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Nanomaterial-mediated CNS Delivery of Diagnostic and Therapeutic Agents

Laura Biddlestone-Thorpe^a, Nicola Marchi^b, Kathy Guo^b, Chaitali Ghosh^b, Damir Janigro^b, Kristoffer Valerie^{a,c}, and Hu Yang^{c,d,*}

^aDepartment of Radiation Oncology, Virginia Commonwealth University, Richmond, VA 23298, USA

^bCerebrovascular Research Center, Cleveland Clinic Foundation, Cleveland, OH 44195, USA

^cMassey Cancer Center, Virginia Commonwealth University, Richmond, VA 23298, USA

^dDepartment of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23284, USA

Abstract

Research into the diagnosis and treatment of central nervous system (CNS) diseases has been enhanced by rapid advances in nanotechnology and an expansion in the library of nanostructured carriers. This review discusses the latest applications of nanomaterials in the CNS with an emphasis on brain tumors. Novel administration routes and transport mechanisms for nanomaterial-mediated CNS delivery of diagnostic and therapeutic agents to bypass or cross the blood brain barrier (BBB) are also discussed. These include temporary disruption of the BBB, use of impregnated polymers (polymer wafers), convection-enhanced delivery (CED), and intranasal delivery. Moreover, an *in vitro* BBB model capable of mimicking geometrical, cellular and rheological features of the human cerebrovasculature has been developed. This is a useful tool that can be used for screening CNS nanoparticles or therapeutics prior to *in vivo* and clinical investigation. A discussion of this novel model is included.

Keywords

blood-brain barrier (BBB); brain cancer; central nervous system (CNS); convection enhanced delivery (CED); flow-based *in vitro* BBB model; nanoparticle

1. Introduction

Recent development of nanotechnology in pharmaceutical and biomedical research has led to the creation of a number of nanostructured diagnostic and therapeutic agents, which could benefit the treatment of many central nervous system (CNS) diseases. Until recently,

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*Corresponding author. Hu Yang, Department of Biomedical Engineering, Virginia Commonwealth University, 401 West Main Street, P.O. Box 843067, Richmond, VA 23284, USA. Tel.: +1 804 8285459; Fax: +1 804 8284454; hyang2@vcu.edu.

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application of nanotechnology in the CNS has been primarily focused on brain cancer because of life-threatening risks associated with this disease. An efficient drug delivery to the brain tumor mass remains a challenging clinical problem. In particular, the blood-brain barrier (BBB), the blood cerebral spinal fluid, and blood-tumor barrier all hamper the successful treatment of brain tumors by severely limiting access of therapeutic or diagnostic agents into the brain [1]. To overcome these limitations, several types of nanoparticles such as linear polymers, hyperbranched polymers, dendrimers, liposomes and micelles have been synthesized or engineered [2]. To bypass or cross the BBB more effectively, novel administration routes and transport mechanisms for nanoparticle-mediated CNS delivery have been actively explored. These include temporary disruption of the BBB to increase permeability, the use of impregnated polymers for local drug administration, convection-enhanced delivery (CED), and intranasal delivery. This review begins with a brief introduction to the BBB and then discusses the latest application of nanoparticles for the treatment and diagnosis of CNS diseases in the context of brain tumors. New routes employed for the administration of nanoparticles are also described in detail. Given the complexity of the CNS and presence of the BBB, screening and pre-optimizing nanoparticles-based agents designed to be administered systemically using an *in vitro* model could be a suitable approach prior to *in vivo* and clinical examination. A discussion of a dynamic *in vitro* (DIV) BBB model capable of mimicking features of the human BBB is included in this review.

2. The blood-brain barrier

Some speculate that strong selective pressures must have existed to allow such a complex structure as the BBB to evolve. The CNS has no lymphatic system or other way of parenchymal drainage and is enclosed within the cranium, a rigid non-expandable structure. A net influx of molecules into the CNS would increase osmolarity and allow water from the vasculature to enter the brain, leading to an elevation of intracranial pressure. Evolution of the BBB fortunately makes large increases in intracranial pressure rare occurrences. Importantly, the BBB serves to prevent potentially harmful toxins from reaching the brain.

