Nano-Enabled Delivery of Diverse Payloads Across Complex Biological Barriers

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Abstract

Complex biological barriers are major obstacles for preventing and treating disease. Nano-carriers are designed to overcome such obstacles by enhancing drug delivery through physiochemical barriers and improving therapeutic indices. This review critically examines both biological barriers and nano-carrier payloads for a variety of drug delivery applications. A spectrum of nano-carriers is discussed that have been successfully developed for improving tissue penetration for preventing or treating a range of infectious, inflammatory, and degenerative diseases.

Keywords: nanoparticle, drug delivery, biological barriers, blood brain barrier, tumor microenvironment, vector-borne disease

Graphical Abstract

Nano-carriers functionalized with active and passive targeting strategies enable the efficient delivery of diverse payloads through complex biological barriers to prevent and treat vector-borne diseases, neuronal disorders, and cancer.

1. Introduction

The transformative impact of nanotechnology on drug delivery cannot be overstated. The range of novel nano-carrier systems for disease prevention and treatment includes polymeric nanoparticles, micelles, and liposomes; each can enable the protection and delivery of proteins and nucleic acids [1, 2]. Nano-carriers deliver diverse payloads to intracellular components and reduce toxicity while increasing bioavailability through tissue targeting and by complex biological barrier crossings [1-3]. Nano-enabled approaches have successfully overcome barriers that limit drug delivery. This review addresses complex biological barriers and diverse payloads both of which influence the design of nano-carriers. How novel materials and methodologies can enable payload delivery across such barriers to improve disease outcomes is discussed. We also describe the barriers in the context of how the novel nano-carriers can overcome barrier restrictions and enhance therapeutic delivery and clinical efficacy. The challenges associated with delivering complex payloads through biological barriers are focused on small molecules, biologicals, and nucleic acids. Nanotechnology approaches that facilitate ingress and that can deliver diverse payloads are examined. The chemistry of nanoscale drug carriers, the advantages of and considerations for rationally designing nano-carriers for drug delivery and the targeting mechanisms they can employ are described. Finally, we offer a perspective on the challenges facing the development of nanoscale platforms.

2. Physiochemical barriers in drug delivery

Nanoscale delivery systems are designed to facilitate penetrance of small molecules, proteins, or nucleic acids through physiological barriers to disease sites while minimizing off-target toxicities. Depending on the delivery target, one or more barriers must be overcome for efficacious delivery. Such barriers can take several forms acting as extracellular materials that adhere to and physically hinder diffusion of disease combating moieties. For example, mucosal surfaces, the adsorption of serum proteins, cuticles, and biofilm matrices form significant physical obstructions for drug penetrance [4-9]. Cells are yet another barrier through their formation into polarized monolayers with tight-junction protein complexes. In this way they may limit the paracellular diffusion of therapeutic nano-carriers. As an example, the endothelial cells of the blood-brain barrier (BBB) provide barriers to drug delivery and result in poor permeation

Figure 1. Interaction of nano-based delivery devices with the blood-brain barrier (BBB).

Nano-based delivery devices are composed of a core (filled circles) containing the therapeutic agent and a surface that can be targeted to specific receptors (blue arrows). The nano-carriers also interact with the cellular (circular cells) and protein-based (blue triangles) environment of the circulation where they can undergo internalization or absorption respectively. Nano-carriers interacting with surface receptors (green Y) on the brain capillary endothelial cells (BCECs) can undergo transcytosis across the BBB. Once across the BBB, the nano-carriers can interact with multiple brain cells: astrocytes (blue), neuronal (purple), and microglia cells (green). Monocytes or macrophages in the circulation with internalized nano-carriers (circular cells) have also been shown to deliver particulate cargo to the BCEC and cross the BBB. Research has also focused on disrupting the tight junctions (TJ) of the BBB (lightning bolt), which would allow paracellular transport of the nano-carriers.

of therapeutics to action sites [4, 10]. The up-regulation of efflux pumps [11], reduced or halted cellular metabolism [7, 12], or changes in pressure gradients (such as in tumors) [13] pose additional physiochemical limitations to drug delivery. Here, we highlight formidable physiological barriers (the BBB, the tumor microenvironment, and the cuticle) that need to be overcome for efficacious therapeutic delivery. These barriers were chosen because they pose drug delivery challenges for a myriad of infectious, inflammatory and degenerative disorders. We posit that nano-based devices can bypass such barrier restrictions leading to improved disease outcomes.

2.1 Barriers to brain delivery

Neurological diseases cause ~12% of total worldwide deaths [14]. Successful treatment of Parkinson's, Alzheimer's and Huntington's disease (PD, AD and HD), chronic traumatic encephalopathy (CTE) and brain cancer requires delivery of therapeutics across the BBB. PD, AD, HD and CTE are characterized by a progressive degeneration and death of neurons, resulting in symptoms such as problems with movement and cognition. While current drugs treat symptoms there is no intervention that halts disease progression or alleviates the underlying pathology. As new generations of therapeutics (such as small molecules and proteins) are developed specifically the ability of molecules to cross the BBB have the highest priority.

The BBB is designed to protect the central nervous systems by restricting the diffusion of small molecules into the brain while facilitating the transport of essential nutrients across endothelial cells. Endothelial cells at the interface between the systemic circulation and the brain (Figure 1) express tight junctions between adjoining cells and effectively block paracellular transport of polar solutes from the peripheral circulation to the central nervous system (CNS) [15]. This essentially eliminates all polar small molecule drugs from crossing the BBB [16]. The free diffusion of highly lipophilic molecules from the circulation to the brain is also limited by their molecular weight, typically <400 Da, which makes this pathway unsuitable to deliver essentially all macromolecular pharmaceuticals (peptides, proteins and antibodies) [10, 16]. However, these endothelial cells express transport proteins for essential metabolites to make up for the lack of diffusion to maintain cerebrospinal fluid homeostasis [17]. Receptors on the circulatory side of the brain capillary endothelial cells (BCECs) include low density lipoprotein

receptor (LDR) [18-20], transferrin [21-24], leptins [25], epidermal growth factor [26], diphtheria toxin [27], and insulin [28, 29]; and the potential for active targeting of these receptors may facilitate drug transport across the BBB.

A further challenge to effective CNS therapeutic delivery is that once the BBB is traversed, payloads need to be transported further to the target disease locale. Brain extracellular spaces provide potential diffusion pathways to achieve this. Measures of fluorescent probe diffusion in rat normoxic neocortex demonstrates the movement of particles from extracellular spaces to precise brain sub-regions equivalent to fluid filled 38-64 nm pores [30]. More recent work performed with nanoparticles with a dense layer of polyethylene glycol showed that the actual limit to diffusion is closer to 120 nm [31]. In either event, such extracellular barriers restrict the size of drug delivery vectors through the brain's extracellular spaces.

2.2 *Barriers to tumor killing*

The World Health Organization attributed 8.2 million deaths worldwide to cancer in 2012 [32]. Cancer contributes to roughly 13% of all deaths worldwide and new incidences are expected to increase by 70% over the next two decades [32]. For cancer treatment, systemically delivered agents need to distribute to the tumor site, which includes the need to cross the complex physiological barrier provided by the tumor microenvironment. In addition, chemotherapeutics include a spectrum of payloads (small molecules, antibodies, and nucleic acids) that have their own unique challenges for delivery (Section 3). However, the ability of chemotherapeutics to be delivered to the tumor site is not by itself sufficient to kill cancer cells. For effective tumor killing the agents need to overcome cancer cell resistance mechanisms, immune suppression, and the microenvironment of the tumor [33].

Cancer cells undergo abnormal growth, tending to up-regulate cellular replication and down-regulate cellular death. For example, in work with rat intestinal epithelial cells the overexpression of cyclooxygenase 2, which is elevated in >80% of human colorectal adenocarcinomas, results in increased cellular adhesion and inhibition of apoptosis [34]. Similarly, increased expression of aryl hydrocarbon receptor can induce tumors and may contribute to apoptosis resistance to chemotherapeutic agents in breast cancer cells [35, 36]. Another growth factor, HER2, is overexpressed by 25 to 30 percent in some types of breast cancer cells [37]. Tumors have also been shown to suppress host immunity through the induction of regulatory T-cells and myeloid-derived suppressor cells, which negatively regulate the immune response during tumor progression and secrete soluble factors for immunosuppression [38, 39]. These diverse changes in cell regulation and receptor expression lead to targets for both treatment and nano-based devices. Examples of agents that could be used to counter regulatory T cells include cyclophosphamide [40] and examples of agents that can be targeted to the tumor to eliminate myeloid derived suppressor cells include Sunitinib and 5-Flouraracil [41].

A solid tumor microenvironment contains a heterogeneous population of cancerous and stromal cells embedded in an extracellular matrix. The extracellular matrix consists of highly interconnected networks of collagen fibers which hinder diffusion [42]. However, the ratios and compositions of these cells can vary greatly by tumor type [43]. The tumor vasculature is often leaky and unorganized compared with normal tissue vasculature [12]. A leaky vasculature leads to preferential delivery of therapeutics to the tumor site from the circulation. However, the disorganization of solid tumor lymphatics affects increased interstitial pressure. This can limit convection and diffusion of cancer therapeutics within solid tumors [13]. The disorganization in

vasculature also leads to variability in perfusion and the development of hypoxic and acidic regions within the solid tumor [44].

Nanotechnologies can enhance cancer therapeutic delivery to the tumor site. This can occur by both size and active protein targeting. Some nanoscale delivery devices are designed to release their payload based on the acidic or hypoxic solid tumor regions. This limits off-target effects of cancer therapeutics. Overall, penetration of the solid tumor microenvironment is a formidable barrier to effective cancer cell killing.

2.3 *Barriers to tropical disease prevention and treatment*

Vector-borne diseases have adversely impacted the health of human populations worldwide. Mosquitoes have been known carry and transmit viral disease such as yellow fever, dengue, chikungunya, and West Nile [45]. In addition, vectors are key to the development and transmission of parasites that cause tropical diseases such as malaria and lymphatic filariasis [45-47]. Vector control is a viable and essential strategy to reduce transmission and prevent the occurrence of vector-borne diseases; however, resistance to existing insecticides is a significant impediment and new methods in vector control must be developed [48]. Because many insecticides are delivered topically, the ability to penetrate the exoskeleton, or cuticle, of mosquitoes poses a major challenge in the development of vector control [49].

The mosquito cuticle is a unique exoskeleton consisting of distinct, organized layers. The epicuticle, or outer most layer, provides protection from the environment. Composed of a lipophilic wax mesh as well as many lipids and proteins, the epicuticle prevents water loss and desiccation as well as provides the first point of contact to interact with the environment [50, 51]. Beneath the epicuticle lie the exocuticle and endocuticle layers, which are largely aqueous and composed of organized, cross-linked chitin sheets. Due to this unique structure, it has been noted in the literature that lipophilic and hydrophilic molecules individually have poor diffusion through the mosquito cuticle [50, 52]. However, mixtures of lipophilic solvents and hydrophilic molecules, as well as molecules with both lipophilic and hydrophilic groups, can pass through the cuticle efficiently [5]. Beyond the cuticle, insecticides have to reach a target organ site; just as pharmaceuticals have to travel from the point of entry to a cell or tissue of interest in a vertebrate body, insecticides typically have to traverse the body cavity to reach the nervous system. It is very likely that engineering nano-carriers with specific moieties designed to interact with particular cell types would facilitate uptake and spread in an insect body.

Similar to the mosquito vector by which they are spread, the nematode parasites that cause lymphatic filariasis also have a cuticular exoskeleton. In nematodes, the cuticle is responsible for the primary interface with the environment as well as required for body shape and movement [53, 54]. Similar to mosquitoes, the epicuticle layer is composed of lipid-rich molecules as well as glycoproteins. Beneath the epicuticle is a layer composed of primarily collagen and cuticlins, resulting in an insoluble material formed by cross-linking of cuticlin proteins [53, 54]. While soluble antihelminthic drugs have been shown to pass through the nematode cuticle, the nematode may regulate specific pumps to expel or minimize the treatments effectiveness [11, 55, 56]. Thus, the cuticle has continued to be a challenging barrier to prevent and treat vector-borne diseases and the development of new nano-carriers, as presented in Section 5, have enabled successful disease combating approaches.

3. Diversity of payloads

While a primary consideration in designing nano-carrier technologies for drug delivery is the ability to interact with and cross complex barriers, it is also important to pay attention to the types of payloads in these nano-carriers. A variety of payloads are used in therapeutic applications and include small molecules, proteins, peptides, and nucleic acids. The challenges associated with each payload type are discussed in this section and summarized in Table 1.

