CHAPTER

Brain targeting with lipidic nanocarriers

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CHAPTER OUTLINE

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7.1 INTRODUCTION

7.1.1 BRAIN TARGETING/MEDICAL NEEDS

Central nervous system (CNS) disorders, including brain tumors, neurodegenerative diseases, and cerebrovascular diseases are serious human health threats. Early diagnosis and successful treatment of these diseases is severely hindered because of the existence of the blood–brain barrier (BBB) (Wohlfart et al., 2012).

The BBB is composed of different cell types, including endothelial cells (ECs), astrocytes, microglial cells, and pericytes (Begley, 2004) (Fig. 7.1). The exchange of substances between brain tissue and blood is restricted by both physical (tight junctions) and metabolic (enzymes) barriers (Brasnjevic et al., 2009). Nevertheless, nutrients such as hexoses, amino acids, and neuropeptides are normally transported from blood to brain through specific receptors; besides nutrients, only small ($<$500 Da) and highly lipophilic molecules can cross the BBB (Pardridge, 1998). In fact, almost 100% of macromolecules and more than 98% of small molecules cannot penetrate the BBB (Pardridge, 2007). Therefore, although novel neurotherapeutics are being discovered, they remain ineffective due to the lack of suitable strategies for transport across the BBB.

An ideal methodology for delivery of therapeutics across the BBB should have the following characteristics: be easily controlled; not damage the barrier; be biodegradable and nontoxic; selectively transport drugs across the BBB following systemic administration; target specific sites of intended action in brain; and transport adequate amount of drug to achieve therapeutic brain concentrations, with sufficient duration. Among the various approaches available to-date, nanobiotechnology-based delivery systems provide the best prospects for achieving the ideal transport methodology. To cross the BBB, drugs should preferably be introduced into the circulation by i.v. injection, since other routes of delivery (such as oral, transmucosal, transdermal, pulmonary, etc.) involve additional barriers, thereby the quantity of nanodrug presented at the BBB would be uncertain (Jain, 2012).

Targeted drug delivery systems are promising for treating CNS diseases, due to the following facts: (1) they can deliver drugs into the brain after systemic administration; (2) novel developments in nanobiotechnology contribute to production of effective targeted systems; (3) progress in biology provides strategies to target the brain and reach disease-related pathologies; and (4) microfluidic technologies allow easy and efficient scaling up, for manufacturing under GMP conditions.

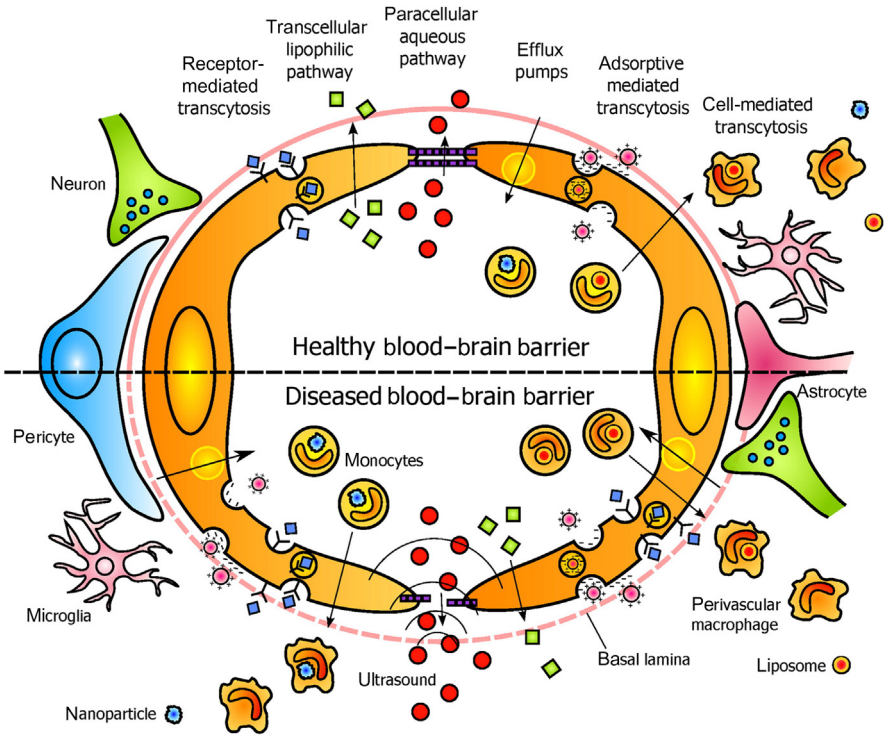

FIGURE 7.1

Schematic representation of BBB physiology and mechanisms of BBB translocation of molecules.

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7.1.2 BLOOD–BRAIN BARRIER PHYSIOLOGY

The main problem for drugs to reach the brain is the BBB, which is formed by ECs that interact with perivascular elements, such as the basal lamina, astrocytes, neurons, and pericytes to compose a functional unit (Fig. 7.1). Special tight junctions between brain epithelial cells are formed and, although all tissues are separated by epithelial cells, such tight junctions are only formed in the brain. The main function of the BBB is to protect the brain and keep it isolated from harmful toxins potentially present in the bloodstream, maintaining homeostasis for the most vital organ of the human body. Tight junctions prevent large molecules as well as several ions from passing to the brain, thus molecules must pass through ECs (Rubin and Staddon, 1999). Because of this, the only molecules that are able to easily transverse the BBB are glucose, oxygen, and carbon dioxide, as well as very lipid-soluble substances.

7.1.3 STRATEGIES FOR TRANSLOCATION OF MOLECULES ACROSS THE BLOOD–BRAIN BARRIER

Most drug molecules reach the brain via systemic blood circulation. To reach effective drug concentrations at CNS sites, it may be necessary to raise systemic drug levels (by enhanced dosing or extended administration), often resulting in increased risk of toxicity. Several strategies have been developed to assist the delivery of therapeutic molecules into the brain, which are categorized as: noninvasive methods; invasive methods (which induce pharmacological disruption of BBB); and other miscellaneous strategies (Guo et al., 2012; Lu et al., 2014; Jain, 2012), the advantages and disadvantages of which are summarized in Table 7.1. It should be emphasized that invasive methods are far from ideal, and the use of endogenous BBB transport systems are safer alternatives (Gabathuler, 2010; Soni et al., 2010; Patel et al., 2009). Numerous review articles are available for more information about the mechanisms of BBB translocation of molecules (Laksitorini et al., 2014; Masserini, 2013; Soni et al., 2010; Pardridge, 2008; Neves et al., 2016).

Noninvasive techniques include: (1) chemical approaches, which rely on chemical structure transformation of drugs to improve their unsatisfactory physicochemical properties, (2) biological approaches, such as receptor/vector-mediated delivery of chimeric peptides, cell-penetrating peptide (CPP)-mediated drug delivery, and viral vectors, and (3) colloidal drug carriers or nanocarriers (NCs) (Lu et al., 2014; Srikanthand and Kessler, 2012).

Chemical approaches rely on certain structural modifications of drug molecules, to improve their physicochemical properties (such as aqueous solubility or membrane penetration). The most applicable modification is the insertion of lipid groups onto polar groups of the drug, as a methodology to enhance passive transport. Another chemical approach to enhance the lipid solubility, and thus BBB passage, is the synthesis of prodrugs. Esterification or amidation of hydroxy-, amino-, or carboxylic acid-containing drugs may enhance their lipid solubility. Prodrugs are converted to their active forms by enzymatic cleavage. Another possibility is to link the drug to a lipid moiety, such as a fatty acid, a glyceride, or a phospholipid. In general, the rationale behind the use of prodrugs is to optimize the absorption, distribution, metabolism, excretion, and unwanted toxicity profile, the so-called absorption, distribution, metabolism, excretion (ADME) properties of drugs (Zawilska et al., 2013). It should be noted that, in cases of chemical modification, drug pharmacokinetic properties are also modified (Bodor and Buchwald, 1999; Dwibhashyam and Nagappa, 2008; Bodor et al., 1992; Salameh et al., 2015).

Biological approaches include conjugation of drugs (especially peptides) with antibodies directed towards antigens residing on the target tissues (receptor/vector-mediated delivery). Conjugation of other ligands (sacharides or lectins) can direct molecules to specific receptors (Begley, 1996; Pardridge, 1986). This strategy is based on chemical coupling of a nontransportable peptide (or other

Table 7.1 Currently Tested Approaches for Delivery of Active Molecules to the CNS/Advantages Disadvantages

Method	Advantages	Disadvantages
Noninvasive Methods		
Chemical/ biological approaches	• Release of active drug in the systemic circulation • Oral administration can lead to significant first pass effect • Possible adverse secondary effects • Efflux transporters at the BBB limits penetration of therapeutics • Penetration of hydrophilic drugs across BBB is limited	• In case of prodrugs: original pharmacokinetic parameters, efficacy and toxicity may be modulated • Poor selectivity and poor tissue retention of some molecules • Lipidization generally increases the volume of distribution, the rate of oxidative metabolism and uptake into other tissues • Increases risk of systemic toxicity
Colloidal drug carriers	• Encapsulate insoluble drugs • Reduced toxicity • High biocompatibility • Simple manufacturing • High drug loads • Biodegradable • Protection of drug from enzymes • Targeted and triggered release	• No colloidal system for BBB targeting has been clinically approved to-date
Invasive Methods		
Intracerebral implants	• Sustained/controlled release kinetics • Tunable release properties • Low invasiveness • Low peak drug release limits tissue damage • Biocompatible • Localized delivery	• Highly traumatic technique • Poor drug penetration • Drug dosage limited by implant size • Increased risk of trauma
Intraventricular intrathecal, interstitial	• High drug concentration in the CNS • Minimal systemic exposure and toxicity	• CNS infection • Catheter obstruction • Inadequate drug distribution
Biological tissue delivery	• The simplest approach to this technique is to implant into the brain a tissue that naturally secretes a desired therapeutic agent	• The survival of foreign tissue grafts may be improved by advancement in techniques for culturing distinct cell types

(*Continued*)

Table 7.1 Currently Tested Approaches for Delivery of Active Molecules to the CNS/Advantages Disadvantages *Continued*

Method	Advantages	Disadvantages
BBB Disruption (Effective Invasive Technique) (Patel et al., 2009)		
CED	• High concentration in brain • CNS-targeted • Lower systemic side effects • Large drug distribution volume • Flexible therapy protocol • Consistent drug concentration	• Invasive • Long infusion times • Unpredictable drug distribution • Potential high intracranial pressures
Osmotic	• Increased BBB permeability by disruption of inter-endothelial Tight Junctions (TJs) for several hours	• Unfavorable toxic/therapeutic ratio often observed with hyperosmotic BBBD
Biomedical	• Less invasive and possibly a more reliable technique for BBBD because it mainly affects the diseased vasculature	
Ultrasound (US)-mediated	• Poor penetration of US through the skull	
Other Methods		
Intranasal delivery	• Drugs with lower molecular weight and higher lipophilicity generally favor rapid intranasal uptake into the CNS	• Reduced efficiency of larger molecular weight drugs • Mucosal irritation caused by nasal pathology should be avoided
Iontophoretic delivery	• Delivers ionized molecules across the BBB	

type of) pharmaceutical to a transportable peptide or protein, which undergoes receptor-mediated or absorptive-mediated transcytosis through the BBB (Kang et al., 1994). Cell-penetrating peptides (CPP) interact with cell surfaces via a receptor independent mechanism and may thus transport the molecules that are coupled to them (Temsamani and Vidal, 2004; Salameh et al., 2015), mediating their delivery.

Colloidal drug carrier methods deliver drugs by the use of Nanocarriers (NCs). Brain delivery of drug-encapsulated NCs can be further enhanced by conjugation of specific ligands to the NC surface. The most extensively studied colloidal drug carriers are polymeric or solid-lipid nanoparticles, or NCs, micelles, liposomes, emulsions, and dendrimers (Fig. 7.2).

Most biological and colloidal drug carrier methods for drug transport into the brain rely on *endogenous BBB transporters*. These transporters are classified into three main categories: carrier-mediated (CMT), active efflux (AET), and receptor-mediated transport (RMT) (Fig. 7.1). The CMT and AET systems are

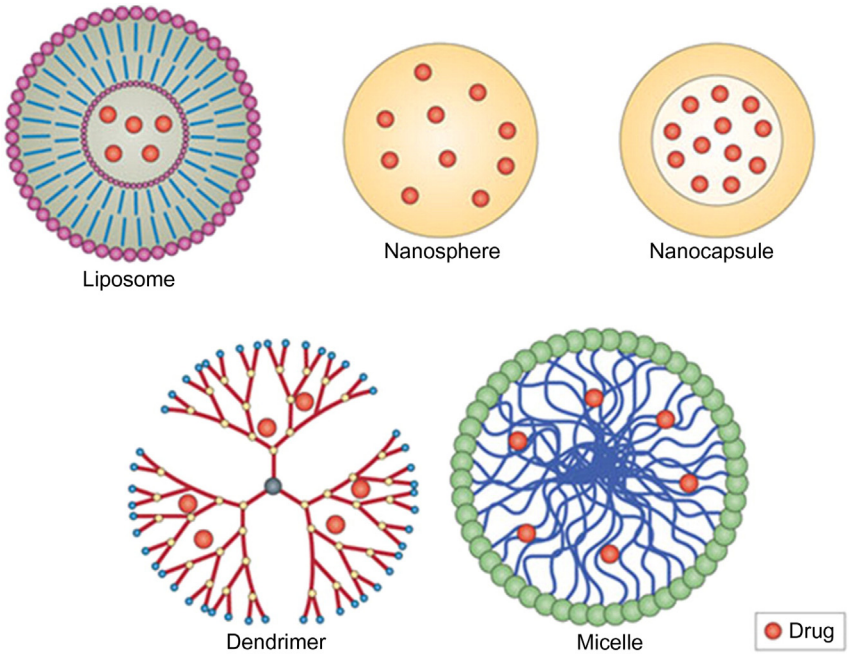


FIGURE 7.2

Graphical representation of the structure of basic types of drug nanocarriers.