Scientific investigation in identifying the BBB dates back to the 19th century. In 1885, Paul Ehrlich, a bacteriologist, observed that aniline dyes intravenously injected into animals colored all organs with the exception of the brain and spinal cord [3, 4]. Today we know that the BBB is composed of microvascular endothelium, basement membrane and neuroglial structures such as astrocytes, pericytes and microglia. The monolayer of microvascular endothelial cells (ECs) lines the intraluminal space of brain capillaries and the endothelial cells are packed close together, forming tight junctions. The EC layer has a luminal (inside) and abluminal (outside) compartment, separated by 300 to 500 nm of cytoplasm between the vascular system and the brain. Tight junctions consist of occludin and claudin adherent junctions and junctional adhesion molecules. There are two fundamental morphological characteristics that separate the brain from peripheral EC. First, the cytoplasm of brain microvascular ECs has rare pinocytotic vesicles—fluid-filled cell membrane invaginations that allow certain compounds to cross the BBB. These ECs also contain a greater concentration of mitochondria meeting the requirements to actively transport molecules from the blood into the brain and vice versa. Second, in addition to the structural integrity of the BBB, there exists an enzymatic surveillance system that metabolizes drugs and other compounds bypassing the structural barrier.

Achieving drug delivery across the BBB requires knowledge of both “barrier” and permeability properties of the brain endothelial cells. In fact, several attempts to outwit the BBB are based on the molecular mimicry of molecules that are normally impermeable (e.g., glucose), yet rapidly and reliably transport across the BBB. This introduces the concept of a

“biochemical BBB”, which is established by transport systems of the BBB. These can be grouped into four types:

1. *Simple diffusion.* Solute travels down a concentration gradient.
2. *Facilitated diffusion.* Solute binds to a specific membrane-spanning protein and like simple diffusion, travels down a concentration gradient.
3. *Simple diffusion via aqueous channel.* Charged ions and solutes are the principal compounds that cross the BBB by this mechanism.
4. *Active transport via protein carrier.* Solutes transport against a concentration gradient. This mechanism requires a change in the affinity of a carrier for the solute and the expenditure of ATP for transport. Vast supplies of mitochondria in the EC are thought to provide the necessary energy for this reaction.

Compounds essential to brain function are regularly transported across the BBB. The glucose transporter system at the BBB is of special importance since glucose is the primary source of energy of the brain and is required for normal brain activity and function. This system is a possible candidate for piggy-backing of molecules into the CNS via a glucose transporter (GLUT). There are five members of the sodium-independent glucose transporters, including GLUT-1 (EC), GLUT-3 (neurons) and GLUT-5 (microglia) in the brain [5]. Each transports 2-deoxyglucose, 3-O-methylglucose, mannose, galactose and glucose across the membrane [5]. GLUT-1 is a 45–55 kDa protein, depending on glycosylation state. It is present in high concentration in EC of arterioles, venules and capillaries and facilitates D-glucose enantiomer movement from the peripheral circulation into the brain.

Another crucial transport system that operates in a similar manner is the system of multiple drug resistance [6]. Multidrug resistance protein (MDR1) has been intensely studied as a possible vehicle for drug delivery. P-glycoprotein (or P-gp, MDR1) is an efflux transporter protein found in EC, astrocytes and microglia. It is expressed on the luminal surface of the endothelial membrane and glia, and prevents toxins from entering into the brain. Many drugs are substrates for MDR1 which limits their accumulation in the brain. Vinca alkaloids, anthracyclines, and taxanes are among the anticancer agents known to be transported by P-gp. Recent work has shown that MDR1 regulation is altered by various disease conditions, and, in turn, diseases of the brain influence MDR1 expression [6, 7]. An abundance of receptors at the surface of the BBB can be utilized by nanoparticles for enhanced brain uptake by coupling with receptor-specific molecules or analogues. Many other molecules such as insulin, insulin-like growth factors (IGF-1 and IGF-2) [8], leptin [9], and transferrin [10] can also get into the brain following receptor-mediated endocytosis. In general, nanoparticles should be used to bypass efflux transport systems present at the luminal side (such as MDR1). Alternatively, nanoparticles could be substrates of those transport mechanisms enhancing the passage of specific molecules (e.g., GLUT-1) across the BBB.

