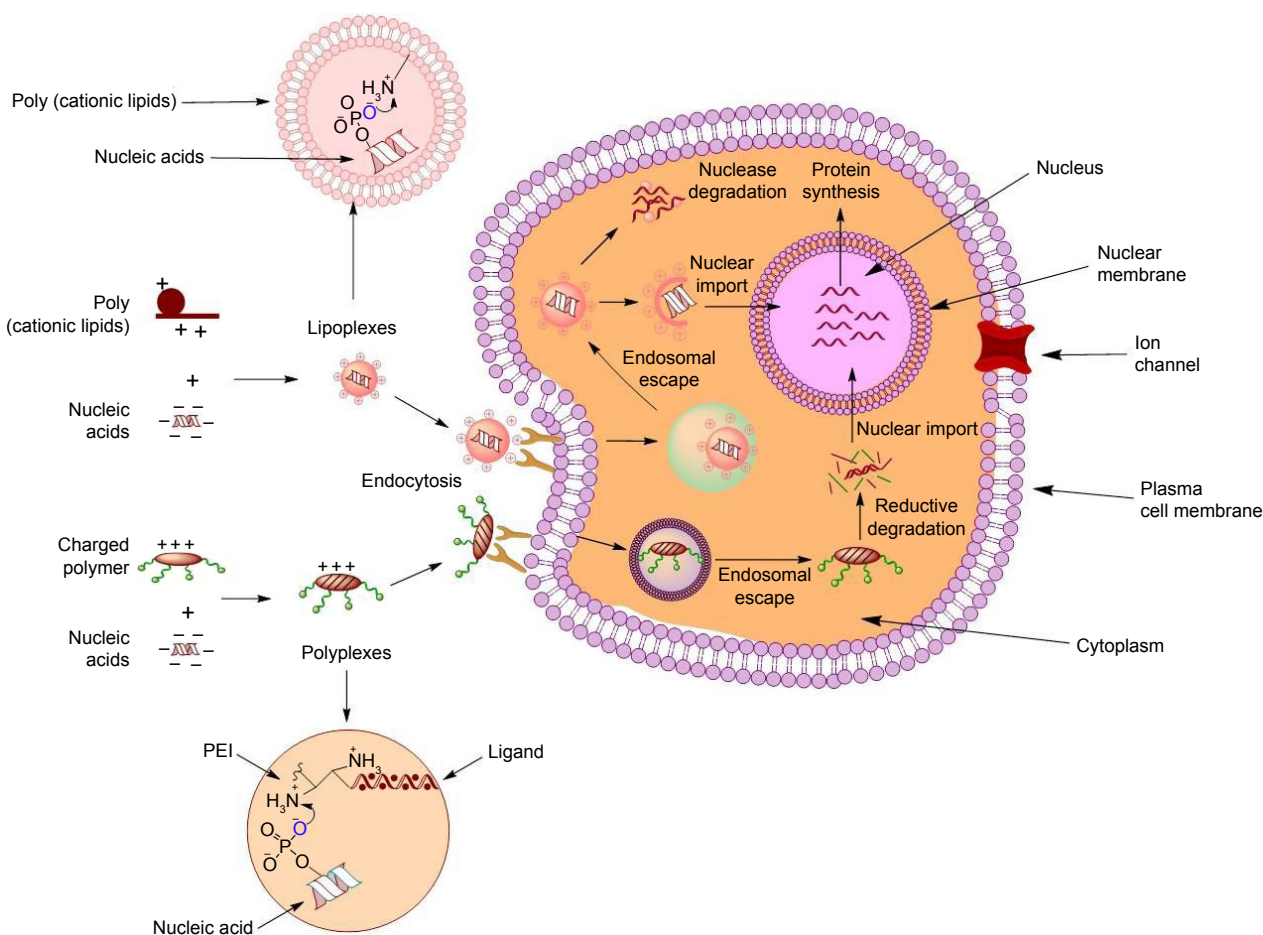


Figure 4 Nonviral gene delivery using lipoplexes and polyplexes. Nucleic acid is complexed with these two types of nonviral delivery systems, and it is internalized through receptor-mediated endocytosis. A large amount of complexes are degraded after their internalization in the endosomal compartments. Only a small fraction enters the nucleus and elicits desired gene expression.

Abbreviation: PEI, polyethylenimine.

larger DNA fragments.⁴⁰ In cancer therapy, it is important to use efficient vectors that can surpass different natural barriers such as extracellular and intracellular membranes, and deliver the genetic material to its target site.⁴¹ In addition, the side effects such as toxicity, mutagenesis and immunogenicity must be avoided by using materials that are biodegradable and compatible with the systems.

Gold nanoparticles

Gold nanoparticles are the key focus of biomedical research due to their physical–chemical properties such as shape, surface area, amphiphilicity, carrier capabilities and biocompatibility. However, because of various drawbacks such as low encapsulation efficiency, poor storage stability and slow endosomal escape, the use of these nanoparticles is limited.⁴² Gold nanoparticles can be conjugated with small strands of RNA such as miRNA and siRNA, or a wide range of small molecules or monoclonal antibodies through physical

and chemical bonds, leading to various sizes. By loading nanoparticles with RNA, the genetic sequence becomes a target for the cancer cell. siRNA and miRNA perform gene silencing, with siRNA inhibiting the translation of mRNA and miRNA cleaving the mRNA.⁴³ Nanoparticles are conjugated with monoclonal antibodies to target a variety of mechanisms, from the simple blocking of the antigen receptor in the effector cells, to cytotoxic action of the cells that express the corresponding antigen. Therapeutic applications include the development of targeted drug delivery systems, hyperthermia, regenerative medicine or radiotherapy. These nanostructures gain attention in various medical fields due to their advantages. Moreover, gold nanoparticles exhibit a low cytotoxicity to the normal cells,⁴⁴ increase the lifespan of the cargo in the bloodstream,⁴³ enable easy size control, improve surface chemistry,⁴⁵ increase therapeutic effects, increase accumulation of drug into the cancer cells and improve pharmacokinetic effects and biodistribution.^{46,47}