Reprinted from Cheng, Y., Morshed, R.A., Auffinger, B., Tobias, A.L., Lesniak, M.S., 2014. Multifunctional nanoparticles for brain tumor imaging and therapy. Adv. Drug Deliv. Rev. 66, 42–57, after permission from Elsevier (Order Number: 4018090184549).

responsible for transport of low-MW-molecules, such as glucose, amino acids, and nucleotides (Gabathuler, 2010), while RMT systems assist the transport of large molecules, such as insulin, transferrin, low-density lipoprotein (LDL), insulin-like growth factor (IGF), and leptin, across the BBB. In RMT, molecules (ligands) bind to specific receptors on the luminal surface of BBB and the receptor–ligand complex is internalized by receptor-mediated endocytosis. The ligand molecule is then transported across the EC and into the brain (Pardridge and Boado, 2012). RMT has been exploited to develop molecules that can efficiently cross the BBB to deliver drugs into the brain. Such molecules can be peptide or proteins with affinity for RMT transporters, or monoclonal antibodies (mAbs) that specifically target RMT receptors (Soni et al., 2010; Pardridge, 2006). Monoclonal antibodies have been integral tools in basic research, due to their high specificity/affinity for target antigens (Aires da Silva et al., 2008). Accordingly recombinant mAbs against RMT receptors have promising potential as "homing devices" for therapeutic brain delivery.

Invasive techniques: In order to avoid toxicity caused by administration if high drug doses, drugs may be directly inserted into the brain tissue by means of

invasive techniques which disrupt the BBB (Wang et al., 2002a,b). Invasive techniques include: (1) intracerebral implants, (2) intraventricular/intrathecal/interstitial delivery, (3) biological tissue delivery, and (4) BBB disruption (BBBD).

Intracerebral implants consist of biodegradable polymeric matrix or reservoir systems utilized for delivery of chemotherapeutics in clinical trials against recurrent high-grade gliomas (Westphal et al., 2003) and in preclinical research (Vukelja et al., 2007; Sheleg et al., 2002; DiMeco et al., 2002), but they are considered as a highly traumatic technique. The increased risk of trauma and poor drug penetration beyond the resection cavity limits their applicability.

Direct drug injection to the intraventricular, intracavitary, or interstitial system is considered as the most appealing technique to circumvent the BBB. High drug concentration in the CNS with minimal systemic exposure and toxicity is the basic advantage of this method. However, CNS infection, catheter obstruction, and inadequate drug distribution are some of the disadvantages (Scheld, 1989).

Releasing drugs from biological tissues is another possibility. The simplest approach is to implant a tissue that naturally secretes the required therapeutic agent. This approach has been extensively applied for treatment of Parkinson's disease (Sladek and Gash, 1988); however, the transplanted tissue cannot survive, due to lack of neovascular innervations.

Finally, several techniques have been applied for *BBBD*, such as *convection-enhanced delivery (CED)*, where drugs are directly administered into the brain parenchyma or tissue by a microinfusion pump, via one or more catheters that are stereotactically placed through cranial holes (DiMeco et al., 2002). Compared with traditional delivery methods, CED has unique characteristics for CNS delivery (Cunningham et al., 2008). Another type of BBBD is *osmotic BBBD*, by temporarily shrinking the BBB ECs with a concentrated sugar solution, leaving drugs to pass into the brain (Neuwelt, 2004). Compared to standard chemotherapy, BBBD procedures deliver 10−100 times higher drug amounts. *Biomedical BBBD* is also possible by using vasoactive compounds that selectively increase the permeability in abnormal brain capillaries (Cloughesy and Black, 1995), while normal brain capillaries resist the effects of these compounds through an "enzymatic barrier" which inactivates the vasoactive agent. *Ultrasound (US)-mediated BBBD* is another strategy that consists of pressure waves of 20 kHz or greater frequencies (Zhao et al., 2008), which is limited by the poor penetration of US through the skull (Guthkelch et al., 1991).

Alternative routes for BBB translocation of drugs include the *intranasal route* (may also be categorized as a noninvasive approach) (Abbott and Romero, 1996), which bypasses the cardiovascular system (Sakane et al., 1995); and *iontophoresis*, a method to deliver ionized molecules across the BBB by an externally applied electric current (Jogani et al., 2008). Drugs with lower molecular weight and higher lipophilicity demonstrate rapid intranasal uptake into the CNS. NCs have also been used for intranasal drug delivery with good results (Eskandari et al., 2011). Limitations of intranasal delivery are: the reduced efficiency for larger MW drugs and the mucosal irritation caused.

Invasive approaches for brain delivery are considered as less patient-friendly, more laborious, and requiring skill, with possible permanent damage to the brain. Consequently, the discovery of attractive strategies for effective delivery of drugs to the brain by noninvasive techniques is of key importance (Chhabra et al., 2015; Lu et al., 2014; Grabrucker et al., 2013).

7.1.4 METHODS TO STUDY BLOOD–BRAIN BARRIER PERMEABILITY OF DRUGS (IN VITRO/IN VIVO)

In order to investigate BBB transport or for screening potential delivery systems for their capability to facilitate drug/imaging agent transport across the BBB, various in vitro systems, cellular models, and animals (wild type (WT) or transgenic (TR)), have been developed to date.

In vitro methods are categorized in two main groups: noncell and cell-based methods. Several noncell methods, such as high-performance liquid chromatography (HPLC) (development of affinity columns); immobilized artificial membranes which mimic the properties of biological membranes; and others, have been used to measure the potential of molecules to be translocated across the BBB. They were moderately successful to rank compounds according to their BBB permeability. However, they were not found suitable for medium- to high-throughput operations. A promising technology is the parallel artificial membrane permeability assay (PAMPA), which provided moderately good correlation with data derived from the human colonic epithelial cell line (Caco-2) and in vivo permeability data. By modifying the lipid composition of the artificial membrane, the basic component of the PAMPA system, it is capable of predicting CNS permeability with reasonable accuracy (Di et al., 2003).

Additionally, *cell-based permeability assays* have been developed and used in BBB permeability prediction studies. Most BBB cellular models are based on cocultures of brain ECs and astrocytes (or glial cells) (Nakagawa et al., 2007). In particular, whereas bovine brain ECs alone only partly recapitulate BBB properties, a coculture system with rat glial cells has been extensively validated as a reference BBB model (Cecchelli et al., 1999). Because these cells express tight junctions as well as numerous membrane transporters, they constitute a valuable alternative or complement to the epithelial cell lines Caco-2 and Madin-Darby canine kidney (MDCK), currently used for drug screening by pharmaceutical industries because of their very high permeability restriction (Garberg et al., 2005). Alternative BBB models are also available, using pig, mouse, rat, or human brain ECs (Antimisiaris et al., 2014). In addition, stable immortalized rat EC lines were produced and validated as in vitro models of the brain endothelium; first the RBE4 cell line, followed by a number of other cell lines. More recently, the human hCMEC/D3 brain EC line, which retains most of the morphological and functional characteristics of brain ECs, was developed. This cell line expresses Tight Junctions

(TJs) and multiple active transporters, receptors and adhesion molecules, even without coculture of glial cells. Permeability across hCMEC/D3 monolayers is significantly decreased when the system is used under three-dimensional flow conditions (Cucullo et al., 2008).

In vivo (and in situ) methods used for evaluation of BBB permeability (Smith, 2003) include determinations of brain/plasma ratio (log BB) and measurements of the permeability surface area product (PS or log PS), from which permeability (P) can be derived provided that the vessel surface area (S) can be estimated. Most pharmaceutical companies generate log BB data in animals (often rat) as part of the standard pharmacokinetic profiling of compounds. Determining the unidirectional influx coefficient (Kin) by using the in situ saline-based perfusion method more accurately reflects the BBB permeation step, effectively isolating the "kinetic" element of drug penetration. Because of accurate quantitation, the Kin (or PS) measurements are considered as "gold standard" references for other methods (Antimisiaris et al., 2014). For drugs acting on CNS target sites (membrane receptors, transporters), the critical concentration is the free concentration in brain interstitial fluid. Brain/plasma ratio measured, especially at longer times and for more lipophilic agents, will be affected by drug distribution into brain lipids and nonspecific binding. Measuring free concentration with a microdialysis probe is possible but technically difficult, and there is a particular problem of the recovery of more lipophilic agents.

For assessment of proposed CNS diagnostic and therapeutic approaches, TR mice models that mimic a range of Alzheimer's disease-related pathologies, Parkinson's disease and glioblastoma (GBM) are currently available. They have been widely used in preclinical testing of potential therapeutics and played a pivotal role in the development of immunotherapies for CNS diseases (Antimisiaris et al., 2014; Antony et al., 2011; Huse and Holland, 2009).

7.1.5 APPROACHES TO TRANSPORT DRUGS ACROSS THE BLOOD–BRAIN BARRIER: A ROLE FOR NANOTECHNOLOGY

Both noninvasive and invasive strategies have been developed to circumvent CNS barriers. As previously mentioned, NCs can be used as efficient drug delivery systems, owing to their advantages over conventional drug delivery systems: (1) drugs can be incorporated within the NCs without any chemical reactions, preserving thus the drug activity and efficacy; (2) NCs provide a protective entity in which the drug can be encapsulated and thus protected from the enzymatic degradation during its journey into the bloodstream (in some cases reducing the first pass effect following oral administration); and (3) NCs can achieve controlled/sustained release and even targeting of the drug following systemic circulation, thus modifying and ameliorating drug distribution kinetics inside the body.

The capability of NCs for controlled release can play a pivotal role in limiting drug concentration in nontargeted sites, thus reducing adverse secondary effects.

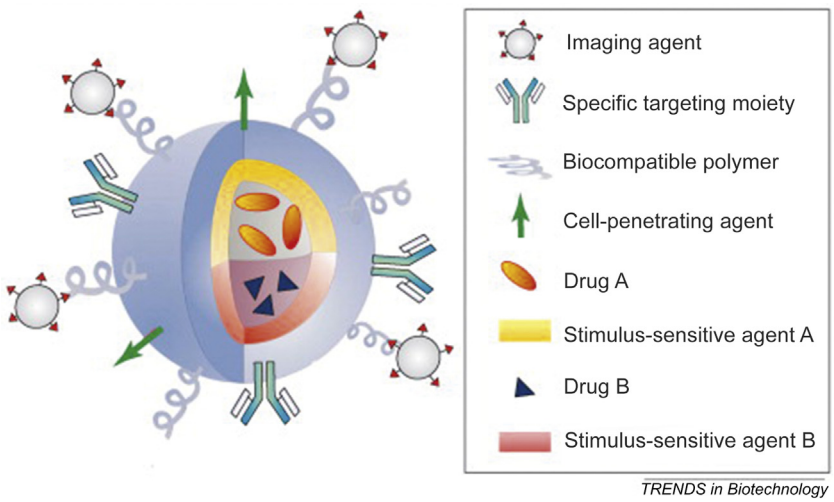


FIGURE 7.3

Schematic representation of multifunctional nanoparticles for brain tumor imaging and therapy applications.

Reprinted from Sanvicens, N., Pilar Marco, M., 2016. Multifunctional nanoparticles – properties and prospects for their use in human medicine. Trends Biotechnol. 26, 8, 425–433 after permission by Elsevier (Order Number: 4018100207296).

The biodegradation of these NCs can be manipulated and time-controlled by the choice of material (depending on the nature of polymer or lipid) used to prepare the NC matrix. Other advantages of using NCs are the enhanced accumulation and capability of targeting the site of action via surface engineering. In fact, the use of surface ligands could be exploited for selective recognition by the receptors expressed on the targeted site or by other triggers (such as magnetic targeting) (Fig. 7.3). The same surface engineering approach allows NCs to preferentially target diseased cells. Furthermore, they can be exploited to interact with specific subcellular organelles to treat organelle-specific diseases. For example, if NCs are designed to be endocytosed by clathrin-mediated endocytosis, they can specifically accumulate and target lysosomes. Protocols for the production of NCs can enable hydrophilic as well as hydrophobic substances to cross the BBB, and to be released from NCs after internalization (Grabrucker et al., 2014). Encapsulation of BBB impermeant drugs could help them reach the brain, since the chemical structure of the drug, which is hindered inside the NCs, has no involvement in the barrier permeation process. NCs can be administered by a variety of routes (including oral, inhalational, and parenteral) (Grabrucker et al., 2011), while their large surface-to-volume ratio permits multiple ligand copies to be attached and thus dramatically increasing their binding affinity via multivalency (Montet et al., 2006).

Organic nanostructured delivery systems are classified into two main categories, *lipid-based nanostructures and polymeric nanostructures*. Lipidic-NCs present several advantages related to their easy production (even at large scale), excellent storage stability, and the possibility of steam sterilization and lyophilization. Moreover, their lipophilic features lead them to the CNS overcoming the BBB by endocytotic mechanisms (Bondì et al., 2012).

7.1.6 GENERAL REQUIREMENTS FOR NANOTECHNOLOGIES TO BE USED FOR BRAIN TARGETING

When designing NCs for clinical applications, it should be clear that their systemic administration generates important modifications. In particular, the nonspecific interaction between NC surface and bloodstream proteins leads to the adsorption of opsonins on NCs, forming the so-called "protein corona." These proteins substantially change the bare material properties determining the removal of NCs from circulation by the reticuloendothelial system (RES) in the spleen and liver. The most common RES-escaping approaches are to formulate NCs with neutral surface charge, by coating their surface with polyethylene glycol (PEG) and to use small size NCs (Provenzale and Silva, 2009). NCs with these features are called "stealth" NCs and can avoid the RES (Gabathuler et al., 2010). Finally, NCs should be nontoxic, biodegradable, biocompatible, noninflammatory, and nonimmunogenic (Lockman et al., 2002). Requirements of NCs for BBB targeting are summarized in Table 7.2.