3. Brain tumors

There are more than 100 types of brain tumors recognized by the World Health Organization (WHO) classified according to histopathological features, genetics, clinical presentation, and malignancy [11, 12]. Gliomas include low-grade, non-malignant (WHO Grades I–II), progressively more malignant, e.g., anaplastic astrocytoma (WHO Grades III), and high-grade malignant brain tumors such as astrocytic gliomas (WHO Grade IV). There are approximately 22,000 new cases of malignant brain tumors in the United States each year [13]. Astrocytic gliomas are either primary *de novo*, or progress from a lower grade over a 5–10 year window. Secondary brain tumors result from tumor metastases originating from peripheral locations such as the lung, breast, or the gastrointestinal tract. Secondary brain

tumors are the most common in adults, accounting for 20–40% of all patients with brain tumors and outnumber primary de novo by at least 10 to 1 [11, 14].

Glioblastoma multiforme (WHO Grade IV) is a devastating form of cancer that appears rapidly without much warning of prior symptoms or antecedent lower grade pathology. Hallmark characteristics of GBM include uncontrolled cell proliferation, diffuse infiltration, and resistance to apoptosis [12]. These features, at least in part, account for GBM's poor prognosis, resistance towards radio- and chemotherapy, and a mean survival of just 12–15 months [12, 15]. The characterization of GBMs by The Cancer Genome Atlas (TCGA) Research Network has revealed several genetic changes in key regulatory genes such as ERBB, PDGFRA, p53, and PI3K/PTEN/AKT; the latter genes play a critical role in cancer progression and response to treatment [16, 17]. Current surgical protocols and radiotherapy are insufficient to control GBM. A plethora of new therapeutic approaches are under investigation, including small molecule radiosensitizers and gene therapy. As the understanding of the molecular mechanisms associated with GBM continues to expand, and more specific and potent drugs are developed, efficient delivery of therapeutic agents to the brain becomes very important. The application of nanoparticles for brain drug delivery has emerged as an important approach. Nanoparticles made from a broad spectrum of materials and in various forms have been developed to deliver therapeutic or diagnostic agents to the brain [2].

4. New routes for CNS delivery of therapeutic and diagnostic nanoparticles

An essential role for nanoparticles is to serve as carriers to help drugs gain increased water stability, better pharmacokinetics, reduced toxicity, and improved therapeutic efficacy [18, 19]. Because of nanoparticle versatility, CNS diagnostic and therapeutic agents can be either physically encapsulated or covalently conjugated to nanoparticles. Nanoparticles can be engineered to mimic the structural features of endogenous molecules to improve transport of individual drug or diagnostic molecules. Receptor-mediated delivery of targeted nanoparticles is a common mechanism for CNS delivery of therapeutics and diagnostic agents. Given that most targeted delivery systems utilize structurally labile bioactive molecules (e.g., antibodies) as targeting ligand, in most cases, nanoparticles can be administered systemically via intravenous (i.v.) injection to avoid first-pass effect and/or significant loss of the bioactivity of functionalized nanoparticles. This route has been adopted as a standard modality [20]. However, i.v. injection can lead to poor patient compliance, particularly among patients suffering from chronic CNS diseases. A less invasive and safer approach to i.v. injections is highly sought. Novel routes and new mechanisms are explored for CNS delivery of nanoparticles, which are discussed in this review.

