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REVIEW ARTICLE

Nanoparticle-Mediated Delivery of Therapeutic Drugs

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Abstract Nanotechnology-based pharmaceutics is a fast emerging field in the diagnosis and therapy of a number of human diseases, including cancer. Nanoparticles offer a stable means to achieve targeted drug delivery to various cells and tissues. They have been investigated for drug delivery to different tumor tissues, to brain where the blood–brain barrier poses a significant problem in the delivery of effective therapeutic molecules, to ocular tissues and also for eliciting immune response via delivery of vaccines. Particularly, the small size of nanoparticles facilitates their easy access to a wide range of cells and tissues. Further, the size of nanoparticles can be controlled and their surface can be modified with desired ligands and receptors to specifically target cells of interest as well as achieve controlled drug release. Research is being carried out on numerous biological and synthetic nanoparticles. Diverse strategies are being developed to improve their stability, specificity and drug delivery efficiency. Nanoparticles have been also used in conjunction with cell-penetrating peptides for efficient drug delivery. Cell-penetrating peptides serve as efficient nanocarriers owing to their inherent ability to cross the plasma membrane barrier and deliver cargo to intracellular targets. Modification of nanoparticles with cell-penetrating peptides further increases their efficacy for increased permeation into varied cells and tissues. The current review focuses on different classes of nanoparticles and their application in the treatment of several types of diseases.

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Key Points

Nanoparticle-based therapeutic drugs are widely used for the treatment of a number of diseases, including cancer.

Ease of modulation of size and tuning of the nanoparticles with various ligands make them effective for formulation into specific drugs with increased therapeutic index and reduced toxicity.

Successful pre-clinical and early phase clinical trials have promised the emergence of nanocarrier-based drugs.

1 Introduction

Pharmaceutical drugs currently under study for treatment of various diseases encounter the constraint of instability and rapid degradation in vivo before reaching the target tissue or organ for therapeutic action. Usually, the administered drug is degraded before reaching the target site, potentiating the need for increased dosage to achieve high local drug concentrations, resulting in systemic toxicity in vivo [1]. Moreover, many pharmaceutical drugs such as oligonucleotides and DNA act on intracellular targets, but the selective plasma membrane barrier prevents the transport of such drugs inside the cell, resulting in low efficacy. The adverse effects and the low bioavailability of the drug at the target site facilitates the need for development of carriers that can promote targeted drug delivery, as well as increased pharmacological activity, with low dosage of the drug.

Nanoparticles are a fast emerging area of nanotechnology with increasing application in the pharmaceutical sector because of the wide variety of modular parameters associated with their usage as efficient drug delivery systems. They generally range in size from a few nanometers to a few hundred nanometers and are being extensively examined for their use in the treatment of cancer, neurodegenerative diseases and other pathological conditions [2, 3]. Nanoparticles can be engineered into 'magic bullets', a concept suggested by Paul Ehrlich several years ago. According to this concept, drugs should effectively act at the intended target site without affecting healthy tissues [4]. Based on this concept, several features are desirable in a nanoparticle-based drug therapeutic, such as multiple moieties for binding different ligands, biocompatibility or non-cytotoxicity, biodegradability, targeted delivery and controlled drug release. Nanoparticles can be engineered to particular sizes, loaded with specific therapeutic drugs, have their surface modified with biocompatible coatings and specific ligands, and can be tailored to target specific cells to achieve increased therapeutic efficiency, drug stability, controlled drug release and reduced toxicity. A wide range of pharmaceutical drugs such as proteins, peptides, oligonucleotides, small interfering RNA (siRNA), and small molecular drugs have been successfully delivered to target sites through conjunction with nanoparticles [3]. Engineering of nanoparticle-based carriers for therapeutic drug delivery has greatly enhanced the efficacy of several therapeutic drugs. Promising results have been observed in the clinical trial phase, while some of the nanoparticle-based therapeutics have also entered the drug market [5].

In the present review, we limit ourselves to the overview of different types of nanoparticles, cell-penetrating peptides as nanocarriers and their role in increasing the therapeutic efficiency of different nanoparticles as well as application of nanoparticles in treatment of certain diseases.

2 Types of Nanoparticles

Nanoparticles can be grouped into different classes according to the source of their origin and composition (Fig. 1). They are briefly described in this section.

2.1 Biological Nanoparticles

As the name "biological nanoparticles" suggests, nanoparticles belonging to this class are synthesized