3.1 *Small molecules*

Small molecule drugs are typically low molecular weight chemically synthesized organic compounds with biological activity. These can be divided into various classes based on their chemical structure and biological function and include, but are not limited to, classes such as neurotransmitters, antimetabolites, DNA intercalators and antibiotics. Neurotransmitters include biogenic amines such as dopamine and serotonin [57]. Antimetabolites are molecules that interfere with essential biosynthetic processes, such as gemcitabine, cytosine arabinoside and paclitaxel, and have been used in cancer therapies [58, 59]. DNA intercalators, such as Doxorubicin, have been utilized in cancer therapy, but has potentially severe health effects [60]. Antibiotics prevent and treat bacterial infections, such as Rifampicin that acts through the inhibition of bacterial RNA polymerase [61]. Among the many other small molecule drugs are antiparasitics, and neuroactive agents.

Many small molecular weight drugs fail to efficiently produce the desired outcome. This could be due to poor bioavailability influenced by the inability of the small molecular weight drug to cross the complex barriers. For example, hydrophilic drugs are not easily transported across the BBB unless they interact with a surface receptor on the circulation side of the endothelium. Additionally, there are concerns with side effects from chemotherapeutics used in cancer treatment and antibiotics used in filariasis due to the high systemic concentrations of drug needed to achieve a therapeutic concentration of drug at the target location [58-60, 62]. The use of nano-based delivery vehicles can overcome complex barrier restrictions and extend the use of small molecular weight drugs. Additionally, nano-based delivery vehicles target specific locations and reduce side effects associated with total drug concentrations. This ability holds the tantalizing promise of enabling the use of small molecules previously discarded as therapeutics due to systemic toxicities.

3.2 *Peptides and proteins*

Protein- and peptide-based therapies have advantages over more traditional medicines. These molecules are capable of performing therapeutic functions, such as acting as receptor agonists or antagonists, or active targeting of ligands (see Section 4.4). Endostatin peptides may be used to inhibit angiogenesis. Neurotrophins such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are neuroprotective [63]. In addition, monoclonal antibodies (trastuzumab) promote cell-cycle inhibitors in cancer treatment [64]. Proteins and peptides also have the added ability of enabling a physiological function. For example, cell penetrating peptides have been designed to mimic viruses by inducing internalization and endosomal escape [65]. Other protein therapies may take advantage of the inherent cell transport and signaling mechanisms. As an example, recombinant hormones such as leptin can act on signaling cascades to treat metabolic disorders such as obesity [66, 67]. Finally, both proteins and peptides typically have high specificity and minimal cross-reactivity, enhancing their safety profile [66].

Protein therapeutics also face many challenges. First, they must be presented in the correct conformation in order to maintain proper function [68]. Second, proteins and peptides may have poor stability and are enzymatically degraded [64, 65, 69]. Third, they may be labile at room temperatures and as such require refrigeration. This incurs expense and poses transport limitations [69]. Fourth, they often have poor bioavailability and are often rapidly cleared by renal filtration [66, 68]. Thus, multiple doses commonly in the form of painful injections are needed to enhance their efficacy. This frequent dosing, however, further increases the cost of biological pharmaceuticals and limits patient compliance [68, 69].

3.3 *Nucleic acids*

The development of new nano-based delivery platforms has revolutionized the use of nucleic acid-based therapies, including gene therapy and RNA interference. During gene therapy, the delivery of DNA to the nucleus facilitates repair of malfunctioning genes or the introduction of new disease combating genes [70]. These new genes are subsequently translated into proteins to restore proper function of abnormal proteins. Similarly, RNA interference (RNAi) involves the delivery of non-coding double stranded (ds)RNA or silencing (si)RNA to the cell cytoplasm [71]. These dsRNA molecules bind to specific, complementary mRNA leading to their cleavage and degradation, ultimately inhibiting protein production of specific genes. In insects, dsRNA-based RNAi has been more successful compared to siRNA, possibly due to the generation of multiple siRNA from dsRNA cleavage [72].

The delivery of nucleic acid molecules is not without its challenges. Its mechanism of action requires the delivery of DNA or RNA molecules to the nucleus or cytoplasm of cells. These molecules are known to be hydrophilic and anionic [70, 73]. These inherent properties, coupled with their generally large size, limit the ability of nucleic acids to cross cellular membranes. Once inside the cell, the therapeutic effects are often short-lived and require frequent, repeated administrations. For example, the therapeutic effect of siRNA only lasts 3-7 days before the siRNA molecules are diluted under therapeutic thresholds [71]. To further limit nucleic acid therapy effectiveness, nucleic acids are quickly cleared from circulation through phagocytosis and kidney filtration [70, 71, 73].

Nucleic acids are also extremely sensitive to degradation by nucleases. For example, exogenous dsRNA is rapidly cleaved by nucleases in the hemolymph of *Manduca sexta* thus imparting resistance against RNAi approaches in insects [74]. In addition, variability caused by the type of the RNAi response induced, i.e., systemic or localized, can impact the overall success of RNAi based interventions [72].

4. **Nano-enabled approaches for delivery of diverse payloads**

Nanotechnology-based vehicles can be highly beneficial in traversing complex barriers for drug delivery because of their ability to overcome the challenges associated with the barriers and by their ability to carry diverse cargo. In particular, nanoparticles and micelles represent two versatile platforms that can be composed of many different materials and that may be tailored to fit a variety of therapeutic applications. In this section, the different types of nano-carrier platforms will be reviewed, important considerations for rational design will be discussed, and surface modification strategies for passive and active targeting will be introduced.

Figure 2: Nano-carriers for treatment of tumors. In normal tissue, blood vessels are composed of tightly aligned endothelial cells. Therefore, only small molecules and particles are able to exit the blood vessel into tissue before draining to the lymphatic system. During tumor development, blood vessels are often disorganized and contain enlarged gap junctions. Therefore, both large and small nano-carriers are able to permeate into tissue, and due to a lack of lymphatic vessels, are retained. The persistence of these nano-carriers within tissue allows for the continuous release of therapeutics at the tumor site. In addition, nano-carriers for cancer treatment can also be functionalized with receptor-targeting molecules or antibodies that enhance uptake into tumor cells.

4.1 Advantages of nano-carriers for drug delivery

As discussed, nanotechnology has played a critical role for payload delivery across complex barriers. Without nanotechnology, a number of therapeutics (including small molecules and nucleic acids) would not prove viable as part of disease treatment strategies. Polymeric and micelle-based nano-carriers have hydrophobic cores capable of encapsulating poorly water soluble drugs and a more hydrophilic surface that can enhance delivery of water insoluble drugs [75-77]. Nanoparticulate drug delivery platforms can also impact payload stability. For example, nucleic acids are quickly degraded by nuclease enzymes. The ability of nano-carriers to encapsulate and prevent exposure to nuclease enzymes ensures that nucleic acids are intact and functional upon delivery [78, 79]. In addition, liposomes and virus based nanoparticles have the advantage of mimicking a protein's natural environment, allowing proteins to be stabilized and expressed in their biologically functional forms [80]. A major advantage for nano-based drug carriers is their capability specific tissues. Passive targeting to tumors can be achieved through the enhanced permeation and retention (EPR) effect (Figure 2). During tumor development, an imbalance of angiogenic factors promotes the rapid growth of vasculature [81]. However, these new vessels are often disorganized, dilated, and contain enlarged gap junctions [2]. Such properties promote the accumulation and retention of nano-based vehicles. This leads to the continual release of therapeutics within the tumor [2, 3, 81]. Whilst the EPR effect has a strong effect in some tumor types, a limitation of the EPR effect is its high degree of heterogeneity between different tumors [82].

Finally, nano-based carriers have reduced the toxicity of pharmaceuticals. For example, systemically delivered therapeutics can often cause undesired side effects on healthy tissues. However, the design of actively targeted nano-carriers results in local administration of drug molecules at target tissues [2, 71]. Furthermore, the sustained or controlled release kinetics enabled by many nano-carrier formulations, combined with the ability to co-encapsulate multiple and diverse therapeutics, also reduces the total number of doses a patient must take [1, 83]. This reduction of dose (and therefore toxicity) has a large impact on the treatment of diseases such as cancer and filarial parasites. As an example, the use of antibiotics during filariasis treatment has been shown to enhance efficacy, however, the observation of adverse effects has prevented their use in children [84].

4.2 *Types of nano-carrier platforms*

4.2.1 *Biodegradable polymer nanoparticles*

Biodegradable polymers are perhaps one of the most well studied materials to design nanoparticles for drug delivery. Biodegradable polymeric nanoparticles for drug delivery can be synthesized from natural or synthetic polymers and can either physically encapsulate or covalently bind drug molecules. After particle formation, the surface properties of nanoparticles can be modified through functionalization, as described below. Several classes of biodegradable polymers have been studied for therapeutic delivery and include polyalkyl cyanoacrylates, polyesters, polyanhydrides, and polyethers. Detailed reviews that provide in-depth information about biodegradable polymers are cited [85-91].

Poly(alkyl cyanoacrylates) have been shown to deliver peptides to the CNS after intravenous injection [92]. Poly(butyl cyanoacrylate) (PBCA) is readily biodegradable with no toxic metabolites [93]. Its rate of degradation can be modified by substitution of the alkyl group, but these substitutions also affect its metabolite toxicity. Therapeutics are typically adsorbed onto the PBCA nanoparticle surface after polymerization. This decouples the release of the drug affecting PBCA degradation and limiting its controlled release.

Polyesters such as poly(lactic-co-glycolic acid) (PLGA) have an extensive safety record, being FDA-approved, and are commonly used in drug delivery applications [85, 94]. One of their most important properties is their low cytotoxicity attributable to their rapid degradation into metabolites that are quickly processed by cells [95]. Even though there is extensive use of polyesters for drug delivery, shortcomings remain for their general use. Notably, all polyesters undergo bulk erosion due to the stability of ester bonds [96, 97], which often results in a rapid, or burst, release of drug payloads [98-102]. The lactic acid component of poly(lactic-co-glycolic acid) (PLGA) can be easily varied between 50 and 95% and the release profiles of encapsulated payloads may be extended [100, 103]. The molecular weight of the polymer can also be varied to marginally control the release of payload [99, 100, 104]. While PLGA promotes stability and sustained release of proteins or molecules [94], the acidity and hydrophobicity of the polymer makes encapsulation of nucleic acids (DNA and RNA) and fragile proteins difficult [105].

Polyanhydrides possess excellent biocompatibility and drug delivery potential, and thus, has fostered significant research with these materials [106]. The Gliadel[®] wafer, a sebacic acid and 1,3 bis(*p*-carboxyphenoxy)propane based wafer encapsulating carmustine, releases the oncolytic agent after tumor resection and is perhaps one of the best translational successes of polyanhydride-based therapies to date [107]. The polyanhydride implants are degraded into biocompatible metabolites and are readily eliminated [108]. Polyanhydride-based devices act as surface eroding materials [96, 97]. By tuning the degree of hydrophobicity of the backbone chemistry, polyanhydrides can be modulated from rapidly degrading (over a period of days) to very slowly degrading (over a period of one year), and as such, control drug release [109-111]. In addition, the monomers released from polyanhydride degradation are not as acidic (pH = 4.2 – 6.5) as those observed during polyester degradation [112, 113]. A less acidic microenvironment combined with surface erosion make polyanhydrides promising carrier materials for fragile payloads; and nanoparticles based on polyanhydrides have been demonstrated to be successful as delivery platforms for proteins, nucleic acids, and small molecules [114-116]. However, polyanhydrides are highly susceptible to hydrolytic degradation with the bond reactivity of the anhydrides being six orders of magnitude greater than polyesters [97].

Synthetically derived and naturally inspired polyethers have been used in polymeric drug delivery for up to 30 years [117-119]. Poly(ethylene glycol) (PEG) and poly(propylene glycol)

(PPG) were used in combination with other nano-carrier platforms and as triblock Pluronics ([PEG]_n-[PPG]_m-[PEG]_n). Attaching PEG to the surface of particles has been shown to prevent protein absorption, reduce phagocytosis by immune cells, and enhance diffusion through the extracellular matrix [8, 31, 120, 121]. Naturally derived polymers such as chitosan, a cationic polysaccharide, have shown promise as drug delivery vehicles [122, 123]. The cationic nature of chitosan has been shown to allow for the complexation of nucleic acids while providing protection from nuclease degradation [73]. In addition, polyethers do not readily undergo hydrolytic degradation since the ether bond is very stable in water. Instead, polyethers can be either degraded by enzymes (chitosanase), through oxidation, or by dissociation prior to excretion.

