Chapter 5

Multifunctional Polymeric Enveloped Nanocarriers: Targeting Extracellular and Intracellular Barriers

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Additional information is available at the end of the chapter

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Abstract

Over the past several years, employment of multifunctional polymeric excipients-based nanoparticles for controlled and targeted drug delivery of therapeutic modalities to mucosal membrane-based organelles and systemic circulation has gained enormous interest. Because they promise to resolve numerous key therapeutical issues associated with current clinical practice including low treatment efficacy and significant side effects. Potential controlled and targeted drug delivery systems, therefore, should be able to overcome not only extracellular barriers but also intracellular barriers. Extracellularly, targeted nanocarriers ought to provide extended circulation time, selective binding to the targeted mucosal tissues, long residence time at the site of absorption, and controlled drug release. Intracellularly, the targeted nanocarriers should offer cellular uptake, cellular localization, and endosomal release. Hence, this chapter will provide an overview of the unique chemistry of multifunctional polymeric enveloped diverse nanocarriers such as dendrimers, semiconducting polymer dots, quantum dots, carbon dots, and magnetic as versatile platform addressing both extracellular and intracellular barriers.

Keywords: polymeric nanocarriers, extracellular drug targeting, intracellular drug targeting, carbon dots, polymer dots, quantum dots, magnetic nanoparticles

1. Introduction

Targeted drug delivery has been massively investigated because of their potential to overcome hurdles of conventional therapy [1]. Administration of drug selectively at desired site ensures the maximum amount of drug to be available at that locality. Moreover, lesser absorption of

drug systemically minimizes the potential for unwanted effects. To target a drug so that it may avoid its uptake by off target tissues or cell and allow its residence at desired site for longer period of time, it is usually escorted with some targeting agent. These targeting molecules can be receptor-specific ligands, vehicles, or biological molecules [2].

Nanocarriers have revolutionized therapeutic approaches by providing numerous means for drug targeting. Nanocarriers have employed various approaches to target drug to a specific organ, tissue, cell, or organelle. The therapeutic efficacy of these carrier systems is highly dependent on their entry into target sites. It has been studied that passage of particles across endothelial cells requires their size to be less than 100 nm approximately [3]. This limits the greater-sized particles to extravagate into tissues having compromised endothelial arrangement. Nanostructures with hydrophobic outer surface have been observed to undergo phagocytosis after being opsonized [4].

Various polymers with diverse chemical nature and various activities and characteristics have been explored to design nanocarriers for delivery of drug molecules. Among them, biodegradable polymers have been of particular interest. Both natural and synthetic biodegradable polymers have been exploited to functionalize nanoparticles exploiting diverse approaches to deliver drug in an effective manner to its target site [1]. Such functionalized polymeric nanostructures (also known as multiplex nanoparticles) have been widely investigated as an effective carrier for a wide variety of drugs as well as biological molecules such as DNA and proteins [1]. Current research has been precisely focused on the use of biodegradable polymers that have shown great promise in modifying the delivery of drug as well as tissue engineering. Such polymers have shown to provide extended and targeted drug release for days to weeks as well as shown great promise for intracellular transport of drugs [5].

Various treatment modalities require intracellular delivery of drug as the causative agent is harboring within cell. This is the case commonly associated with infectious diseases such as tuberculosis, leishmaniasis, and leprosy, where the pathogen invades macrophages. Therefore, complete eradication requires utmost delivery of drug at right concentration to infected cells. Certain other conditions also require delivery of therapeutic agents into cytoplasm where they can target various cellular organelles such as endoplasmic reticulum, mitochondria, nucleus, and lysosomes [6]. This has found particular interest in gene therapy for targeting cellular genome [7] as well as drug targeting for the treatment of cancer and lysosomal storage disease [6]. Intracellular transport has also been widely appreciated for bioimaging and biosensing both *in vitro* and *in vivo*.

2. Intracellular drug targeting

Entry or transport of drug into the cell has never been easy and thus widely explored [6]. Cells offer several mechanisms to allow ingress of drug carriers prominently comprising endocytosis. Internalization of drug into cells may either obey receptor-dependent endocytosis or receptor-independent pathway as shown in **Figure 1**. Combining small drug molecules with macromolecular carriers restricts their entry into highly perfused tissues, thus averting