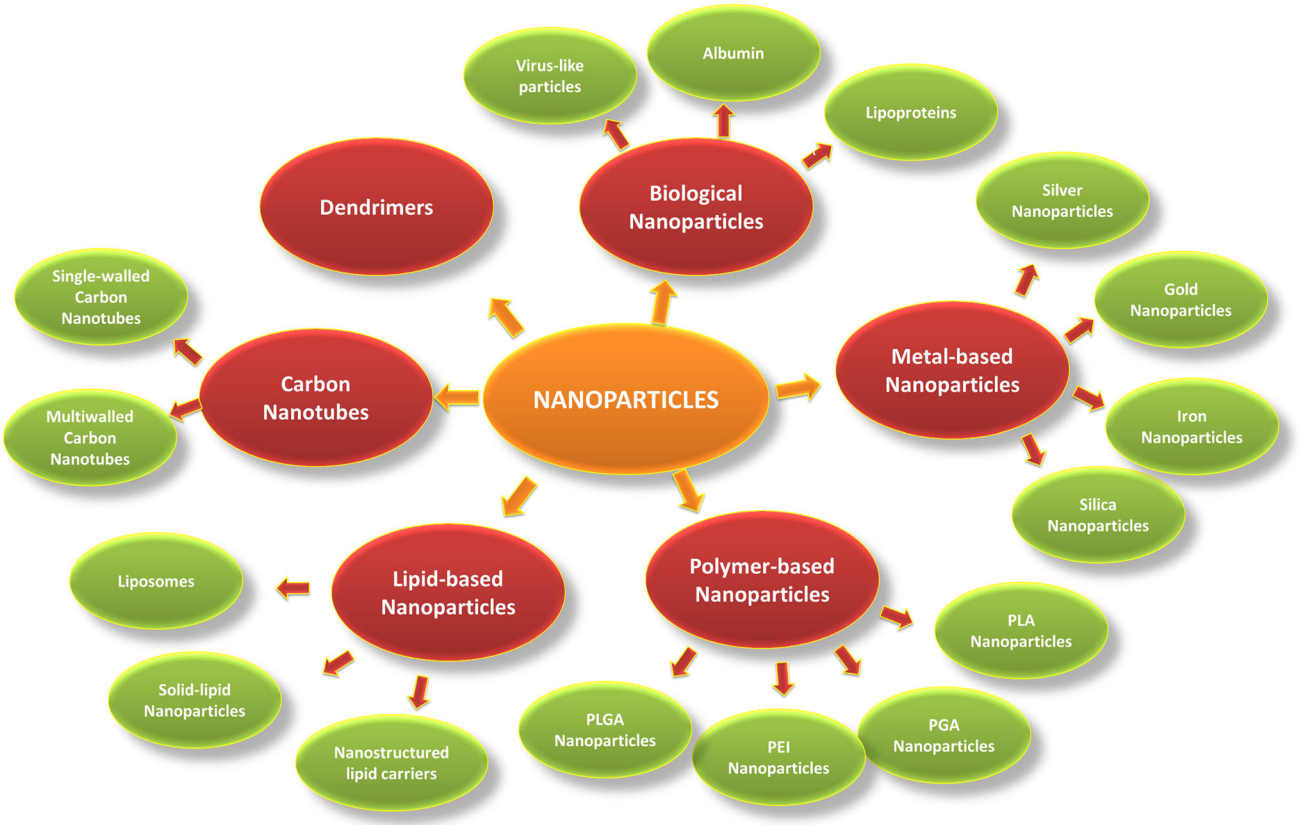

Fig. 1 Pictorial representation of the classification of different nanoparticles used as therapeutics or drug-delivery agents. *PEI* polyethyleinemine, *PGA* polyglycolic acid, *PLA* polylactic acid, *PLGA* poly(lactic-*co*-glycolic acid)

naturally in a biological system. These nanoparticles can form a part of intracellular structures such as exosomes or extracellular molecules (e.g., albumin, lipoproteins and gelatin) [6]. Biological nanoparticles are attractive as pharmaceutical nanocarriers as they are not recognized by the immune system and, therefore, can generally evade the elicitation of an immune response, resulting in increased half-life and bioavailability of the sequestered drug in vivo. Some of the widely used biological nanoparticle-based pharmaceuticals include viruses, albumin and lipoproteins. Viruses possess a capsid protein structure enclosing genetic material in the form of DNA or RNA and attack several species of organisms and replicate inside the host cell. Investigation of viruses and virus-like particles (VLPs) for use as pharmaceuticals in vitro has yielded promising results. Virus capsid proteins have been mainly used for vaccination. Of these, Gardasil® and Cervarix® have been approved by US Food and Drug Administration (FDA) as human papilloma virus (HPV) vaccines for treatment of cervical cancer [7, 8]. They also find application in delivery of certain drugs with intracellular targets that include anticancer drugs such as taxol and DNA vaccine [9, 10]. However, use of viruses or VLPs as nanoparticles or nanopharmaceuticals poses a safety concern because of the potential for eliciting immune response when used for non-vaccine delivery applications in vivo [11]. Therefore, pharmaceuticals based on these nanoparticles need to be thoroughly assessed in the clinical phase for their safety before being approved for widespread use.

Albumin is a high-molecular weight protein found in the blood plasma and serves as a carrier for various biomolecules in the body. Albumin-based nanoparticles have been approved by the US FDA for delivery of anticancer agents. For example, albumin-bound paclitaxel, Abraxane® has been approved for the treatment of metastatic breast cancer, non-small cell lung cancer and pancreatic cancer [12]. Other natural nanoparticles exist in the form of lipoproteins that are the biological carriers of cholesterol and fat in the body. They are composed of lipids and specialized proteins known as apolipoproteins. Lipoproteins form spherical nanoparticles of 7 to >80 nm size. Lipoproteins have generated considerable interest as nanocarriers for certain drugs owing to their ability to carry hydrophobic cargo such as triacylglycerols and cholesterol in the body and target specific cells or tissues such as adipocytes and liver. Modified forms of lipoproteins such as reconstituted high-density lipoproteins (rHDLs) have been used as contrast agents, wherein contrast-generating agents are attached to the protein constituent of the lipoproteins or loaded in their hydrophobic core [13]. Examples include rHDLs containing chelated paramagnetic ions as a contrast agent for imaging of atherosclerotic plaques, and HDL incorporated with gold, iron oxide or quantum dot nanocrystals for biomedical imaging [14, 15]. Besides this, lipoproteins have been also used for delivery of therapeutic drugs such as antitumoral drug into hepatoma cells and for siRNA delivery for the treatment of tumor angiogenesis [16, 17].