The therapeutic efficiency of NCs can further be improved by surface functionalization. Whereas monofunctional NCs provide a single function, multifunctional MCs combine different functionalities in a single stable construct (Fig. 7.3). Consequently, in principle, a multifunctional NC could achieve physical stability, biocompatibility, nonimmunogenicity, reduced toxicity, and also be

Table 7.2 General Requirements for Lipidic-NCs Intended for BBB Targeting

- Scalable and cost effective with regard to manufacturing process/inexpensive
- Small particle diameter < 100 nm
- Nontoxic, biodegradable, biocompatible
- Physical stability in blood (no aggregation)
- Noninflammatory/nonimmunogenic/avoidance of the MPS (no opsonization)/prolonged blood circulation time
- Nonthrombogenic
- Encapsulation of both hydrophilic and lipophilic drugs
- Amenable to small molecules, peptides, proteins or nucleic acids
- BBB-targeted (use of cell surface ligands)
- Minimal nanoparticle excipient which may induce drug alteration (chemical degradation/alteration, protein denaturation)

able to diagnose a BBB disease either by encapsulating or conjugating fluorescent dyes or MRI contrast agents (on their surface). Multifunctional NCs could also be conjugated with targeting ligands or antibodies to efficiently target BBB. The drug of interest could be either encapsulated into the NCs cavity (or incorporated in the lipid layer) or attached onto its surface (Chatterjee et al., 2014). A schematic presentation of the different functions possible within platform multifunctional NC systems is shown in Fig. 7.3.

7.2 LIPIDIC CARRIERS FOR BRAIN TARGETING

As mentioned above, receptor-mediated endocytosis (e.g., antibody or protein drug conjugates) (Fu et al., 2012) has been applied to facilitate drug transport across the BBB. The low BBB permeability of many drug molecules, especially biologicals, has driven research to the development of novel NC types (Seelig et al., 1994; Sarkar et al., 2011; Gabathuler, 2010; Banks et al., 2011; Yang, 2010; Kumari et al., 2010).

7.2.1 LIPIDIC NANOCARRIER TYPES

A wide range of organic NCs have been designed for drug delivery purposes. These include liposomes (Liu et al., 2014a,b), solid-lipid nanocarriers (SLNs) (Blasi et al., 2007), nanostructured lipid carriers (NLCs), polymer-based NCs (Tosi et al., 2008), protein-based NCs (Zensi et al., 2009), and other configurations, such as micelles and dendrimers (Fig. 7.2) (Ambade et al., 2005; Naseri et al., 2015). Herein, we will focus more on lepidic NCs, and mainly on liposomes, SLNs, NLCs which have been applied and used in most cases, and less on a few other types of lipid-based monolayer NCs. Furthermore, extracellular vesicles (EVs), particularly exosomes, which are being intensively exploited recently for drug targeting, are described. Structural characteristics, advantages, and disadvantages of lepidic NC types are summarized in Table 7.3.

Liposomes consist of an aqueous core surrounded by phospholipid bilayer(s). The lipid bilayer is formed so that the hydrophobic tails of lipids interact with each other internally and their hydrophilic heads are aligned outwards. This leads to the formation of vesicles with an aqueous core surrounded by a hydrophobic layer, which is further surrounded by the outermost hydrophilic heads of the lipids. Liposomes have been used for BBB targeting, owing to their advantages such as prevention of drug degradation, low toxicity, and encapsulation of hydrophilic and lipophilic drugs, etc. Surface-modification methods have been applied to increase the brain distribution of drug-loaded liposomes (Pardridge, 1999). Delivery of Cisplatin in brain tumors with increased concentration, increased cytotoxic effect, and significant increase in brain uptake of Amphotericin B have been demonstrated by

Table 7.3 Overview of Lipidic Nanocarrriers Tested Up-to-Date for Drug Delivery/Targeting Applications

Components/ structure	Advantages	Disadvantages
Liposomes (25–200 nm)		
Phospholipids, sphingolipids, cholesterol, PEGylated lipids (phospholipid bilayer/ aqueous core)	• Control release • Biocompatibility • Simple manufacturing • High drug loads • Nontoxic • Biodegradable • Nonimmunogenic • Targeted or triggered release • Can be administered through various routes • Possibility for triggered drug release	• Variable kinetics/distribution processes • Relatively less stable/rapid elimination by RES/lack of the ability for sustained release (PEG is required) • Fast degradation of lipids • Low encapsulation efficacy • Intermembrane transfer • Cell interactions or adsorption • Poor stability • Expensive
SLN (50–1000 nm)		
Pure triglycerides, glyceride mixtures, waxes, physiological lipids or lipid molecules (solid lipid core/surfactant coating)	• Good release profile • Targeted release • High drug content • Lipophilic and hydrophilic drugs • Excellent biocompatibility • Safe (avoid organic solvents during construction) • Easy preparation, scale up, sterilization • Less expensive than polymeric NCs • Easier validation and regulatory approvals • Drug stability, prolonged release/physical stability • Chemical versatility • Nontoxic lipids • Low toxicity (due to low solubility) • Stability can be further improved by coating • Production in powder form	• Lipid particle growth (by agglomeration) • Tendency for gelation • Unexpected dynamics of polymorphic transitions • Unpredictable gelation tendency and inherent low incorporation rates (resulting from the crystalline structure of the solid lipid) • Sometimes burst release of the drug (by coagulation) • Often expulsion of the loaded drug solution • Use of surfactants during construction

(*Continued*)

Table 7.3 Overview of Lipidic Nanocarrriers Tested Up-to-Date for Drug Delivery/Targeting Applications *Continued*

Components/ structure	Advantages	Disadvantages
NLC (100–500 nm) (Second Generation SLN)		
Pure triglycerides, saturated and unsaturated fatty acids, waxes (Blend of solid and liquid lipid phases)	• Can be formulated as foams, liquids, creams, and sprays • Nontoxic and nonirritant • Easily applied to skin/ mucous membranes • NLCs vs SLN: higher loading for some drugs, prevention of drug expulsion during storage • High tolerability and low toxicity (low solubility). Prevention of leakage • Multifunctionalized	• They are susceptible to Oswald ripening • Surface charge has a marked effect on stability • Variable kinetics of distribution processes and clearance • The use of surfactants during their construction
Cubosomes (100–300 nm)		
• Amphiphilic lipids GMO and phytantriol) • Stabilizers (Poloxamer 407)	• Loaded with hydrophilic, lipophilic, amphiphilic drugs • High payloads • Protect drugs from degradation • Enable sustained and targeted release-colloidally and/or thermodynamically stable for long time • Biocompatible • Biodegradable • Considered as nontoxic-prepared by simple method • Cubic phases are more bioadhesive in nature (topical /mucosal delivery of drugs)	• They do not offer controlled drug delivery on their own • It is very difficult to load water soluble active ingredients during the formation of cubosomes (due to large amounts of water already present in it) • Large scale production is sometimes difficult because of high viscosity
Niosomes		
Nonionic synthetic surfactants self-assembly (closed bilayer structures delimitating one or more internal aqueous compartments)	• Low cost • Great chemical and physical stability • Extended shelf-life • Wide formulation versatility • Sterilization	• Physical instability • Aggregation • Fusion • Leaking of entrapped drug • Hydrolysis of encapsulated drugs which limiting the shelf life of the dispersion

(*Continued*)

Table 7.3 Overview of Lipidic Nanocarrriers Tested Up-to-Date for Drug Delivery/Targeting Applications *Continued*

Components/ structure	Advantages	Disadvantages
	• Insertion of suitable surfactants functionalized to interact with biological targets • Conjugation of targeting ligands • Entrap drugs with wide range of solubility • Loaded with hydrophilic, lipophilic and amphiphilic drugs • Osmotically active and stable • Surfactants are biodegradable, biocompatible, and nonimmunogenic	• The use of surfactants during their construction
Exosomes (40–100 nm)		
Proteins, lipids: phospholipids, cholesterol, nucleic acids	• Small size (up to 100 nm) • Nonimmunogenic (due to similar composition as bodies own cells) • They have the ability to target tissues • Biocompatibility and minimal or no inherent toxicity	• Therapeutic effect, production efficiency and reproducibility of liposomal preparations is much higher than that of the current exosome standards • Exosome-based drug delivery still lacks substantial experimental validation in vivo

liposomal encapsulation. Limitations of liposomes include low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components, and poor storage stability (Garg et al., 2015). Moreover, conventional liposomes are characterized by lower plasma circulation times as a result of elimination by the RES. Extended circulation time can be achieved by decreasing their size (100 nm) or by modification of their surface with PEG (i.e., stealth liposomes) (Chhabra et al., 2015). Several recent review articles describe liposomes for BBB targeting (Bellavance et al., 2010; Ramos et al., 2011; Rueda and Cruz, 2016; Garg et al., 2015; Maussang et al., 2016; Craparo et al., 2011; Alam et al., 2010; Jain and Jain, 2015; Domínguez et al., 2014; Larsen et al., 2014).

Recent examples of BBB targeting by liposomes include multifunctional liposomes functionalized with: (1) drugs, peptides or other molecules that enable a specific targeting (e.g., curcumin which targets Aβ-peptides) onto their surface, by incorporation of a pre-synthesized lipid-drug derivative during liposome formation, or by chemical conjugation of the drug on preformed liposomes bearing appropriate groups. Liposome encapsulating or being conjugated by curcumin derivatives have been extensively tested, for therapy and diagnostic applications (Lazar et al., 2013; Mourtas et al., 2011); (2) mAbs or peptides (e.g., transactivating transcriptor, TAT) which enable specific targeting. Liposomes can be loaded with drugs/fluorescent molecules/other particles (e.g., magnetic nanoparticles for diagnosis).

Recent studies with different types of liposomes and other types of lipidic-NCs are summarized in Table 7.4.

Other classes of lipid-NCs include SLNs and lipid nanostructured carriers (LNC), with an average diameter of 40−1000 nm and spherical morphology. Both SLNs and LNCs are composed of solid phase lipids and surfactants. The dispersed phase is solid fat, and the surfactants are used as emulsifiers. Lipid components of SLNs are solid at both body and ambient temperature and can be highly purified triglycerides, complex glyceride mixtures, or even waxes. Surfactants are used in concentrations from 0.5% to 5% to enhance stability. The proper selection of lipids and surfactants can affect the NC physicochemical properties (Naseri et al., 2015). *SLNs* were developed at the beginning of the 1990s as alternative colloidal carriers to emulsions, liposomes, and polymeric NCs. The physical stability of SLNs, the use of bioacceptable and biodegradable lipids, the size in the nanometer range, and the protection of the encapsulated drug are promising features for parenteral administration. SLNs may be used for both hydrophilic and hydrophobic drugs (Das and Chaudhury, 2011). However, the drug-loading capacity of conventional SLNs is limited by low solubility of some drugs in the lipid melt and by drug expulsion after polymorphic transition. In order to incorporate hydrophilic compounds, they need to be combined with amphiphilic polymers to form hybrid polymer-lipid-NCs. PEGylated SLNs have been widely investigated for brain drug delivery (Chhabra et al., 2015; Wong et al., 2007). SLNs and PEGylated SLNs are readily taken up by brain tissues because of their lipidic nature. Accordingly, SLNs are promising candidates for CNS drug delivery (Kaur et al., 2008) and have been used for brain-targeted delivery of many therapeutics, such as doxorubicin (Agarwal et al., 2011), docetaxel (Venishetty et al., 2013a,b), quercetin (Dhawan et al., 2011), atazanavir (Chattopadhyay et al., 2008), quinine (Gupta et al., 2007), and camptothecin (Martins et al., 2012). More detailed information can also be found elsewhere (Patel et al., 2013; Gastaldi et al., 2014).

Nanostructured lipid carriers (NLCs) are often referred to as second generation SLNs. In NLCs, the lipid phase is composed of both solid (fat) and liquid (oil) lipids, improving the stability and drug-loading capacity, and preventing drug leakage during storage (Naseri et al., 2015). In more detail, NLCs are composed of a liquid, oily core

Table 7.4 Examples of the Various Lipidic-NC Types Incorporating Low or Large MW or Large-MW Active Molecules From the Recent Literature

Advantages/Results	Targeting Ligand	Active	Reference
Liposomes			
High affinity for amyloid deposits, delayed Ab1-42 aggregation	Anti-TrF	Curcumin	Mourtas et al. (2014)
Internalized into BBB endothelial cells, and cross-BBB models	dCDX and dA7R	DOX	Ying et al. (2016)
Enhance delivery of encapsulated drugs to brain	+ GSH	Ribavirin	Maussang et al. (2016)
Enhanced transport across BBB, and inhibited multidrug resistance	Tf	Vincristin/TET	Song et al. (2016)
Inhibited MT-3 tumors significantly better than free oxp (in vivo)	Angiopep (peptide)	Oxaliplatin	Orthmann et al. (2016)
Transport across BBB and enhanced in vivo anti-ischemic stroke ability in MCAO rats	T7 peptide, stroke homing peptide	tZL006	Zhao et al. (2016)
Targeted liposome were active for treatment of brain glioma	PTDHIV-1 peptide	Epirubicin and celecoxib	Ju et al. (2016)
Efficient targeting of brain gliomas in vitro and in vivo	TF/TAT	DOX and PTX	Chen et al. (2016)
Significant accumulation into brain tumor, and antitumor efficacy in glioma-bearing mice	PEI, somatostatin analog vapreotide	Vinorelbine and TET	Li et al. (2016)
Transport through the BBB by transcytosis	GLU	Sertraline	Harbi et al. (2016)
BBB transport & MRI contrast for detection of early stage glioma	Interleukin-13		Liu et al. (2016)
Tf(α-M) liposome improved brain delivery of α-M	Tf	α-Mangostin	Chen et al. (2016)
LIP destroy glioblastoma cells and glioblastoma neovasculature	GLU, cRGD	Epirubicin	Zhang et al. (2015)
High transport ability across BBB, destroyed glioma and brain cancer stem cells (CSCs) and vasculogenic mimicry (VM) channels	TR peptid-cRGD, TH peptide	Paclitaxel	Shi et al. (2015)
Increased uptake and in vivo enhanced transport across the BBB	T7/peptide	ZL 006	Wang et al. (2015)
Comigration across BBB model. TFF3 was delivered to the brain	cRGD	TFF3	Qin et al. (2014, 2015)
Median survival of tumor-bearing mice was significantly prolonged	TAT, transferrin	DOX	Zong et al. (2014)
High affinity for amyloids, delayed Aβ1-42 peptide aggregation	Antitransferin mAb, lipid-PEG-curcumin	–	Mourtas et al. (2014)
Efficiently delivered into the brain and accumulated in glioma	Synthetic R8-RGD	Paclitaxel (PTX)	Liu et al. (2014a,b)