4.2.2 *Micelles and core-shell nanoparticles*

Micelles (also called core-shell nanoparticles) are a class of drug delivery platform that is self-assembled from block copolymers [2, 3]. Typically, hydrophobic interactions among hydrophilic and hydrophobic segments of block copolymers drive self-assembly, although electrostatic interactions may play a role as well [124]. Commonly, the hydrophobic segments in micellar block copolymers contain polyesters or poly (amino acids) [124, 125]. Similar to the polyesters discussed in the previous section, poly (amino acids) are known to be both biodegradable and biocompatible. In addition, the structure of poly (amino acids) can be varied to control the degradation of the particle [124]. Polyethers, such as PEG or chitosan, are the most common hydrophilic segment in designing amphiphilic block copolymers for micellar drug delivery [124, 125].

Micelles are biocompatible and biodegradable, making them excellent candidates for drug delivery. Due to the amphiphilicity of these polymers, the particle contains a hydrophobic core which can encapsulate water-insoluble payloads [2, 3]. However, the shell remains hydrophilic, allowing the carriers (although encapsulating water-insoluble molecules) to remain water-soluble and avoid clearance by the reticuloendothelial system [2]. In addition, the use of a core-shell system enables high loading of therapeutics into the particle that remains protected until arrival at the targeted location [126]. While the hydrophobic core of micelles is generally not regarded as suitable for the encapsulation and delivery of nucleic acids; however, nucleic acids can be complexed with polyion micelles [70, 73]. In this method, negatively charged nucleic acids form a core with the cationic segments of a block copolymer, while the remaining block copolymer segments form the hydrophilic shell. Finally, these core-shell or micellar systems have the advantage of multi-functional properties. For example, core-shell nanoparticles containing magnetic or fluorescent cores may provide theranostic capabilities that combine bioimaging with therapeutic delivery [127].

4.2.3 *Dendrimers*

Dendrimers represent another type of nanoparticulate drug delivery platform that while similar to micelles retain several distinct characteristics. Dendrimers are composed of synthetic, repeatedly branched polymer macromolecules that extend from a central core [2, 128, 129]. Dendrimers are characterized into classes by their repeating unit, generation number, and degree of repetitive branching. Perhaps the most common dendrimers studied for drug delivery are poly(amidoamine) (PAMAM), poly(ethylenimine) (PEI), and polypropyleneimine (PPI), although polypeptides have been explored in recent studies [128, 130]. Particularly, PAMAM and PEI are suitable as gene delivery vectors [131, 132]. Due to their cationic nature, these

polymers are capable of condensing DNA into small, particulate complexes. In addition, their cationic surface charge is beneficial for efficient binding to and uptake by cells [131, 132]. PAMAM and PPI have been also demonstrated to be pH responsive, which may be useful in limiting therapeutic release to the acidic microenvironments of tumors [130]. However, PAMAM and PPI dendrimers often require modification to avoid liver retention and toxicity [128].

Due to their structure, dendrimers have several unique advantages in drug delivery applications. The functional group at the end of each dendrimer branch makes them conducive to controlled surface modification. In addition, dendrimers can encapsulate drug within their cores (similar to micelles) but lack the disperse molecular weights of amphiphilic block copolymers. Instead, dendrimers are typically monodisperse and small in size (30 nm or less) [2, 128, 129]. Thus, dendrimers are adept at high loading of therapeutics for maximum efficacy, yet capable of deep tissue penetration due to their small size.

4.2.4 *Liposomes*

While generally less stable than polymeric or dendrimer-based nano-carriers, liposomes offer several advantages for drug delivery. Liposomes are composed of self-assembling lipid bilayers enclosing a central, aqueous core [2, 70]. Although liposomes can be formed from either natural or synthetic lipids, phospholipids are the most commonly used in drug delivery applications [133]. Phospholipids typically assemble into membranes, and additional layers can be added to the liposome to control the release rate of encapsulated payloads [133]. Due to the nature of liposomes, their membrane is highly permeable to hydrophobic molecules. However, incorporation of other molecules within the bilayer, such as cholesterol, has been shown to improve the retention of hydrophobic molecules by inducing dense packing of the phospholipid membrane [133-135]. In addition, PEGylation has been demonstrated to improve the half-life of liposomes within circulation as well as reduce their aggregation [135]. Doxil, which is PEGylated liposomal doxorubicin, was the first FDA-approved nanotherapeutic and is used to treat specific forms of cancer [136]. Stimuli-responsive liposomes can be triggered to release encapsulated therapeutics by heat, ultrasound, light, and pH, increasing therapeutic effects on target organs or tissues [137].

4.2.5 *Virus based nanoparticles*

Virus based nanoparticles represent a new alternative to drug delivery development that is similar to liposome technology. Virus based nanoparticles for drug delivery are typically composed of plant-based viruses, such as tobacco mosaic virus, and bacteriophages that cannot replicate in and pose no hazard to humans [138]. While the use of virus based nanoparticles has been shown to stabilize drug molecules similar to other carriers, virus based nanoparticles have the distinct advantage of expressing biologically functional forms of proteins and peptides on their surfaces [2, 80]. This can be greatly advantageous in achieving targeting of specific cells, for example in cancer therapeutics, and reduce toxicity to healthy tissues.

4.3 *Design considerations for nano-carriers*

Despite the advantages of nano-carrier-based platforms in delivering diverse payloads across complex barriers, several considerations must be taken into account to ensure their success. To begin with, nanoparticle delivery systems should be biodegradable, biocompatible, and non-toxic. The degradation of nano-carriers prevents the need for surgical removal after use

(necessary in some implants), as well as enables controlled release of encapsulated payloads. For example, in surface-eroding polymers (such as polyanhydrides) the rate of polymer degradation is directly proportional to the rate of payload release [139]. By altering the composition (and therefore hydrophobicity) of the polyanhydride the biodegradation and release can be tailored for each application. In addition, nanoparticles composed of biodegradable, amphiphilic block copolymers can also be used to modulate the release kinetics of encapsulated payloads [126].

While biodegradable nano-carriers may alleviate toxicities by limiting accumulation within tissue any inherent toxicities of the degradable polymers must be considered. For example, while cationic polymers have been useful in gene delivery applications, the excess positive charge of these nanoparticles may destabilize cell membranes resulting in cytotoxicity [140]. In addition, the specificity of nanoparticles designed for cytotoxic applications, such as the killing of cancerous cells, is important as healthy tissues may be damaged by non-specific, non-target cellular delivery.

The size of the nano-carrier must also be designed with the therapeutic application in mind. The biodistribution of particulate carriers is largely influenced by size as larger carriers may form depots and smaller carriers generally disperse well throughout tissues [141-143]. Additionally, smaller nano-carriers typically have a higher rate of uptake and are useful for delivery to intracellular targets [141, 143, 144]. The size of particulate antigen carriers has also been shown to have a direct effect on the magnitude of antigen-specific immune responses stimulated with smaller particulates generating stronger responses than larger particulates [145]. Finally, while the rate of drug release can be controlled by the surface to volume ratio of particles, the amount or dose of drug molecules is also limited by particle size.

The surface charge and charge density of nano-carrier platforms is also integral to the design of a successful delivery system. As an example, the surface charge of carrier platforms is very important to deliver nucleic acids. Positively charged carriers can more easily form complexes with negatively charged DNA or siRNA [71, 146]. In addition, this positive charge may increase interaction with the cell membrane and enhance uptake of the encapsulated payloads [147]. However, excess positive charge may lead to increased binding to non-target cells, and therefore, the charge density should be carefully considered during nanoparticle design [140]. Finally, increase in charge density increases serum protein adsorption and may influence the half-life of circulating nanoparticles [148].

The shape of the nano-carrier can also impact their targeting capabilities. Polyethylene glycol nanoparticles of various shapes, sizes, and charge exhibited different biodistribution and persistence profiles when administered to mosquito larvae and adults [147, 149]. Typically, negatively particles had a higher *in vivo* persistence compared to positively charged particles except for long, rod-shaped particles where no difference was observed.

4.4 Targeting and surface modification

In many cases surface modification and active targeting have been employed to expand the functionality of nano-based delivery devices beyond their size and core materials. These modifications are used to reduce clearance, improve target specificity, and in some cases add therapeutic triggering to the delivery device.

Polyethylene glycols are often used as surface modifiers to alter the surface property of the core nano-carrier. The surface attachment of PEG is generally accomplished through conjugation to the core polymer either pre- or post-particle synthesis. The pre-particle covalent attachment of PEG has been used in multiple nano-based systems to form a block copolymer,

shifting the particle synthesis towards the formation of micelles [150]. In addition, PEG may be tethered to the lipid bilayer during liposome formation by conjugating a portion of the lipid component with PEG [151]. Post-particle surface modification with PEG is often performed to establish a hydrophilic corona around the nano-carrier. This is typically performed by covalent conjugation between functional groups on the surface of the particle and complementary functional groups on the end of a specific molecular weight PEG. This corona minimizes the absorption of extracellular materials on the nano-carrier and reduces clearance [8, 152].

During particle synthesis surfactants are introduced to stabilize particle formation. For example, Pluronic[®], poly(vinyl alcohol) (PVA) and to a lesser extent human serum albumin (HSA), are used as stabilizers in many nanoparticle formation synthesis [153]. While both PVA and HSA are biocompatible, PVA is not biodegradable [154]. The use of these stabilizers controls the size of the particles synthesized, reduces the polydispersity of the synthesized particle size, and enhances drug encapsulation efficiency. However, the inclusion of these surfactants can alter the surface properties of the nano-carrier core which can be important in determining therapeutic efficacy [155].

Surface modification of nano-based delivery devices can facilitate active cell targeting. Decoration moieties can be attached directly to the functional groups of the particle surface or to the end of a space to extend the active targeting moiety beyond a PEG corona. Endogenous ligands, peptides derived from endogenous ligands, and antibodies against specifically expressed receptors have all been used as active targeting ligands. In addition, the specificity of active targeting strategies may limit side effects of non-target cells. In cancer applications, ligands or antibodies can be used to target nano-carriers to tumor cells, avoiding the effect of chemotherapeutics on healthy tissues [156].

Two issues related to active targeting of nano-based delivery devices are the blocking of targeting moieties by proteins and the need for the nano-based device to be in close proximity to the intended target. The specificity of active targeting on nano-based delivery devices can be blocked by the adsorption of a protein corona on their surface [157]. This leads to the need for balanced particle shielding, which can be achieved with a PEG corona associated with the quantity of active targeting ligands. Despite their specificity, nano-carriers must be near their intended targets to facilitate receptor-ligand recognition [156]. Due to this issue, many nano-carriers take advantage of both passive (EPR effect) and active targeting strategies to effectively deliver cancer therapeutics (Figure 2).

5. Case studies

Despite the challenge of delivering therapeutics across complex biological barriers, recent research has successfully developed several promising nano-carrier platforms that do exactly that (Table 2). In this section, we present a few success stories of how nano-enabled delivery systems have been used to carry diverse payloads across the BBB, the tumor, and insect barriers to enhance therapeutic efficacy.

5.1. *Nanoscale carriers for BBB penetration*

Nano-based carriers have been developed to encapsulate diverse payloads, cross the BBB and improve CNS delivery. Various strategies including active targeting, cellular transport, and nasal delivery have been utilized to improve the efficacy of nano-based carrier delivery across the BBB [158]. A brief description of some of these promising strategies follow and are summarized in Table 2.

The active targeting of a surface expressed receptor on the circulation side of the BCECs and inducing receptor-mediated transcytosis is a viable approach in crossing the BBB. Two limitations to receptor-mediated endothelial transcytosis are the quantity of receptors on the BCEC (limiting the amount of transport) and the expression of receptors on cells other than BCECs; the latter severely limits the specificity of brain delivery and particle-receptor mediated transcytosis [159]. Glutathione-targeted PEGylated liposomes were observed to enhance BBB transport of **a peptide cargo**, an anti-amyloid antibody fragment [160], and lactoferrin-targeted PEG-PLA nanoparticles were also shown to enhance BBB delivery **of a peptide cargo**, urocortin [24]. Recently, **the small molecule** dopamine loaded into poly(lactic-co-glycolic acid) (PLGA) nanoformulations were shown to improve functional deficits in animal models of Parkinson's disease [161]. Surface modification of chitosan nanoparticles with anti-transferrin receptor-1 antibodies enabled these particles to cross the BBB and deliver basic fibroblast growth factor, **a protein**, or the caspase-3 inhibitor z-DEVD-FMK, **a peptide**, in the brain parenchyma significantly decreasing the infarct volume in a mouse model of stroke [162]. This combined approach using chitosan nanoparticles and antibodies against key transport proteins could be extended to insulin receptor, folate receptor, and low density lipoprotein receptor.