Biological nanoparticles are bioinspiration for the rational design of nanocarriers to achieve efficient drug delivery based on their physical size, receptor-binding attributes and efficient cargo transport properties [11]. Understanding the mechanism of interaction of biological nanoparticles with target cells and tissues and transport of various biomolecules in the body would help in the engineering of bioinspired and biomimetic nanoparticles which can overcome specific limitations related to synthetic nanoparticles, such as drug stability and bioavailability.

2.2 Metal-Based Nanoparticles

Various nanoparticles for pharmaceutical applications have been designed with metals and metal oxides forming the core of the nanostructured complex. In fact, metal-based nanoparticles have been indigenously used in Ayurveda, an Indian traditional form of medicine, in the form of Bhasms that are metallic preparations of herbal extracts with high medicinal value [18]. The most commonly employed metal-based nanoparticles for therapeutic applications include gold, iron, silica and silver nanoparticles. They have been of therapeutic interest owing to their small size (generally limited to 100 nm), ease of synthesis, and surface modifications, as well as light absorbing and scattering properties, which potentiates their use as biosensors [19, 20]. Metal-based nanoparticles find application as antimicrobial drugs, optical contrast agents, drug delivery vehicles and in cancer imaging [19, 21, 22].

Silver nanoparticles have been extensively studied for their toxic effects on different microorganisms, including Gram-negative and Gram-positive bacteria, fungi and virus [23–25]. The antibacterial activity of metal-based nanoparticles has been mainly attributed to the pore-forming ability of nanoparticles in bacterial membranes, resulting in increased membrane permeability of the bacterial cells and subsequent cell death [26]. Silver nanoparticles have been also found to result in loss of DNA replication ability and inactivation of certain proteins in bacteria [27, 28]. The mechanism of antifungal activity of silver nanoparticles has also been found to be similar to that of their antibacterial activity, as scanning electron microscopy studies have shown the accumulation of silver nanoparticles in the fungal cell wall, loss of structural integrity of the cells and also cell cycle arrest resulting in deformation of *Candida albicans* cells [24]. Metal nanoparticles have been also studied for their antiviral efficacy. Metal-based nanoparticles inhibit viral replication or prevent the entry of virus inside the host cell [25]. It has been demonstrated by Lara

et al. [29] that silver nanoparticles inhibit HIV-1 by interacting with the CD4 binding domain of the gp120 glycoprotein receptor present on the envelope of HIV-1 virus and prevents interaction between the glycoprotein envelope and target cell membrane receptors, thus, effectively inhibiting viral fusion and infectivity [29]. Nanoparticles can also bind to viral DNA and inhibit viral replication and protein synthesis, as evident from the study of Lu et al. [30]. The study showed that silver nanoparticles inhibit hepatitis B virus (HBV) replication and synthesis of extracellular virions by binding to HBV DNA.

Metal-based nanoparticles have been also used for cancer cell imaging because of their strong surface plasmon resonance properties [21]. Gold nanoparticles are particularly used for biomedical imaging of cancer cells. For example, Huang et al. [31] have used gold nanorods conjugated to anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibodies. The conjugated nanoparticles specifically attach to the surface receptors of the malignant cells [31]. Due to the strong absorption and scattering of light by gold nanorods in the near infrared region, imaging of malignant cells can be done using simple dark-field microscopy. Similarly, in another example, gold nanoparticles have been used as a nanotheranostic tool to simultaneously detect and inhibit tumor growth in the mouse model [32]. The nanotheranostic approach employs the use of gold nanoparticles coated with Raman reporters and cetuximab, a monoclonal antibody that specifically targets EGFR. The antibody conjugate binds to the EGFR abundantly present on the cancer cells, thereby, blocking the signal cascade that leads to their increased proliferation, while simultaneously allowing the spectroscopic detection of tumors through Raman reporters coated on the surface of gold nanoparticles.

Ease of surface modification of metal nanoparticles also facilitates their use in drug delivery to various cells and tissues. The examples of drugs include antibiotics such as ampicillin, streptomycin and kanamycin for treatment of various intracellular infections, anticancer drugs such as cisplatin and methotrexate and proteins such as insulin [33–36]. Gold nanoparticles have been recently developed into multifunctional carriers with an increased ability to deliver siRNA for gene silencing in both in vitro and in vivo models [37]. Conde et al. [38], in their studies with lung cancer mice models, reported that delivery of siRNA by engineered gold nanoparticles resulted in silencing of the target oncogene, thereby, suppressing tumor cell proliferation and extending survival of tumor-bearing mice. In another interesting strategy, gold nanoparticles were functionalized with fluorophore-labeled hairpin DNA such that the fluorescence was quenched when present in close proximity to the gold nanoparticle [39]. Fluorescence of the nanocomplex is restored only when it binds to the complementary target. These nanoparticle complexes, called gold nanobeacons, have been shown to silence endogenous microRNA (miRNA) with simultaneous tracking of intracellular silencing events, promising their effectiveness in cancer theranostics.

Although metal-based nanoparticles have been of considerable interest as pharmaceutical agents, they cause biological toxicity in vivo when administered at high concentration [40–42]. Metal-based nanoparticles need to be thoroughly assessed for their cytotoxicity and systemic side effects. Optimum strategies need to be devised for reducing the toxic effects of the metal-based therapeutics.