Increase BBB targeting potential in vitro	Anti-TfR mAb & ApoE3	–	Markoutsa et al. (2014)
Transport across the BBB and distribution in brain glioma	Folate and transferrin	DOX	Gao et al. (2013)
Brain targeting in vivo and ex vivo	MAN	–	Hao et al. (2013)
Lf-LIPs showed better brain targeting in vitro and in vivo	Lactoferrin	99mTc-BMEDA	Huang et al. (2013)
GLU1000-LIP exhibited the strongest brain delivery capacity	GLUT400, 1000–2000	Coumarin-6	Xie et al. (2012)
Improved daunorubicin circulation and transport across the BBB	Man, transferrin	Daunorubicin	Ying et al. (2011)
Improved therapeutic efficacy on brain glioma in vitro and in vivo	TAT-cholesterol	DOX	Qin et al. (2011)
Significant transport across BBB, and extended median survival in the brain glioma-bearing rats	Epirubicin, transferrin	Tamoxifen (TAM)	Tian et al. (2010)
Considerable increase (10-fold) in drugs in the brain parenchyma	+	Citicoline	Ramos et al. (2011)
Strongly internalized in cultured cell lines	+	Oregon green	Bellavance et al. (2010)
SLN			
Increased permeability of etoposide across the BBB	8314MAb & AEGFR	Etoposide (ETP)	Kuo and Lee (2016)
Potential to deliver drug to brain tumor	Lactoferin (Lf)	Docetaxel (DTX)	Singh et al. (2016)
Higher AUC and Cmax of the Baicalin	Anti-TfR mAb	Baicalin	Liu et al. (2015)
Enhanced efficacy of conjugated slns. Increased DTX in brain	MAN	Docetaxel (DTX)	Singh et al. (2015)
Increase permeation of coumarin-6 (in respect to coumarin-6 alone)	–	Paclitaxel	Chirio et al. (2014)
HBMECs could internalize SQV-CSLNs	Cholesterol	Saquinavir (SQV)	Kuo and Wang (2014)
Increased permeation through hCMEC/D3 monolayer	–	DOX	
Improved drug brain targeting	83-14 MAb	Saquinavir (SQV)	Kuo and Ko (2013a); Kuo and Shih-Huang (2013b)
Increased brain permeation of folate-grafted docetaxel/ketoconazole	Folic acid	DTX and ketoconazole	Venishetty et al. (2013a,b)
Enhanced the permeability across the BBB	83-14 MAb	Carmustine	Kuo and Ko (2013a); Kuo and Shih-Huang (2013b)

(*Continued*)

Table 7.4 Examples of the Various Lipidic-NC Types Incorporating Low or Large MW or Large-MW Active Molecules From the Recent Literature *Continued*

Advantages/Results	Targeting Ligand	Active	Reference
Higher affinity to porcine brain capillary endothelial cells	–	Camptothecin	Martins et al. (2012)
Confirmed localization of drug in brain after intranasal delivery	–	Risperidone	Patel et al. (2011)
AUC and MRT of clozapine slns in brain were significantly higher	–	Clozapine	Manjunath, Venkateswarlu (2005)
Drug and metabolite detected in the brain only by SLN	–	Idarubicin	Zara et al. (2002)
Exosomes			
Exosomes for brain delivery across the BBB	–	Paclitaxel/DOX	Yang et al. (2015)
Wide distribution of in the brain	–	Catalase	Haney et al. (2015a,b)
Other NC Types			
LN can effectively inhibit growth of C6 glioma cells in vitro	Tween 80, compritol and Precirol	Edelfosine	Estella-Hermoso de Mendoza et al. (2011)
High baicalein accumulations in the cerebral cortex and brain stem	–	Baicalein	Tsai et al. (2012)
Maximum uptake in BCECs and effective permeation of BBB	Lactoferin (Lf)	Curcumin	Meng et al. (2015)
Effective system to deliver drugs to the brain	(TAT) peptide	HES, NAR, GSH	Wen et al. (2014)
Highest in vitro inhibitory effect, cell apoptosis and cell uptake	Folic acid, cRGDfK	Paclitaxel	Agrawala et al. (2015)
Trancytosis across a BBB model	59-r Peptide, MCF-7	DOX	Sánchez-Purrà et al. (2016)
Dynorphin-B peptide brain delivery	–	Dynorphin-B	Bragagni et al. (2014)
Significantly higher brain uptake of peptide	NPG	VAS	Dufes et al. (2004)
High DOX brain concentration achieved after 60 min	NPG	DOX	Bragagni et al. (2012)

(medium-chain triglycerides) surrounded by hydrophilic (PEG660-hydroxystearate) and lipophilic (phosphatidylethanolamine and phosphatidylcholine) surfactants; they are obtained without organic solvents but with pharmaceutically acceptable excipients. PEG derivatives on the NLC surface improve their pharmacokinetics. Paclitaxel-loaded NLCs led to a significant accumulation of anticancer molecules in the brain and the therapeutic efficiency of doxorubicin-loaded liposomes was evaluated in patients bearing recurrent high-grade gliomas. In order to optimize their biodistribution, these vectors were decorated by site-specific biomolecules-recognizing target tissues (Beduneaua et al., 2007). Baicalein-loaded NLCs were intravenously administered to rats and the plasma levels of baicalein in NLCs was much higher (with a longer half-life) than those of the free drug. In the brain, NLCs conferred higher baicalein accumulations in the cerebral cortex and brain stem, compared to the aqueous solution (Tsai et al., 2012). Lactoferrin-targeted NLCs loaded with curcumin (Cur) were designed for brain targeting. An Alzheimer's disease (AD) rat model was employed to evaluate the therapeutic effects and results indicated that the targeted NLCs conferred maximum stability as they moved across the BBB. Ex vivo imaging studies demonstrated that Lf-mNLC could effectively permeate BBB and preferentially accumulate in the brain (Meng et al., 2015).

A list of targeting ligands used to enhance the brain targeting of various NCs, is summarized in Table 7.5.

Another type of NC recently exploited for drug targeting to the brain is *extracellular vesicles/exosomes*. EVs are membrane-based structures which serve as vehicles to carry different types of cellular cargo (e.g., lipids, proteins, receptors, and other molecules) to recipient cells (Lai et al., 2013). EVs are categorized based on their intracellular origin as apoptotic bodies, microvesicles, and exosomes (Schiller et al., 2008). Exosomes differ from the other EVs due to their origin (they are derived from endosomes (Qin et al., 2014)) and their size (40−100 nm) (Fig. 7.4A). Exosomes are composed of various types of proteins, such as major histocompatibility complex (MHC)-II, integrin, tetraspanins, heat shock protein (Hsp), Ras-related protein (Rab), etc., and contain various types of lipids, such as sphingomyelin, and cholesterol (Fig. 7.4B). Exosomes also contain nucleic acids (NAs), including miRNA, mRNA, and noncoding RNAs (Vlassov et al., 2012). They can be isolated from several types of extracellular fluids including blood, urine, amniotic fluid, saliva, and cerebrospinal fluid (Vlassov et al., 2012; Witwer et al., 2013; Jia et al., 2014), and offer important advantages as drug delivery vehicles mainly due to their small size (up to 100 nm) and nonimmunogenicity. Moreover, exosomes have the ability to target tissues (Johnsen et al., 2014).

The use of exosomes for drug delivery to BBB has recently been pursued by encapsulating anticancer drugs and demonstrating their ability to deliver drugs across the BBB (Yang et al., 2015). A recent study showed that exosomes loaded with catalase was successfully delivered across the BBB, resulting in an improved disease state in *Parkinson's disease* (PD) (Haney et al., 2015a,b). Exosomal

Table 7.5 Examples of Ligands Tested Up-to-Date for Targeting of Lipidic-NCs to the Brain. Ligands are Categorized According to the Targeting Approach (Receptor, Active Efflux, and Transporter-Mediated)

Receptor-Mediated Transporters	Active Efflux Transporters	Carrier-Mediated Transporters
Transferrin receptor (TfR)	Adenosine triphosphate binding cassette (ABC) transporter, subfamily B, member 1 (P-glycoprotein)	Glucose transporter, member 1 (GLUT1)
Insulin receptor (INSR)	ABC transporter, subfamily G, member 2 (ABCG2)	Large neutral amino acid (CAT1)
Low-density lipoprotein receptor – related protein (LDL)	Organic anion transporter (OAT or SLC22)	
Nicotinic acetylcholine receptor	Organic anion-transporting polypeptide (OATP or SLC21)	Cationic amino acid transporter, member 1 (CAT1)
Insulin-like growth factor	Glutamic acid amino acid transporter (EAAT or SLC1)	Monocarboxylic acid transporter, member 1 (MCT1)
Receptor (IGF1R & IGF2R)		
Diphtheria toxin receptor	Taurine transporter (TAUT or SLC6)	Concentrative nucleoside
		Transporter (CNT2)
Scavenger receptor B1 type (SCARB1)	Thiamine transporter/thiamine	Choline transporter (CHT)
Leptin receptor (LEPR)		Nucleobase transporter (NBT)
Fc like growth factor receptor (FCGRT)		
Trans-activating transduction protein		
Folate receptor		
Glycoside receptor		
Lactoferrin receptor		

curcumin has been examined for its anti-inflammatory properties (Ha et al., 2016). NA delivery to the brain by exosomes is discussed below.

7.3 OTHER LIPIDIC NANOCARRIERS

Self-assembling amphiphilic biocompatible lipid-NCs are monolayer membrane structures that consist of lipids and stabilizing surfactants. Although such NCs are

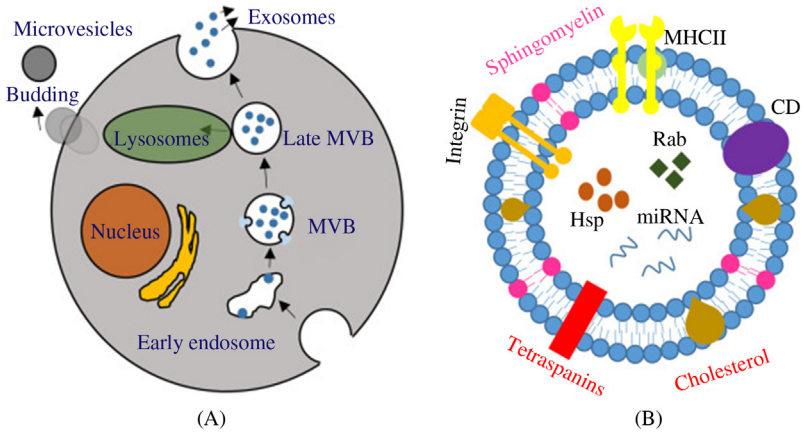


FIGURE 7.4

Graphical representation of the formation of exosomes (A) and basic components of exosomes (B).

The figure is reprinted from: Ha, D., Yang, N., Nadithen, V., 2016. Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges, Acta Pharmaceut. Sin. B 6 (4), 287–296, after permission by Elsevier.

not very popular for brain drug delivery, there are a few recent publications. Oral administration of edelfosine in Tween 80–coated Compritol and Precirol lipid nanocarriers (LNs) showed accumulation of the drug in brain tissue, due to the inhibition of P-glycoprotein by Tween 80 (Estella-Hermoso de Mendoza et al., 2011). Curcumin and piperine coloaded glyceryl monooleate (GMO) NCs were also able to cross the BBB and rescue rotenone-induced motor coordination impairment in a PD mice model (Kundu et al., 2016).

Cubosomes (Fig. 7.5) are "crystalline liquid phase" nanostructures, which differ from other drug carrier such as liposomes due to their cubic shape. Their size ranges from 100 to 300 nm (Pan et al., 2013; Seo and Kim, 2013; Rarokar et al., 2016). Two commonly used lipids for cubosome construction are GMO (referred to as monoolein), and phytantriol (PHYT). Poloxamer 407 (P407) is the most-applied surfactant in cubosomes. PEGylated lipids with reduced cytotoxicity have been used to engineer and stabilize PHYT-based cubosomes (Zhai et al., 2015). Cubosomes attract attention for drug delivery because of significant advantages (Table 7.3), such as the possibility of being loaded with various drugs (different types of hydrophilic, lipophilic, and amphiphilic drugs), the protection of drugs from physical and enzymatic degradation, and potential for sustained release of incorporated drugs (Karami and Hamidi, 2016; Rizwan et al., 2007; Bei et al., 2010; Anbarasan et al., 2015; Tilekar et al., 2014). Recently, it was shown that drugs incorporated in polymeric NCs demonstrate increased BBB permeability and brain uptake when functionalized with Tween 80, due to plasma

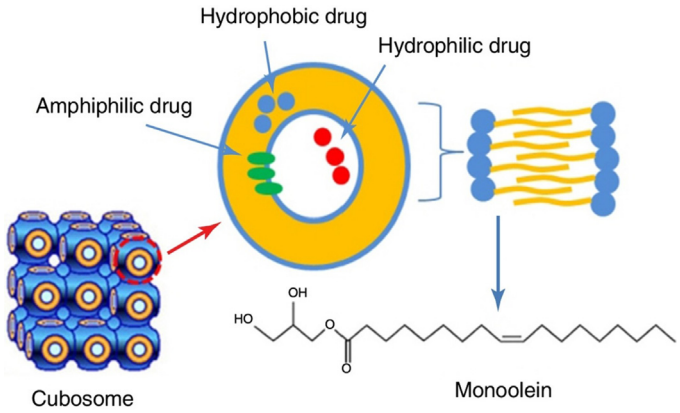


FIGURE 7.5

Cubosome structure and components.