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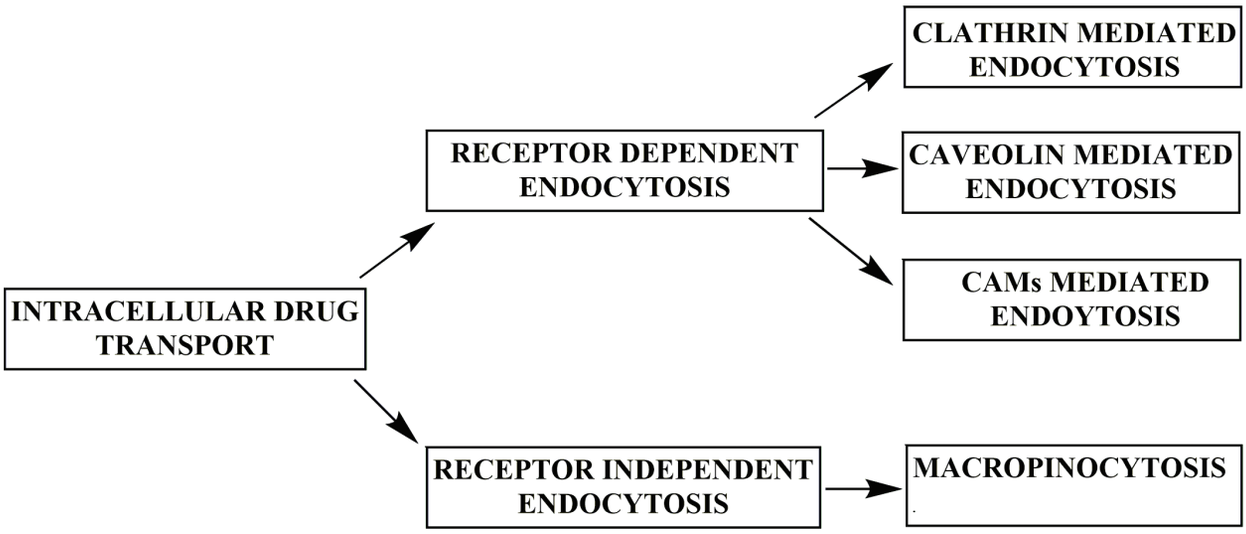

Figure 1. Pathways adopted for intracellular drug transport.

untoward side effects [2]. Macromolecules (proteins, peptides, DNA, etc.) are not allowed by lipophilic biological membranes to enter into cytoplasm. Such molecules may follow active transport involving endocytosis via cell surface receptors [6]. This receptor-mediated endocytosis offers a faster course for drug internalization in contrast to untargeted conjugates. These receptors have been located on surface of cells as well as accompany intracellular membranes [8].

2.1. Clathrin-associated, receptor-mediated endocytosis

The bases on which molecules are sorted to follow either clathrin-dependent or clathrin-independent pay are still not fully understood. However, it has been found that some specialized lipid domains are involved in membrane organization, sorting, and signal transduction in clathrin-independent endocytosis [9]. Ligand-associated drug carriers may bind to specific cell receptors that get assembled into particular areas of plasma membrane termed as coated pits. These regions (diameter 0.1 μm approx.) have been explained as plasma membrane invaginations with fuzzy cytoplasmic coat. This coat is mainly composed of clathrin protein present at cytoplasmic periphery of membrane and serves as major route for cellular internalization [10] as depicted in **Figure 2**. These coated pits allow intracellular vesicle formation in less than 1 min of time that is much faster than other mechanisms of endocytosis. A protein known as adaptin is responsible for polymerization of clathrin in the form of polyhedral lattice scaffold by binding with cell surface receptors. Two other proteins amphiphysin and endophilin get neighboring membrane into close vicinity. Dynamin (a cytosolic GTPase) gathers around the neckline of budding vesicle followed by its scission and intracellular discharge [2]. After intracellular entry of vesicle, an uncoating protein (heat shock protein; hsc70) causes the clathrin coat to shed of. At this stage, endocytosis trafficking of endosomal content decides the fate of therapeutic agent delivered. Endosomes may end up into lysosomes that may lead to degradation of drug or may be safely released into cytoplasm to reach desired organelle [6].