4.1. Osmotic disruption of the blood-brain barrier

In general, drugs greater than 400 Da in molecular mass with low lipid solubility are unable to cross the BBB. Transient disruption of the BBB can be achieved with intra-carotid administration of hyperosmolar agents such as mannitol; the latter removes water from ECs resulting in shrinkage and loss of tight junction functionality. An alternative to this approach is the administration of the bradykinin agonist RMP-7 that directly disrupts the BBB. Both mannitol and RMP-7 increase drug penetration into the brain parenchyma of animals [21, 22].

A recent study by Reme *et al.* reported that polysorbate 80 (PS80)-coated poly(n-butylcyano-acrylate) nanoparticles (PBCA-NP) causes a reversible dose-dependent disruption of the BBB *in vitro* [23]. The authors confirmed this result by measuring the permeability of ¹⁴C-sucrose and high-molecular-weight biomarker FITC-albumin. This

finding suggests PS80-coated nanoparticles alone can be used as a BBB “opener” in addition to functioning as an efficient carrier for drug delivery to the brain. The mechanism underlying PS80-coated nanoparticle mediated delivery across the BBB remains controversial. Several studies suggested that PS80 may selectively adsorb plasma proteins such as apolipoproteins E and B or apolipoproteins A–I, promoting receptor-mediated endocytosis across the BBB [24, 25]. Subsequently, the nanoparticles release the drug which diffuses into the brain parenchyma. When PS80-coated nanoparticles were loaded with doxorubicin, a significant anti-tumor effect was achieved in glioblastoma-bearing rats [26]. Other types of nanoparticles such as poly(lactide-co-glycolide) PLGA coated with PS80 or poloxamer 188 (pluronic F-68) improved the delivery of doxorubicin and loperamide to the brain and resulted in pronounced pharmacological effects [24]. Overall, PLGA nanoparticles coated with poloxamer 188 appear to be more efficient in delivering drugs to the brain than PS80. A clear understanding of the mechanism underlying the transport of surfactant-coated nanoparticles across the BBB is still lacking.

4.2. Polymer wafers

Gliadel[®], currently used in the clinic, consists of bis-chloroethylnitrosourea (carmustine) polymer wafers that are placed in the resection cavity after tumor excision. This technology is considered the gold standard of intra-cerebral drug therapy [27]. Clinical trials have indicated that Gliadel[®] as a successful adjunct to surgery and radiation increasing survival of GBM patients up to two months [1]. The success of Gliadel[®] wafers has led to clinical reconsiderations of cancer drugs that were once excluded due to systemic toxicity or scarce BBB permeability. An example of a revisited drug is taxol, an agent that prevents microtubule assembly/breakdown leading to cell cycle arrest and apoptosis. Systemic administration of taxol failed in several clinical trials against malignant glioma because the maximum tolerated dose (MTD) was reached before achieving any clinical benefit [28, 29]. The use of biodegradable taxol polymers was demonstrated to ensure safe delivery of taxol into the CNS in an animal model of malignant glioma [30–32].

Another example of a revisited drug is camptothecin (CPT), a topoisomerase I inhibitor. While early clinical trials were halted due to unexpected systemic toxicity, local delivery of CPT (or the water-soluble analogs irinotecan and topotecan) can now be achieved using polymers. In a series of *in vivo* experiments, more than half of animals treated with CPT-loaded biodegradable polyanhydride polymer were long term survivors showing no evidence of systemic toxicity or side effects [33–35]. These results demonstrate that the use of polymers for local delivery significantly decreases systemic toxicity without compromising effective drug concentration reaching the CNS. Despite the considerable attractiveness and simplicity of the Gliadel[®] technology, one remaining concern is the insufficient penetration depth in the brain seen with this particular drug [36]. Although this strategy has not been applied to deliver nanostructured agents to the brain, it is reasonably expected that nanoparticle carriers can be designed to help drugs gain deeper penetration into the tumor. Exploratory studies are needed to demonstrate the efficiency of this route delivering nanoparticle-based agents for treating or diagnosing malignant brain tumors.