Liposomes

Liposomes were the first colloidal drug carriers⁴⁸ used in gene therapy⁴⁹ and are used for targeted delivery of natural or synthetic chemotherapeutics. They consist of a phospholipid bilayer surface enclosing an aqueous core.^{8,50,51} Liposomes are closed spherical vesicles⁵² and can encapsulate both hydrophilic and hydrophobic drugs,⁵³ which can be released through diffusion or cell internalization. Liposomes have a structure of hydrophilic heads stabilized by surfactants, and multiple hydrophobic tails (Figure 5).⁸ Due to this structure, aqueous hydrophilic components can be entrapped in the interior, while the lipophilic components can be incorporated between the lipid bilayers. These carriers are attractive for drug designing due to their biodegradability, biocompatibility, low toxicity, ability to encapsulate multicomponents⁵⁴ and ease of surface manipulation, and have been approved for multiple clinical trials.⁸ However, liposomes have several disadvantages such as low encapsulation efficiency, poor storage stability, easy oxidation of liposomal phospholipids and short release time.⁵⁴

Liposomes are widely used to carry the drug inside the lipid bilayer and transfer the contents to plasma proteins by diffusion.⁵⁵ Liposomes have been clinically used to improve drug

delivery to tumor sites and diminish the side effects of chemotherapy or antimicrobial therapies,⁵⁶ as well as to enhance specificity to injurious sites.⁴⁸ The stability of liposomes is influenced by the lipid composition and structure, and this contributes to the optimization of liposomal product design.²⁹ The stability of liposomal nanostructures includes multiple aspects, such as colloidal and biological stability. Should colloidal stability lack, liposomes form larger-sized particles, and their efficiency as delivery systems is reduced.⁴⁸

Encapsulation of drugs into liposomes has allowed the delivery of therapeutic agents to the target⁵⁷ and also avoided their uptake by the reticuloendothelial system.^{58,59} Due to specific stimuli present at the tumor site, the liposomes are able to target the tumor cells and release the chemotherapeutic agents, which are encapsulated into the nanoparticles.⁶⁰ Liposomal encapsulation of doxorubicin, a topoisomerase II inhibitor,⁵⁸ changes its pharmacology and pharmacokinetics and leads to enhanced drug delivery into tumor sites⁶¹ and reduced toxic effects, in comparison to classical treatments.⁵⁹ Liposomes release their load in the tumor vessels, which then diffuse through the vascular sites into the distal tumor areas.⁶² Tissue distribution and pharmacokinetic profile of doxorubicin are altered by the

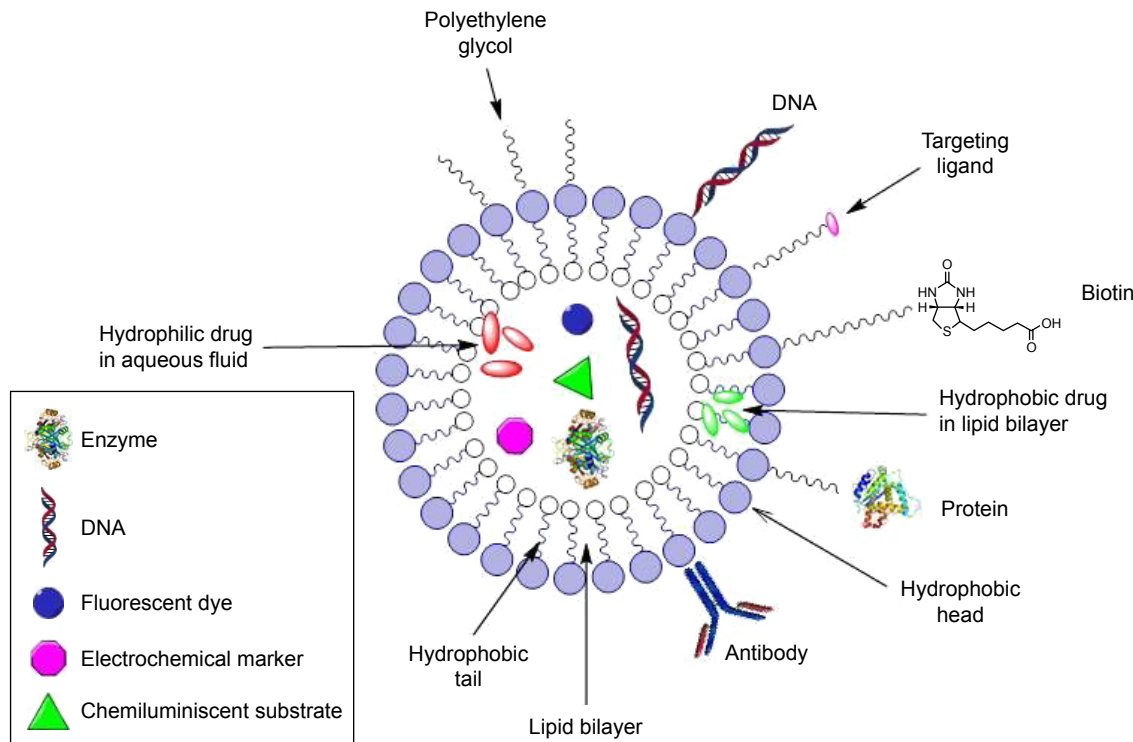


Figure 5 Structure of liposomes. Liposomes are colloidal drug carriers consisting of a phospholipid bilayer surface enclosing an aqueous core. Hydrophilic components can be entrapped inside the aqueous core, while the lipophilic components can be incorporated between the lipid bilayers. On the liposomes surface, different particles that target the interest cells can be attached. To avoid the immune system response, the liposomes surface is loaded with a polymer called polyethylene glycol. Thus, the cargo is protected and is discharged into the target cells.