Another strategy to traverse the BBB involves the use of innate immune cells. For example, mononuclear phagocytes (MP; monocyte, macrophages and dendritic cells) can be used to carry drug and/or protein-based payload(s) across the BBB. MP-mediated transcytosis was first demonstrated with serotonin-carrying liposomes [163]. Experiments performed with **the protein** catalase-loaded into polymeric nanoparticles also showed enhanced brain delivery when the nano-carriers were pre-loaded into MPs [164].

The nasal pathway has also been investigated as an alternate route for drug delivery to the cerebrospinal fluid and has received considerable attention recently [165-169]. Drugs delivered to the olfactory region of the nose could lead to direct delivery to the CNS if it can transport across both the nasal epithelium and the arachnoid membrane that houses the olfactory nerves [170]. Multiple studies have shown enhanced nose-to-brain transport of nanoparticles when compared to intravenous administration. When chitosan nanoparticles were loaded with β -cyclodextran complexed to estradiol and administered both intranasally and intravenously to rats, significantly higher amounts of drug were measured in the CSF of the animals that were administered the treatment intranasally [171]. A comparative evaluation of intranasal vs. intravenous administration of nanoformulated cyclosporine showed superior pharmacokinetic profile and CNS bioavailability for the intranasal route [172]. Notably, nasal delivery of nanoformulated cyclosporine limited off-target organ exposure. In addition, chitosan and methyl- β -cyclodextran particles have also been shown to enable enhanced nose-to-brain transport of deferoxamine mesylate, **a small molecular weight payload** [173]. While the nose to brain route is an attractive option for delivering drugs to the CNS, the nasal mucous membrane and constant clearance of foreign material by the nasal cilia limit the bioavailability of the nanoparticles. These aspects must be factored in when designing a drug therapy using nanoparticles as a delivery platform through intranasal route.

Finally, research has focused into disrupting the BBB to enhance therapeutic transport [174]. A combination of MRI-guided focused ultrasound to disrupt the BBB, fluorescently-labeled PEG-PLGA nanoparticles, and albumin microbubbles can bring nanoparticles to the brain [175]. Liposomal doxorubicin transport across the BBB was also observed to significantly increase with focused ultrasound BBB disruption [176].

5.2. *Nanoscale carriers for cancer therapy*

Nano-based cancer therapy vehicles include active targeting of the tumor and address the difficulties of overcoming the tumor microenvironment. The biodistribution of therapeutics from the administration site to the tumor is a barrier to effective treatment in some forms of cancer. This section discusses the design of multifunctional nano-carriers with target specificity that overcomes the solid tumor microenvironment.

Glutathione-responsive release from chitosan-based nano-carriers has demonstrated promising results against cancer. While biodistribution studies showed that nanoparticles localized in the liver, spleen, and tumor sites, the improved redox sensitivity of the nano-carrier resulted in preferential release of cargo in the tumor environment [177]. A folate-targeted and pH-triggered micelle was designed to improve the delivery of the small molecular weight drug doxorubicin to the tumor microenvironment. The micelles were composed of a PEG hydrophilic block and a polypeptide (Asp-Hyd) hydrophobic block. *In vitro* results showed greater growth inhibition of KB cells with the folate-targeted pH sensitive micelles when compared to the non-targeted pH sensitive micelle [178]. This was attributed to the greater degree of cellular internalization of the targeted micelle. When KB tumor bearing mice were administered free drug and micelle-formulated drug (with and without folate modification) intravenously it was observed that the pH-triggered micelles were less toxic than the free drug, but required a higher effective dose for tumor suppression. However, it was observed that the folate-targeted pH-triggered micelles were both less toxic and had a lower effective dose than the free drug, supporting the selectivity of the targeting together with the specificity of the drug release [179].

Photo-triggered tumor vascular treatment in tumor-bearing mice enhanced the efficacy of subsequently injected PEG liposomal paclitaxel, a small molecular weight payload. The enhanced efficacy of the PEG liposomal paclitaxel was only observed in mice bearing tumors with low permeability in their vasculature [180]. Antinucleolin aptamer targeted liposomes containing doxorubicin and ammonium bicarbonate were designed to treat doxorubicin-resistant breast cancer cells that overexpress nucleolin receptors. Tumors in animals treated with the targeted liposomes had significantly higher doxorubicin signal, which increased when external heat was applied. However, only when external heat was applied at the tumor site was tumor growth suppressed [181].

Nano-based devices can also be used to mount anti-tumor responses and activate cytotoxic T cells. These devices can be used to deliver both tumor-associated antigens, peptides, and siRNA, a nucleic acid payload, to overcome the immunosuppressive nature of tumors and kill the tumor. In a recent study a model tumor antigen, ovalbumin (Ova), was encapsulated into polyanhydride microparticles. Mice were primed and boosted via intraperitoneal injections of Ova-loaded nanoparticles and challenged with a subcutaneous injection of Ova-expressing tumor cells. Animals administered the nanoparticle formulation displayed enhanced survival upon challenge compared to animals that received soluble antigen adjuvanted with a TLR agonist [182].

5.3. *Nanoparticle-based approaches for vector and parasite control*

New approaches in drug delivery have improved the treatment of nematode parasites; however, further research is necessary to achieve drug delivery through the insect cuticle. Nanoparticles provide a unique opportunity to deliver insecticidal molecules directly through cuticle pores into the open circulatory system of the insect. For example, mosquitoes topically exposed to deltamethrin-functionalized silver nanoparticles showed 95% mortality within 12 h of

exposure. Furthermore, the content of silver within the hemolymph was enhanced by 600% in nanoparticle-administered mosquitoes [183].

PEG hydrogels have been explored as potential delivery tools as well; this chemistry distributes to a number of tissues and organs in *Anopheles gambiae*, the African malaria mosquito, in larval and adult stages [147, 149]. Topical application of fluorescent PEG hydrogel nanoparticles to the head of adult mosquitoes showed penetrance to head tissue, proboscis, and alimentary tract following one day post-exposure. In addition, surface charge dependency was observed where 100% of mosquitoes exposed to negatively charged particles exhibited fluorescence in contrast to only 40% mosquitoes exposed to positively charged particles. Moreover, the intensity of fluorescence in the mosquitoes exposed to negatively charged particles was much higher than those treated with positively charged particles [149]. Interestingly, when the same PEG nanoparticles were topically administered to the thorax or the abdomen, very low penetrance in the tissue was observed.

Nanoparticles are suitable carriers for RNA and could be a useful addition to the arsenal of chemistries currently used for vector control (Figure 3). The delivery of nucleic acids is limited due to their ready degradation and the need for specific delivery to cell compartments. Polymeric nanoparticles, specifically, increase the stability and uptake of siRNA as a trigger for RNAi. For example, Zhang et al. encapsulated dsRNA against chitin synthase genes into nanoparticles and fed them to mosquito larvae to observe a 34% reduction in chitin production [184]. Because chitin is important for maintenance of the cuticular exoskeleton, the cuticle was found to be increasingly permeable and susceptible to pesticides [184]. Future research may also improve the ability of nanoparticles to directly cross the cuticle via topical application.

Studies have also recently shown that nanoparticles have the potential to significantly advance prevention and treatment of lymphatic filariasis. Ivermectin encapsulated in chitosan-alginate nanoparticles reduced the recovery of *B. malayi* adult worms post-treatment [185]. In addition, PLGA nanoparticles have been used to encapsulate ivermectin and demonstrated elimination of filarial worm parasites from circulation with dose sparing effects [186]. Recent

Figure 3: Nano-carriers for the control of vector-borne diseases. Mosquito vectors carry and transmit a wide variety of diseases such as malaria and lymphatic filariasis. However, the ability to penetrate the cuticle, a unique exoskeleton composed of lipids, proteins, and layered chitin sheets, poses a major challenge to the delivery of insecticides for vector control. Nano-carriers represent a promising technology for crossing the cuticle and their properties, such as size, shape, and charge can be tailored to

enable optimal breaching of the cuticle and delivery of cargo. Once through the cuticle, the nano-carriers can distribute to target tissues where they provide sustained release of encapsulated payloads, ultimately leading to the destruction of the cuticle and/or mosquito vector.

studies from our laboratories have demonstrated success in using polyanhydride nanoparticles encapsulating anti-filarial drugs and antibiotics to kill parasites with several thousand fold dose-sparing [187]. Imaging of filarial worms incubated *in vitro* with drug-loaded polyanhydride nanoparticles indicated that these nanoparticles penetrated the worm cuticle and persist, in contrast to soluble drugs [187]. It has been speculated that encapsulation of drugs into particles may reduce the ability of filarial worms to regulate mechanisms (i.e., changes in glutamate-gated chloride channels or ABC transporter expression) that enable recovery of the worm via the expulsion of soluble drugs [11, 55, 56]. The polyanhydride nanoparticle-based delivery system successfully penetrated the cuticle and provided sustained release of anti-filarial drugs and antibiotics in nematode parasites, resulting in enhanced and rapid killing of these worms with a significantly reduced dose [187].

6. Perspectives and future outlook

Despite the challenges posed by complex physiological barriers, nanotechnology has paved the way towards the design of drug delivery systems with diverse cargos that can enhance therapeutic efficacy. However, new challenges have arisen and future research will need to rationally design nano-carriers for enhanced delivery of diverse therapeutic molecules. For example, nano-carriers that successfully cross the BBB have several more obstacles to circumvent. Once across the BBB, nano-carriers must localize to specific pathological sites within the brain without inducing inflammation. In addition, nano-carriers with payloads targeted to intracellular organelles (e.g., mitochondria) need to be efficiently taken up by non-phagocytic cells, such as neurons. Such new challenges provide major opportunities in terms of integrating materials science, surface functionalization, cell biology, and nanotechnology.

While many nano-carriers for cancer therapy have successfully employed active targeting strategies, other factors within the tumor microenvironment should be taken into consideration. For example, the lack of lymphatic vessels can often lead to increased interstitial fluid pressure, which may reduce the ability of nano-carriers to localize to tumor tissues. In addition, the extracellular tumor environment is known to be acidic [2], and thus may affect the stability of nano-carrier systems and the sustained release of active therapeutic molecules. Finally, cancer nano-therapeutics should also consider tumor metastasis to treat cancerous cells in various anatomical locations as well as those cells not associated with tumor environments. Once again, these new challenges require highly cross-disciplinary approaches.

The development of nano-carrier systems will need to be designed with an eye towards field applications. As an example, delivery platforms developed for disease vector control must persist in a liquid substrate in tropical environments (i.e., high temperature, intense UV exposure), which may affect their stability. In addition, therapeutic resistance is a growing challenge for many diseases, including vector-borne diseases. For example, the therapeutics available for treatment of lymphatic filariasis is limited, and repeated exposure to therapeutics without complete clearance of infection may eventually lead to parasitic resistance. Thus, nano-carrier approaches must enhance efficacy to ensure complete clearance of infection, and perhaps, prevent reoccurrence. In this context, a “systems nanomedicine” approach is needed, wherein issues such as field applicability, cost, and transportation logistics need to be considered much more upstream than has been done before and incorporated into the earliest stages of conceptualization of the nanomedicine product(s).

Finally, scale-up and cGMP production of nanoscale devices is a major hurdle that needs to be overcome to enable regulatory approval. Researchers developing nano-carriers that can

cross complex barriers need to partner with practitioners and evaluate the feasibility of producing large doses of lead candidate nanomedicines under pharmaceutically relevant conditions. These partnerships are critical to translate the novel advances made in the laboratory to the clinic. In this context, a systems nanomedicine approach will enable creating more robust nanomedicine products rapidly, at lesser cost, and that are readily field-deployable. The importance of close interactions with the regulatory agencies at all stages of this process cannot be overstated.

In summary, advances in nanomedicine are poised to overcome the formidable challenges associated with delivering diverse cargos across complex biological barriers and have set the stage for the design of a new generation of nanomedicine products. The translation of these exciting products to the clinic will transform how we treat disease.