2.3 Polymer-Based Nanoparticles

Several polymer-based nanoparticles have been used for biomedical applications. They possess an advantage of biodegradability and biocompatibility compared with metal-based nanoparticles. Various polymer-based pharmaceuticals include chitosan, gelatin, polylactic acid (PLA), polyglycolic acid (PGA), polyethyleinemine (PEI) and copolymers such as poly(lactic-*co*-glycolic acid) (PLGA). The polymer-based nanoparticles are suitable for encapsulation or entrapment of various pharmaceutical drugs and allow surface modifications with various ligands. Polymer-based coatings have been also used in conjunction with other nanoparticles to improve their systemic circulation in blood and for improved biodistribution. Polyethylene glycol (PEG) is the most common polymer used for the surface coating of various inorganic nanoparticles as it provides improved stability and reduced immunogenicity to the nanocarrier complex. It has been approved by the US FDA for human use [43]. It was observed by Panagi et al. [44] that PEGylated PLGA nanoparticles have longer half-lives in blood circulation than non-PEGylated PLGA nanoparticles,which exhibited rapid clearance from the blood circulation, indicating that a polymer coating on the surface of various nanoparticles indeed increases the stability and circulation time of the nanoparticle in vivo. PEGylated nanoparticles have been approved for therapeutic use. These include PEGylated liposomes loaded with doxorubicin (Doxil®) and a methoxy poly (ethylene glycol)-poly (lactide) co-polymer (mPEG-PLA) loaded with paclitaxel (Genoxol-PM) [45, 46]. Similar to metal-based nanoparticles, polymeric nanoparticles have been also used as nanocarriers for various cargo molecules such as magnetic resonance imaging (MRI) contrast agents and various anticancer drugs, and for gene therapy [2, 43, 47].

Chitosan is another widely used natural polysaccharide with increased biocompatibility and non-toxicity. The polymer has been already approved by FDA for wound dressing [48]. Chitosan-based nanocarriers have been used for delivery of various drugs including proteins, genes,

siRNA and various small molecular drugs [49–52]. Although polymer-based nanoparticles have advanced rapidly and several of them are in clinical trials, the transportation and distribution of these nanoparticles in various tissues and organs needs to be closely assessed for biological effects other than their intended use, for their safe administration as therapeutic drugs in humans.

2.4 Lipid-Based Nanoparticles

Liposomes, nanostructured lipid carriers (NLCs) and solid-lipid nanoparticles (SLNs) are the lipid-based nanopharmaceuticals that find applications as nanocarriers. Liposomes are spherical lipid bilayer structures composed of primarily amphipathic phospholipids. Liposomes are attractive as drug carriers owing to the ease of their synthesis and surface tunability for increased stability and biocompatibility. A few liposome-based nanoparticulate drugs have been already approved by the US FDA, and some of them are in clinical development. Examples include Doxil®, a liposomal doxorubicin for treatment of metastatic breast cancer and ovarian cancer; DaunoXome®, liposomal daunorubicin for the treatment of HIV-related Kaposi's sarcoma; Epaxal, a virosomal vaccine for hepatitis A infection; and AmBisome, a liposomal formulation of amphotericin B for the treatment of fungal infections [53, 54]. Besides liposomes, SLNs and NLCs also serve as nanoparticulate formulations for the delivery of various drugs. They are composed of a solid hydrophobic lipid core enclosed by a phospholipid monolayer. The hydrophobic solid core enables the sequestration of hydrophobic drugs and controlled release of the drug at the target site, with low systemic toxicity. SLN- and NLC-based drug formulations have been successfully tested for drug delivery via parenteral, topical, oral, ocular and intranasal routes [53]. Lipid-based nanoparticles, owing to their increased biocompatibility and biodegradability, are being widely studied for various drug delivery applications. However, extensive research needs to be carried out before the lipid-based drugs are released into the market for clinical use.

2.5 Carbon Nanotubes

Carbon nanotubes (CNTs) are allotropic forms of carbon in which the graphene sheets are rolled into cylindrical tubes with a diameter in the nanoscale range. There are two categories of CNTs depending on the number of sheets rolled into cylindrical structures, namely, single-walled CNTs (SWCNTs) and multiwalled CNTs (MWCNTs) [55]. The large inner volume of CNTs facilitates loading of various small biomolecules, and their external surface can be functionally modified for efficient delivery of various therapeutic drugs. However, CNTs bear the limitation of incompatibility with biological systems due to a lack of solubility and the toxicity caused by the hydrophobic surface [56]. Therefore, CNTs need to be functionalized in order to render them efficient for drug delivery. Both covalent and non-covalent modes of functionalization are being carried out to render CNTs more soluble and effective as nanocarriers [57]. Covalent functionalization includes covalent attachment of bioactive ligands onto the surface of CNTs through a chemical reaction, and non-covalent functionalization involves adsorption or interaction of different functional groups with the CNT surface through hydrophobic interactions or Van der Waals interactions. CNTs have been investigated for the treatment of various types of cancer, including brain cancer, ovarian cancer, liver cancer and cervical cancer [58–61]. CNTs have also been employed as drug delivery vectors for the treatment of infectious diseases. For instance, Pruthi et al. [62] developed mannosylated MWCNTs loaded with amphotericin drug, AmBitubes with site-specific delivery to the macrophage cell line. However, drug delivery via CNTs needs to be thoroughly investigated as CNTs have been implicated in inducing cytotoxic effects in vivo, leading to induction of oxidative stress, inflammatory responses, increased permeability of the cell plasma membrane, DNA damage and mutations [55].