The figure is reprinted from Karami, Z., Hamidi, M., 2016. Cubosomes: remarkable drug delivery potential. Drug Discov. Today 21 (5), 789–801, after Permission from Elsevier (Order Number: 4018130072714).

lipoproteins ApoB and ApoE adsorption to Tween 80 (Kreuter et al., 2002; Michaelis et al., 2006; Kreuter et al., 2007; Wagner et al., 2012). In this context, the fact that Tween 80 coating stabilizes PHYT cubosomes creates the possibility for their future application for drug transport to the brain (Azhari et al., 2016).

Hybrids of polymeric and lipidic-NCs have also been proposed for brain targeting applications. As an example, TAT-MPLs, developed by magnetic (PLGA)/lipid nanocarriers (MPLs) conjugated to TAT peptide and encapsulating hesperidin, naringin, and glutathione, were tested were found to accumulate in cells in higher levels than control MPLs. Accumulation of QD-loaded, fluorescein isothiocyanate (FITC)-labeled TAT-MPLs was dose- and time-dependent, showing that TAT-conjugated MPLs are effective systems to deliver drugs to the brain (Wen et al., 2014). Polymer-lipid hybrid nanocarriers (PLNs) decorated with folic acid (F) and cyclo-[Arg-Gly-Asp-d-Phe-Lys], loaded with paclitaxel (PtxR-FPLNs) were found to efficiently cross the BBB (Agrawala et al., 2015).

Niosomes are colloidal systems consisted of nonionic surfactants, able to self-assemble into closed bilayer structures (Bragagni et al., 2012) (Table 7.3). They have a similar structure with liposomes, and are thus defined as "nonionic liposomes," while they demonstrate important benefits, such as greater chemical and physical stability, extended shelf life, lower cost, formulation versatility, easier sterilization (Uchegbu and Vyas, 1998; Chandu et al., 2012; Varun et al., 2012). Conjugation of ligands targeting the BBB on niosomes surfaces could allows development of BBB-targeted NCs (Sankar et al., 2009). Dynorphin-B niosomes showed a pronounced antinociceptive effect proving their effectiveness for peptide brain delivery (Bragagni et al., 2014). Encapsulation of the vasoactive intestinal peptide

into glucose-bearing niosomes, functionalized with *N*-palmitoylglucosamine (NPG) resulted in higher brain uptake of the peptide compared to control niosomes (Dufes et al., 2004). Doxorubicin was encapsulated into NPG-bearing niosomes and applied for brain uptake (Bragagni et al., 2012).

The main characteristics of the basic all lipidic-NCs are summarized in Table 7.3 (Chhabra et al., 2015; Naseri et al., 2015; Salunkhe et al., 2015; Mehnert and Mäder, 2001; Puri et al., 2009).

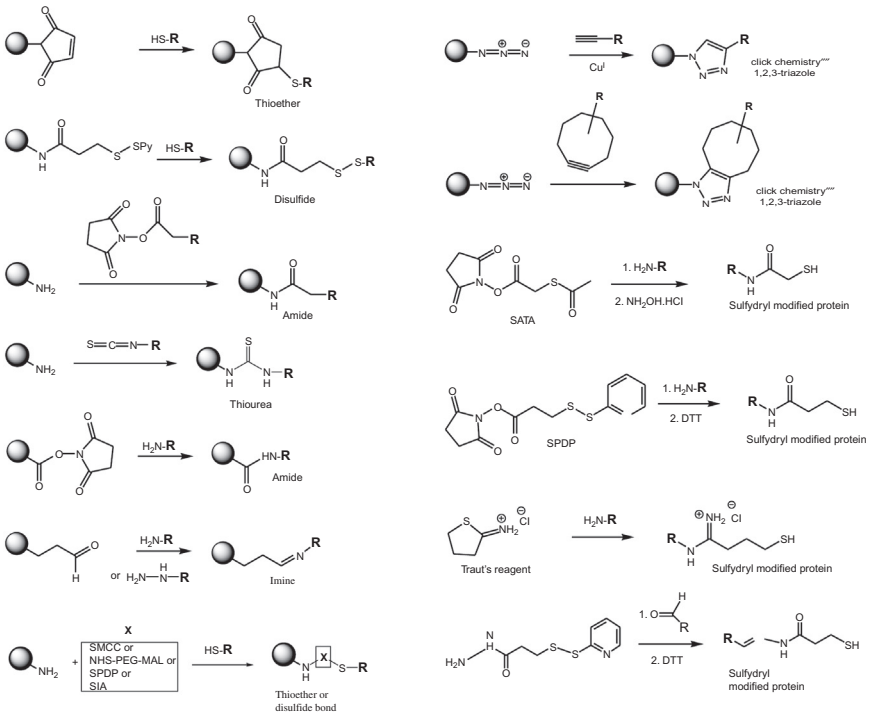
7.3.1 TARGETING APPROACHES

Although the BBB is intact, there are many receptors and carriers that are overexpressed on the BBB which can mediate the transport of specific ligands and their cargoes. Additionally, the membrane of the BBB is negatively charged and shows high affinity to positively charged compounds, which could trigger the cell internalization processes, mediating NC penetration through the BBB (Gao, 2016; Salunkhe et al., 2015; Joseph and Saha, 2013; Wong et al., 2012; Neves et al., 2016).

Chemistries to attach an active ligand on the NP surface (Scheme 7.1) are divided into covalent and noncovalent. In general, noncovalent strategies do not enable control over the composition and size and of the nanoparticulate system and of course suffer from possible extraction of the noncovalently molecule after iv injection of the NC system. On the other hand, covalent attachment of a ligand makes it more difficult to detach from the NP surface (Sanvicens and Marco, 2008).

Several *covalent coupling* strategies have been developed. The most common methods use bifunctional PEG molecules, containing a hydrophobic lipid anchor at one end (embedded into the lipid bilayer) and a crosslinker at the other. The lipid part of the bifunctional PEG could be a phospholipid (Huwyler et al., 2008; Maruyama et al., 1997) while the crosslinker could be reactive amine, such as the *N*-hydroxysuccinimide group (NHS) derivative. A carboxy-group can be also selected, that after appropriate chemical activation, could react with the amine-ligand to be attached. Thiol-reactive groups, such as the maleimide and pyridyldithiopropionylamine derivatives (Mercadal et al., 2000) have also been used. Examples of reactions are presented in Scheme 7.1.

Other possibilities include imine formation between an aldehyde and the so-called "clickchemistry" (Beduneaua et al., 2007; Airoldi et al., 2014; Mourtas et al., 2011). Bifunctional PEG can be inserted into the lipid NC either by pre-insertion procedure or by post-insertion (Uster et al., 1996; Beduneau et al., 2007). By applying these methods covalent functionalization of NCs either with the maleimide or amide method have been described. Several methods for thiolation of peptides have been adopted (Barbet et al., 1981; Duncan et al., 1983; Harasym et al., 1998; Domen et al., 1990; Melton, 1996; Ansell et al., 1996). Other strategies include preformed lipid-PEG-folate conjugates, cholesterol-PEG-folate (Guo et al., 2000), DPPE-PEG-folate and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE)-PEG-folate incorporated into SLN (Venishetty et al., 2013a) and



SCHEME 7.1

Representative bioconjugation protocols of dyes, drugs, peptides, mAbs and methods to introduce sulfydryl groups on amine and aldehyde ligands.

liposomes (Lee and Low, 1995; Saul et al., 2003). A novel DSPE-PEG-glutathione (GSH)-folate lipid has also been synthesized (Liu et al., 2011) while a post-insertion procedure was also used to incorporate DSPE-PEG-thiamine into SLN NCs (Lockman et al., 2003). More detailed methods for NC functionalization can be found elsewhere (Béduneaua et al., 2007; Yu et al., 2012; Rahim et al., 2013; Haun et al., 2010; Hudlikar et al., 2016; Wang et al., 2014; Marqués-Gallego and de Kroon, 2014).

Noncovalent coupling: Linkage strategies using avidin or streptavidin (SA)/biotin affinity has been extensively used in NCs (Green, 1990; Kang et al., 1995; Pardridge, 1995). The avidin or SA is activated with *N*-maleimidobenzoyl-*N*-hydroxysuccinimide and then reacted with sulfhydryl groups previously introduced into the protein, leading to the formation of a thioether bond. In parallel, biotinylated NCs are prepared. Lipid-PEG-biotin constructs such as DSPE-PEG-biotin are introduced into liposomes (Schnyder et al., 2005). SA was also associated to cationic SLN via electrostatic interactions (Pedersen et al., 2006). Another approach involved ligand biotinylation by NHS-PEG-biotin (on protein-bearing primary amine groups)

(Phillips et al., 1994). Liposomes with covalently coupled mAb gave higher values of uptake and permeability across the BBB model used (Salvati et al., 2013a,b,c).

7.4 CARRIERS FOR NUCLEIC ACID DELIVERY

7.4.1 INTRODUCTION TO NUCLEIC ACIDS

Using NAs as drugs is an important future direction of molecular medicine. Nonviral gene therapy or other NA therapies have been proposed to treat the most serious diseases which require systemic administration of genes that should subsequently enter target cells affected by genetic diseases, viral infections or cancer. To-date, the production of effective gene delivery vectors is the bottleneck limiting the success of gene-based drugs in clinical trials. Naked NAs can be delivered locally into specific organs such as muscle or liver by physical methods, such as electroporation or hydrodynamic injection. However, these methods are not applicable for systemic gene delivery or are unrealistic for commercial gene therapy (Li and Szoka, 2007).

Formulation technologies for encapsulating NAs into nanosized lipidic carriers for in vivo administration are summarized below. NAs include plasmid DNA, siRNA (or miRNAs) and antisense oligonucleotides (ONs). These NAs have a high linear negative charge density hence the initial formulation parameters can be similar for the various NAs in spite of large differences in their molecular weight. However, molecular weight differences among the various NA drugs may require formulation modifications. Some NA-types can interfere with mRNA molecules to silence protein expression, and many of these structures have been extensively investigated in brain diseases (Burnett and Rossi, 2012; Davidson and McCray, 2011; Kanasty et al., 2013; Kim and Rossi, 2007).

The main barriers to NA-based therapeutics, include a successful in vivo delivery strategy designed to satisfy the following major criteria: (1) the method should protect NAs from degradation by nucleases; (2) it should help NAs cross the cell membrane, escape from endosome and finally enter either the nucleus or the cytoplasm, depending on their mechanisms of action; (3) it should have no or fewer side effects caused by either NAs or the method itself; and (4) it should prolong their circulation time and prevent nonspecific disposition of NAs to facilitate their delivery to the target cells (Zhu and Mahato, 2010). The success of gene therapy depends largely on the development of a vehicle or vector that can efficiently and effectively deliver genetic material to target cells and obtain sufficient levels of gene expression in vivo with minimal toxicity. Virus-derived vectors for gene therapy are efficient in gene delivery and transfer, but safety issues limit their use in gene therapy. Naked RNAs or DNAs are unstable under physiological conditions, resulting in enzymatic degradation by endogenous nucleases and clearance by the RES, and additionally they are not favorable for uptake by cells, because of their anionic surface. Off-target effects of genes often lead to

unwanted toxicities in normal tissues. Furthermore, immune stimulation upon injection hinders further development of new gene therapies.

7.4.2 CURRENT LIPIDIC VECTORS FOR PLASMID DNA DELIVERY

Cationic lipid/DNA complexes (lipoplexes) and cationic polymer/DNA complexes (polyplexes) used in gene therapy are based on the hypothesis that the complexes adsorb more effectively to the anionic plasma membrane of mammalian cells via electrostatic interactions. Compared with other nonviral delivery systems, including anionic liposomes that encapsulate NAs, lipoplexes and polyplexes tend to mediate a higher level of transfection in numerous cell lines. The mechanisms of complex formation, binding, and entry into cells have been studied extensively. The successful delivery of DNA depends on a number of factors. These include the chemical structure of the cationic reagents, the supramolecular structure of lipoplexes and polyplexes, their interactions with cell membranes, their internalization and intracellular localization, the release of DNA from the cationic carriers, and the role of helper lipids in cationic liposomes (Ilarduyaa et al., 2010).

Lipoplexes-cationic lipids were introduced as carriers for delivery of NAs over two decades ago (Zhao and Huang, 2014). They are still the major carriers for gene delivery, because they can be easily synthesized and extensively facilitated by modifying each of their constituent domains. Cationic lipids can be used as vectors to condense and deliver anionic NAs through electrostatic interactions. These nanostructured complexes, called *lipoplexes* (Fig. 7.6) have shown to be extremely useful vehicles in gene therapy. The liposomes typically contain at least two components: a cationic lipid and a neutral lipid, which is sometimes called helper lipid. Cationic lipids are amphiphilic molecules containing a positively charged polar headgroup linked to a hydrophobic domain via a connector. DOPE and cholesterol are often used as neutral lipids. On its own, DOPE forms inverted hexagonal HII (nonbilayer) phase structures at neutral pH and physiologic temperatures. When combined with a cationic lipid, however, it can participate in bilayer formation.

Cationic lipids with various types of hydrocarbon chains have been studied thoroughly. The most common types of chain lengths are C8:0 to C18:1. The use of mono-unsaturated fatty acid chains have resulted in higher transfection, possibly because of their influence on enhancing membrane fluidity (Ilarduyaa et al., 2010).