PEGylated liposomal doxorubicin, which decreases the rate of left ventricular cardiac dysfunction and symptomatic congestive heart failure.^{58,63}

Hybrid systems

The polymer–lipid hybrid system is a mixture of polymeric nanoparticles and liposomes. The components involved in the hybrid system design present interesting features for potential use in cancer therapy. The core of the hybrid system consists of a biodegradable hydrophobic polymer that allows the encapsulation of water-soluble drugs and thus assures a continuous release. To increase the circulation time in the bloodstream and avoid immune system response, the hybrid system is coated with a hydrophilic shell. Between the hydrophobic core and the hydrophilic shell, the system has a lipid monolayer that prevents diffusion of encapsulated drugs and reduces water penetration inside the nanoparticles.⁶⁴ Chavanpatil et al developed a polymer–surfactant hybrid system for encapsulation of water-soluble drugs and enhancing their release, consisting of polymer (sodium alginate) and dioctylsodium sulfosuccinate (Aerosol OT [AOT], which is an anionic surfactant) forming AOT-alginate nanoparticles.⁶⁵ Bellocq et al developed a polymer–cyclodextrin hybrid system for siRNA delivery. This system was made by condensation of a polycation cyclodextrin with nucleic acid and coating with polyethylene glycol (PEG) to enhance the stability in biological fluids.⁶⁶ Wong et al presented a polymer–lipid hybrid nanoparticle containing cationic doxorubicin, anionic soybean oil-based polymer and stearic acid for water dispensing of the nanoparticle.⁶⁷

Hybrid systems designed from noble metals are promising anticancer agents used in diagnostics and anticancer therapy. Shmarakov et al have tested bimetallic silver–gold (Ag–Au) nanoparticles on lung cancer cells and observed that Ag–Au NPs may serve as a suitable prototype to develop anticancer agents and drug vehicles.⁶⁸ Fakhri et al used in their experiments silver–gold (Ag–Au) bimetallic nanoparticles and demonstrated that they have antiproliferative effects on the human lung cancer cell line A549 and the human breast cancer cell line MCF-7.⁶⁹ Mittal et al synthesized silver–selenium (Ag–Se) bimetallic nanoparticles functionalized with quercetin and gallic acid and showed their potential anti-tumor activity against Dalton's lymphoma cells.⁷⁰ Wu et al showed that silver–gold bimetallic nanostructures present significant cytotoxic effects against the breast cancer cell line MCF-7.⁷¹ Another study presented the effects of gold–platinum (Au–Pt) bimetallic nanoparticles on cervical cancer, and Alshatwi et al studied the potential cytotoxic effects of bimetallic nanoparticles.⁷²

Dendrimers

Dendrimers are well-organized nanoscopic macromolecules and have an essential role in the emerging field of nanomedicine. Due to their high water solubility, biocompatibility, polyvalence and precise molecular weight, dendrimers are gaining considerable attention in modern biomedicine. These characteristics make them ideal carriers for drug delivery and targeting applications. They have the ability to interact with cell membranes, cell organelles and proteins. In addition, dendrimers with cationic surface tend to interact with the lipid bilayer, facilitating increased permeability and decreased integrity of biological membranes. Interaction between dendrimers and cell membrane determines the mechanism that causes the leakage of cytosol proteins.⁷³ Cell toxicity is determined by the number of end groups and surface charges. It was shown that cationic dendrimers such as poly-amidoamine, polypropylenimine and poly-L-lysine expose toxicity in a dose-dependent manner. To prevent the toxicity of dendrimers, the surface groups of cationic dendrimers are modified with neutral molecules. The positive charge of end groups may interact with the negative charge of the membrane, which increases the permeability and facilitates the intracellular delivery of agents. In the case of cationic dendrimers with high charge density, the interaction with the membrane may result in the disruption of membrane integrity and the leakage of important intracellular components which finally causes cell death and toxicity.⁷⁴ Through physical and chemical bonds, dendrimers interact with different classes of drugs, and they can be used for the incorporation of hydrophobic/hydrophilic molecules inside their empty cavities through nonbonding interactions. Another alternative is to attach the drug molecule to its periphery, thus obtaining a complex. The complex is formed due to the electrostatic interactions or conjugation between the drug and the dendrimers. Moreover, the covalent conjugation of drugs to dendrimers may include PEG, p-amino benzoic acid, p-amino hippuric acid and lauryl chains or biodegradable linkages including amide or ester bonds. These conjugates have been found to increase the stability of drugs and blood resistance time, and cause enhanced therapeutic action.⁷⁵

Carbon nanotubes

CNTs have cylindrical shape and belong to the fullerene family of carbon allotropes,⁷⁵ consisting of a hexagonal arrangement of sp²-hybridized carbon atoms. The wall of CNTs is formed from single or multiple layers of graphene sheets. When a single sheet is rolled up, it forms single-walled carbon nanotubes, and multiwalled carbon nanotubes are

obtained by rolling up more than one sheet.⁷⁶ CNTs display abilities for drug loading on the surface or in the inner core through covalent and non-covalent interactions. These nanoparticles are able to immobilize therapeutic agents such as drugs, proteins, DNA and antibodies on the outer wall, or encapsulate them inside the nanotubes, decreasing the cytotoxicity for healthy tissues. Due to their nanoneedle-like structure, carbon nanoparticles are efficiently taken up and translocated into the cytoplasm of target cells without causing cell death. Their applications are limited due to the fact that CNTs are hydrophobic in nature and insoluble in water, and are accumulated in internal organs, having a low degradation rate.⁷⁷ To eliminate the undesirable effects and to facilitate their use in medical applications, various methods of functionalization of CNTs, such as adsorption and electrostatic and covalent interaction, are used. To increase the systemic retention, circulation time and the solubility of CNTs, a hydrophilic biocompatible polymer with neutral charge such as PEG or polyethylene oxide is used.^{78,79}