2.6 Dendrimers

Dendrimers are synthetic, immensely branched nanoscopic macromolecules that form a tree-like structure. The tree-like branching of dendrimers is characterized by the presence of peripheral groups at each cascade point that makes them highly versatile and highly functional nanomaterials. A wide range of targeting moieties has been attached to the dendrimers to achieve site-specific delivery of drugs. These include folic acid, antibodies, peptides and sugar groups [63]. Dendrimers find applications in the targeted delivery of various anticancer drugs (such as paclitaxel and doxorubicin) and the anti-HIV drug zidovudine; for gene transfection with oligo-DNA and siRNA; and as imaging agents [64–69]. Dendrimer-based drugs have entered clinical trials. For instance, Starpharma has developed a poly(l-lysine) dendrimer-based antimicrobial agent, Vivagel® (SPL7013), for the treatment of bacterial vaginosis, that is currently undergoing phase III clinical trials (http://www.clinicaltrials.gov, identifier: NCT01577537). Further, dendrimers are more amenable to tuning and systematic engineering of their structure with respect to their size, shape and surface chemistry for specific targeting through a wide range of drug administration routes [70]. Various toxicological studies have revealed that anionic dendrimers are non-toxic compared with cationic ones and functionalization of dendrimers drastically reduces their toxicity

[71, 72]. Dendrimers could serve as sophisticated highly functional nanocarriers for various therapeutic drugs provided their cytotoxicity can be mitigated strategically.

3 Cell-Penetrating Peptides and Nanoparticles

Cell-penetrating peptides (CPPs) are small peptides, generally 5–30 amino acids in length, and possess the ability to cross biological membranes and deliver various conjugated cargoes into the cells. They were first discovered 20 years ago when it was observed that the trans-activating regulatory protein (Tat) of HIV and the third alpha-helix of antennapedia homeodomain protein (penetratin) were readily taken up by cells in vitro [73, 74]. Since then many CPPs with capability to deliver cargoes intracellularly in the form of proteins, peptides, nucleic acids, nanoparticles and small molecular drugs have been characterized [75–79]. CPPs have been used as nanocarriers for various living cells, including plant cells, where they have been used for gene delivery into gametophytic cells [80]. However, the mechanism by which these peptides enter the cell still remains elusive. Two major pathways have been proposed for their entry into cells; one is direct translocation, and the other pathway is endocytosis [81]. Although biophysical studies have indicated that both pathways may be involved in the uptake of cell-penetrating peptides, the mechanism of uptake of the peptide and peptide-conjugated cargoes needs to be elucidated to facilitate effective CPP-mediated drug delivery into cells. Nevertheless, CPPs have been used as nanocarriers themselves as well as in conjunction with various nanoparticles to achieve efficient cellular drug delivery both in vitro and in vivo [77, 82–84].

CPPs form nanoparticle-like structures upon interaction with the plasma membrane, as is evident from the study of Padari et al. [85] with $S4_{13}$-PV peptide. It has been shown that the peptide forms nanoparticle-like spherical structures upon interaction with cell surface glycosaminoglycans and then interacts with plasma membrane to gain entry into the cells. The study indicates that CPPs also behave as nanoparticles upon aggregation and thus could be used to facilitate delivery of various drugs into cells by tweaking their properties based on charge and stability. Liu et al. [86] have designed CPP-based core-shell nanoparticles comprising a hydrophobic cholesterol core and hydrophilic cationic peptide shell consisting of Tat peptide. The nanoparticle complex exhibited antimicrobial activity against various types of Gram-positive bacteria, fungi and yeasts. The complex was able to cross the blood–brain barrier effectively and inhibit *Streptococcus aureus* infection in a mouse model [86].

CPPs have been used as carriers to transport various nanoparticles to the desired target. For example, solid lipid nanoparticles have been modified with CPPs such as octaarginine and IRQ peptide for improved oral delivery of protein drugs, insulin and salmon calcitonin, respectively [87, 88]. CPPs have been also used to deliver quantum dots into various tissues [89]. Quantum dots are nanocrystals of semiconductor material, with their size ranging from 2 to 10 nm. Quantum dots can be excited to emit various color fluorescence and, therefore, find use in various biological applications, such as immunofluorescence assays, intracellular labeling and in vivo imaging. However, their use is limited owing to their low permeability into cells, and this can be overcome by conjugating them to CPPs. For example, Tat peptide has been used to enhance the delivery of CdS:Mn/ZnS quantum dots across the blood–brain barrier into the parenchymal cells of the brain, enabling the successful imaging of brain cells [90]. A few examples of some of the CPPs that have been reported to be effective as therapeutic carriers for various nanoparticles and drugs have been listed in Table 1.