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References

- [1] O.C. Farokhzad, R. Langer, Impact of nanotechnology on drug delivery, *ACS Nano*. 3 (2009) 16-20.
- [2] K. Cho, X. Wang, S. Nie, Z. Chen, D.M. Shin, Therapeutic nanoparticles for drug delivery in cancer, *Clin. Cancer Res.* 14 (2008) 1310-1316.
- [3] R. Singh, J.W. Lillard, Jr., Nanoparticle-based targeted drug delivery, *Exp. Mol. Pathol.* 86 (2009) 215-223.
- [4] J.S. Patton, J.D. Brain, L.A. Davies, J. Fiegel, M. Gumbleton, K.-J. Kim, et al., The Particle has Landed - Characterizing the Fate of Inhaled Pharmaceuticals, *J. Aero. Med. Pul. Drug Del.* 23 (2010) S-71 - S-87.
- [5] H. Hurst, Permeability of insect cuticle, *Nature*. 145 (1940) 462-463.
- [6] T.R. Thrimawithana, S. Young, C.R. Bunt, C. Green, R.G. Alany, Drug delivery to the posterior segment of the eye, *Drug Discov. Today*. 16 (2011) 270-277.
- [7] J.L. del Pozo, R. Patel, The challenge of treating biofilm-associated bacterial infections, *Clin. Pharmacol. Ther.* 82 (2007) 204-209.
- [8] C.D. Walkey, J.B. Olsen, H. Guo, A. Emili, W.C.W. Chan, Nanoparticle size and surface chemistry determine serum protein adsorption and macrophage uptake, *J. Am. Chem. Soc.* 134 (2012) 2139-2147.

- [9] M.J. Haney, N.L. Klyachko, Y. Zhao, R. Gupta, E.G. Plotnikova, Z. He, et al., Exosomes as drug delivery vehicles for Parkinson's disease therapy, *J. Control. Release.* 207 (2015) 18-30.
- [10] W.M. Pardridge, Drug transport across the blood-brain barrier, *J. Cereb. Blood Flow Metab.* 32 (2012) 1959-1972.
- [11] S.T. Laing, A. Ivens, V. Butler, S.P. Ravikumar, R. Laing, D.J. Woods, et al., The Transcriptional Response of *Caenorhabditis elegans* to Ivermectin Exposure Identifies Novel Genes Involved in the Response to Reduced Food Intake, *PLoS One.* 7 (2012).
- [12] R.K. Jain, Determinants of tumor blood flow: a review, *Cancer Res.* 48 (1998) 2641-2658.
- [13] C.H. Heldin, K. Rubin, K. Pietras, A. Ostman, High interstitial fluid pressure - an obstacle in cancer therapy, *Nat. Rev. Cancer.* 4 (2004) 806-813.
- [14] Neurological Disorders: Public Health Challenges, World Health Organization, Switzerland, 2006.
- [15] D.J. Begley, Delivery of therapeutic agents to the central nervous system: the problems and the possibilities, *Pharmacol. Ther.* 104 (2004) 29-45.
- [16] W.M. Pardridge, Blood-brain barrier delivery, *Drug Discovery Today.* 12 (2007) 54-61.
- [17] I. Tamai, A. Tsuji, Transporter-mediated permeation of drugs across the blood-brain barrier, *J. Pharm. Sci.* 89 (2000) 1371-1388.
- [18] H.R. Kim, K. Andrieux, S. Gil, M. Taverna, H. Chacun, D. Desmaele, et al., Translocation of poly(ethylene glycol-co-hexadecyl)cyanoacrylate nanoparticles into rat brain endothelial cells: role of apolipoproteins in receptor-mediated endocytosis, *Biomacromolecules.* 8 (2007) 793-799.
- [19] J. Kreuter, D. Shamenkov, V. Petrov, P. Ramge, K. Cychutek, C. Koch-Brandt, et al., Apolipoprotein-mediated transport of nanoparticle-bound drugs across the blood-brain barrier, *J. Drug Target.* 10 (2002) 317-325.
- [20] K. Michaelis, M.M. Hoffmann, S. Dreis, E. Herbert, R.N. Alyautdin, M. Michaelis, et al., Covalent linkage of apolipoprotein E to albumin nanoparticles strongly enhances drug transport into the brain, *J. Pharmacol. Exp. Ther.* 317 (2006) 1246-1253.
- [21] K. Ulbrich, T. Hekmatara, E. Herbert, J. Kreuter, Transferrin- and transferrin-receptor-antibody-modified nanoparticles enable drug delivery across the blood-brain barrier (BBB), *Eur. J. Pharm. Biopharm.* 71 (2009) 251-256.
- [22] Y. Aktas, M. Yemisci, K. Andrieux, R.N. Gursoy, M.J. Alonso, E. Fernandez-Megia, et al., Development and brain delivery of chitosan-PEG nanoparticles functionalized with the monoclonal antibody OX26, *Bioconjugate Chem.* 16 (2005) 1503-1511.
- [23] S. Skarlatos, T. Yoshikawa, W.M. Pardridge, Transport of [¹²⁵I]transferrin through the rat blood-brain barrier, *Brain Res.* 683 (1995) 164-171.
- [24] K. Hu, Y. Shi, W. Jiang, J. Han, S. Huang, X. Jiang, Lactoferrin conjugated PEG-PLGA nanoparticles for brain delivery: Preparation, characterization and efficacy in Parkinson's disease, *Int. J. Pharm.* 415 (2011) 273-283.
- [25] G. Tosi, L. Badiali, B. Ruozzi, A.V. Vergoni, L. Bondioli, A. Ferrari, et al., Can leptin-derived sequence-modified nanoparticles be suitable tools for brain delivery?, *Nanomedicine.* 7 (2012) 365-382.
- [26] W. Pan, A.J. Kastin, Entry of EGF into brain is rapid and saturable, *Peptides.* 20 (1999) 1091-1098.

- [27] P. Gaillard, J., C.C. Visser, A.G. de Boer, Targeted delivery across the blood-brain barrier, *Expert Opin. Drug Deliv.* 2 (2005) 299-309.
- [28] K. Ulbrich, T. Knobloch, J. Kreuter, Targeting the insulin receptor: nanoparticles for drug delivery across the blood-brain barrier (BBB), *J. Drug Target.* 19 (2011) 125-132.
- [29] W.A. Banks, J.B. Owen, M.A. Erickson, Insulin in the brain: there and back again, *Pharmacol. Ther.* 136 (2012) 82-93.
- [30] R.G. Thorne, C. Nicholson, In vivo diffusion analysis with quantum dots and dextrans predicts the width of brain extracellular space, *Proc. Natl. Acad. Sci. U. S. A.* 103 (2006) 5567-5572.
- [31] E.A. Nance, G.F. Woodworth, K.A. Sailor, T.-Y. Shih, Q. Xu, G. Swaminathan, et al., A dense poly(ethylene glycol) coating improves penetration of large polymeric nanoparticles within brain tissue, *Sci Transl Med.* 4 (2012) 149RA119.
- [32] B.W. Stewart, C.P. Wild, *World Cancer Report 2014*, 2014.
- [33] I.A. Khawar, J.H. Kim, H.-J. Kuh, Improving drug delivery to solid tumors: priming the tumor microenvironment, *J. Control. Release.* 201 (2015) 78-89.
- [34] M. Tsujii, R.N. DuBois, Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2, *Cell.* 83 (1995) 493-501.
- [35] P. Andersson, J. McGuire, C. Rubio, K. Gradin, Whitelaw, M. L., S. Pettersson, A. Hanberg, et al., A constitutively active dioxin/aryl hydrocarbon receptor induces stomach tumors, *Proc. Natl. Acad. Sci. U. S. A.* 99 (2002) 9990-9995.
- [36] K. Bekki, H. Vogel, W. Li, T. Ito, C. Sweeney, T. Haarmann-Stemmann, et al., The aryl hydrocarbon receptor (AhR) mediates resistance to apoptosis induced in breast cancer cells, *Pestic. Biochem. Physiol.* 120 (2015) 5-13.
- [37] D.J. Slamon, G.M. Clark, S.G. Wong, W.J. Levin, A. Ullrich, W.L. McGuire, Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene, *Science.* 235 (1987) 177-182.
- [38] Y.S. Khaled, B.J. Ammori, E. Elkord, Myeloid-derived suppressor cells in cancer: recent progress and prospects, *Immunol. Cell Bio.* 91 (2013) 493-502.
- [39] A. Kano, Tumor cell secretion of soluble factor(s) for specific immunosuppression, *Sci. Rep.* 5 (2015) 1-8.
- [40] R. Goforth, A.K. Salem, X.Y. Zhu, S. Miles, X.Q. Zhang, J.H. Lee, et al., Immune stimulatory antigen loaded particles combined with depletion of regulatory T-cells induce potent tumor specific immunity in a mouse model of melanoma, *Cancer Immunol. Immunother.* 58 (2009) 517-530.
- [41] S.M. Geary, C.D. Lemke, D.M. Lubaroff, A.K. Salem, The Combination of a Low-Dose Chemotherapeutic Agent, 5-Fluorouracil, and an Adenoviral Tumor Vaccine Has a Synergistic Benefit on Survival in a Tumor Model System, *PLoS One.* 8 (2013).
- [42] R.K. Jain, T. Stylianopoulos, Delivering nanomedicine to solid tumors, *Nat Rev Clin Oncol.* 7 (2010) 653-664.
- [43] H. Ohtani, Stromal reaction in cancer tissue: pathophysiologic significance of the expression of matrix-degrading enzymes in relation to matrix turnover and immune/inflammatory reactions, *Pathol. Int.* 48 (1998) 1-9.
- [44] O. Tredan, C.M. Galmarini, K. Patel, I.F. Tannock, Drug resistance and the solid tumor microenvironment, *J. Natl. Cancer Inst.* 99 (2007) 1441-1454.
- [45] World Health Organization, Health topics: malaria. 2015. Available at <http://www.who.int/topics/malaria/en/>. (Accessed April 2015).

- [46] Centers for Disease Control and Prevention, Parasites - lymphatic filariasis. 2013. Available at <http://www.cdc.gov/parasites/lymphaticfilariasis/>. (Accessed April 2015).
- [47] World Health Organization, Health topics: filariasis. 2015. Available at <http://www.who.int/topics/filariasis/en/>. (Accessed April 2015).
- [48] J. Hemingway, B.J. Beaty, M. Rowland, T.W. Scott, B.L. Sharp, The Innovative Vector Control Consortium: improved control of mosquito-borne diseases, *Trends Parasitol.* 22 (2006) 308-312.
- [49] J.E. Webb, R.A. Green, On the penetration of insecticides through the insect cuticle, *J. Exp. Biol.* 22 (1945) 8-20.
- [50] M. Rockstein, *The physiology of insecta*, 2nd ed, Elsevier Science, 1973.
- [51] V.B. Wigglesworth, The insect cuticle, *Biol. Rev.* 23 (1948) 408-451.
- [52] R.F. Chapman, *The insects: structure and function*, 5th ed, Cambridge University Press, 2012.
- [53] A.P. Page, G. Stepek, A.D. Winter, D. Pertab, Enzymology of the nematode cuticle: A potential drug target?, *Int. J. Parasitol. Drugs Drug Resist.* 4 (2014) 133-141.
- [54] WormBook: The online review of *C. elegans* biology, WormBook Research Community, Pasadena, CA, 2005.
- [55] C.E. James, M.W. Davey, Increased expression of ABC transport proteins is associated with ivermectin resistance in the model nematode *Caenorhabditis elegans*, *Int. J. Parasitol.* 39 (2009) 213-220.
- [56] R.K. Prichard, Ivermectin resistance and overview of the Consortium for Anthelmintic Resistance SNPs, *Expert Opin. Drug Discov.* 2 (2007) S41-S52.
- [57] S. Fahn, L.R. Libsch, R.W. Cutler, Monoamines in the human neostriatum: topographic distribution in normals and in Parkinson's disease and their role in akinesia, rigidity, chorea, and tremor, *J. Neurol. Sci.* 14 (1971) 427-455.
- [58] L. Li, B. Fridley, K. Kalari, G. Jenkins, A. Batzler, S. Safgren, et al., Gemcitabine and cytosine arabinoside cytotoxicity: association with lymphoblastoid cell expression, *Cancer Res.* 68 (2008) 7050-7058.
- [59] E.K. Rowinsky, E.A. Eisenhauer, V. Chaudhry, S.G. Arbuck, R.C. Donehower, Clinical toxicities encountered with paclitaxel (Taxol), *Semin. Oncol.* 20 (1993) 1-15.
- [60] M.R. Bristow, P.D. Thompson, R.P. Martin, J.W. Mason, M.E. Billingham, D.C. Harrison, Early anthracycline cardiotoxicity, *Am. J. Med.* 65 (1978) 823-832.
- [61] E.A. Campbell, N. Korzheva, A. Mustaev, K. Murakami, S. Nair, A. Goldfarb, et al., Structural mechanism for rifampicin inhibition of bacterial RNA polymerase, *Cell.* 104 (2001) 901-912.
- [62] M.J. Taylor, A. Hoerauf, M. Bockarie, Lymphatic filariasis and onchocerciasis, *Lancet.* 376 (2010) 1175-1185.
- [63] S.K. Tiwari, R.K. Chaturvedi, Peptide therapeutics in neurodegenerative disorders, *Curr. Med. Chem.* 21 (2014) 2610-2631.
- [64] V.P. Torchilin, A.N. Lukyanov, Peptide and protein drug delivery to and into tumors: challenges and solutions, *Drug Discov. Today.* 8 (2003) 259-266.
- [65] R. Juliano, Challenges to macromolecular drug delivery, *Biochem. Soc. Trans.* 35 (2007) 41-43.
- [66] X. Yi, D.S. Manickam, A. Brynskikh, A.V. Kabanov, Agile delivery of protein therapeutics to CNS, *J. Control. Release.* 190 (2014) 637-663.