4.3. Convection-enhanced delivery

Direct delivery of therapeutic or diagnostic nanoparticles into the brain guarantees high intra-tumoral concentrations and a decreased risk of systemic toxicity [15]. Because most nanostructured agents can be formulated the same way as those small-molecular-weight therapeutic or diagnostic agents, routes (e.g., convection-enhanced delivery, CED) used for local delivery of small molecules can also be extended to deliver nanoparticulate delivery systems.

Similar to polymer wafers, CED is used to directly deliver a given drug to the tumor site bypassing the BBB [37]. CED relies on applying continuous positive pressure through a pump to administer the drug via a cannula directly to the tumor site. Standard treatment of GBM starts with surgery and, frequently, the insertion of a cannula or catheter into the cavity for drainage and future biopsies. Therefore, a drug can be administered through these devices. An example of a mouse brain infused with a food dye in the left hemisphere by CED is shown in Figure 1. Several factors influence tissue distribution of the drug by CED. In addition to drug half-life and tissue binding properties, drug flow-rate, volume; size, shape, and placement of the cannula are also important factors [18, 38]. Brain tissue permits bulk flow of small- and large-molecular-weight agents to disperse along several paths. Over extended periods of time, diffusion, metabolic process and loss of drug within the capillaries determine the net distribution. Catheter-induced tissue damage and backflow of drug adjacent to the catheter will also affect drug distribution. Backflow during CED can be a problem as it limits drug delivery to target tissues and increase the potential for widespread neurotoxicity [21]. A reduction in the diameter of the cannula reduces backflow [39].

Problems associated with the use of very fine catheters were addressed with the development of step-down catheters which have wide enough diameters to maintain rigidity and small enough to reduce backflow [40]. Development of multiple-hole catheters failed to improve drug distribution and instead increased the incidence of backflow. This was concluded from a series of experiments conducted using agarose blocks that showed only the proximal ports were able to deliver drugs [41]. Hollow fiber multihole catheters with multiple fenestrations have many advantages over more conventional catheters [42]. Not only did multiple fenestrations increase the surface area of brain tissue into which the drug was infused but the size and porosity of the hollow fibers allowed for a more uniform delivery along the entire length of the hollow fiber catheter segment. Combined, these unique features greatly reduced the backflow of the hollow fiber catheter. Another approach involves the use of ultrafine, tissue-compatible, anti-reflux catheters that are inserted into a guidance sheath to minimize tissue damage. This technique allows for accurate placement of a reduced, small-end diameter catheter that is resistant to backflow and also allows for drug delivery to be shaped to better conform to the treatment volume [18].

An attractive approach is to combine CED with nanoparticle technology. CED of nanoparticle would maximize administration and uniformity of drug delivery to the tumor site and also extend treatment over longer periods of time. Hadjipanayis *et al.* coupled a GBM-specific antibody to magnetic iron oxide nanoparticles (IONP; 10 nm in core size) (Figure 2) and administered those nanoparticles using CED for targeted MRI imaging of GBM [43]. With the help of the coupled antibody, IONP can selectively bind to the epidermal growth factor receptor (EGFR) deletion mutant (EGFRvIII) on human GBM. The IONPs coupled with EGFRvIII antibody not only enabled MRI contrast enhancement but led to a significant increase in survival. Mice implanted with highly tumorigenic U87ΔEGFRvIII intracranial xenografts had a median survival of 19 days in contrast to the control (untreated) group with a median survival of only 11 days.

CED of liposomes or PEGylated liposomes encapsulating chemotherapeutic drugs was effective in treating intracranial rodent brain tumor xenografts [44]. To understand the transport of drug-loaded liposomes and examine possible pathological changes in primate brain after repeated CED, Krauze *et al.* studied CED of liposomes loaded with MRI contrast agent Gadoteridol (GDL) [45]. They investigated particle distribution in the corona radiata, putamen and brainstem in adult male Cynomolgus monkeys subjected to repeated CED infusion. Their studies showed that all regions showed a linear relationship between infusion and distribution volume in consecutive infusions. Using hematoxylin and eosin staining (Figure 3, upper panel), signs of increased intracellular space in the corresponding

distribution areas of liposomes were observed and verified with rhodamine-liposomes (Figure 3, bottom panel). This approach was demonstrated to be safe given the fact that neither necrosis inflammatory reaction nor microgliosis was found. Recently, PLGA nanoparticles encapsulated with CPT were administered by CED into the brains of rats [46]. This approach resulted in prolonged release of CPT beyond 50 days and significantly improved survival compared to animals treated with CPT alone.