By modulating the ratio of cationic lipids and NAs, the excess cationic coating was able to facilitate the binding of vectors with negatively charged cell surfaces, and furthermore interruption with endosomal membrane to help cytoplasmic delivery of NAs. However, lipoplex suspensions are known to be unstable in aqueous suspension for long-term storage, especially with respect to hydrolysis and size stability (Fehring et al., 2014). DNA can be encapsulated in liposomal formulations by thin film, reverse-phase evaporation and asymmetric liposome formation methods (Levine et al., 2013). It is well accepted that the polar

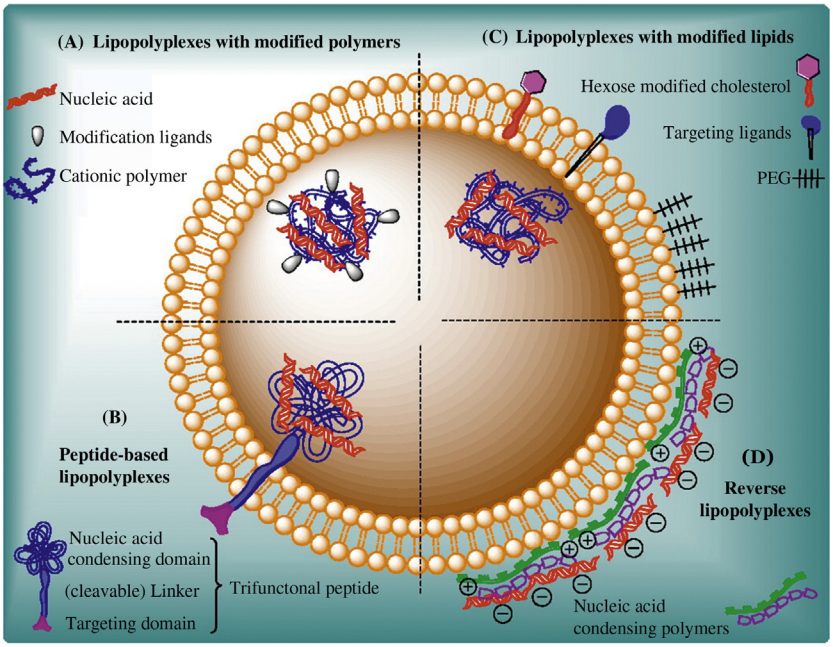


FIGURE 7.6

Graphical representation of the structure of different types of lipoplexes. The schematic structure of lipopolyplexes (LPPs), one of the most efficient nonviral vectors. (A) Lipopolyplexes with modified polymers, (B) peptide-based lipopolyplexes, (C) lopopolyplexes with modified lipids, (D) reverse lipopolyplexes.

Reprinted for Rezaee, M., Kazemi Oskuee, R., Nassirli, H., Malaekeh-Nikouei, B., 2016. Progress in the development of lipopolyplexes as efficient nonviral gene delivery systems. J. Controll. Release 236, 1–14, after permission by Elsevier (Order Number: 4018111068865).

headgroup, hydrophobic moiety, and linker are three important constituent domains for cationic lipids. While hydrophobic regions, including the length and the degrees of nonsaturation of the alkyl chains, are relatively similar, the structure and component of polar headgroup and linkers are substantially different. The polar hydrophilic headgroup is positively charged, usually through the protonation of one or several amino groups.

Originally lipoplexes were proposed to form either large aggregates surrounded by thin fibers, or condensed DNA coated by a lipid bilayer (Felgner et al., 1987). The simplest case is one where the liposomes adhere to the DNA strand like "beads on a string," similar to the structure of micelle complexes with oppositely charged polyelectrolytes. Hexagonally packed DNA coated by lipid was also proposed (Fig. 7.7). In particular, a novel new "sliding columnar phase" was predicted, in which the positional coherence between DNA molecules in adjacent layers is lost, without destroying the orientation of the chains from layer

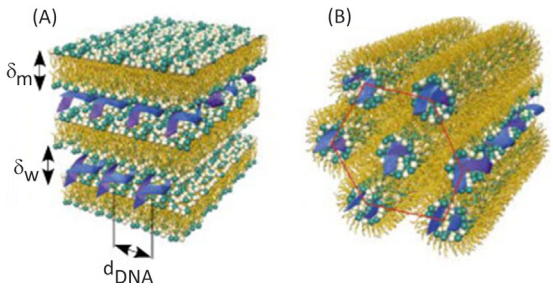


FIGURE 7.7

Schematic representation of the structure of lateral (A) and hexagonal (B) lipoplex conformations.

Reprinted from Ilarduya, C.T., Sun, Y., Düzgüneş, N., Gene delivery by lipoplexes and polyplexes. Eur. J. Pharmaceut. Sci. 40 (3), 159–170, after permission by Elsevier (Order Number: 4018121048291).

to layer. X-ray diffraction studies confirmed the two types of structures observed in plain lipoplexes (Ilarduyaa et al., 2010), the multilamellar structure, with DNA monolayers sandwiched between cationic membranes, and the inverted hexagonal structure, sometimes called the inverted "honeycomb" phase, with DNA encapsulated within cationic lipid monolayer tubes.

7.5 POLYPLEXES

Cationic polymers include natural DNA-binding proteins, such as histones, synthetic polypeptides, poly(ethylenimine) (PEI), cationic dendrimers, 2-dimethyl (aminoethyl) methacrylate, or carbohydrate-based polymers such as chitosan. Poly(l-lysine) and PEI are among the most widely studied polymers for gene delivery. Cationic polymers can be combined with DNA to form a particulate complex, and can condense DNA molecules to a relatively small size, compared to cationic liposomes. This can be crucial for gene transfer, as small particle size may be favorable for improving transfection efficacy, particularly in vivo. The most potent polyplex formulations have reached efficiencies of viral vectors, although far more particles per cell are required for successful transfection (Curiel et al., 1991).

The formulation of polyplexes plays an important role in both their transfection efficiency and stability. Polyplex formation is usually kinetically controlled; it is performed at ionic strengths where the polycation/polyanion association is rapid and almost irreversible. The sequence of addition of components during the complexation procedure influences the resulting polyplex size, as well as their transfection efficiency. Condensation of plasmid DNA or RNA into stable complexes by PEI is considered to rely predominantly on electrostatic interactions. Since approximately 90% of its charged groups must be neutralized

to condense DNA, a N/P ratio of about 2−3 is necessary to achieve stable complexes, using branched or linear PEI (Ilarduyaa et al., 2010). The composition of the medium is also important in the outcome of complexation. PEI/DNA complexes formulated in saline solution have sizes that depend on the medium ionic strength.

Lipidoids mixed with polyanionic NA (SNALPs). Ionizable lipids and lipidoids provide an advanced delivery platform for gene therapy that can self-assemble into NCs when mixed with polyanionic NAs. Ionizable cationic lipids with modulated p*K*a values increase NA payload and enhance the therapeutic efficacy of gene therapy. At low pH conditions, ionizable lipids will become positive charged, resulting in high NAs loading, but after injection where the pH is above the p*K*a of the ionizable lipids, the surface of the LNPs has an almost neutral charge that can evade RES uptake, improve circulation, and reduce toxicity (Tam et al., 2013). Once nanoparticles are internalized into endosomes, where pH is lower than the p*K*a of the lipids, the amino group of the ionizable lipid becomes protonated and associates with the anionic endosomal lipids, facilitating endosome escape. Lipidoids and lipids share many of the physicochemical properties that drive the formation of liposomes for gene delivery. However, lipidoids are easy to synthesize and purify and do not require a colipid for efficient DNA delivery. These advantages make high-throughput combinatorial synthesis of lipidoids possible and allow for rapid in vitro screening of thousands of potential drug delivery candidates.

Gene-lipid conjugates-NA conjugation could improve in vivo pharmacokinetic behavior of genetic materials, providing an alternative approach for gene therapy (Christensen et al., 2013). Conjugating the lipids to the site of NAs without loss of bioactivity is the key step for modification (Behlke, 2008). Exogenous siRNA can activate the innate immune system through toll-like receptors (TLRs), but introduction of 2′-*O*-methyl (2′OMe) to nucleotide can inhibit the TLR-associated inflammatory response (Judge et al., 2006). Hydrophobic lipids can also be attached to siRNAs to change their biodistribution, extend circulation time, and facilitate direct cellular uptake (Zhao and Huang, 2014).

7.5.1 DELIVERY OF OLIGONUCLEOTIDE AND RNA WITH LIPIDIC-NANOCARRIERS

NAs such as antisense oligodeoxynucleotides (oligos), anti-microRNA (anti-miR) and siRNA are typically in the range 15−21 bases in length and bind to their target RNAs with perfect base-pairing. These molecules have therapeutic potential, especially in silencing disease-causing or disease-associated genes that are not amenable to conventional therapeutics such as small molecules or monoclonal antibodies (Ashizawa and Cortes, 2015).

Lipid carriers used for RNA delivery are usually cationic lipoplexes/liposomes or solid-lipid nanoparticles. siRNA-lipid formulations can be categorized in four major

categories, similar to those mentioned above for plasmid DNA, depending on their physical structure and surface properties (Fig. 7.8): (1) lipoplexes, or cationic liposomes, (2) stable NA-lipid particles (SNALPs), (3) lipopolyplexes, a more complex type of lipoplexes (or multifunctional-envelope-type-nano devices (MENDs)), and (4) membrane-core NPs (MCNPs) (Xia et al., 2016). *Lipoplexes* generally have multilamellar structures (Weisman et al., 2004), lipids form multiple bilayers and siRNAs are embedded between adjacent lipid bilayers. Lipoplexes can only be formed from cationic liposomes and are easily synthesized by simply mixing cationic liposomes and siRNAs at expected ratios. In this process, siRNAs first induce the aggregation of liposomes and the rupture of lipid membranes, and then the ruptured membranes wrap around the siRNA-coated liposomes to form multilamellar structure (Weisman et al., 2004). The synthesis of lipoplexes is convenient and robust, and cationic liposome formulations such as lipofectin, lipofectamine, and LipoRNAiMAX, form efficient lipoplexes used in in vitro gene delivery studies (Lee et al., 2012). In *SNALPs*, siRNAs are loaded in the interior of liposomes (close to the inner membrane due to the electrostatic attraction) (Semple et al., 2010) with high efficiency. The surface charge of SNALPs is nearly neutral. Some SNALPs are

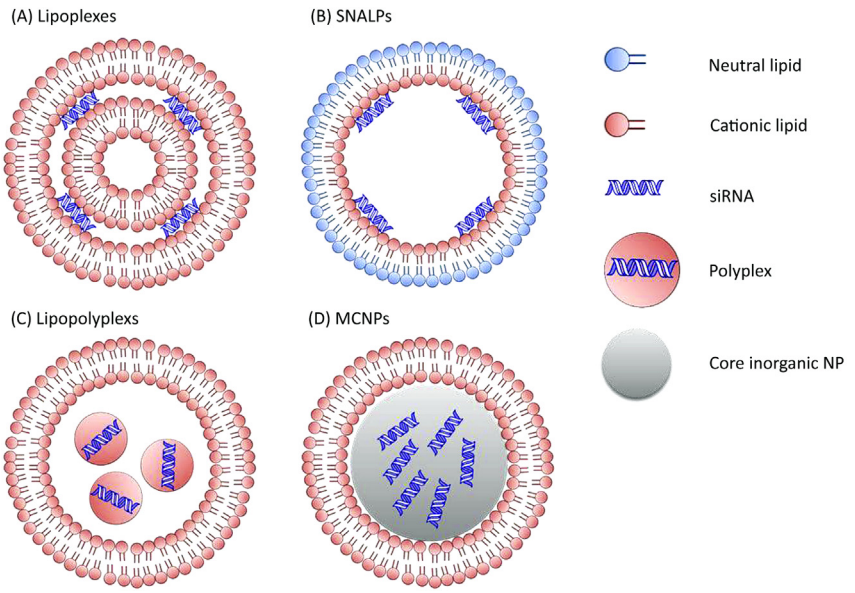


FIGURE 7.8

Structure of four types of liposomes or lipid-NPs used for the delivery of siRNAs. (A) Lipoplexes; (B) SNALPs; (C) lipopolyplexes; (D) MCNPs.

Figure is reprinted from Xia, Y., Tian, J., Chen, X., 2016. Effect of surface properties on liposomal siRNA delivery. Biomaterials 79, 56–68, after permission from Elsevier (Order Number: 4018121312385).

currently in clinical trials (Kanasty et al., 2013). Only liposomes containing acidic pH-sensitive lipids (e.g., cationic at pH = 4 and neutral at pH = 7.4) can form SNALPs. *Lipopolyplexes* are liposomes containing polyplexes (cationic polymer/RNA complexes), as described above for plasmid DNA (Gao and Huang, 2008) (Fig. 7.6). Lipopolyplexes are synthesized by coating the cationic lipid bilayer onto anionic polyplexes (Chen et al., 2009) or vice-versa. PEGylation is often needed to increase their stability. Lipopolyplexes can also be synthesized by direct hydration of lipid membranes with polyplex dispersions (e.g., MEND) (Hatakeyama et al., 2011; Hatakeyama et al., 2011).

A membrane/core nanocarrier (*MCNP*) has a core-shell-like structure with one or more inorganic NCs as core and a lipid bilayer as shell. The core could be porous calcium phosphate or silica NCs, loaded with high amount of siRNAs. In an MCNP, lipid bilayer could be either symmetric (neutral bilayer or cationic bilayer) (Li et al., 2010) or asymmetric (anionic innerlayer and cationic outerlayer) (Li et al., 2012). MCNPs with symmetric bilayers are generally synthesized in two steps (Li et al., 2010; Ashley et al., 2012): inorganic NPs are synthesized, siRNAs are loaded onto the porous surface of NPs, and lipid bilayers are coated on the preformed inorganic NPs. For asymmetric MCNPs (Li et al., 2012), the procedure is similar except that innerlayer and outerlayer are coated sequentially. Symmetric MCNPs can also be synthesized by in situ mineralization of CaP in the interior of preformed siRNA-loaded liposomes (Khatri et al., 2014). Since the liposome structure is supported by the inner solid NC core, MCNPs are more stable than hollow liposomes. Sometimes, the size of MCNPs depends on the size of the core NCs and MCNPs can be very small ($\sim$30 nm) (Li et al., 2012). A smaller size of gene carriers can be beneficial in penetrating tissues and in the cellular uptake, leading to a higher gene silencing efficiency. However, the synthesis of MCNPs is rather complex, due to the need of co-synthesis of inorganic NCs.