Functionalization and applications of nanoparticles in chemotherapeutic delivery

In this section, we summarize some examples of nanomaterials used for nanoparticle functionalization. We also describe some chemotherapeutic agents used in cancer therapy and their applications. Nanoparticles can be functionalized with amine, carboxylic acid, *p*-nitrophenyl carbonyl, thiol, maleimide, aldehyde or any ligand that can be attached to the surface,⁹⁸ small molecule, dendrimers, biomolecules and polymers.⁹⁹ In addition, they can be labeled with different types of radioisotopes such as fluorine-18, copper-64, arsenic-72, germanium-69 and zirconium-89. Design and synthesis of radiolabeled nanoparticles is a technique that is faster, easier, more stable and more specific for the next generation of nano-oncology.¹⁰⁰ Liposome changes are performed when needed to attach large targeting molecules, such as proteins, but due to conformational changes, this may cause a decrease in their targeting efficiency. If the target molecule is attached too close to the liposomal bilayer, the stability of the liposome may be altered. To avoid these disadvantages, longer PEG moieties are used, which contain functional groups, and due to steric reasons, may influence the targeting efficiency. Nanoparticles encapsulate the chemotherapeutic agent and are functionalized with specific ligands that are directed to the specific molecule found on the cancer cell. These particular surface markers are used for the development of novel target ligands.¹⁰¹ The above-mentioned

markers need to be overexpressed on the surface of cancer cells, leading to the selective delivery of the cytotoxic drugs only to these cells.¹⁰² These targeted deliveries reduce the toxic effect of the chemotherapeutic agents on healthy cells, and allow the delivery of higher doses of agents to cancer cells. To avoid the interaction with the innate immune cells, nanoparticles are coated with a bio-polymer, such as PEG, that keeps them from being recognized and cleared by the mononuclear phagocyte system.⁶ PEG functionalization is used in a wide range of nanomedical applications due to its flexible structure, non-reactivity, low toxicity^{103,104} and solubility in water^{104–107} and polar and nonpolar solvents^{51,105} such as tetrahydrofuran, chloroform, dimethylsulfoxide¹⁰⁵ or methanol.^{105,106} PEG is a hydrophilic polymer^{106–111} with terminal hydroxyl groups¹⁰⁶ that covalently attach to the nanoparticles' surface and form a protective shell, which inhibits the binding of serum proteins to the liposome,¹¹² increasing liposome circulation time,^{49,109} preventing particle aggregation and protecting the liposomes from opsonization and the mononuclear phagocyte system.⁴⁸

A wide range of ligands can be incorporated on the surface of nanoparticles, allowing them to be used for detecting biomolecules or cells, diagnosis of diseases and intracellular delivery. Nanoparticles functionalized with ligands show different affinity to proteins and cell surface molecules. Nanoparticles functionalized with positively charged ligands present higher internalization into cells when compared to neutral and negatively charged ones.⁹⁹ Positively charged magnetic iron oxide (Fe_2O_4) nanoparticles coated with dendritic guanidine head groups were found to be efficient in HIV-1 trans-activator detection.¹¹³ Amine-functionalized mesoporous silica nanoparticles are used as gene delivery systems for both *in vitro* and *in vivo* studies.¹¹⁴ Nanoparticles functionalized with oligonucleotides have offered new opportunities in molecular diagnostics, such as the detection of nucleic acids.¹¹⁵ For example, oligonucleotide-Au nanoparticles hybridized with fluorophore-labeled complementary sequences are used to visualize and quantify mRNA in living cells.¹¹⁶ Nanoparticles can also be coated with proteins and antibodies that bind to the surface receptors, providing targeted delivery. Nanoparticles coated with Herceptin, a small molecule that targets the Her-2 receptor, assure a useful tool for targeting particles for imaging and photothermal therapy. Magnetic nanoparticles coated with specific proteins can be used as imaging agents, because they can bind to specific tumor tissues.¹¹⁷

Pharmacokinetic and pharmacodynamic profiles of nanoparticle therapeutic agents are dependent on physical

aspects such as size, shape, surface charge and PEGylation (Table 2).

Gold nanoparticles are conjugated with different types of agents such as drugs, monoclonal antibodies, aptamers, peptides, RNA transcripts (siRNA and miRNA), fluorophores, PEG and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid for chelating ⁶⁴Cu, or small natural or synthetic molecules.¹³⁴ Dendrimers can be functionalized with various targeting moieties such as folic acid, peptides, monoclonal antibodies and sugar groups.⁷³ Anthracyclines (ANTs) are cancer drugs effectively used for treating malignant neoplasms.¹³⁵ Doxorubicin, an amphiphilic drug with small molecular weight,^{136,137} is an effective ANT antibiotic^{27,138} used for the treatment of multiple types of cancers. Doxorubicin's advantages include high response rate, increased time to disease progression and low therapeutic index. It is not recommended for patients with high risks of developing cardiac toxicity^{139–142} with adjacent congestive heart failure, arrhythmias or conductivity dysfunction.¹³⁷ The high rates of adverse drug reactions limit the use of this drug. The adverse effects are dose-dependent and are characterized by injuries in the liver, brain, kidney¹⁴³ and gastrointestinal tract.¹⁴⁴ Enhancing strategies for protection from these severe side effects¹⁴⁵ by reducing the cumulative dose of cardiotoxic agents,¹⁴⁶ while increasing the cytotoxic effects on malignant cells,¹⁴⁵ has become a high priority. Drugs affect DNA intercalation and helicase inhibition