Protein transduction domains of trans-activating transcriptor (TAT) peptide can cross cell membranes without relying on transporter- and receptor-mediated endocytosis [47]. Green fluorescent protein (GFP) plasmid delivered by liposomes coupled to TAT was expressed more exclusively by brain tumors following intra-tumoral injection. In contrast, no detectable GFP expression was found in the normal brain tissues adjacent to the tumor [48]. Delivery of these types of liposomes may be improved and precisely controlled with CED. Overall, drug-loaded nanoparticles in combination with CED may have general applicability for treating brain tumors such as glioma.

4.4. Intranasal delivery

Intranasal administration has emerged as a compelling method for delivering drugs or diagnostic agents to the brain as it bypasses the BBB non-invasively [49]. Mechanisms of intranasal drug delivery to the CNS remain to be elucidated. Evidence suggests that nerves connecting the nasal passages to the brain and spinal cord as well as the vasculature, cerebrospinal fluid, and lymphatic system contribute to transportation of molecules to the CNS following adsorption from the nasal mucosa [50]. The major route of intranasal delivery is the olfactory nerve pathway. Drugs travel via the olfactory nerve axons, accumulate in olfactory bulbs (OB), and diffuse into the brain [50]. This administration route has been exploited for pain management, particularly post-operative pain and moderate-to-severe cancer pain due to ease of delivery and high brain uptake [51, 52].

Evidence suggests that intranasal delivery is a feasible approach for delivery of nanostructured therapeutics to the brain, achieving higher drug concentrations compared to intranasally administered free drugs. A liposomal delivery system was formulated to deliver rivastigmine intranasally for the management of Alzheimer's disease [53]. This modality achieved a higher drug concentration and a longer half-life in the brain compared to intranasal free drug or the oral route. When the area-under-the-curve (AUC) values of these groups were compared, the intranasal liposome group had five-fold higher AUC when compared to orally taken free drug and an almost three-fold higher value when compared to free drug administered intranasally [53].

In vivo toxicity and immunogenicity of intranasally administered nanoparticles have not been adequately addressed in the past. In a recent report, Liu *et al.* tested the levels of amino acid neurotransmitters (i.e., glutamate (Glu), aspartate (Asp), γ -aminobutyric acid (GABA), taurine (Tau) and glycine (Gly) and cytokines as well as enzyme activities in OB and brain after intranasal administration of wheat germ agglutinin (WGA) conjugated poly(ethylene glycol)-poly(lactic acid) (PEG-PLA) nanoparticles (WGA-NP) [54]. Although WGA-NP had no effect on the amino acid neurotransmitter levels in the rat OB, a slight excitotoxicity was observed, as demonstrated by increased Glu level. After 7-day continuous intranasal instillation, WGA-NP increased lactate dehydrogenase (LDH) activity by 18%. An increase in LDH activity indicates cellular damage related to the loss of mitochondrial function. It was observed that neither the cholinergic nerve system nor cytokine levels were influenced suggesting WGA-NP is a safe carrier system for intranasal delivery of CNS therapeutics.