- [67] W.A. Banks, S.A. Farr, J.E. Morley, The effects of high fat diets on the blood-brain barrier transport of leptin: failure or adaptation?, *Physiol. Behav.* 88 (2006) 244-248.
- [68] R. Lax, C. Meenan, Challenges for therapeutic peptides part 1: on the inside, looking out, *Innovations in Pharmaceutical Technology.* 42 (2012) 54-56.
- [69] R. Lax, C. Meenan, Challenges for therapeutic peptides part 2: delivery systems, *Innovations in Pharmaceutical Technology.* 43 (2012) 42-45.
- [70] K. Remaut, N.N. Sanders, B.G. De Geest, K. Braeckmans, J. Demeester, S.C. De Smedt, Nucleic acid delivery: Where material sciences and bio-sciences meet, *Mater. Sci. Eng. R-Rep.* 58 (2007) 117-161.
- [71] K.A. Whitehead, R. Langer, D.G. Anderson, Knocking down barriers: advances in siRNA delivery, *Nat. Rev. Drug Discov.* 9 (2010) 412-412.
- [72] J.G. Scott, K. Michel, L.C. Bartholomay, B.D. Siegfried, W.B. Hunter, G. Smagghe, et al., Towards the elements of successful insect RNAi, *J. Insect Physiol.* 59 (2013) 1212-1221.
- [73] H. Ragelle, G. Vandermeulen, V. Preat, Chitosan-based siRNA delivery systems, *J. Control. Release.* 172 (2013) 207-218.
- [74] J.S. Garbutt, X. Belles, E.H. Richards, S.E. Reynolds, Persistence of double-stranded RNA in insect hemolymph as a potential determiner of RNA interference success: evidence from *Manduca sexta* and *Blattella germanica*, *J. Insect Physiol.* 59 (2013) 171-178.
- [75] A.N. Lukyanov, V.P. Torchilin, Micelles from lipid derivatives of water-soluble polymers as delivery systems for poorly soluble drugs, *Adv. Drug Deliv. Rev.* 56 (2004) 1273-1289.
- [76] L. Zhang, J.M. Chan, F.X. Gu, J.-W. Rhee, A.Z. Wang, A.F. Radovic-Moreno, et al., Self-assembled lipid-polymer hybrid nanoparticles: A robust drug delivery platform, *ACS Nano.* 2 (2008) 1696-1702.
- [77] V.P. Torchilin, Targeted polymeric micelles for delivery of poorly soluble drugs, *Cell. Mol. Life Sci.* 61 (2004) 2549-2559.
- [78] H.-Q. Mao, K. Roy, V.L. Troung-Le, K.A. Janes, K.Y. Lin, Y. Wang, et al., Chitosan-DNA nanoparticles as gene carriers: synthesis, characterization and transfection efficiency, *J. Control. Release.* 70 (2001) 399-421.
- [79] S. Gao, F. Dagnaes-Hansen, E.J.B. Nielsen, J. Wengel, F. Besenbacher, K.A. Howard, et al., The Effect of Chemical Modification and Nanoparticle Formulation on Stability and Biodistribution of siRNA in Mice, *Mol. Ther.* 17 (2009) 1225-1233.
- [80] N.F. Steinmetz, Viral nanoparticles as platforms for next-generation therapeutics and imaging devices, *Nanomedicine.* 6 (2010) 634-641.
- [81] F. Kratz, A. Warnecke, Finding the optimal balance: challenges of improving conventional cancer chemotherapy using suitable combinations with nano-sized drug delivery systems, *J. Control. Release.* 164 (2012) 221-235.
- [82] A.E. Hansen, A.L. Petersen, J.R. Henriksen, B. Boerresen, P. Rasmussen, D.R. Elema, et al., Positron Emission Tomography Based Elucidation of the Enhanced Permeability and Retention Effect in Dogs with Cancer Using Copper-64 Liposomes, *ACS Nano.* 9 (2015) 6985-6995.
- [83] S. Acharya, S.K. Sahoo, PLGA nanoparticles containing various anticancer agents and tumour delivery by EPR effect, *Adv. Drug Deliv. Rev.* 63 (2011) 170-183.

- [84] A. Hoerauf, K. Pfarr, S. Mand, A.Y. Debrah, S. Specht, Filariasis in Africa-treatment challenges and prospects, *Clin. Microbiol. Infect.* 17 (2011) 977-985.
- [85] A. Kumari, S.K. Yadav, S.C. Yadav, Biodegradable polymeric nanoparticles based drug delivery systems, *Colloids Surf. B.* 75 (2010) 1-18.
- [86] S. Doppalapudi, A. Jain, W. Khan, A. Domb, Biodegradable polymers - an overview, *Polym. Adv. Technol.* 25 (2014) 427-435.
- [87] B. Felice, M. Prabhakaran, A. Rodriguez, S. Ramakrishna, Drug delivery vehicles on a nano-engineering perspective, *Mater. Sci. Eng. C: Mater. Biol. Appl.* 41 (2014) 178-195.
- [88] B. Guo, L. Glavas, A. Albertsson, Biodegradable and electrically conducting polymers for biomedical applications, *Prog. Polymer Sci.* 38 (2013) 1263-1286.
- [89] J. Nicolas, S. Mura, D. Brambilla, N. Mackiewicz, P. Couvreur, Design, functionalization strategies and biomedical applications of targeted biodegradable/biocompatible polymer-based nanocarriers for drug delivery, *Chem. Soc. Rev.* 42 (2013) 1147-1235.
- [90] J. Panyam, V. Labhasetwar, Biodegradable nanoparticles for drug and gene delivery to cells and tissues, *Adv. Drug Del. Rev.* 64 (2012) 61-71.
- [91] S. Tammam, H. Azzay, A. Lamprecht, Biodegradable particulate carrier formulation and tuning for targeted drug delivery, *J. Biomed. Nanotechnol.* 11 (2015) 555-577.
- [92] J. Kreuter, R.N. Alyautdin, D.A. Kharkevich, A.A. Ivanov, Passage of peptides through the blood-brain barrier with colloidal polymer particles (nanoparticles), *Brain Res.* 674 (1995) 171-174.
- [93] L. Grislain, P. Couvreur, V. Lenaerts, M. Roland, D. Deprez-Decampeneere, P. Speiser, Pharmacokinetics and distribution of a biodegradable drug carrier, *Int. J. Pharm.* 15 (1983) 335-345.
- [94] J. Panyam, V. Labhasetwar, Biodegradable nanoparticles for drug and gene delivery to cells and tissue, *Adv. Drug Deliv. Rev.* 64 (2012) 61-71.
- [95] P.A. Gunatillake, R. Adhikari, Biodegradable synthetic polymers for tissue engineering, *Eur Cell Mater.* 5 (2003) 1-16; discussion 16.
- [96] J.A. Tamada, R. Langer, Erosion kinetics of hydrolytically degradable polymers, *Proc. Natl. Acad. Sci. U. S. A.* 90 (1993) 552-556.
- [97] F. von Burkersroda, L. Schedl, A. Gopferich, Why degradable polymers undergo surface erosion or bulk erosion, *Biomaterials.* 23 (2002) 4221-4231.
- [98] A. Budhian, S.J. Siegel, K.I. Winey, Controlling the in vitro release profiles for a system of haloperidol-loaded PLGA nanoparticles, *Int. J. Pharm.* 346 (2008) 151-159.
- [99] T.S. Kashi, S. Eskandarion, M. Esfandyari-Manesh, S.M. Marashi, N. Samadi, S.M. Fatemi, et al., Improved drug loading and antibacterial activity of minocycline-loaded PLGA nanoparticles prepared by solid/oil/water ion pairing method, *Int. J. Nanomed.* 7 (2012) 221-234.
- [100] T. Musumeci, C.A. Ventura, I. Giannone, B. Ruozi, L. Montenegro, R. Pignatello, et al., PLA/PLGA nanoparticles for sustained release of docetaxel, *Int. J. Pharm.* 325 (2006) 172-179.
- [101] G. Tosi, L. Costantino, F. Rivasi, B. Ruozi, E. Leo, A.V. Vergoni, et al., Targeting the central nervous system: in vivo experiments with peptide-derivatized nanoparticles loaded with Loperamide and Rhodamine-123, *J. Control. Release.* 122 (2007) 1-9.
- [102] S. Wohlfart, A.S. Khalansky, S. Gelperina, O. Maksimenko, C. Bernreuther, M. Glatzel, et al., Efficient chemotherapy of rat glioblastoma using doxorubicin-loaded PLGA nanoparticles with different stabilizers, *PLoS One.* 6 (2011) e19121.

- [103] Y. Wang, P. Challa, D.L. Epstein, F. Yuan, Controlled release of ethacrynic acid from poly(lactide-co-glycolide) films for glaucoma treatment, *Biomaterials*. 25 (2004) 4279-4285.
- [104] T. Sadat, J. Kashi, S. Eskandarion, M. Esfandyari-Manesh, S. Mahmoud, A. Marashi, et al., Improved drug loading and antibacterial activity on minocycline-loaded PLGA nanoparticles prepared by solid/oil/water ion pairing method, *Int. J. Nanomed.* 7 (2012) 221-234.
- [105] K. Fu, D.W. Pack, A.M. Klibanov, R. Langer, Visual evidence of acidic environment within degrading poly(lactic-co-glycolic acid) (PLGA) microspheres, *Pharm. Res.* 17 (2000) 100-106.
- [106] H.B. Rosen, J. Chang, G.E. Wnek, R.J. Linhardt, R. Langer, Bioerodible polyanhydrides for controlled drug delivery, *Biomaterials*. 4 (1983) 131-133.
- [107] M. Westphal, Z. Ram, V. Riddle, D. Hilt, E. Bortey, Gliadel (R) wafer in initial surgery for malignant glioma: long-term follow-up of a multicenter controlled trial, *Acta Neurochir.* 148 (2006) 269-275.
- [108] A.J. Domb, M. Rock, J. Schwartz, C. Perkin, G. Yipchuk, B. Broxup, et al., Metabolic disposition and elimination studies of a radiolabelled biodegradable polymeric implant in the rat brain, *Biomaterials*. 15 (1994) 681-688.
- [109] Y. Tabata, R. Langer, Polyanhydride microspheres that display near-constant release of water-soluble model drug compounds, *Pharm. Res.* 10 (1993) 391-399.
- [110] J.P. Jain, S. Modi, N. Kumar, Hydroxy fatty acid based polyanhydride as drug delivery system: synthesis, characterization, *in vitro* degradation, drug release, and biocompatibility, *J Biomed. Mater. Res. A*. 84 (2008) 740-752.
- [111] L. Shieh, J. Tamada, I. Chen, J. Pang, A. Domb, R. Langer, Erosion of a new family of biodegradable polyanhydrides, *J. Biomed. Mater. Res.* 28 (1994) 1465-1475.
- [112] B.A. Pfeifer, J.A. Burdick, R. Langer, Formulation and surface modification of poly(ester-anhydride) micro- and nanospheres, *Biomaterials*. 26 (2005) 117-124.
- [113] A.S. Determan, J.H. Wilson, M.J. Kipper, M.J. Wannemuehler, B. Narasimhan, Protein stability in the presence of polymer degradation products: consequences for controlled release formulations, *Biomaterials*. 27 (2006) 3312-3320.
- [114] K.A. Ross, H. Loyd, W. Wu, L. Huntimer, S. Ahmed, A. Sambol, et al., Polyanhydride-based H5 hemagglutinin influenza nanovaccines elicit protective virus neutralizing titers and cell-mediated immunity, *Int. J. Nanomed.* 10 (2015) 229-243.
- [115] M.P. Torres, A.S. Determan, G.L. Anderson, S.K. Mallapragada, B. Narasimhan, Amphiphilic polyanhydrides for protein stabilization and release, *Biomaterials*. 28 (2007) 108-116.
- [116] B.D. Ulery, D. Kumar, A.E. Ramer-Tait, D.W. Metzger, M.J. Wannemuehler, B. Narasimhan, Design of a protective single-dose intranasal nanoparticle-based vaccine platform for respiratory infectious diseases, *PLoS One*. 6 (2011) e17642.
- [117] M. Donbrow, Y. Samoilov, Controlled release of tripeleennamine and other drugs dispersed in ethyl cellulose PEG films, *J. Pharm. Pharmacol.* 28 Suppl (1976) 23P.
- [118] F.L. Meyskens, Jr., V. Graham, M. Chvapil, R.T. Dorr, D.S. Alberts, E.A. Surwit, A phase I trial of beta-all-trans-retinoic acid delivered via a collagen sponge and a cervical cap for mild or moderate intraepithelial cervical neoplasia, *J. Natl. Cancer Inst.* 71 (1983) 921-925.