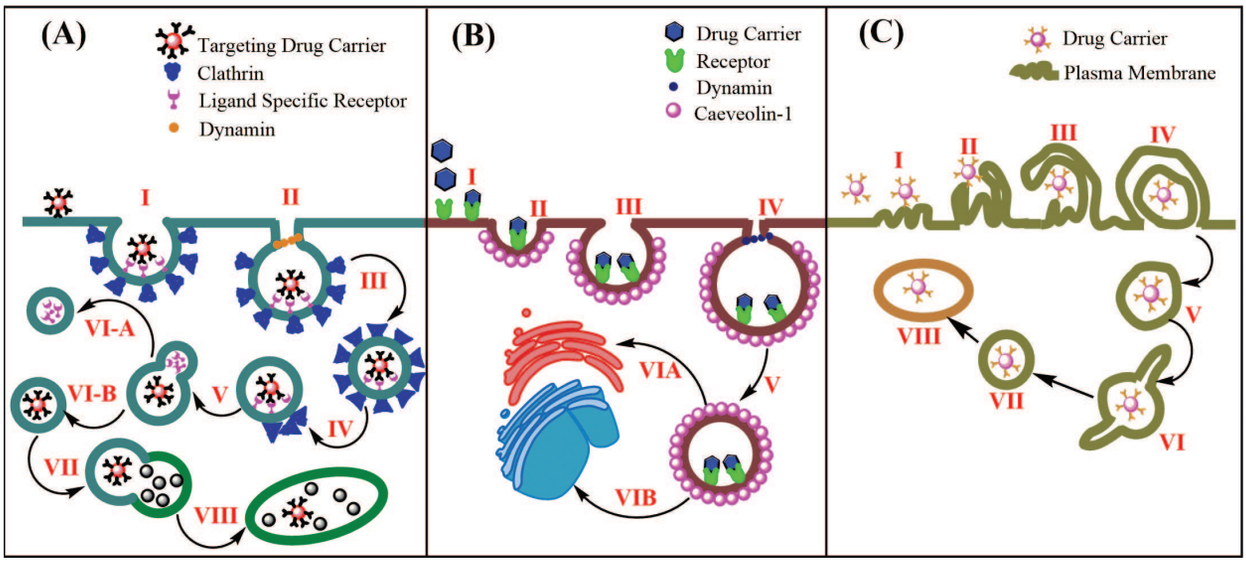

Figure 2. (A) Clathrin-dependent receptor-mediated endocytosis, I—entry of targeting drug carrier in clathrin-coated pits and binding with ligand-specific receptor; II—dynamin-associated endocytosis; III—formation of clathrin-coated vesicle; IV—shedding of clathrin coat; V—early endosomal sorting and uncoupling of ligand-receptor; VI-A—formation of late endosome; VI-B—formation of transport vesicle; VII—fusion of lysosome with transport vesicle; VIII—formation of endolysosome. (B) Caveolin-dependent, receptor-mediated endocytosis, I—interaction of ligand with receptor; II—movement of receptor ligand complex toward caveolar invagination; III—retention of receptor ligand complex in caveolar invagination; IV—dynamin-associated caveolar endocytosis; V—formation of caveosome; VI-A and VI-B—transport of drug carrier to endoplasmic reticulum or Golgi apparatus, respectively. (C) Macropinocytosis, I—movement of drug carrier toward membrane ruffling; II—rearrangement of cytoskeleton, folding of ruffle around drug carrier; IV—internalization; V—formation of macropinosome; VI—early maturation of macropinosome; VII—late endosome; VIII—endolysosome.

2.2. Cell adhesion molecule (CAM)-mediated endocytosis

Drug targeting has also been investigated using cell adhesion molecules (CAMs). Recently, integrins and cadherins have been found to internalize their ligand into intracellular milieu. Thus, many cell adhesion peptides such as arginylglycylaspartic acid (RGD) [11] and peptides derived from intercellular adhesion molecule 1 (ICAM-1) and lymphocyte function-associated antigen 1 (LFA-1) sequences that bind to specific integrins have been extensively investigated for targeting tumor and vascular endothelial cells and suppressed progression of autoimmune disorders [12]. CAMs undergo cellular internalization while they are recycled via clathrin-coated pits and thus can be useful in cell-specific drug targeting through specific peptides. Such peptides are usually derived from proteins comprising extracellular matrix, immunoglobulins superfamily, and integrins. Integrins have also been associated with cellular uptake of certain viruses and bacteria through surface interactions at unique regions and initiate transduction pathways [13].

2.3. Caveolin-dependent, receptor-mediated endocytosis

Another version for cellular internalization of drug carriers is caveolin-mediated endocytosis. This process is sensitive to temperature and also dependent on ATP and sulphydral reagents [14]. Caveolin-1 is a protein associated with flask-shaped invaginations making up greater

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than 10% of endothelial cell membrane. Only cells expressing caveolin-1 protein develop caveolar invaginations [15]. Ligand after association with plasma membrane moves along it toward caveolar invagination, where being retained for some time internalization occurs via certain unidentified receptor as shown in **Figure 2**. The presence of GTPase dynamin has suggested involvement of caveolae in membrane internalization [9].

Folic acid, cholesterol, albumin complexes, and serum lipoproteins are commonly encountered ligands internalized via caveolae-dependent endocytosis. These ligands have been considered as attractive candidates for drug targeting especially to intracellular organelles. Caveolar vesicles after getting internalized fuse with caveosomes following delivery of content at subcellular level bypassing acidic and degradative milieu of lysosomes [15]. Another mechanism suggested for caveolae-dependent internalization is 'potocytosis' that implies diffusion of smaller moieties into cytoplasm after interacting caveolae without membrane internalization [16]. One other associated pathway for cells without caveolae expression is 'lipid rafts'. These flat structures are composed of lipid- and protein-based assemblies that allow receptor-specific ligands to anchor on raft domain [15].

2.4. Macropinocytosis

Macropinocytosis employs distinct mechanism to transport molecules inside the cell without any direct coordination with receptors [17]. Macropinocytosis begins with actin polymerization at surface of cell membrane that is regulated by tyrosine kinase, epidermal growth factor, and platelet-derived growth factor receptors. This leads to increased ruffling at membrane surface and subsequent formation of macropinosomes (**Figure 2**). It involves absorption of molecules present in extracellular fluid (ECF) and seems to be a slower process as compared to RME. This process has sometimes shown to accompany receptor-mediated endocytosis; thus, absorption of receptor bound ligand and molecules in ECF may occur through clathrin-coated vesicles side by side. After entry of fluid vesicles, they are supposed to follow usual endolysosomal trafficking pathway [18]. Negative charge on membrane surface naturally favors positively charged molecules to reside there and eventually get internalized through fluid phase endocytosis. This phenomenon has been exploited by researches for intracellular delivery of drugs [19].

3. Extracellular drug targeting

Many targeting approaches utilize such mechanisms that exploit extracellular barriers to ensure efficient delivery of drugs. Nanocarriers following intravenous administration are rapidly recognized by reticuloendothelial system (liver and spleen macrophages), making it difficult for drug to reach its site of action at the minimum effective concentration [20]. This owes to opsonization of particle surface with certain plasma proteins (albumin, apolipoprotein-E, etc.) that make them recognizable by body's immune system and thus are rapidly evacuated from circulation. Therefore, the extent of opsonization will determine the fate of nanoparticles *in vivo* [21].