CPPs in conjugation with nanoparticles have been also used for the delivery of nucleic acids such as DNA and siRNA. Application of charged nucleic acids for the treatment of various diseases is restricted by their poor cellular uptake. Conjugation of nucleic acids with CPPs enhances their uptake into cells and protects them from cellular nucleases, providing stability to the nucleotides. Hu et al. [91] have designed a mannosylated CPP conjugated with a low-molecular weight polyethyleimine group which is able to deliver DNA with high efficiency into the dendritic cell line. CPPs enhance delivery efficiency of gene-loaded nanoparticles, as evident from the study of Zhao et al. [92], wherein the KALA peptide was used to enhance the uptake of $CaCO_3$-conjugated p53 plasmid and doxorubicin into HeLa cells. The studies indicate that modification of nanoparticles with CPPs enhances the drug or gene delivery efficiency of nanoparticles by several folds as they can be easily delivered into the cells by crossing the plasma membrane barrier.

Additional modifications of CPP-based nanoparticles are being carried out to enhance the drug stability, bioavailability as well as controlled release of the drug at the target site. Multifunctional envelope type nanodevice (MEND) is one such improved nanocarrier system which integrates various functional devices into a single system. It was first developed by Kogure et al. [93] on the principle of 'programmed packaging'. MEND comprises a nucleic acid core complexed with a polycation which is coated with a lipid envelope. This nanostructure is further modified with functional devices such as CPPs for enhanced cell permeability, cleavable PEG to evade the host-defense mechanism, ligands for specific targeting of the drug-loaded nanocomplex and fusogenic lipids to enhance endosomal escape [94]. MEND has been used to improve the delivery of various biomolecules, including genes, siRNA, proteins

Table 1 Examples of CPPs used in conjunction with varied nanoparticles for delivery of therapeutic drugs

CPP	Sequence	Nanoparticle	Drug	Application	References
Tat	CGGGGYGRKKRRQRRR	PLGA-PEG	Flurbiprofen	Ocular delivery	[84]
Tat (48–57)	GRKKRRQRRRCG	Methoxy PEG/PCL diblock copolymers	Anti-VEGF siRNA	Gene-silencing for tumor inhibition	[133]
Polyarginine	RRRRRRRRR (R9)	Mesoporous silica nanoparticles	Doxorubicin	Tumor inhibition in vitro and in vivo	[83]
	RRRRRRRRRRR (R11)	NLCs	Spantide II Ketoprofen	Topical drug delivery	[134]
Penetratin	CRQIKIWFQNRRMKWKK	HPMA polymer	Insulin	Oral delivery	[135]
CSK	CSKSSDYQC	Solid lipid nanoparticles	Salmon calcitonin	Oral delivery	[88]
IRQ	IRQRRRR				
KALA	WEAKLAKALAKALAKHLAKALAKALKACEA	Calcium carbonate-based nanoparticles	p53 plasmid and doxorubicin hydrochloride	In vitro drug delivery	[92]
LNP	KKRTLRKNDRKKRC	Dendrigraft poly-L-lysines dendrimers and PEG	Plasmid DNA encoding inhibitor of growth 4	Glioma gene therapy	[136]
LMWP	VSRRRRRRGGRRRR	PLGA	Doxorubicin	Multidrug-resistant breast cancer	[137]

CPP cell-penetrating peptides, *HPMA* *N*-(2-hydroxypropyl) methacrylamide, *LMWP* low-molecular weight protamine, *LNP* LIM Kinase 2 nucleolar translocation signal peptide, *NLCs* nanostructured lipid carriers, *PCL* polycaprolactone, *PEG* polyethylene glycol, *PLGA* poly(lactic-*co*-glycolic acid), *VEGF* vascular endothelial growth factor, *siRNA* small interfering RNA, *Tat* trans-activating regulatory protein

and small molecules such as doxorubicin, to intracellular compartments [95–99]. MEND has provided a novel means of integrating various nanotechnological tools into a single device to achieve efficient and stable delivery of drugs to intracellular target sites. However, the safety of such devices needs to be assessed at clinical levels to take the drug-based therapeutics to the pharmaceutical market.

4 Biomedical Application for Engineered Nanoparticles

Nanoparticles have been used to deliver various drugs in the form of proteins, peptides, siRNA, and genes to specific cells for treatment of various diseases, including cancer. Nanoparticle-based pharmaceuticals that have been approved by the FDA and those in clinical trials have been reviewed elsewhere [100–102].

4.1 Nanoparticle-Mediated Delivery for the Treatment of Cancer

Nanoparticles have been widely investigated for their effectiveness in the treatment of different types of cancer, and some of them have entered clinical trials. SGT-53 nanoparticles are composed of cationic liposomes loaded with the plasmid encoding *p53* gene for effective treatment of primary and systemic tumors [103]. The nanocomplex is coated with an anti-transferrin receptor single-chain antibody fragment that specifically targets cancer cells expressing transferrin glycoprotein receptor. The complex has been shown to be effective against different primary and metastatic tumors by specifically delivering *p53* transgene into the tumor cells, resulting in reduction in tumor growth and tumor regression [104, 105]. The nanocomplex is currently undergoing clinical phase I trials, with promising results as observed in human subjects with various cancers [103]. Similarly, nanoparticle albumin-bound paclitaxel (nab-paclitaxel) has entered clinical phase II/III trials for the treatment of metastatic breast cancer, metastatic adenocarcinoma of pancreas and metastatic urothelial carcinoma [106–109]. Since paclitaxel is highly lipophilic, albumin is effective in solubilizing paclitaxel, and it is several times more effective and less toxic against a range of metastatic tumors compared with the organic solvent-based counterparts of paclitaxel such as polyoxyethylated castor oil (Cremophore® EL) solubilized paclitaxel (CrEL-paclitaxel) [109]. Another nanoparticle-based formulation of an anticancer drug, paclitaxel, currently under clinical trials is Genexol-PM, developed by

Samyang Co., Seoul, Korea [110]. It is a lyophilized polymeric micelle-based formulation of paclitaxel and has been shown to be effective for treatment of metastatic breast cancer. The phase II study of the nanoparticle-based complex showed it to be effective in patients with metastatic breast tumor. Also, increased efficacy and less acute toxicity has been observed among cancer patients [111].