Although the literature on intranasal drug delivery for brain tumors is still scarce, it was recently explored as an alternative for administering anticancer therapeutics for the

treatment of brain tumors [55]. Shingaki *et al.* evaluated intranasal transport of methotrexate (MTX) to brain tumors and observed significant direct transport of MTX from the nasal cavity to both the cerebrospinal fluid (CSF) and the brain [55]. Tumor-bearing rats received nasal chemotherapy of MTX three times at two-day intervals. Two days after the third treatment, brain tumor weight was reduced by 80% compared to control. Direct transport from nose to brain may help reduce dose and toxicity of the anticancer drug. Nanoparticle interactions within the CNS, effects of anticancer drugs on brain tumors and transport pathways should be investigated. It was noted that repeated dosing via intranasal administration of MTX results in higher drug brain concentrations [55]. Thus, pharmacokinetics/pharmacodynamics (PK/PD) of intranasally administered anticancer drugs should be profiled to predict outcomes and to reduce non-specific toxicity effects. Intranasal delivery of nanoparticle-based anticancer drugs are expected to be advantageous over intranasal delivery of free drugs because nanoparticles can carry a high drug payload, selectively target brain tumors and enhance permeability and retention (EPR) to penetrate deeper to the brain reducing drug resistance. Although extensive application of intranasal delivery of nanoparticles has mainly centered on vaccine delivery [56], brain imaging [57], and the treatment of chronic CNS diseases such as psychosis [58] other than brain tumors, increased research activity in applying intranasal delivery for nanoparticle-based anticancer drugs is expected in the foreseeable future.

5. The art of modeling the human BBB

A clinically acceptable brain drug delivery system has to undergo multiple phases of studies and trials. It is necessary to test several important delivery properties of a new brain drug delivery system such as efficiency of macromolecular system crossing the BBB at the early stage with the use of an *in vitro* BBB model before a full investigation is conducted. *In vitro* BBB models can exclude many uncontrolled factors and parameters *in vivo*, enabling elucidation of the structure-function relationship of brain-targeted nanoparticles for optimization of their targeting and delivery efficiency.

In the past, many attempts have been made to model the human BBB and to “isolate in a dish” all the necessary components to replicate the mechanisms regulating drug and BBB passage under physiological or pathological conditions. Virtually all attempts to reproduce the BBB in the laboratory have been limited by geometrical (*e.g.*, cells cultured on a flat surface), rheological (*e.g.*, lack of intra-luminal flow [59, 60]) and biological (*e.g.*, use of rodent cells, or cell lines with cell cycle impediment precluding differentiation) nature. Similar difficulties have arisen when trying to mimic “diseased” BBB. Human cerebrovascular diseases are present throughout all age groups. Thus, the goal of creating true disease model systems is imperative.

5.1. Rationale for modeling phenotypic characteristics of the human BBB

Whilst the “barrier” properties dictate new strategies for trans-BBB drug delivery, the mechanisms naturally used to transport molecules can also be used for drug delivery. A paradoxical situation exists whereby it is necessary to overcome the BBB in diseases such as brain cancer and epilepsy, both of which are associated with leaky BBB [61–63]. To further complicate the anatomical and functional definition of the BBB, it has become apparent that in human brain pathology the BBB serves as a conduit for intravascular signals and circulating white blood cells and platelets. Many of the factors that regulate permeability under normal conditions are altered during pathological conditions resulting in enhanced vascular permeability and edema formation. Disruption of the BBB occurs in many neurological diseases, including trauma, inflammatory and autoimmune disease, infections, cerebrovascular disease, neurodegenerative disease, epilepsy, and neoplasia. Head trauma alters transporter activity that may inhibit essential compounds from crossing the BBB while

permitting pathogens and toxins. Lymphoid surveillance of the CNS occurs via lymphatic vessels like olfactory nerves or arachnoid granulations. Thus, in spite of its location at the blood-brain interface, the potential impact of BBB cells is more widespread than initially believed. In addition, peripheral incidents affecting the circulatory/heart – brain axis will inevitably interfere with BBB function. It is therefore essential to take into account and reproduce these pathological features when testing the biodistribution and CNS availability of the next brain therapeutic.