Recognition of nanoparticles by RES and their uptake by macrophages can be avoided by modifying surface properties of these carrier systems. One of such modifications is by making surface of nanoparticles to be very hydrophilic. This avoids adsorption of opsonins and ensures nanoparticles to pass unrecognized by RES [22]. Surface of nanoparticles has been made more hydrophilic by increasing the thickness of coating layer of poloxamer and poloxamine. Coating layers up to 10 nm were considered necessary to bypass RES [23].

Many drug molecules have shown poor penetration across the blood-brain barrier due to their inherent nature. These molecules have been successfully delivered to the brain when incorporated into nanocarriers [24]. Coated nanoparticles have been studied for delivery of drug into the brain. Polybutylcyanoacrylate (PBCA) nanoparticles coated with polysorbate-80 have been studied to improve penetration of drug across the blood-brain barrier [25]. In a study, transport of nanoparticles across BBB was investigated. Penetration of nanoparticles across BBB was three to four times increased when charged nanoparticles were coated with dipalmitoyl phosphatidyl choline and cholesterol-based lipid bilayer [26]. Multifunctional nanoparticles have also been investigated for delivery of proteins and peptides. These biological molecules are associated with rapid degradation at acidic pH of GIT and by activity of proteolytic enzymes that owe to their shorter half-life. Moreover, lesser partition coefficient and diffusivity make their movement difficult across biological membranes. These limitations can be conquered using functionalized polymeric nanoparticulate drug delivery systems [27]. Properties of PLGA matrices have been modified through hydrogel systems to deliver proteins and peptides. Bovine serum albumin was loaded in poly vinyl alcohol nanoparticles, which were then incorporated into PLGA microspheres and characterized to release the protein for more than 2 months [28]. Poly(isobutyl cyanoacrylate) nanocapsules have also been investigated for oral delivery of insulin [29].

Sustained release of drugs has also been achieved using various functionalized nanocarriers. Poly DL-lactic acid (PLA) nanoparticles have been used to provide sustained release of savoxepine following intramuscular and intravenous administrations [30]. Damge and his coworkers have successfully developed nanocapsules for sustained delivery of insulin. About 100 U/kg of insulin-loaded nanocapsules were effective to reduce blood glucose level for 6 days by 25% on oral administration to diabetic rats [31]. Colloidal suspension of docetaxel-loaded nanospheres has been prepared using PLA and PLGA to study sustained release of drug after intravenous administration [32]. PEG-grafted polyamidoamine (PAMAM) dendrimers were used to control the release of adriamycin and methotrexate [33]. Mu and Feng have used d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) to prepare controlled release paclitaxel nanoparticles with high encapsulation efficiency [34].

4. Nanocarriers for intracellular and extracellular targeting

Scientists have extensively explored a wide range of drug carriers at nanoscale extending from highly simple to complex geometries. Other than polymeric nanostructures, several lipids-based structures have been investigated for drug delivery, among which liposomes hold a noticeable status. Owing to their resemblance to lipid bilayer structure, they have been

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studied for a wide range of therapeutic applications (antivirals, antimicrobials, antitubercular drugs, biological molecules, and gene therapeutics) by offering enhanced accumulation and reduced toxicity at targeted site. However, reduced stability and trouble in immobilization of vector molecules on exterior of liposomes have led to exploration of other more useful nanocarriers [35]. Nanodots have exhibited great promise in therapeutics owing to diverse physicochemical properties, functionalization opportunities, and contenting stability attributes, which make them excellent candidate for bioimaging along with drug delivery. They have also been found substantially successful in photodynamic therapy in treatment of tumors [36]. Further, our discussion will be focused on multifunctional polymeric nanocarriers including dendrimers, various nanodot structures, and magnetic nanoparticles with reference to their application in intracellular and extracellular targeting for diagnostics and therapeutics.

4.1. Dendrimers

Dendrimers are three-dimensional, globular structures consisting of highly branched, repeating, and controllable peripheral functionalities originating from a central core [37]. These structures are assembled in layered fashion from core by repetition of two sequential reaction steps. Origination of a new branching point in layer leads to creation of a new generation (denoted as G). Thus, a regular dendrimer structure is usually composed of three major elements, i.e., a central core, branched units, and surface groups [38]. Such a diverse structure and nanometric size range has pulled them to massive exploration as potential carrier for drug delivery to targeted regions. The chemistry of dendrimers offers several modes for incorporation of drugs. Most commonly, drug is linked either covalently or noncovalently to dendrimers. A drug can be noncovalently introduced into dendrimer by simple encapsulation method that is mostly used to enhance solubilization of lipophilic drugs in aqueous media. Charged drugs such as DNA, RNA, or siRNA can also be incorporated into dendrimers through noncovalent electrostatic interactions. Thus, drug can be conjugated in this manner to both the internal and external regions of dendrimer. Covalent incorporation involves formation of stable bonds between drug and dendrimers [39].