4.2 Nanoparticles for Targeted Delivery to the Brain

The blood–brain barrier poses a challenge for the delivery of therapeutic agents for treatment of several neurodegenerative diseases and neurological cancer. Drugs targeting brain cells are ineffective mainly because of their inability to cross the blood–brain barrier. Also, the drugs that are able to cross the physical barrier are restricted by their ineffective distribution in the target tissue and, therefore, exhibit limited efficacy. Various drug formulations composed of nanoparticles are being evaluated for their efficiency in crossing the blood–brain barrier and delivering the drug effectively to target cells or tissues in brain. Recently, Tat peptide-modified gold nanoparticles were used as a platform to deliver an anticancer drug, doxorubicin, and imaging agents such as Gd^{3+} contrast agents to brain tumor tissues in mice [112]. Increased survival rate in mice treated with nanoparticle-complexed doxorubicin was observed when compared with those treated with free doxorubicin. Further, the peptide-nanoparticle complex was also effective in delivering the Gd^{3+} contrast agent as observed by enhanced brain tumor imaging and prolonged retention time of Gd^{3+} chelates. More recently, a dual-functional nanoparticle loaded with H102 peptide, a β-sheet breaker peptide, was developed to specifically target Alzheimer's disease (AD) brain lesions [113]. The nanocomplex comprises of a PEG–PLA complex modified with a brain-targeting peptide, named TGN, and a peptide with increased affinity for Aβ42 peptide, named QSH. The nanocomplex was further loaded with H102 peptide drug, which is effective in interfering with the β-sheets within Aβ peptide deposition, accumulation and oligomerization, which leads to cognitive impairment in AD. The peptide drug-loaded nanoparticle complex has been reported to be effective in delivering the drug to the AD lesions in the mouse model. Thus, release of drugs by nanocarriers forms a novel mechanism by which drugs can be delivered specifically to the target tissues in the central nervous system.

Although nanoparticle-based drugs effective for treatment of cancer have entered various phases of clinical trials, the nanoparticles that are effective for treatment of neurodegenerative diseases are yet to reach the clinical trial stage. This may be attributed to the impediment of drugs in crossing the blood–brain barrier as well as successful delivery to the specific cells of the brain. Nevertheless, successful pre-clinical studies of nanoparticle-mediated therapeutic drugs for treatment of neurodegenerative diseases as well as neurological cancers provide a stable ground for further clinical evaluation of such drugs.

4.3 Nanoparticles for Ophthalmic Delivery

Ophthalmic drug delivery mainly comprises drugs in the form of eye drops. However, the major limitation of the current pharmaceuticals in the administration of drugs to the eyes for treatment of several fungal diseases and cancer is the inefficient penetration of drugs through the corneal layer and, therefore, significant loss in the dosage of administered drugs [114]. Non-viral drug delivery-based nanoparticle systems are preferable routes for administration of ophthalmic drugs because of safety concerns. Recently, polymethylmethacrylate nanoparticles loaded with the chemotherapeutic drug carboplatin have been tested for their efficacy in patients suffering from intraocular retinoblastoma, a cancer of the retina [115]. Increased transportation of nanoparticle-based carboplatin across the sclera to the ocular tissue was observed in patients with a sustained release of the chemotherapeutic drug from nanoparticles. The study indicates the effectiveness of nanoparticles in mediating sustained and stable drug delivery to the ophthalmic tissues in the eye without systemic side effects.

Nanoparticles have been also tested for correcting blindness through delivery of specific genes that help in regulating the expression of essential enzymes such as retinal pigment epithelium protein 65 (Rpe65), which controls the availability of a photochemical, 11 cis-retinal, involved in vision [116]. A nanoparticle-based complex consisting of a liposome–protamine–DNA complex was modified with a cell-penetrating peptide and a nuclear localization signal. The modified complex was then used for the delivery of *Rpe65* gene to *Rpe65* knockout mice and subsequently led to in vivo correction of blindness through preservation of cone cells. Similarly, a CPP-based novel peptide for ocular delivery (POD) has been used along with PEG for ocular delivery of transgene into murine retinal pigment epithelium. The plasmid DNA is able to significantly reduce apoptosis of retinal cells after exposure to blue light, indicating that CPP-based nanocarriers can rescue retinal cells from light-induced degeneration [117]. Nanoparticle-based ocular drugs have advantages over conventional ophthalmic therapeutics in that they prevent the pre-corneal drug loss and facilitate sustained release of drugs to the target intra-ocular tissues for prolonged periods that greatly enhance their bioavailability and efficacy at

the target site. However, nanoparticle-based ophthalmic drugs need to be further assessed for their safety and systemic cytotoxicity. Further optimization of drug loading capacity and drug-release kinetics of the nanoparticle-based pharmaceuticals is required for establishing their success as eye therapeutics.