causing a cytotoxic mechanism,¹³⁸ DNA cross-linking and free-radical formation.¹⁴⁷ Tumor growth is inhibited by triggering cell apoptosis or inhibition of angiogenesis.¹⁴⁸ Doxorubicin interacts with dsDNA and nucleic acids, causing single- and double-strand breaks, but at the same time, it increases cell membrane permeability and inactivates membrane receptors.¹³⁵ Damage induced by free radicals affects the heart through high oxidative metabolism and low level of antioxidant defenses, and regarding the liver, free radicals induce cell death and tissue damage.¹⁴³ Through active internalization, doxorubicin exhibits accelerated intracellular trafficking in vivo. Greater accumulation of doxorubicin relies on internalization of carriers, which triggers a mechanism for fast release of drugs in cancer cells.

The delivery of chemotherapeutics into cancer cells is based on liposomal penetration into the tumor interstitia and release of therapeutic agents. Poor penetration leads to low antitumor efficacy. Through nanoparticles and targeting ligands with low affinity, intratumor distributions can be improved. Intracellular trafficking of the released agent is affected by the pH values and size of the agent. For doxorubicin, a weak base that limits the permeability of the cell membranes leads to decreased endosomal pH values and allows diffusion through the endosomal membrane to the cytoplasm.¹⁴⁹ Another problem during therapy is the development of resistance to treatment. Doxorubicin is a substrate for various ATP-binding cassette membrane pumps, such

Table 2 Nanoparticle functionalization and physicochemical characteristics

Nanoparticle characteristics	Advantages	Disadvantages
PEGylation	Increased circulation time protecting from opsonization, improved biocompatibility and low immunogenicity	Blood clearance is accelerated due to production of PEG-specific antibody: "PEG dilemma" ^{98,100,118–121}
Size	The optimal size of nanoparticles is between 30 and 50 nm, which improves accumulation in tumor	Nanoparticles of <10-nm diameter are rapidly dispersed in lymph nodes and eliminated by fast renal clearance Nanoparticles of >100-nm diameter are accumulated at the injection site or trapped by the spleen, lung and liver macrophages ^{120,122–125}
Surface charge	The optimal surface charge of nanoparticles is neutral; negative-charged nanoparticles have extended bloodstream lifetime and improved tumor uptake	Positive charge facilitates the binding of nanoparticles to the cell membrane and might strengthen the nonspecific binding of vectors to normal tissue, and promote platelet aggregation and hemolysis If the zeta potential is between –25 and +25 mV, the nanoparticles are considered unstable after long-term storage ^{120,126–129}
Shape	Shapes of particles minimize phagocytosis, leading to longer blood lifetimes and improve the ability of drug delivery systems to reach tumor tissues	Extravasation ability is dependent on morphological characteristics of the tumor ^{120,125,130,131}
Stimulus-responsive delivery systems	Using magnetic field, the characteristics can be improved to promote the accumulation of nanoparticles carrying the therapeutic agents in tumors or thermosensitive polymeric delivery systems and local hyperthermia	Sensitive to tumor environment and have the feasibility of triggering systems ^{120,132,133}
Modification with a target moiety	Cause strong binding and internalization of nanoparticles into cancer cells more efficiently	Targeted nanoparticles can lose their specificity due to shielding with protein corona, and have low tumor uptake ^{120,132,133}

Abbreviation: PEG, polyethylene glycol.

as P-glycoprotein and multidrug resistance-related proteins. As a result of the overexpression of these transporters, doxorubicin can be removed from cancer cells, reducing its intracellular accumulation.¹⁵⁰

Doxorubicinol, a doxorubicin metabolite, is cytotoxic, being responsible for the adverse effects. To alleviate acute toxicities¹⁵¹ and improve antitumor effect, the chemotherapeutic agent is encapsulated into a PEGylated liposome. This clinical product, Doxil (liposomal doxorubicin), is an anticancer drug²⁸ that shows increased microvascular permeability of the tumor with a prolonged duration of circulation in plasma and decreased distribution volume,¹⁵² increasing tumor uptake through EPR effect.^{137,151} To avoid drug interaction with plasma proteins such as opsonins, high-density lipoproteins and low-density lipoproteins, or elimination by macrophages, liposomes' surface is conjugated with PEG. The external part of Doxil is formed from a lipid bilayer coated with PEG, and the molecules of doxorubicin are encapsulated into the aqueous core^{137,153} with better pharmacokinetic profile.¹⁰⁹ Doxil is the first nanodrug approved by the US Food and Drug Administration, and is extensively used in the clinic for cancer patients.²⁸ Through lipid bilayer stabilization and steric hindrance, the interaction with the plasma proteins is decreased, and the recognition by the macrophages is diminished.¹³ Doxil is used to treat AIDS-related Kaposi's sarcoma, metastatic breast cancer and ovarian cancer,^{11,136,151} with a better toxicity profile and reduced incidence of cardiotoxicity.^{154,155} At high dose or short dosing intervals, some forms of toxicity, including acute infusion reaction, mucositis, palmar-plantar erythrodysesthesia,¹³⁹ nausea or vomiting, are present.¹⁵³ Doxorubicin is deposited in the liver, spleen and tumor.^{154,155} An attractive strategy to increase tumor growth via modulating different signaling pathways is to combine multiple drugs with different mechanisms of actions.¹⁴⁸

miRNA and siRNA delivery systems for applications in cancer therapy

In the last decades, alternative delivery systems functionalized with RNA (miRNA and siRNA) have gained increasing attention. Here, we summarize the mechanism of action and the benefits of such delivery systems in cancer therapy. At the moment, these delivery systems are intensively studied in *in vitro*/*in vivo* experiments and also in clinical trials.