- [119] Y. Kawashima, T. Handa, A. Kasai, H. Takenaka, S.Y. Lin, Y. Ando, Novel method for the preparation of controlled-release theophylline granules coated with a polyelectrolyte complex of sodium polyphosphate-chitosan, *J. Pharm. Sci.* 74 (1985) 264-268.
- [120] F. Meng, G.H.M. Engbers, J. Feijen, Polyethylene glycol-grafted polystyrene particles, *J Biomed Mater Res.* 70A (2004) 49-58.
- [121] J. Zhou, T.R. Patel, R.W. Sirianni, G. Strohbehn, M.-Q. Zheng, N. Duong, et al., Highly penetrative, drug-loaded nanocarriers improve treatment of glioblastoma, *Proc. Natl. Acad. Sci. U. S. A.* 110 (2013) 11751-11756.
- [122] H.T. Ta, C.R. Dass, D.E. Dunstan, Injectable chitosan hydrogels for localised cancer therapy, *J. Control. Release.* 126 (2008) 205-216.
- [123] M.F. Shamji, P. Hwang, R.W. Bullock, S.B. Adams, D.L. Nettles, L.A. Setton, Release and activity of anti-TNF α therapeutics from injectable chitosan preparations for local drug delivery, *J. Biomed. Mater. Res. B.* 90 (2009) 319-326.
- [124] G. Gaucher, M.H. Dufresne, V.P. Sant, N. Kang, D. Maysinger, J.C. Leroux, Block copolymer micelles: preparation, characterization and application in drug delivery, *J. Control. Release.* 109 (2005) 169-188.
- [125] V.P. Torchilin, Micellar nanocarriers: pharmaceutical perspectives, *Pharm. Res.* 24 (2007) 1-16.
- [126] A.Z. Wang, R. Langer, O.C. Farokhzad, Nanoparticle delivery of cancer drugs, *Annu. Rev. Med.* 63 (2012) 185-198.
- [127] W. Scharlt, Current directions in core-shell nanoparticle design, *Nanoscale.* 2 (2010) 829-843.
- [128] E.R. Gillies, J.M.J. Frechet, Dendrimers and dendritic polymers in drug delivery, *Drug Discov. Today.* 10 (2005) 35-43.
- [129] A. Quintana, E. Raczka, L. Piehler, I. Lee, A. Myc, I. Majoros, et al., Design and function of a dendrimer-based therapeutic nanodevice targeted to tumor cells through the folate receptor, *Pharm. Res.* 19 (2002) 1310-1316.
- [130] P. Kesharwani, A.K. Iyer, Recent advances in dendrimer-based nanovectors for tumor-targeted drug and gene delivery, *Drug Discov. Today.* 20 (2015) 536-547.
- [131] D.W. Pack, A.S. Hoffman, S. Pun, P.S. Stayton, Design and development of polymers for gene delivery, *Nat. Rev. Drug Discov.* 4 (2005) 581-593.
- [132] C. Tros de Ilarduya, Y. Sun, N. Duzgunes, Gene delivery by lipoplexes and polyplexes, *Eur. J. Pharm. Sci.* 40 (2010) 159-170.
- [133] G. Bozzuto, A. Molinari, Liposomes as nanomedical devices, *Int. J. Nanomed.* 10 (2015) 975-999.
- [134] M.-L. Briuglia, C. Rotella, A. McFarlane, D. Lamprou, Influence of cholesterol on liposome stability and on in vitro drug release, *Drug Deliv. Transl. Res.* (2015) 1-12.
- [135] J. Mérian, R. De Souza, Y. Dou, S.N. Ekdawi, F. Ravenelle, C. Allen, Development of a liposome formulation for improved biodistribution and tumor accumulation of pentamidine for oncology applications, *Int. J. Pharm.* 488 (2015) 154-164.
- [136] H.-I. Chang, M.-K. Yeh, Clinical development of liposome-based drugs: formulation, characterization, and therapeutic efficacy, *Int. J. Nanomed.* 7 (2012) 49-60.
- [137] F. Movahedi, R.G. Hu, D.L. Becker, C. Xu, Stimuli-responsive liposomes for the delivery of nucleic acid therapeutics, *Nanomedicine.* (2015).
- [138] H.E. van Kan-Davelaar, J.C. van Hest, J.J. Cornelissen, M.S. Koay, Using viruses as nanomedicines, *Br. J. Pharmacol.* 171 (2014) 4001-4009.

- [139] A.S. Determan, B.G. Trewyn, V.S. Lin, M. Nilsen-Hamilton, B. Narasimhan, Encapsulation, stabilization, and release of BSA-FITC from polyanhydride microspheres, *J. Control. Release.* 100 (2004) 97-109.
- [140] S. Honary, F. Zahir, Effect of zeta potential on the properties of nano-drug delivery systems - a review (part 2), *Trop. J. Pharm. Res.* 12 (2013) 265-273.
- [141] T.M. Brenza, L.K. Petersen, Y. Zhang, L.M. Huntimer, A.E. Ramer-Tait, J.M. Hostetter, et al., Pulmonary biodistribution and cellular uptake of intranasally administered monodisperse particles, *Pharm. Res.* 32 (2015) 1368-1382.
- [142] L. Huntimer, A.E. Ramer-Tait, L.K. Petersen, K.A. Ross, K.A. Walz, C. Wang, et al., Evaluation of biocompatibility and administration site reactogenicity of polyanhydride-particle-based platform for vaccine delivery, *Adv. Healthc. Mater.* 2 (2013) 369-378.
- [143] C. He, Y. Hu, L. Yin, C. Tang, C. Yin, Effects of particle size and surface charge on cellular uptake and biodistribution of polymeric nanoparticles, *Biomaterials.* 31 (2010) 3657-3666.
- [144] Y. Phanse, P. Lueth, A.E. Ramer-Tait, B.R. Carrillo-Conde, M.J. Wannemuehler, B. Narasimhan, et al., Cellular Internalization Mechanisms of Polyanhydride Particles: Implications for Rational Design of Drug Delivery Vehicles. (Submitted 2015).
- [145] V.B. Joshi, S.M. Geary, A.K. Salem, Biodegradable Particles as Vaccine Delivery Systems: Size Matters, *AAPS Journal.* 15 (2013) 85-94.
- [146] S.C. Semple, A. Akinc, J. Chen, A.P. Sandhu, B.L. Mui, C.K. Cho, et al., Rational design of cationic lipids for siRNA delivery, *Nat. Biotechnol.* 28 (2010) 172-U118.
- [147] Y. Phanse, B.M. Dunphy, J.L. Perry, P.M. Airs, C.C. Paquette, J.O. Carlson, et al., Biodistribution and Toxicity Studies of PRINT Hydrogel Nanoparticles in Mosquito Larvae and Cells, *PLoS Negl. Trop. Dis.* 9 (2015) e0003735.
- [148] A. Gessner, A. Lieske, B.R. Paulke, R.H. Müller, Influence of surface charge density on protein adsorption on polymeric nanoparticles: analysis by two-dimensional electrophoresis, *Eur. J. Pharm. Biopharm.* 54 (2002) 165-170.
- [149] C.C. Paquette, Y. Phanse, J.L. Perry, I. Sanchez-Vargas, P.M. Airs, B.M. Dunphy, et al., Biodistribution and trafficking of hydrogel nanoparticles in adult mosquitoes, *PLoS Negl. Trop. Dis.* 9 (2015) e0003745.
- [150] F. Jia, X. Liu, L. Li, S. Mallapragada, B. Narasimhan, Q. Wang, Multifunctional nanoparticles for targeted delivery of immune activating and cancer therapeutic agents, *J. Control. Release.* 172 (2013) 1020-1034.
- [151] B. Ozpolat, A.K. Sood, G. Lopez-Berestein, Liposomal siRNA nanocarriers for cancer therapy, *Adv. Drug Delivery Rev.* 66 (2014) 110-116.
- [152] F. Meng, G.H.M. Engbers, J. Feijen, Polyethylene glycol-grafted polystyrene particles, *J. Biomed. Mater. Res. A.* 70 (2004) 49-58.
- [153] C.E. Astete, C.M. Sabliov, Synthesis and characterization of PLGA nanoparticles, *J. Biomater. Sc. Polymer Edn.* 17 (2006) 247-289.
- [154] S. Wohlfart, S. Gelperina, J. Kreuter, Transport of drugs across the blood-brain barrier by nanoparticles, *J. Control. Release.* 161 (2012) 264-273.
- [155] N. Voigt, P. Henrich-Noack, S. Kockentiedt, W. Hintz, J. Tomas, B.A. Sabel, Surfactants, not size or zeta-potential influence blood-brain barrier passage of polymeric nanoparticles, *Eur. J. Pharm. Biopharm.* 87 (2014) 19-29.

- [156] N. Bertrand, J. Wu, X. Xu, N. Kamaly, O.C. Farokhzad, Cancer nanotechnology: the impact of passive and active targeting in the era of modern cancer biology, *Adv. Drug Deliv. Rev.* 66 (2014) 2-25.
- [157] A. Salvati, A.S. Pitek, M.P. Monopoli, K. Prapainop, F.B. Bombelli, D.R. Hristov, et al., Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface, *Nat. Nanotechnol.* 8 (2013) 137-143.
- [158] S.K. Mallapragada, T.M. Brenza, J.M. McMillan, B. Narasimhan, D.S. Sakaguchi, A.D. Sharma, et al., Enabling nanomaterial, nanofabrication and cellular technologies for nanoneuromedicines, *Nanomedicine.* 11 (2015) 715-729.
- [159] R. Gabathuler, Approaches to transport therapeutic drugs across the blood-brain barrier to treat brain diseases, *Neurobiol. Dis.* 37 (2010) 48-57.
- [160] M. Rotman, M.M. Welling, A. Bunschoten, M.E. de Backer, J. Rip, R.J.A. Nabuurs, et al., Enhanced glutathione PEGylated liposomal brain delivery of an anti-amyloid single domain antibody fragment in a mouse model for Alzheimer's disease, *J. Control. Release.* 203 (2015) 40-50.
- [161] R. Pahuja, K. Seth, A. Shukla, R.K. Shukla, P. Bhatnagar, L.K.S. Chauhan, et al., Trans-Blood Brain Barrier Delivery of Dopamine-Loaded Nanoparticles Reverses Functional Deficits in Parkinsonian Rats, *ACS Nano.* 9 (2015) 4850-4871.
- [162] M. Yemisci, S. Caban, Y. Gursoy-Ozdemir, S. Lule, R. Novoa-Carballal, R. Riguera, et al., Systemically administered brain-targeted nanoparticles transport peptides across the blood-brain barrier and provide neuroprotection, *J. Cereb. Blood Flow Metab.* 35 (2015) 469-475.
- [163] E. Afergan, H. Epstein, R. Dahan, N. Koroukhov, K. Rohekar, H.D. Danenberg, et al., Delivery of serotonin to the brain by monocytes following phagocytosis of liposomes, *J. Control. Release.* 132 (2008) 84-90.
- [164] Y. Zhao, M.J. Haney, V. Mahajan, B.C. Reiner, A. Dunaevsky, R.L. Mosley, et al., Active targeted macrophage-mediated delivery of catalase to affected brain regions in models of Parkinson's disease, *J. Nanomed. Nanotechnol.* (2011) S4.
- [165] P. Calvo, B. Gouritin, H. Chacun, D. Desmaele, J. D'Angelo, J.-P. Noel, et al., Long-circulating PEGylated polycyanoacrylate nanoparticles as new drug carrier for brain delivery, *Pharm. Res.* 18 (2001) 1157-1166.
- [166] K. Gao, X. Jiang, Influence of particle size on transport of methotrexate across blood brain barrier by polysorbate 80-coated polybutylcyanoacrylate nanoparticles, *Int. J. Pharm.* 310 (2006) 213-219.
- [167] C.L. Graff, G.M. Pollack, Nasal drug administration: potential for targeted central nervous system delivery, *J. Pharm. Sci.* 94 (2005) 1187-1195.
- [168] K.-I. Ogawara, K. Furumoto, Y. Takakura, M. Hashida, K. Higaki, T. Kimura, Surface hydrophobicity of particles is not necessarily the most important determinant in their *in vivo* disposition after intravenous administration in rats, *J. Control. Res.* 77 (2001) 191-198.
- [169] L. Shah, S. Yadav, M. Amiji, Nanotechnology for CNS delivery of bio-therapeutic agents, *Drug Deliv. Transl. Res.* 3 (2013) 336-351.
- [170] A. Mistry, S. Stolnik, L. Illum, Nanoparticles for direct nose-to-brain delivery of drugs, *Int. J. Pharm.* 2009 (2009) 146-157.