Recent research is focused on development of synthetic carriers for delivery of genetic material with low cytotoxicity, highly efficient delivery, and minimal lysosomal degradation [40]. Gene delivery using polyamidoamine (PAMAM) dendrimers has been studied by Haensler and Szoka. Covalent linking of dendrimer with GALA peptide resulted in improved transfection efficiency [41]. Polypropylene imine (PPI) dendrimers enjoying low generations have also shown capacity as DNA carrier for gene transfection with lower cytotoxic potential [42]. Dendrimers have also been explored for delivery of chemotherapeutic agents. Quintana and coworkers have developed PAMAM dendrimers composed of ethylenediamine core. Methotrexate along with targeting ligand and fluorescein was covalently attached to dendritic surface. Experimental data confirmed highly specific binding with KB cell line with 100% improved cytotoxic response as compared to free drug [43]. In another study, siRNA was incorporated in PPI dendrimer that was stabilized using cross-linker to cage the preformed siRNA-dendrimer nanoparticle. PEG layer was applied over this nanostructure that further utilized luteinizing hormone releasing hormone (LHRH) to guide siRNA-loaded nanoparticles

to tumor cells. *In-vivo* studies suggested highly specific tumor targeting with improved accumulation of siRNA in cytoplasm of cells and effective gene silencing [44].

Dendritic structures have also inspired boron neutron capture therapy for tumor targeting. To kill tumor cells, it is necessary for ^{10}B to reach intracellular concentration of at least 10^9 atoms/cell. This tumor-specific delivery at desired concentrations has been achieved through use of boronated antibodies. Epidermal growth factor receptors (EGFR) that are overexpressed at surface of glioma cells have been targeted by ^{10}B PAMAM dendrimers. Dendrimers after being covalently linked to epidermal growth factor were effectively internalized by endocytosis with substantial accumulation of ^{10}B in lysosomes of cells *in vitro* [45]. However, *in-vivo* studies demonstrated uptake of boron carriers by the liver and less level of accumulation in C6 glioma cells. To address uptake of boronated dendritic conjugates by the liver, scientists have exploited the steric effect provided by polyethylene oxide (PEO) chains. Such PEO-shielded boronated PAMAM dendrimers showed lesser uptake of conjugate by the liver. However, uptake of PEO-shielded dendritic conjugates by liver was increased with an increase in the number of PEO chains [46].

Exploration of dendrimers in photodynamic therapy (PDT) has also captured great interest. Therapy employs a photosensitizing agent that upon exposure to light of specific wavelength causes irreversible photo-chemical or photo-biological damage to tumor cells. Dendrimers on suitable peripheral functionalization can be promising carrier for photosensitizers. Eighteen ALA (5-aminolevulinic acid) units have been conjugated with dendrimer through amide linkage. These ALA-conjugated dendrimers exhibited increased cellular level of protoporphyrin IX (PIX) and thus showed increased cytotoxicity on exposure of radiations in PAM 212 tumorigenic cell lines [47]. Because of increased tissue permeability to near IR or IR light, the photodynamic system with high absorbance at longer wavelength is extremely attractive. To exploit this feature, aluminum-phthalocyanines polymer conjugates have been designed with the maximum absorption observed at 675 nm [48]. A two-photon approach also has great potential to target deeper tissues with near IR laser. Multivalent character of dendrimers has the capacity to accommodate several two-photon absorbing moieties to porphyrin core. Excitation of chromophores at 780 nm resulted in generation of increased singlet oxygen luminescence [49].

4.2. Semiconductor polymer dots

Semiconducting polymer dots (Pdots), also described as organic nanodots or conjugated polymeric nanoparticles (CPNs), have emerged as promising fluorescent probes due to their exceptional brightness, high quantum yield, nonblinking, photo-stability, and faster emission rate. Pdots, particularly, refer to small semiconducting polymeric nanoparticles and have shown remarkable conduction properties due to the presence of highly delocalized π-conjugated backbone [50]. Pdots prepared by miniemulsion method usually produced polymeric particles with size ranging from 40 to 500 nm depending on nature of polymer and concentration of surfactant. Reprecipitation method yields Pdots in the range of 5–30 nm. Size usually can be modified depending on biological application; however, most bioimaging and assays require smaller nanoparticle. Brightness and photo-stability of fluorescent Pdots appear to increase with size increment but have also exhibited higher steric hindrance,

decreasing their target specificity and binding affinity [51]. These carriers have widely investigated for their outstanding potential for bioimaging and biosensing both *in vitro* and *in vivo*. Major advantage lies in lesser cytotoxicity as was observed in the case of inorganic nanocrystals or quantum dots (Qdots) that were also observed to be associated with genotoxic and epigenetic effects in mammalian cells even at minute concentrations [52].

Biological applications of fluorescent probes can be controlled by manipulating surface chemistry. Usually, aqueous solubility is the primary requirement for these particles to perform biological functions efficiently. Such modifications have been carried out by incorporating charged molecules in polymer side chains [53]. Current research has much interest in development of various multifunctional Pdots that offer a wide range of biological applications due to easy preparation and diverse chemical dynamics. Conjugated polymers on the basis of varying structure of backbone can be distinguished into four major categories including poly-(fluorine), poly-(p-phenylene vinylene), poly-(p-phenylene ethynylene), and poly-(thiophene). These polymeric backbones can be further functionalized to incorporate desired characteristics [54]. Wang and coworkers have developed water-soluble conjugated polymer (polythiophene) with tyrosin kinase inhibitor (lapatinib)-modified side chains for plasma membrane imaging by targeting intracellular regions of transmembrane proteins [55]. Scientists have prepared conjugated polymer nanoparticles for both drug delivery and cell imaging together via exploiting electrostatic interactions among cationic conjugated polymers and anionic functionalities. Doxorubicin was delivered to target cancer cells by conjugating it with cationic fluorescent PFO and anionic poly-(L-glutamic acid) that allowed to monitor drug release through 'turn on' fluorescent signal generated by PFO [56].