7.5.2 EXOSOMES FOR NUCLEIC ACID TARGETING

As mentioned above, due to their remarkable easy transport from cell to cell and their impressive organotropism (Hoshino et al., 2015; Liu et al., 2016), EVs are currently under intensive investigation as therapeutic targets (Vader et al., 2014) and diagnostic biomarkers (Van der Meel et al., 2014). EVs include different categories of vesicles, mainly apoptotic bodies, microvesicles and exosomes (Van der Pol et al., 2012). Following exosome isolation from parental cells or body fluids, they are characterized based on their protein contents which indicate their endosomal origin (Raposo and Stoorvogel, 2013). As mentioned, interfering RNA delivery is of great interest for the treatment of different types of cancer (Lin et al., 2014; Møller et al., 2013; Henriksen et al., 2014), thereby most of the current studies involving RNA interference (RNAi) and exosomes are related to cancer. In most cases loading of siRNAs (or miRNAs) is carried out by techniques

used in molecular biology and biotechnology, such as electroporation or incubation (Alvarez-Erviti et al., 2011). Other chemical based transfection methods using commercial transfection reagents have also been used to load exosomes with siRNA (Wahlgren et al., 2012; Shtam et al., 2013). The most widely used approach for loading therapeutic cargo into exosomes is by transfecting exosome donor cells to overexpress a certain gene product that the cell will package into the exosome lumen or membrane for secretion (Ohno et al., 2013; Katakowski et al., 2013). Cell activation as a means of achieving exosome loading has also been studied (Xin et al., 2012).

Several preclinical studies have been carried out using exosomes and RNAi molecules. Downregulation of mRNA expression of the housekeeping gene Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and the AD-associated gene BACE1, was observed in neurons following targeted delivery of siRNA-enriched exosomes (Alvarez-Erviti et al., 2011). In another study, hydrophobically modified siRNAs (hsiRNAs) targeting Huntington mRNA were loaded into exosomes and then efficiently internalized by mouse primary cortical neurons promoting dose-dependent silencing of Huntington mRNA and protein (Didiot et al., 2016). Several examples with miRNAs also exist. Mesenchymal stem cell (MSC)-derived exosomes with a high expression of tumor suppressor miRNA, inhibited tumor growth in a xenograft model of GBM. Also MSC exosomes delivered anti-miRs for the knockdown of the oncogenic miRNA, miR-9, in GBM cells in vitro, increased the GBM cell sensitivity to chemotherapeutic treatment with temozolomide (Munoz et al., 2013).

Examples of the various lipidic-NC types incorporating NAs (plasmid DNA or RNA or ONs) from the recent literature are presented in Table 7.6.

7.6 DISEASES TARGETED

Because of the continuously aging population neurodegenerative diseases, are becoming more prevalent. Nanosized carriers have an influential role in therapeutics for brain disorders, especially in overcoming and facilitating enhanced treatment options (Kanwar et al., 2012a,b)

The diseases that affect the brain and the CNS can be divided into three categories, *neurodegenerative* diseases, *neuroinflammatory* diseases, and *neoplastic* diseases, and this division is followed (below) in the description of recent efforts applying lipidic-NCs for disease treatment and/or diagnosis. Some examples of lipidic-NCs designed as therapeutics for neurodegenerative diseases are also summarized in Table 7.7.

7.6.1 NEURODEGENERATIVE DISEASES

The precise causes and mechanisms of neurodegeneration are unknown. A significant risk factor for developing neurodegeneration is aging (Tanner, 1992), and this

Table 7.6 Examples of the Various Lipidic-NC Types Incorporating Nucleic Acids (Plasmid DNA or RNA or Oligonucleotides (ON)) From the Recent Literature

Lipidic Nanocarrier	Advantages/ Disadvantages	N.A.	Targeting Ligand	Disease	Result	Reference
Cationic liposomes	(+) high level of transfection easily synthesized easily modified	Plasmid psv-B-galactosidase	–	Delivery to mice brain	Protection of DNAse digestion	Ghaly et al. (2013)
	(–) Need for colipid; unstable in aqueous solutions; low size stability	Plasmid Pci-Luc	Peptide P (K16GACLPHKSMPCG)	Brain gene therapy	Delivery of contrast agents and genes for real-time monitoring of gene therapy in the brain	Writer et al. (2012)
		Plasmid pci-Luc/ plasmid pegfp-N1	Neurotensin-targeting peptide	MS, AD	Widespread dispersal and effective gene expression in brains	Kenny et al. (2013)
		Plasmids encoding the BDNF proteins	Transferrin (Tf)	In vivo targeted gene therapy	Delivering brain derived neurotrophic factor (BDNF)	Chen et al. (2014)
		150-Base-pair genomic fragment	–	Glioma	New antitumor and anti-metastasis treatment for human glioma	Wang et al. (2013)
		Heavy chain ferritin specific-sirnas	–	Glioma	Reduced tumor growth to a degree comparable to that seen with carmustine	Shim et al. (2013)
		Cas9:sgrna	–	DNA recombination in vivo	Gene recombination and genome editing with efficiencies greater than 70%	Wang et al. (2016)
		Plasmid DNA	–	Brain targeting	Protection from DNAse digestion	Wang and Ghaly, (2013)

(*Continued*)

Table 7.6 Examples of the Various Lipidic-NC Types Incorporating Nucleic Acids (Plasmid DNA or RNA or Oligonucleotides (ON)) From the Recent Literature *Continued*

Lipidic Nanocarrier	Advantages/ Disadvantages	N.A.	Targeting Ligand	Disease	Result	Reference
Neutral liposomes	(+) Easily synthesized; good transfection efficacy; no toxicity	Luciferase-encoding pDNA	–	Brain targeting/gene therapy	High gene expression levels in brain	Akita et al. (2015)
	(–) Big size of carriers	Plasmid DNA encoding for GUSB	TfRMab	Type VII mucopolysaccharidosis (MPS)	GUSB enzyme activity at high levels	Boado, Pardridge (2011)
		TH expression plasmid driven by the Gfap brain-specific promoter	OX26 MAb to target the rat TfR	Parkinson's disease	Reduction of TH levels	Boado, Pardridge (2011)
		Pcdna3-Luc	–	Increased gene expression in the brain	Increased gene expression firefly luciferase plasmid in the brain at the focused-US exposure site	Negishi et al. (2015)
		Plasmid DNA for EGFR	rat 8D3 MAb/HIRMAb	Intracranial human brain cancer/Glioma	100% increase in survival time of mice with intracranial human brain cancer	Zhang et al. (2004b)
Lipoplexes	(+) High level of transfection	Pc27/pegfp	OX26/CTX	Brain glioma	High cell transfection and increased transport of plasmid DNA across the BBB	Yue et al. (2014)
	(–) Need for colipid; unstable in aqueous solutions; low size stability	Plasmid encoding both luciferase Pluc-N3 gene (marker) and the GDNF gene (therapeutic)	–	CNS diseases	Luciferase and GDNF protein expression/ potential to achieve noninvasive and targeted gene delivery for treatment of CNS diseases	Lin et al. (2015)

		Noncoding plasmid DNA (pmb75.6)	–	Encephalitic arboviral infection	Significant protective effect against encephalitic arboviral infection	Logue et al. (2010)
		Plasmid pegfp-N1 containing the gene GFP	Peptide Y and ligand YL, and ligand YSL	Brain gene therapy	Theranostic delivery of genes and contrast agents to allow monitoring by MRI	Tagalakis et al. (2014a,b)
		BACE1 sirna	16-Lysine peptide	Alzheimer's disease	Silencing efficiency of PRLAP1 with no cytotoxicity	Tagalakis et al. (2014a,b)
		Sirna against MGMT	–	GBM	TMZ-resistant glioma-initiating cells with increased DNA repair and drug efflux capabilities could be efficiently transduced with MGMT-siRNA	Kato et al. (2010)
		Sirna	RVG-9/RVM-9r	Creutzfeldt-Jakob	Knockdown PrPC expression and greatly decrease PrPRES in chronically prion infected neuronal cells	Pulford et al. (2010)
Lipopolyplexes	(+) Small size; improved transfection efficacy; protection against degradation	Plasmid DNA encoding luciferase	ApoE	In vivo targeted gene therapy	Higher transgene expression	Tamaru et al. (2014)

(*Continued*)

Table 7.6 Examples of the Various Lipidic-NC Types Incorporating Nucleic Acids (Plasmid DNA or RNA or Oligonucleotides (ON)) From the Recent Literature *Continued*

Lipidic Nanocarrier	Advantages/ Disadvantages	N.A.	Targeting Ligand	Disease	Result	Reference
SLN	(+) High long-term stability; easily attaching ligands; low toxicity; N.A. protection	Sirna targeting human c-Met	–	U-87MG human GBM cells	Successfully controlled tumor via intravenous administration of the siRNA complex in the orthotopic GBM xenograft tumor model	Jin et al. (2011)
SNALPs	(+) Enhanced therapeutic efficacy; high NA-loading; improved circulation; reduced toxicity; no colipid required	GFP and luciferase-encoding plasmids	TATp	U87-MG tumors	Increased gene expression	MacKay et al. (2008)
		Anti-mir-21 oligonucleotides	CTX	GBM	Increased mRNA and protein levels and no signs of systemic immunogenicity	Costa et al. (2015)
		Antisense oligonucleotides (ASOS) or small interfering RNAs (siRNAs)	Chlorotoxin (CTX)	GBM	Enhanced particle internalization into intracranial tumors and nanocarrier-mediated miR-21 silencing	Costa et al. (2013)

Table 7.7 Examples of the Various NCs Used as CNS Disease Therapeutics, From the Recent Literature

Disease	Molecules	Target	Lipidic-NC Type	Result	Reference
Parkinson's disease	Tyrosine hydroxylase gene	TfR	PEGylated liposome decorated with OX26 ab agains TfR	Reduction of TH levels	Zhang et al. (2003), Zhang et al. (2004a)
Brain tumors	DO-FUdR	ND	Drug incorporated in solid-lipid nanoparticles	Antitumor activity	Wang et al. (2002a,b)
	Doxorubicin	TMEM30A transmembrane protein	Liposomes decorated with FC5 ligand	BBB enhance transport and aanticancer activity	Gabathuler (2010)
	shRNAs against EGFR	Insuline receptor/ transferrin receptor	PEGylated immunoliposomes associated to TfR ab and insulin receptor Ab	Enhance transport across BBB and increased survival in mice with intracranial tumors	Boado et al., (2007), Pardridge (2004)
Cerebral ischemia	NGF gene	TfR	Lipoplexes decorated with transferrin	Induced transgenic protein levels	da Cruz et al. (2005)
Infectious diseases	AntiVIH drugs	ND	Drug associated to liposomes	Increased brain levels of anti-HIV drugs	Dusserre et al. (1995)
	AntiVIH drugs	ND	Drug associated to micelles	Increased brain levels of anti-HIV drugs	Spitzenberger et al. (2007)
	Diminazenediaceturate	LDL receptor via Apo E enrichement	Lipid-drug conjugate	Increased brain levels of diminazenediaceturate	Gessner et al. (2001)
Mucopolysacharidosis	Beta-glucuronidase gene	TfR	Liposomes associated to TfR Ab	Enhanced BBB transport	Zhang et al. (2008)

(*Continued*)

Table 7.7 Examples of the Various NCs Used as CNS Disease Therapeutics, From the Recent Literature *Continued*

Disease	Molecules	Target	Lipidic-NC Type	Result	Reference
Alzheimer's disease	Ferrulic acid	–	SLN	Reduction of ROS and cytochrome c-apoptosis	Picone et al. (2009)
	Quercetin	–	SLN	Increase of therapeutic efficacy of quercetin	Dhawan et al. (2011)
	Cardiolipin		SLN	Disaggregation of Aβ	Gobbi et al. (2010)
	Curcumin	–	Liposomes	High affinity for Aβ	Mourtas et al. (2011)
	PA		Liposomes	Disaggregation of Aβ	Gobbi et al. (2010)
	AβMAb	MAb	Liposomes	Increased binding to Aβ1-42 peptides	Markoutsa et al. (2012), Canovi et al. (2011)
	PA LIP +	MAb	Liposomes	Increased binding to Aβ1-42 peptides	Salvati et al. (2013a,b,c)
	–	MAb + peptide	Liposomes	Enhanced BBB transport	Markoutsa et al. (2014)

has gained special attention because of the continuously aging populations in developed countries. Aging has also been found to be associated with neuronal death in various regions of the brain (Finch and Day, 1994). At autopsy, aged individuals demonstrate the hallmarks of neurodegeneration in the brain, such as Lewy bodies in PD and the neurofibrillary tangles in AD (Gibb et al., 1989). Various factors have been linked to neuronal damage (Kurtland, 1988; Relja, 2011; Waldmeier, 2003; Kanwar et al., 2009; Clarke, 1990). Between neurodegenerative disease:

AD is one of the commonest for which only symptomatic therapy is available. Dementia is the primary clinical symptom noticed, along with impaired learning and cognition (Nowacek et al., 2009). The brains of AD patients show a characteristic pattern of β-amyloid (Aβ) plaques and neurofibrillary tangles that are considered to be pathological hallmarks of disease and progression (Scheuner et al., 1996; Swartz et al., 1999). After diagnosis, the amount of Aβ deposited and its neuroanatomical location reflects the extent of neuronal damage (Karran et al., 2011). Other proteins associated with the pathology are neurofibrillary tangles, which become cytotoxic if hyperphosphorylated (Imbimbo et al., 2005). Several NCs have been found to increase the bioavailability and efficacy of different AD therapeutic agents. Various types of liposomal formulations with high affinity for Aβ peptides (monomer, oligomers, or fibrils) have been developed. The ligands used for Aβ targeting vary from lipids as cardiolipin or phospatidic acid (Gobbi et al., 2010), curcumin or curcumin derivatives (Mourtas et al., 2011; Lazar et al., 2013; Taylor et al., 2011) as well as a monoclonal antibodies against Aβ1−42 (Markoutsa et al., 2012). In some cases the liposomes were further engineered to incorporate a second functionality in order to also enhance their transport across the BBB and thus target Aβ plaques in the brain. A peptide derivative of ApoE 3 (known to target the apolipoprotein receptor overexpressed on BBB cells) (Re et al., 2011), or an antitransferin receptor monoclonal antibody (Markoutsa et al., 2012; Salvati et al., 2013a,b,c) were used. All liposome types were demonstrated to have increased Aβ binding affinity (compared to the free ligands and nonligand control liposomes) explained by multivalent effects and most of them significantly retarded or blocked Aβ aggregation. Several of these nanoliposome types are currently under in vivo investigation for their therapeutic effects on AD.