4.4 Nanoparticles for Vaccine Delivery

Nanoparticles also find application in delivery of protein and DNA vaccines for triggering immune response by antigen presenting cells (APCs) of the host immune system. Dendritic cells have been an effective target for the delivery of vaccines as they form a part of both innate and acquired immunity and play a central role in triggering immune response after coming in contact with an antigen [118, 119]. Polymeric nanoparticles such as PLGA, liposomes and virus-like nanoparticles have been studied for delivery of vaccines [120–122]. For example, PLGA nanoparticles have been used for mucosal immunization against hepatitis B [120]. Recently, Tahamtan et al. [123] showed that chitosan-based nanoparticles are able to deliver tumor-specific antigen in the form of HPV-16 DNA vaccine for treatment of cervical cancer caused by HPV. The nanoparticle-based DNA vaccine is efficient in triggering immune response by activation of antigen-specific CD8+ T-lymphocytes and eliciting interferon responses in mice when compared with naked DNA vaccine. Nanoparticle-based vaccines have advantages over conventional vaccines as they can facilitate controlled and sustained release of encapsulated adjuvant/antigen at the target site, resulting in a long-lasting immune response [124, 125]. Use of nanoparticles for vaccine delivery also enables surface modification with different ligands that bind to specific receptors on APCs as well as behave as adjuvants. The strategy has been employed by Fukasawa et al. [126], wherein oligomannose-coated liposomes for delivery of HIV-1 glycoprotein gp120 peptide have been developed. They are able to elicit immune response in mice compared with non-coated liposomes, indicating that the surface modification of liposomes enabled their dual application as adjuvant as well as nanocarriers [126].

5 Nanotoxicity and Regulation

Although several nanoparticle-based therapeutic drugs have been approved by the US FDA and several others have entered clinical development, toxicity concerns are still a major hurdle for their success. Recent reports shed light on adverse effects of nanotoxicity at organ, tissue, cellular and protein levels [127]. Factors governing the toxicity of nanoparticles include their size, shape, surface modification, chemical composition and physico-electrochemical properties. Nanoparticles have been particularly implicated in cardiovascular and pulmonary toxicities as a result of inhalation of these ultra-fine substances [128]. Bhabra et al. [129] have shown that cobalt–chromium nanoparticles can induce DNA damage in cells without crossing the plasma membrane barrier mediated through gap junctions. Metal-based nanoparticles have been shown to interact with a number of different proteins and enzymes and lead to generation of reactive oxygen species through interference with the antioxidant defense mechanism. This in turn leads to the induction of inflammatory response, thus resulting in apoptosis or necrosis [127]. Silver and copper nanoparticles have been shown to induce oxidative stress through generation of free radicals and disrupt the endothelial cell membrane after crossing the blood–brain barrier [130]. Nanoparticles have been also observed to cross the blood–brain barrier and trigger alterations in the central nervous system [131]. Thus, keeping in view several toxicity effects of nanoparticles arising at cellular and sub-cellular levels, stringent evaluation of nanoparticle-based therapeutics needs to be carried out at molecular, cellular and systemic levels. Rigorous risk and impact assessment of newly introduced nanoparticles should be carried out, and diverse tools for timely regulation and updation of data for risk management of nanoparticle-based therapeutics should be developed. Although several databases are available concerning the toxicity and risk of engineered nanomaterials, a comprehensive and critical database for the influence of nanoparticles on human health and the environment should be developed, as this will help in realizing the potential risk to the safety aspect of various nanoparticles. The strategy will aid in engineering nanoparticles and lead to their optimal use in therapeutics.

6 Conclusion and Perspectives

Nanoparticle-based pharmaceuticals are generating great interest among researchers. Several pharmaceutical nanocarriers have already entered clinical trials, and some have already reached the market. Nanoparticles, owing to their small size, are the preferred as drug vehicles. Their ease of synthesis and production in bulk makes them cost effective. Increased bioavailability and biodistribution of the drug at the target site of action is essential for effective therapeutic treatment of different types of diseases. Effective sequestering of drugs with nanoparticles, along with their modification with specific ligands, facilitates targeted drug delivery. This effectively increases the therapeutic efficiency of the desired drug molecule. Cell-penetrating peptides conjugated to nanoparticles offer a means of increasing the uptake of nanocomplex into cells of interest

and to deliver the drug molecules to the intracellular targets. Engineered nanoparticle devices such as MEND have the potential to contribute in the field of gene therapy as numerous diseases, including cancer, can be treated by nuclear gene delivery, thus, achieving the desired therapeutic effect with long-term efficacy. Encapsulating or loading pharmaceutical drugs inside nanoparticles not only increases their stability but also facilitates increased efficacy at the target site, thus, reducing the need for increased drug dosage and subsequent toxicity. However, nanotoxicity resulting from the use of nanoparticles needs to be assessed thoroughly since administration of the nanosized carrier molecules in vivo can result in their permeation through blood capillaries and distribution in non-targeted tissues and organs, resulting in unwarranted side effects. Nanocomplexes can also cross the blood–brain barrier and can cause neurological disturbances. Therefore, effective screening and careful systemic studies need to be carried out before releasing the nanoparticle-based drugs into the pharmaceutical market. Apart from nanotoxicity, another aspect to be taken into consideration while developing nanoparticle-based pharmaceuticals is their synthesis and production in bulk. Different scale-up technologies are being developed for their large-scale synthesis [132]. Cost optimization along with the demands for market supply need to be fulfilled in order to render nanoparticle-based therapeutics more suitable for clinical use.

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