Apart from cellular imaging, Pdots have also been investigated for delivery of DNA and siRNA. It has been found that nucleic acid carrying a negative charge can be easily incorporated into positively charged semiconductor Pdots. Silva and coworkers have demonstrated the delivery of siRNA using fluorescent CPNs for posttranscriptional gene silencing in plant protoplast without any significant impact on cellular viability in 72 h. They also explained the delivery of siRNA to specifically targeted genes in NTCesA-1 pathway associated with cellulose biosynthesis using CPNs [57]. Moon and associates have developed loosely aggregated CPNs for delivery of siRNA for transfection into HELA cells. siRNA-loaded CPNs caused downregulation of actin b protein with a transfection efficiency of 94% [58].

Scientists have also investigated the role of conjugated polymers for their antimicrobial potential. Cationic, light-absorbing, conjugated polyelectrolytes were studied for their activity against Gram-positive bacterial spores and Gram-negative bacterial strains. The study suggested that conjugated poly-electrolytes formed surface coating on both bacterial types and caused light-induced bactericidal activity [59]. Electrostatic interactions between negatively charged cell surfaces and oppositely charged markers have also been exploited against microbial and cancerous cells. Cationic CPNs have been designed through electrostatic interaction between positively charged PBF and negatively charged SDPA (disodium salt 3,3′-dithiodipropionic acid). These nanoparticles on photoexcitation by white light sensitized production of reactive oxygen species that effectively killed surrounding tumor and bacterial cells along with fluorescent imaging of cellular uptake of these particles [60]. Electrostatic interactions

have also been exploited for delivery of doxorubicin through multifunctional CPNs. CPNs (50 nm approx.) with excellent photo-stability and quantum yield but lower cytotoxicity have been prepared by combination of cationic PFO and anionic poly(L-glutamic acid) followed by conjugation with doxorubicin. This carrier offered targeted release of drug in cancer cells along with concurrent examination of drug release via self-luminescence activity [56].

4.3. Carbon dots

Carbon dots (CDs), also known as carbon nanoparticles or carbon quantum dots, are quasi-spherical fluorescent nanoparticles, gaining excessive attention because of unique optical nature, biocompatibility, low cytotoxicity, and simplistic synthesis [61]. These particles were accidently discovered while electrophoretic purification of single-walled carbon nanotubes (SWCNTs) synthesized using arc discharge process. CDs are usually defined as zero-dimensional particles with size range lying around 10 nm. Various synthetic approaches have been investigated for preparation of CDs with efficient photoluminescence, longer wavelength, and multicolor tunable emission [62]. Several types of carbon materials have been engaged to prepare CDs including graphite, activated carbon, carbon nanotubes (CNTs), and nano-diamond using top-down approach [63]. Bottom-up approach has employed citrate, biomolecules, and polymer-silica nanocomposites to prepare CDs using a variety of reaction conditions [64]. CDs have been addressed as safe and biocompatible substitutes to quantum dots that offer better brightness, photo-stability, and lower cytotoxicity both *in vitro* and *in vivo* [65].

Fluorescent CDs have expressed great potential in the field of biosensing, imaging, and photodynamic therapy as well as gene and drug delivery. CDs can be employed for *in-vitro* and *in-vivo* cell imaging using both one- and two-photon excitations. Yang and coworkers have demonstrated biomolecule surface-modified fluorescent carbon dots for *in-vivo* cell imaging along with good biocompatibility and less cytotoxicity [66]. Fluorescent CDs with surface modified with PEG were also studied for *in-vivo* biocompatibility and cytotoxicity through fluorescence imaging [67]. Luo and coworkers have extensively reviewed the optical imaging of carbon dots both *in vitro* and *in vivo* [65]. Various functionalized CDs have been studied for fluorescent imaging of plasma membrane and cytoplasm of COS7 cells, BGC823 cells, MG-63 cells, A549 cells, and HEPG-2 cells [68]. Scientists have also demonstrated uptake of CDs by endosomes and lysosomes in fluorescent imaging of HELA cells [69]. Besides these investigations, some studies have reported distribution of CDs in entire cell including nucleus [70]. CDs have also been explored for cellular imaging and labeling of *E. coli* [63].

CDs have also been investigated as biosensors in various research studies. Fluorescent carbon dots when conjugated with *N*-(2-aminoethyl)-*N*, *N*, *N*-tris (pyridin-2-yl methyl) ethane-1, 2-diamine have been studied to detect intracellular Cu^{+2} ions with greater specificity and stability [71]. In another study, fluorescent CDs have been used for detection of metal ions. Scientists have prepared carbon dots from citric acid as a carbon source in the presence of PEI for intracellular imaging and detection of Cu^{+2} ions [72]. Besides metal ion detection, COOH- or OH-functionalized CDs have been used as a receptor to detect change in hydrogen ion concentration. This fluorescent C dot probe has been successfully investigated to detect change in pH of 6–8 range in A549 and LLC-MK2 cells [73]. CDs have been studied for detection of glucose as its transport is associated with certain anomalies such as diabetes and cancer [74].