PD is another neurodegenerative disease associated with loss of striatal and dopaminergic neurons in the substantia nigra that coordinate motor movements, along with intracellular inclusions of abnormal proteins called Lewy bodies (Michael and Robert, 1996; Gibb et al., 1991). The etiology of PD is rather unclear, with a pathology of misfolding and aggregation of proteins, oxidative stress, neuroinflammation, and loss of integrity of the BBB. Mutations are also involved in the disease, and four proteins, i.e., α-synuclein, parkin, DJ-1 and PINK1, are found to be associated with its inheritance. A possible mechanism correlated with the disease generation is the ability of Lewy bodies to self-aggregate (Kholodilov et al.). Free radicals have a significant role in the pathogenesis of neurodegenerative diseases, including PD (Mcnaught et al., 2003).

The first drug used clinically was the dopamine precursor L-Dopa, that contrarily to dopamine itself, crosses the BBB by using a large amino acid transporter (Wade and Katzman, 1975). On the other hand, in a Trojan Horse approach resulted in normalized striatal tyrosine hydroxylase levels and reversed functional signs in a Parkinson model. A tyrosine hydroxylase gene empowered by a nervous system-specific promoter was injected, carried by PEGylated liposomes decorated with OX26 antibody against TfR (Zhang et al., 2003, 2004a). Additionally successful delivery of erythropoietin (Zhou et al., 2011) and glial derived neurotrophic factor (GDNF) (Fu et al., 2010) was achieved by joining these therapeutic proteins to mice anti-TfR antibodies.

7.6.2 NEUROINFLAMMATORY DISEASES

Neuroinflammatory processes are mediated principally by the key players of the brain's innate immune system, i.e., microglial cells, that are only equipped with dedicated antigen-presenting potential, and have been found to be involved in several CNS disorders, including AD, multiple sclerosis (MS), and infections, which substantiates their pathological role.

MS is a chronic demyelinating inflammatory CNS disease characterized by immune attack directed towards myelin, the protective neuronal sheath (Lucchinetti et al., 2000). It affects more than one million people globally, and patients present with symptoms of weakness, ataxia, fatigue, sensory and vision loss, and impaired memory. The severity of these symptoms is determined by the amount of demyelination present, and the more severe the damage, the less the neurons can communicate with each other. The exact etiology of the disease remains elusive.

Prion diseases are invariably fatal neurodegenerative disorders affecting primarily sheep (Scrapie), cattle (bovine spongiform encephalopathy), cervids (chronic wasting disease) and humans (Creutzfeldt-Jakob and fatal familial insomnia). The prion hypothesis asserts that TSEs are caused by a misfolded, protease resistant isoform (PrPRES) of a cellular prion protein (PrPC) that is present on many mammalian cells but most highly expressed on neurons and glial cells in the CNS (Pulford et al., 2010). Presently, no effective therapies have been developed to treat TSEs. The two most recent studies used RNAi, for silencing specific genes in biological systems to decrease PrPC within the CNS.

7.6.3 NEUROPLASTIC DISEASES

Neuroplastic diseases refer to a wide range of brain tumors. There is a multitude of neoplasms that originate in the brain, arising either from neural elements or resulting from metastasis of primary tumors situated elsewhere in the body, including gliomas, medulloblastomas, and primary CNS lymphomas (Kanwar et al., 2012a,b). Astrocytomas are considered to be relatively benign, but often undergo mutation,

advancing to high-grade astrocytoma or glioblastoma multiforme, the most aggressive and malignant form of astrocytoma (Baumann and Zumwalt, 1989).

Targeting the transmembrane protein TMEM30A, the ligand FC5, drives liposomes though the BBB to release doxorubicin into CNS (Gabathuler, 2010). PEGylated immunoliposomes targeted to TfR 14 (Boado et al., 2007), and delivery of shRNAs expression vectors against the epidermal growth factor receptor (EGFR) increased the survival in mice with intracranial tumors (Boado et al., 2007; Pardridge, 2004; Zhang et al., 2004b). Despite no direct CNS targeting, it has been possible to increase intracranial levels of anticancer 3′5′-dioctanoyl-5-fluoro-2′deoxyuridine (DO-FUdR), by incorporation into SLNs (Wang et al., 2002a,b). The nerve growth factor (NGF) gene has been introduced into the CNS while inside lipoplexes decorated with the TfR natural ligand, transferrin (da Cruz et al., 2005).

7.6.4 OTHER DISEASES

Other types of CNS-located diseases include infectious diseases, such as HIV, trypanosomiasis and specific syndromes. The brain levels of different anti-HIV drugs have been increased several folds through association with liposomes (foscarnet, Dusserre et al., 1995) and micelles, (Spitzenberger et al., 2007). Furthermore, second stage African trypanosomiasis was treated by conjugating the active water-soluble drug to liposomes using polysorbate 80 as surfactant (Olbrich et al., 2002). BBB crossing has been successfully achieved for therapy of Hurler's syndrome (mucopolysacharidosis), using the mouse anti-TfR antibody associated to a liposome with beta-glucuronidase gene (Zhang et al., 2008) or fused to the alpha-L-iduronidase enzyme (Boado et al., 2008).

7.7 CURRENT LIMITATIONS OF THE DIFFERENT LIPIDIC NANOCARRIERS FOR BRAIN TARGETING AND FUTURE PERSPECTIVES

Many factors can affect the targeting efficiency of NCs, such as particle size, surface properties, ligand properties, and ligand density. Improved brain-targeted systems can be developed through optimization. For example, Pang et al. found that the optimizal number of OX26 molecules per NC is 34. However, it is not easy to control the homogenious attachment of ligands on each particle.

The particle size of NCs also substantially affects their in vivo behavior and distribution. Larger sized NCs are trapped by lungs and smaller NCs tend to be eliminated by kidneys. Particle size also affects BBB permeability. For example, the cutoff pore size of the U87 brain tumor model is 7−100 nm (Karim et al., 2016) which means that NCs that are larger than 100 nm would have difficulty accessing U87 brain tumors. Another study showed that gold NCs of 70 nm demonstrated best penetration through

bEnd.3 monolayers among 20−110 nm gold NCs (Shilo et al., 2015). Additionally, particle size can affect the elimination and penetration in diseases tissues (Kibria et al., 2013; Hirsjärvi et al., 2013; Popović et al., 2010; Zan et al., 2014), as well as tumor penetration and retention (Zan et al., 2014; Ruan et al., 2015a,b). For most biodegradable NCs, the size range is wide; a "mean particle size of 200 nm" actually refers to "a mixture of particles that range from 50 nm to 400 nm." The response to physical barriers of such NC dispersions is hard to predict and control. A recently developed technique named particle replication in non wetting templates (PRINTs) has the ability to produce uniform NCs (Rolland et al., 2005), which may partly solve such problems. However, no ligand-modified PRINT particles were prepared yet. Microfluidic techniques are good alternative methods for precise formation of lipidic-NCs.

The protein corona may also seriously hinder the targeting potential of NCs. A protein corona is formed as soon as NCs are introduced into biological fluids (Nel et al., 2009), by serum proteins (opsonin and dyopsonins) (Aggarwal et al., 2009). In addition to the alteration of the distribution of NPs, the protein corona may cover the targeting ligand and hinder the specific reaction between ligands and targets (Salvati et al., 2013a,b,c). A consistent result was provided by Mirshafiee et al. (2013) using bicyclononyne and azide as model interaction. Unfortunately, most studies on brain-targeted drug delivery systems did not evaluate the formation and influence of protein coronas.

Another problem is the off-target potential of brain-targeted delivery. The basis of ligand-modified NPs for brain-targeted delivery is that these ligands can specifically react with their receptors or transporters and these receptors and transporters are highly and specifically expressed on the BBB and/or brain diseased cells. As discussed above, the protein corona may attenuate the specific reaction between ligand and receptor/transporter. In addition, the expression of receptors/transporters in other tissues could also lead to off-target effects. Unfortunately, most, if not all of the receptors/transporters are also expressed on normal cells. For example, the Tf receptor is overexpressed on BBB and brain tumor cells, but it is additionally expressed on almost all cells at various levels (Ponka and Lok, 1999). Therefore, it is hard to avoid off-target effects when using endogenous receptors/transporters as targets. However, labeling target cells with exogenous ligands may avoid the off-target effect. 9-Azido sialic acid could be incorporated into brain because the metabolism of brain cells requires a high level of sialic acid. The systemic administration of an alkyne-functionalized biotin probe could then be labeled onto brain cells due to the azide-alkyne click reaction, resulting in specific imaging of brain sialoglycansin living animals (Xie et al., 2016).

In spite of the various problems stated above, novel nanostructured delivery systems to target the brain are continuously being developed. To meet all the required criteria for brain-targeted NCs, as have been mentioned in Table 7.2, researchers are turning more and more to nature for inspiration. It is well known that some naturally occurring particles are in-born vehicles to target the brain, such as the Rabies virus. Exosomes can also circulate and deliver their contents

to distant target cells, in some cases in the brain. Currently, substantial efforts are being made to isolate the key features of such natural carriers, and use them for construction of bioengineered or biomimetic NCs. With advancements in molecular biology, deeper recognition of the mechanism for homing of such natural nanostructures to the brain can be elucidated and used for development of future brain vehicles either reconstructed from natural vehicles or (more feasibly) developed as artificial mimics of the natural vehicle-types.

ABBREVIATIONS

A	avidin
AD	Alzheimer's disease
AET	active efflux transport
ApoB	apolipoprotein B
ApoE	apolipoprotein E
APP	amyloid precursor protein
AUC	area under the curve
BBB	blood−brain barrier
BBBD	BBB disruption
BCECs	brain capillary endothelial cells
BCNU	carmustine
CAT	cationic amino acid transporter
CD	cluster of differentiation
CED	convection-enhanced delivery
Chol/CHOL	cholesterol
CHT	choline transporter
CMT	carrier-mediated transport
CNS	central nervous system
CNT	concentrative nucleoside transporter
CPP	cell-penetrating peptide
CSCs	cancer stem cells
Cur/CUR	curcumin
Dox/DOX	doxorubicin
DPPE	1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine
DSPE	1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine
DTPA	diethylenetriaminepentacetate
DTX	docetaxel
ECs	endothelial cells
EDC	1-ethyl-3-(dimethylaminopropyl) carbodiimide
ETP	etoposide
EV	extracellular vesicle
F	folic acid/folate
FCGR	Fc like growth factor receptor
FITC	fluorescein isothiocyanate

GBM	glioblastoma
GDNF	glial derived neurotrophic factor
Glu/GLU	glucopyranoside/glucose
GLUT	glucose transporter
GMO	glyceryl monooleate
GSH	glutathione
HBMECs	human brain microvascular endothelial cells
hCMECs	human cardiac microvascular endothelial cells
HES	peptide encapsulating hesperidin
HPLC	high-performance liquid chromatography
Hsp	heat shock protein
IAMs	immobilized artificial membranes
IGF	insulin-like growth factor
IGFR	insulin-like growth factor receptor
IL	interleukin
IL-13Rα2	interleukin-13 receptor alpha 2
INSR	insulin receptor
IRs	insulin receptors
ISF	interstitial fluid
LDL	low-density lipoprotein
LEPR	leptin receptor
Lf	lactoferrin
LNPs/LNs	lipidic nanoparticles
LPs/LIPs	liposomes
mAbs	monoclonal antibodies
MAL/mal	maleimide
MAN	*P*-aminophenyl-α-d-mannopyranoside
MCF	MCF cancer cells
MCNPs	membrane-core NPs
MCT	monocarboxylic acid transporter
MENDs	multifunctional-envelope-type-nano devices
MHC-II	major histocompatibility complex
miRNA	microRNA
mRNA	messenger RNA
MRT	mean residence time
MTD	maximal tolerated dose
MTH	molecular Trojan horses
MVB	multivesicular bodies
NA	nucleic acid
NAR	naringin
NBT	nucleobase transporter
NC(s)	nanocarrier(s)
NHS	*N*-hydroxysuccinimide group
NLC	nano-structure lipid carriers
NP(s)	nanoparticle(s)
NPG	*N*-palmitoyl glucosamine

NRP	neuropilin
OAT	organic anion transporter
OATP	organic anion-transporting polypeptide
ON	antisense oligonucleotide
P	permeability
P407	poloxamer 407
PAMPA	parallel artificial membrane permeability assay
PAMPA	parallel artificial membrane permeability assay
PC	prostate cancer
PD	Parkinson's disease
PEG	polyethylene glycol
PEI	polyethylenimine
PEO	polyethyleneoxide
p-gp	p-glycoprotein
PHYT	phytantriol
PLGA	D,L-lactide-co-glycolide
PLNs	polymer-lipid hybrid nanocarriers
PPO	polypropylenoxide
PrPC	cellular prion protein
PTX	paclitaxel
RES	reticuloendothelial system
RISC	RNA-induced silencing complex
RMT	receptor-mediated transcytosis
RSP	risperidone
S	surface area
SA	streptavidin
SATA	*N*-succinimidyl-2-mercapto-(*S*-acetyl)acetic acid
SCAR	scavenger receptor
Ser-HCl	sertraline
SHp	stroke homing peptide
si RNA	small interfering RNA
SIA	*N*-succinimidyl iodoacetate
SLN	solid-lipid nanocarriers
SNALPs	stable nucleic acid-lipid particles
SolC24	SolulanC24
SPDP	*N*-succinimidyl-3(2-pyridyldithio) propionate
SPR	surface plasmon resonance
SQV	saquinavir
sulfo-SMCC	sulfosuccinimidyl-4-(maleimidomethyl) cyclohexane-1-carboxylate
TAM	tamoxifen
TAT	trans-activating transcriptor
TAUT	taurine transporter
TET	tetrandrine
Tf/TF	transferrin
TFF	trefoil factor
TfR	transferrin receptor

TR	transgenic
TSEs	transmissible spongiform encephalopathies
US	ultrasound
VAP	vapreotide
VEGFR	vascular endothelial growth factor receptor
VIP	vasoactive intestinal peptide
WT	wild type
α-M	α-mangostin

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