miRNAs are short molecules of noncoding RNAs consisting of 20–24 nucleotides that exhibit important roles in all biological pathways in multicellular organisms, including mammals,^{156–158} and are responsible for the regulation of post-transcriptional gene expression.^{45,159} miRNAs are necessary

for normal cellular processes, being deregulated in almost all the diseases, including cancer.^{42,158} Mature miRNAs are processed from hairpin structures into pre-miRNA precursors (60–100 nucleotides). Mature miRNAs are obtained after RNase III Dicer recognition and cleavage into small dsRNA duplexes.¹⁶⁰ miRNAs are involved in many processes, such as development, cell proliferation, apoptosis, tumorigenesis,⁴⁵ cell cycle control, differentiation, migration and metabolism.¹⁵⁶ miRNAs can act similar to oncogenes or tumor suppressor genes.^{45,160–164}

In mammals, miRNAs binding to the 3'-untranslated region halts translation and results in mRNA degradation.¹⁵⁹ Dysregulation of miRNAs causes gene expression imbalance that is related to the dysregulation of key cellular pathways.¹⁶⁵ In recent published papers, different types of miRNAs were used in the medical field as therapeutic agents loaded on the surface of nanoparticles (Table 3).

A great applicability of miRNAs is provided by the use of artificial structures able to restore the normal expression level of a gene, called miRNA mimics and anti-miRNAs (anti-miRs).¹⁶⁴ miRNA mimics are RNA duplexes that are identical to the mature miRNA sequence. An miRNA mimic is designed to have the function of the endogenous miRNA, attempting to restore its loss of function as a tumor suppressor. Anti-miRs are synthetic-modified oligonucleotides that help to explain the function of miRNAs and their targets.^{164,183} They are complementary to the mature miRNAs or their precursors, designed to block their function in RNA-induced silencing complex (Figure 6).¹⁸⁴

siRNAs are short RNA duplexes that gained attention due to their role in gene regulation, making them targets for drug discovery and development. siRNAs act by their ability to specifically inhibit a target gene.¹⁸⁵ Delivery systems are designed to protect the cargo from premature nuclease degradation.¹⁸⁵ miRNA and siRNA have an intracellular site of action, but due to their intrinsic properties such as hydrophilic nature, high molecular weight and negative charge, the permeability for the cellular membrane is decreased. Both types of RNA molecules are used for many disorders, and due to their similar physicochemical properties and intracellular site of action, similar delivery technologies can be used for both transcripts.¹⁸⁶ Therapeutic applications of siRNAs rely on their local delivery to the specific tissue or tumor site. In cancer therapy, the systemic delivery of siRNA has become a major topic and faces many challenges such as interaction with specific gene targets, obtaining pharmacologically effective levels, stability in circulation, improved cellular uptake, monitoring the distribution and therapeutic efficacies. Due to these challenges, novel delivery

Table 3 Relevant examples of miRNAs loaded on nanoparticle surfaces used for medical applications

Delivery system	miRNA	miRNA type	Cancer
Lipid vesicle			
Cationic lipoplex	miR-29b, miR-113b	Mimic	Lung ^{166,167}
Lipid-based nanoparticles	miR-34a, miR-143 cluster, miR-145 cluster	Mimic	Pancreas ¹⁶⁸
Liposomes coated with $\alpha v \beta 3$ -targeted nanoparticles	miR-132	Anti-miR	Breast ¹⁶⁹
Neutral lipid	miR-34a	Mimic	Lymphoma and prostate
	miR-34a, let7		Lung ¹⁷⁰⁻¹⁷³
Lactosylate gramicidin-coated lipid nanoparticles	miR-155	Anti-miR	Hepatocellular carcinoma ¹⁷⁴
Liposome-polycation-hyaluronic acid	miR-34a	Mimic	Melanoma
	miR-296	Anti-miR	Angiogenesis ^{175,176}
Cationic polymers			
PU-PEI	miR-145	Mimic	Lung ¹⁷⁷
CD-PEI coated with RGD-targeting peptide	miR-34a	Mimic	Lung ¹⁷⁸
Dendrimers			
PAMAM nanoparticles coated with 5-FU	miR-21	Anti-miR	Glioma ¹⁷⁹
Gold nanoparticles	miR-31	Mimic	Neuroblastoma
	miR-1323	Anti-miR	Ovarian ⁴²
Encapsulation			
PLGA nanoparticles	miR-155	Anti-miR	Lymphoma ¹⁸⁰
Silica nanoparticles			
Coated with cell surface antigen GD2	miR-34a	Mimic	Neuroblastoma ¹⁸¹
Gold nanoparticles			
-	miR-29	Anti-miR	HeLa cells ¹⁶⁵
Magnetic nanoparticles			
Conjugated with fluorescence and ASI4aa aptamer-targeting nucleolin	miR-221	Anti-miR	Astrocytoma ¹⁸²

Abbreviations: miRNAs, microRNAs; anti-miR, anti-miRNA; PU-PEI, polyurethane-polyethylenimine; CD-PEI, cyclodextrin-polyethylenimine; PAMAM, polyamidoamine; 5-FU, 5-fluorouracil; PLGA, poly-lactic-co-glycolic acid; RGD, arginine-glycine-aspartic; GD2, ganglioside antigen.