Scientists have also demonstrated use of CDs for detection of DNA. ssDNA was immobilized on CDs that can get hybridized with required complementary DNA molecule to form dsDNA followed by desorption from CDs and quantification of fluorescence [75]. CDs-dsDNA complex has also been investigated to detect histones. The strong interaction between DNA and histone causing the detachment of DNA from CD that turned on the signal for native fluorescence of CD [76]. CDs conjugated with gold and silver nanostructures have been explored as electro-chemiluminescence (ECL) immuno-sensing devices for detection of prostate-specific antigen (PSA) [77]. CDs have also been conjugated with chlorin e6 (Ce6) photosensitizer for efficient intracellular transport of photosensitizer, longer circulation time, and homing ability in tumor cells. These conjugates revealed excellent stability, biocompatibility with least cytotoxicity and exhibited tremendous bioimaging and homing ability in subcutaneous MGC-803 xenografts in nude mice [78].

Another therapeutic application associated with CDs is gene and drug delivery to targeted cells. pH-responsive, COOH-functionalized CDs caped on surface of mesoporous silica nanoparticles (MSPs) have been studied for intracellular tracking and delivery of doxorubicin with strong luminescence and low cytotoxicity both *in vitro* and *in vivo* [79]. CDs-conjugated mesoporous silica nanoparticles capped with PEG have been investigate for tracking controlled release of doxorubicin through quantifiable fluorescent intensity in HELA cells [80]. In another study, hollow CDs have been prepared from bovine serum albumin for pH-dependent delivery of doxorubicin and its rapid intracellular uptake. Such functionalized hollow CDs have been regarded suitable for bioimaging and targeted drug delivery [81]. Quinolone-conjugated fluorescent CDs have also been explored for *in-vitro* cellular imaging and delivery of drug to cancer cells [82].

4.4. Quantum dots

Quantum dots (Qdots) are inorganic, semiconductor, fluorescent nanostructures composed of II–IV or III–V group elements. They are crystal structures with size smaller than excitation Bohr radius (few nanometers), and these physical dimensions are controllable by time, temperature, and molecules (ligands) used in their synthesis [83]. Qdots in the range of 2–6 nm are of especial interest due to resemblance of their dimensions with biomolecules and have also shown to display strong dimension-dependent electrical and optical characters. Other distinct features include necessity of few Qdots to generate a detectable signal and minimal photo-bleaching property [84]. The idea of quantum confinement is responsible for unique optical and electronic characteristic of Qdots. Both group II–IV and III–V Qdots have been synthesized with relatively lower quantum yield and greater size difference. Higher quantum yield and better luminescence were observed when CdSe core was capped on surface with ZnS or CdS (higher band gap) [85].

The optical character of Qdots has been associated with the interactions among electrons, holes, and surrounding environment. Qdots undergo absorption of photon when excitation energy surpasses band gap where electrons jump from valence band toward conduction band. The presence of multiple electronic states at elevated energy level offers excitation at relatively lower wavelengths across UV-visible spectra. Emission wavelength can be tuned among the region of blue and near infrared (NIR) wavelength by manipulating size

and composition of Qdots. This feature allows simultaneous excitation of multicolor Qdots with single light source that makes them excellent candidate for biological application. Bioconjugation and functionalization of Qdots have increased the spectrum of their activities [36]. Qdots have been widely investigated for *in-vitro* and *in-vivo* imaging at molecular and cellular levels, to study intracellular trafficking as well as tumor targeting [86]. Quantum dots have been studied in immunofluorescence assays for detection of biological molecules and labeling of tissues and cells. NIR fluorescent nanoprobes conjugated with copolymer grafts of poly(L-lysine) and methoxy-polyethylene glycol succinate for *in-vivo* imaging of tumor related lysosomal protease activity. These probes successfully detected small-sized solid tumors with higher NIR signals and to examine specific enzyme activity [87]. Qdots have also explored to study the modifications in erythrocyte membranes caused by plasmodium invasion in malaria via immuno-cytochemical studies [88]. Jaiswal and coworkers have demonstrated multicolor imaging of Qdots-labeled live cells. They explained two approaches for cell labeling; either through intracellular uptake of Qdots by endocytic mechanism or use of antibody-conjugated quantum dots specific to cell surface proteins [89]. Parak and associates have used colloidal Qdots to study metastatic potential of cancer cells due to their photochemical stable nature and to study the mechanism of motility and migration of cancer cells. Uptake of nanocrystals was explained to occur through pinocytosis and phagocytosis [90].

Qdots have also been explored to prevent their nonspecific uptake by RES. Molecular markers expressed by blood vessels have been exploited to target nanocarriers toward specific tissues or organs. This strategy has been employed to target lung tumor cells using functionalized Qdots in mice [91]. Surface of quantum dots has been functionalized with COOH, NH2, and streptavidin that was further derivitized using PEG. PEG-conjugated Qdots decreased nonspecific uptake by RES, while COOH- and NH2-functionalized Qdots without PEG showed improved intracellular uptake among various cell types [92]. Qdots have also been explored for gene delivery and gene silencing. Sponge proton-coated Qdot-siRNA has been studied to improve gene silencing efficiency and reduced cytotoxicity in MDA-MB231 cells. These nanocarriers also allowed intracellular tracking and localization of siRNA delivery and transfection [93]. In another study, siRNA transfection was performed using Qdots. siRNA-Qdots exhibited greater photo-stability and tunable optical characteristics. This method was developed to observe the function of T-cadherin in intercellular communication [94].