- [171] Y.Y. Wang, S.K. Lai, J.S. Suk, A. Pace, R. Cone, J. Hanes, Addressing the PEG mucoadhesivity paradox to engineer nanoparticles that "slip" through the human mucus barrier, *Angew. Chem. Int. Ed. Engl.* 47 (2008) 9726-9729.
- [172] S. Yadav, F. Gattacceca, R. Panicucci, M.M. Amiji, Comparative Biodistribution and Pharmacokinetic Analysis of Cyclosporine-A in the Brain upon Intranasal or Intravenous Administration in an Oil-in-Water Nanoemulsion Formulation, *Mol. Pharm.* 12 (2015) 1523-1533.
- [173] G. Rassu, E. Soddu, M. Cossu, A. Brundu, G. Carri, N. Marchetti, et al., Solid microparticles based on chitosan or methyl-B-cyclodextrin: a first formulative approach to increase the nose-to-brain transport of deferoxamine mesylate, *J. Control. Release.* 201 (2015) 68-77.
- [174] F.-Y. Yang, G.-L. Lin, S.-C. Horng, T.-K. Chang, S.-Y. Wu, T.-T. Wong, Pulsed high-intensity focused ultrasound enhances the relative permeability of the bloodtumor barrier in a glioma-bearing rat model, *IEEE Trans. Ultrason. Ferroelectr. Freq. Control.* 58 (2011) 964-970.
- [175] E. Nance, K. Timbie, G.W. Miller, J. Song, C. Louttit, A.L. Klibanov, et al., Non-invasive delivery of stealth, brain-penetrating nanoparticles across the blood-brain barrier using MRI-guided focused ultrasound, *J. Control. Release.* 189 (2014) 123-132.
- [176] M. Aryal, N. Vykhodtseva, Y.-Z. Zhang, N. McDannold, Multiple sessions of liposomal doxorubicin delivery via focused ultrasound mediated blood-brain barrier disruption: a safety study, *J. Control. Release.* 204 (2015) 60-69.
- [177] Y.-W. Hu, Y.-Z. Du, N. Liu, X. Liu, T.-T. Meng, B.-L. Cheng, et al., Selective redox-responsive drug release in tumor cells mediated by chitosan based glycolipid-like nanocarrier, *J. Control. Release.* 206 (2015) 91-100.
- [178] Y. Bae, W.-D. Jang, N. Nishiyama, S. Fukushima, K. Kataoka, Multifunctional polymeric micelles with folate-mediated cancer cell targeting and pH-triggered drug releasing properties for active intracellular drug delivery, *Mol. BioSyst.* 1 (2005) 242-250.
- [179] Y. Bae, N. Nishiyama, K. Kataoka, In vivo antitumor activity of the folate-conjugated pH-sensitive polymeric micelle selectively releasing adriamycin in the intracellular acidic compartments, *Bioconjugate Chem.* 18 (2007) 1131-1139.
- [180] T. Araki, K.-I. Ogawara, H. Suzuki, R. Kawai, T.-I. Watanabe, T. Ono, et al., Augmented EPR effect by photo-triggered tumor vascular treatment improved therapeutic efficacy of liposomal paclitaxel in mice bearing tumors with low permeable vasculature, *J. Control. Release.* 200 (2015) 106-114.
- [181] Z.-X. Liao, E.-Y. Chuang, C.-C. Lin, Y.-C. Ho, K.-J. Lin, P.-Y. Cheng, et al., An AS1411 aptamer-conjugated liposomal system containing a bubble-generating agent for tumor-specific chemotherapy that overcomes multidrug resistance, *J. Control. Release.* 208 (2015) 42-51.
- [182] V.B. Joshi, S.M. Geary, B.R. Carrillo-Conde, B. Narasimhan, A.K. Salem, Characterizing the antitumor response in mice treated with antigen-loaded polyanhydride microparticles, *Acta Biomater.* 9 (2013) 5583-5589.
- [183] A. Sooresh, H. Kwon, R. Taylor, P. Pietrantonio, M. Pine, C.M. Sayes, Surface functionalization of silver nanoparticles: novel applications for insect vector control, *ACS Appl Mater Interfaces.* 3 (2011) 3779-3787.

- [184] X. Zhang, J. Zhang, K.Y. Zhu, Chitosan/double-stranded RNA nanoparticle-mediated RNA interference to silence chitin synthase genes through larval feeding in the African malaria mosquito (*Anopheles gambiae*), *Insect Mol. Biol.* 19 (2010) 683-693.
- [185] M. Ali, M. Afzal, M. Verma, S. Misra-Bhattacharya, F.J. Ahmad, A.K. Dinda, Improved antifilarial activity of ivermectin in chitosan-alginate nanoparticles against human lymphatic filarial parasite, *Brugia malayi*, *Parasitol. Res.* 112 (2013) 2933-2943.
- [186] M. Ali, M. Afzal, M. Verma, S.M. Bhattacharya, F.J. Ahmad, M. Samim, et al., Therapeutic efficacy of poly (lactic-co-glycolic acid) nanoparticles encapsulated ivermectin (nano-ivermectin) against brugian filariasis in experimental rodent model, *Parasitol. Res.* 113 (2014) 681-691.
- [187] A.M. Binnebose, S.L. Haughney, R.L. Martin, P. Imerman, B. Narasimhan, B.H. Bellaire, Polyanhydride nanoparticle delivery platform dramatically enhances killing of filarial worms, Submitted to *PLoS Neglected Trop. Dis.* (2015).
- [188] A.B. Madhankumar, B. Slagle-Webb, X. Wang, Q.X. Yang, D.A. Antonetti, P.A. Miller, et al., Efficacy of interleukin-13 receptor-targeted liposomal doxorubicin in the intracranial brain tumor model, *Mol. Cancer Ther.* 8 (2009) 648-654.
- [189] M.J. Serramia, S. Alvarez, E. Fuentes-Paniagua, M.I. Clemente, J. Sanchez-Nieves, R. Gomez, et al., In vivo delivery of siRNA to the brain by carbosilane dendrimer, *J. Control. Release.* 200 (2015) 60-70.
- [190] K. Hu, J. Li, Y. Shen, S. Lu, X. Gao, Q. Zhang, et al., Lactoferrin-conjugated PEG-PLA nanoparticles with improved brain delivery: in vitro and in vivo evaluations, *J. Control. Release.* 134 (2009) 55-61.
- [191] J.-Y. Kim, W.I. Choi, Y.H. Kim, G. Tae, Brain-targeted delivery of protein using chitosan- and RVG peptide-conjugated, pluronic-based nano-carrier, *Biomaterials.* 34 (2013) 1170-1178.
- [192] K.S. Rao, M.K. Reddy, J.L. Horning, V. Labhasetwar, TAT-conjugated nanoparticles for the CNS delivery of anti-HIV drugs, *Biomaterials.* 29 (2008) 4429-4438.
- [193] H. Xin, X. Sha, X. Jiang, W. Zhang, L. Chen, X. Fang, Anti-glioblastoma efficacy and safety of paclitaxel-loading Angiopep-conjugated dual targeting PEG-PCL nanoparticles, *Biomaterials.* 33 (2012) 8167-8176.
- [194] H. Karatas, Y. Aktas, Y. Gursoy-Ozdemir, E. Bodur, M. Yemisci, S. Caban, et al., A nanomedicine transports a peptide caspase-3 inhibitor across the blood-brain barrier and provides neuroprotection, *J. Neurosci.* 29 (2009) 13761-13769.
- [195] J.Y. Kang, M. Eggert, S. Mouli, I. Ajuffali, X. Fu, B. Nie, et al., Pharmacokinetics, antitumor and cardioprotective effects of liposome-encapsulated phenylaminoethyl selenide in human prostate cancer rodent models, *Pharm. Res.* 32 (2015) 852-862.

Table 1. Barriers to therapeutic efficacy of drugs

Type of Drug	Treatments	Complexities and Barriers	Ref.
Small molecule	Neuro-active agents Chemotherapeutics Antibiotics Antiparasitics	Hydrophobic formulation Inability to cross barriers High systemic concentrations for treatment Off-target toxicity	[62, 179]
Peptides and proteins	Receptor agonists or antagonists Recombinant hormones Antigens	Conformational stability Enzymatic degradation Thermal stability Poor bioavailability Rapid clearance Off-target binding	[64-66, 68, 69]
Nucleic acids	Gene therapy RNA interference (dsRNA, siRNA)	Inability to cross cellular membranes Rapid clearance Poor stability Inefficient delivery to target site	[2, 70, 71, 73, 74]

Table 2. Nano-enabled approaches for enhanced *in vivo* therapeutic efficacy

Nano-carrier Platform	Drug	Barrier	Targeting Strategy	Physiological Response	Ref.
PEG-Liposome	Radiolabel	BBB	Glutathione and amyloid-targeted antibody fragments	Label concentration	[160]
Liposome	Doxorubicin	BBB	Intranasal administration and IL-13 receptor	Survival	[188]
Liposome	DOX-NP®	BBB, Tumor	Micro bubble assisted focused ultrasound disruption	Drug concentration	[176]
Dendrimer	siRNA	BBB	Retro-orbital venous plexus administration	Gene silencing	[189]
Polymer	Dalargin	BBB	Apolipoprotein	Percent of max effect	[19]
Polymer	Doxorubicin	BBB, Tumor	Lecithin	Drug concentration	[102]
PEG-Polymer	Coumarin-6	BBB	Lactoferrin	Dye concentration	[190]
Polymer	Loperamide	BBB	g7 peptide	Percent of max effect	[101]
Pluronic	β -galactosidase	BBB	RVG29	Drug concentration	[191]
Polymer	Deferoxamine mesylate	BBB	Intranasal administration	Drug concentration	[173]
Polymer	Ritonavir	BBB	TAT peptide	Drug concentration	[192]
PEG-Polymer	Paclitaxel	BBB, Tumor	Angiopep	Inhibition ratio of tumor; mean survival time	[193]
PEG-Polymer	Peptide	BBB	Antibody to transferrin receptor	Target inhibition	[194]
Liposome	Paclitaxel	Tumor	Photosensitizer in nanoparticle (PEG-polymer)	Tumor volume	[180]
Micelle	Paclitaxel	Tumor	Redox responsive polymer	Tumor volume	[177]
Liposome	Phenyl-2-aminoethyl selenide	Tumor	Reduction of cardiotoxicity from soluble doxorubicin	Survival	[195]
Polymer	dsRNA	Cuticle	RNAi	Reduction in chitin production	[184]
Polymer	Ivermectin	Cuticle	Nematode cuticle penetration	Dose sparing	[186, 187]

Graphical Abstract

Nano-carriers functionalized with active and passive targeting strategies enable the efficient delivery of diverse payloads through complex biological barriers to prevent and treat vector-borne diseases, neuronal disorders, and cancer.