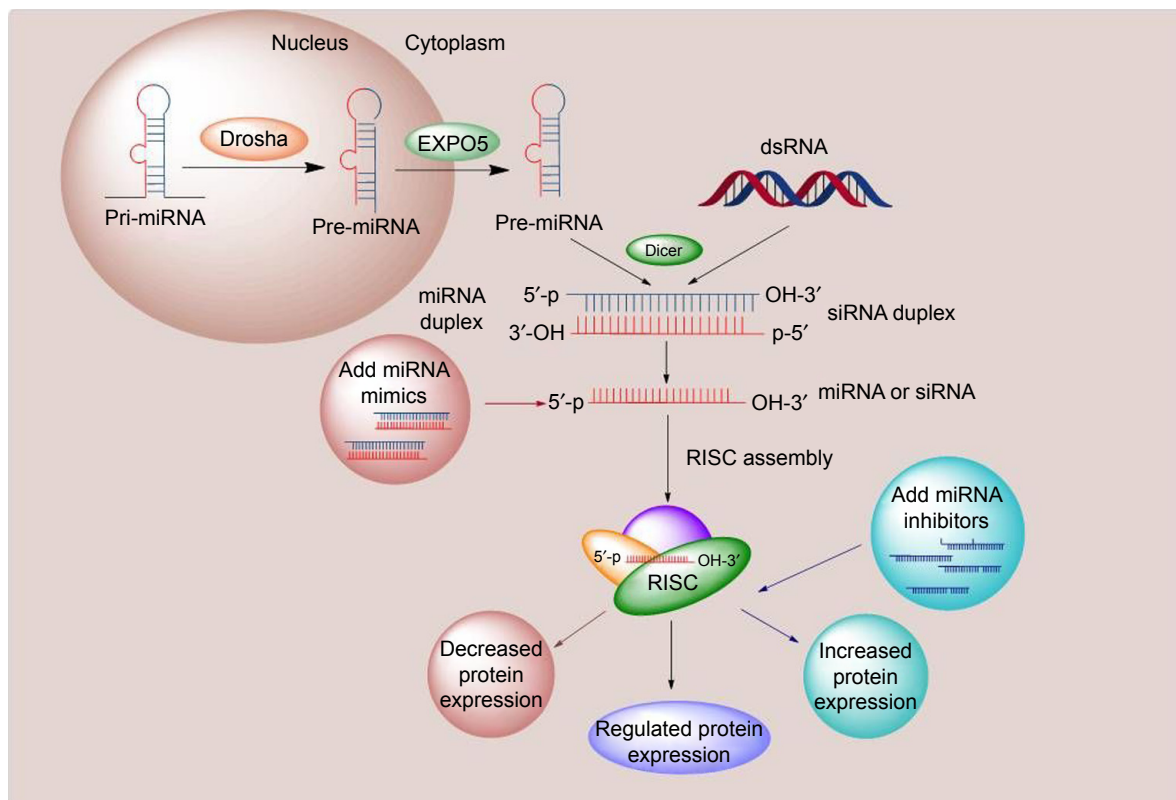


Figure 6 miRNA and siRNA mechanism. miRNA is first transcribed in the nucleus as primary miRNA and then is activated by the RNase III Drosha to create precursor miRNA. The siRNA mechanism starts from dsRNA being transferred into cytoplasm. miRNA mimic involves the reintroduction of a tumor suppressor miRNA to restore a loss of function. Anti-miRNA traps the endogenous miRNA in a configuration that is unable to be processed by RISC.

Abbreviations: miRNA, microRNA; siRNA, small interfering RNA; dsRNA, double-stranded RNA; RISC, RNA-induced silencing complex; pri-miRNA, primary microRNA; pre-miRNA, precursor microRNA; expo5, exportin-5.

Table 4 Different types of delivery systems used for siRNA delivery

siRNA-based delivery systems	Type of delivery systems	Advantages	Disadvantages
Liposome	DOTAP cationic liposomes, PEGylated DOTAP cationic liposomes, PIL, RGD-modified DOTAP cationic liposomes	High transfection efficiency, pharmacokinetic properties are improved; toxicity and immunogenicity are decreased; protection from the enzymatic degradation and renal release is reduced; conjugated with different types of specific ligands	Cationic lipid caused cell toxicity; not always validated on in vivo tests ¹⁸⁵
Nanoparticle	PEGylated nanoparticles; calcium phosphate nanoparticles; chitosan nanoparticles; PEI nanoparticles; MR-sensitive liposome-entrapped siRNA nanoparticles	Increased circulation time; pharmacokinetics, pharmacodynamics, biodistribution and toxicology are improved	Reticuloendothelial system recognized nanoparticles bigger than 100 nm and having a short half-life ¹⁸⁷
Dendrimer	PPI, PAMAM, dendrosomes, PAMAM-PEG-PLL	Internal cavities encapsulate siRNA; having accessible terminal functional groups that conjugate ligands; circulation half-life is improved due to the higher-branched dendrimers; molecular weight is controlled	Increased cytotoxicity; limited for clinical use ¹⁸⁶
Carbon nanotube	SWNT and MWNT	Controlled and targeted RNA delivery; having the ability to penetrate the cells due to the needle mechanism	Accumulation in internal organs, low degradability and excretion rate ¹⁸⁸

Abbreviations: siRNA, small interfering RNA; DOTAP, N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium; PIL, PEGylated immunoliposome; PEI, polyethylenimine; MR, magnetic resonance; PPI, polypropylenimine; PAMAM, polyamidoamine; PEG, polyethylene glycol; PLL, poly-L-lysine; SWNT, single-walled carbon nanotube; MWNT, multiwalled carbon nanotube; DOTAP, N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate; RNA, ribonucleic acid; RGD, arginine-glycine-aspartic.