Chitosan-folate-encapsulated ZnO Qdots have been prepared for delivery of anticancer agent doxorubicin with enhanced and longer photo-stability of Qdots. This nanocarrier showed an initial rapid release followed by controlled liberation of drug [95]. Doxorubicin-loaded, immuno-liposome-based quantum dots were modified with HER2scFv for targeted delivery of drug to SKB-3 and MCF-7 cells with overexpressed HER2. These Qdot-IL conjugates exhibited receptor-dependent endocytosis in target cells but not in control MFC-7 cells. They also showed longer circulation of Qdots, and their localization in tumor models was confirmed by florescence imaging [96]. Cadmium telluride-incorporated Qdots with PEI functionalization for tracking of plasmid DNA in mice were designed. After intravenous injection, these structures showed rapid accumulation in the lungs, spleen, and liver.

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PEG functionalization caused improved circulation time and rapid accumulation in cancer cells [97].

4.5. Magnetic nanocarriers

Magnetic nanoparticles (MNPs) are one of other fascinating elements of nanotechnology. Their nanometric dimension, biocompatibility, nontoxicity, and surplus accumulation in targeted cells or tissues justify intensive research in this subject matter. MNPs are mostly composed of ferromagnetic material such as ferrous or ferric oxide core with limited use of cobalt and nickel [98]. Magnetic properties are associated with movement of subatomic particles including electrons, holes, protons, and positive-/negative-charged ions. These materials respond to external stimulus of magnetic field and orient themselves according to magnetic moment. This magnetic behavior has been exploited for both *in-vitro* and *in-vivo* biomedical applications [99]. Magnetic nanoparticles have also been suggested for labeling cells in tissue engineering as they can be easily handled using magnets. Streptavidin-functionalized paramagnetic particles in combination with antibodies have been investigated for magnetic field-guided retroviral infection *in vitro* [100].

Magnetic nanoparticles have the ability to cause ablation of tumor cells via generation of heat. AC magnetic field causes the magnetic particles dispersed in target cells or tissues to get heated. This heat is rapidly disseminated to diseased cells, and if 42°C (therapeutic temperature threshold) can be maintained for 30 min, tumor cells get destroyed. However, this thermal ablation may be associated with undesirable concurrent killing of healthy cells [101]. Hase and coworkers have used ferromagnetic heating in combination of hepatic arterial embolization to study heat induction of ferromagnetic implants on VX2 hepatic cancer in rabbits. Results indicated extensive degeneration of tumor cells suggesting a suitable therapeutic strategy for localized hepatic carcinomas with little damage to healthy parenchyma of the liver because of selective heat induction [102].

Various functionalized magnetic nanocarriers have been investigated for targeted delivery of therapeutic agents. Magnetic drug carriers were designed either by using a magnetic core with surface coated with polymer or magnetic particles precipitated within porous polymeric composite. Such modifications have been studied to protect magnetic particle from harsh physiological vicinity and also to guide the drug carrier to desired location. Magnetic field-guided uncharged magnetic nanoparticles have been investigated for intracerebral targeting of rat glioma-2 in male (Fisher 344) rats. These magnetic nanoparticles (10–20 nm) exhibited greater uptake in brain tumor cells as compared to larger size (1 μm) magnetic particles [103]. In another study, iron oxide core was coated with oleic acid and subsequent coat of PEG-oleic acid for sustained release of doxorubicin and as MRI contrast. These modified magnetic nanoparticles were further conjugated with antibodies for active targeting of MFC-7 cells. These MNPs showed better MRI contrast with longer circulation time. They exhibited sustained release of drug with enhanced antiproliferative effect [104]. Doxorubicin-loaded monodisperse mesoporous single crystal iron oxide nanoparticles have also been developed as a promising carrier with improved drug loading and delivery [105].

5. Conclusion

Multifunctional nanocarriers offer a wide spectrum of biological applications exploiting both extracellular and intracellular barriers. These polymeric nanostructures have successfully improved the efficacy and safety of molecules delivered for various diagnostic and therapeutic purposes. Such nanocarriers have propounded unique physicochemical properties that have overall augmented the pharmacokinetic and pharmacodynamics parameters of drugs owing to versatility in their dimensions and surface functionalization. Bioavailability of many drug molecules that was compromised due to uptake by RES has been enhanced by exploiting these nanodevices. They have also offered long circulation time with release of drug molecules in a controlled or sustained manner with substantially fewer adverse effects. Nanocarriers have also shown exceptional promise in cellular imaging and diagnosis. By using various functionalization techniques, fluorescent probes have been directed to target tissues and cells to study site-specific delivery as well as intracellular trafficking of targeted molecules. Thus, they have been exploited to perform dual role of cell imaging along with drug delivery. Nanocarriers have also been successfully employed for gene transfection and gene silencing as well as *in-vitro* and *in-vivo* detection of biological molecules. Most recent therapeutic strategies under research seem substantially captivated in various dot structures for improved delivery of therapeutic agents, and the same is the case for magnetic nanoparticles that also have offered incredibly assuring results. However, much work is yet to be accomplished to prepare a successful commercial candidate with an ultimate therapeutic spectrum. Critical *in-vivo* cytotoxic behavior of these nanocarriers and untoward effects on normal physiological processes still requires intensive exploration. Some drug-loaded coated nanoparticles have been subjected to preliminary human trials after display of promising outcomes in animal studies but will even so require a long while for appearance in clinical market.

Conflict of interest

There is no conflict of interests among authors.

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