vehicles such as lipid-, polymer- and nanoparticles were developed for the systemic delivery of siRNAs (Table 4). These delivery systems have been tested on animal models, and some disadvantages regarding toxicity, immune and inflammatory responses gene-control and gene-targeting issues were observed.¹⁸⁷

siRNA and miRNA present similar physicochemical properties but distinct functions (Table 5). They are short RNA duplexes that produce gene-silencing effects by targeting mRNA(s), but their mechanism of action and requirements for sequence design and therapeutic applications are different. For clinical development, they present some disadvantages, such as poor stability in vivo, delivery challenges and off-target effects.¹⁸⁶

Nanoscale immunotherapy

Tumor immunotherapy has been shown to complement available treatment modalities to fight severe cases of relapsed disease, to provide so far unseen clinical results. This novel treatment method is based on the use of in vitro-modified adoptively transferred T lymphocytes to express an artificial signaling molecule named CAR which would specifically redirect the modified lymphocytes to the surface antigens expressed only by malignant cells.¹⁹⁰ CARs typically encode an extracellular antibody-derived domain to bind a surface antigen linked to an intracellular signaling domain that mediates T cell activation such as TCR ζ chain, and various co-stimulatory domains such as CD28 or 4-1BB intracellular chains.¹⁹¹ In principle, any surface antigen can be targeted

Table 5 Comparison of general properties between miRNA and siRNA

Nucleic acids	Occurrence	Configuration/length	Complementarity to target mRNA	Action	Function	Clinical uses
miRNA	Plants and animals	Single stranded/19–25 nt	Not exactly a perfect match, only at seed level; a single miRNA may target up to hundreds of mRNAs	Inhibit translation of mRNA	Modulators of gene expression regulation	Used as drug targets or biomarkers and diagnostic tools ^{186,189}
siRNA	Plants and lower animals	Double stranded/21–22 nt	Perfect match, siRNAs knock down specific genes, with minor off-target exceptions	Cleavage of mRNA	Act as gene-silencing guardians	Used to knock down specific genes, with application in clinical trials as therapeutic agents ^{186,189}

Abbreviations: miRNA, microRNA; siRNA, small interfering RNA; nt, nucleotides.

with CAR in a non-HLA-restricted manner. A large number of CARs targeting diverse tumors have been recently developed, and there are ongoing multiple clinical trials. One of the most successful examples of CAR-based immunotherapy is the treatment of B cell acute and chronic leukemia by targeting the cell surface antigen CD19.¹⁹² However, in spite of these highly encouraging results, major questions remain unresolved. The major problem with CAR-based therapy is the unpredictable treatment responses. A subset of patients encounter limited or missing expansion of infused CAR T cells that might be caused by inefficient activation of CAR T cell in combination with immunosuppressive environment within the tumor stroma. In other patients, there is a complete depletion of all B type-lymphocytes also including the nonmalignant ones.

Advanced medicinal products constitute a group of novel treatment modalities and are rapidly being developed by scientific communities worldwide.¹⁹³ Recently, CAR-based cancer immunotherapy was listed as one of the breakthroughs of the year 2016, and this method is tested in many clinical studies in the US and EU for the therapy of many types of cancer. Adoptive T cell therapy is an important approach to cancer treatment that assumes the infusion of tumor-specific T cells.¹⁹⁴ T cells for adoptive therapy of patients with hematological malignancies can originate from an allogeneic donor. Several techniques have been developed to remove alloreactive T cells from grafts of stem cells to reduce the risk of graft-versus-host disease (GVHD). It has been shown recently that alloreactivity stems mainly from naïve T cells (CD45RA+) and not from memory T cells. Depletion of graft cells from naïve T cells could be a new approach for GVHD prophylaxis. Moreover, the depletion procedure sustained the T cell reactivity to pathogens. The elimination of all CD45RA+ cells also depletes naïve CD4+ Treg cells and CD45RA+ effector memory T cells (TEMRA). TEMRA cells are expanded in subjects chronically infected with HCMV,¹⁹⁵ and they represent senescent and terminally differentiated (CD27-, CD28-, CD57+) cytotoxic T cells which produce large amounts of granzyme and IFN gamma and have a low proliferative potential, and thus, the presence of such cells in expanded antiviral T cells is disadvantageous. The nanoscale immunotherapy represents a fundamental starting point for all forms of cancer.

Conclusion and perspectives

Nanotechnology is a field with high applicability in basic and translational medicine. Nanomedicine relies on various

nanostructure designs, which are conjugated with a wide range of specific targeting agents used for clinical applications, like early diagnosis or disease treatment. The specific agents are attached to the nanoparticles' surface, which promote the accumulation and delivery of those agents in the neoplastic tissue. Nanomedicine's aim is to replace the chemotherapeutic drugs that are highly invasive or nonspecific with particular targeting agents with potential in detection, diagnosis, imaging, targeted delivery and controlled release of therapeutic cargo. In clinical trials, delivery system-based therapies meet many obstacles such as therapeutic molecules stability, decreased nonspecific inflammation, controlled release of therapeutic molecules, specificity and efficiency of the delivery systems. To enhance the stability of therapeutic molecules and to decrease the nonspecific immunogenicity, chemical modifications are required, while for efficient and specific delivery, tailored carriers are needed. RNA therapeutics exhibit great potential in clinical trials, while needing advanced delivery strategies to perform and play their roles in cancer therapy. There are some important component designs, such as PEGylated, tumor-specific ligand that coats the nanoparticles in combination with other light-, thermal-, pH- or magnetic-sensitive components, that enhance the precision, specificity and efficiency of therapeutic molecules to act on tumor sites and tumor cells. Also, a great potential is offered by biochemical modifications that increase the potency and decrease the off-target effects and other side effects of therapeutic drugs, allowing the implementation of new personalized drugs in clinical use.

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Disclosure

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