5.2. Dynamic *in vitro* blood-brain barrier model

Given how much is known about the BBB's role in pharmacology and neurobiology/neuropathology, it is not surprising that the research field of BBB modeling is a crowded one. In the past decade, a flow-based dynamic *in vitro* BBB (DIV-BBB) system was successfully used to reproduce the key features of the *in vivo* BBB [59, 60, 64–66], including the creation of cell-to-cell tightness, matching ratio of luminal to abluminal volume found *in vivo*, and functional transport and metabolic processes regulating drug blood-to-brain passage. Exposure of brain ECs to laminar flow has been shown to affect cell differentiation, tight-junction formation, and the regulation of the cell cycle leading to mitotic arrest [59, 60]. Remarkably, *in vivo*, cerebral blood flow is coupled to the levels of energy substrates (*e.g.*, pO₂, pCO₂) and regulates vessel caliber and cell adhesion to the vascular BBB wall. Using the DIV-BBB model, brain endothelial cells can be co-cultured with other brain-derived cell (astrocytes [67], neurons [68], and smooth muscle), and exposed intraluminally to serum components, white blood cells and even platelets. The latter is significant since the BBB is at the interface between the brain and the peripheral circulation systems. It is therefore important to expose BBB cells to “fluids” (*e.g.*, serum of CSF) or cells (*e.g.*, white blood cells) that are likely to play critical roles in the pathophysiological changes affecting the BBB under disease-conditions [69] (Figure 4). The DIV-BBB model enables the determination of the role of individual variables modulating BBB function, including the effect of lack or decreased luminal flow, oxygen or glucose (Figure 5).

Owing to its comprehensive set up, the DIV-BBB has allowed for pharmacological studies (*e.g.*, drug brain permeability) and has accelerated the investigation of the mechanisms underlying BBB modification under pathological conditions. Noteworthy, the BBB itself is increasingly becoming a therapeutic target in several brain disorders, underscoring the significance of an appropriate BBB *in vitro* model. For example, even when *in vivo* rheological and structural features are strictly considered, appropriate cell phenotypes need to be used (Figure 5). In other words, *in vitro* models work best if a disease specific *in vitro* BBB is generated. For example, reduced drug efficacy due to impaired delivery to the neuronal target represents the conceptual basis of drug resistance in epilepsy displayed by BBB endothelial cells. Using human brain specimens we identified cells responsible for drug resistance and then incorporated them into an appropriate *in vitro* model [70–72]. This becomes possible when primary brain endothelial and glial cells can be isolated from surgical specimens and cultured using the DIV-BBB [72 - 74]. The latter approach allows for a personalized medicine approach where *in vitro* BBBs can be generated from patients' surgical material (Figure 4). Further validation of the use of the DIV-BBB tailored for specific human CNS diseases will allow for the characterization of inter-patient heterogeneity of pharmacologic response of the brain in relation to patient outcome. Altogether, the DIV-BBB model will facilitate BBB permeability studies of brain drugs and therapeutic and diagnostic nanoparticles in a disease-specific manner. Other advantages using this *in vitro* model are apparent: cost-effective, simple setup, and well-defined conditions with excellent repeatability. Furthermore, by excluding many uncontrolled factors and parameters *in vivo*, this new model is ideal for revealing the structure-function

relationship of brain-targeted nanoparticles for optimization of their targeting and delivery efficiency.

6. Future Perspectives

Emerging therapeutic targets for a variety of cancers including brain cancer are those broadly referred to as members of the DNA damage response (DDR). The DDR provides excellent targets for the treatment of GBM that could be realized with nanotechnology. There are now quite a few small molecule inhibitors targeting the DDR. These include inhibitors of poly-(ADP)-ribose polymerase (PARP), ataxia telangeictasia (A-T) mutated (ATM) kinase, A-T and RAD3-related (ATR) kinase, and the checkpoint kinases CHK1 and CHK2 [75–77]. Many cellular processes are affected when the DDR is inhibited, including DNA damage checkpoints, apoptosis, and DNA repair. Most of these drugs are only in the developing and pre-clinical testing stages except for several PARP inhibitors (PARPi) that are in phase I–III trials for breast, ovarian, lung, and head & neck cancer. For glioma, the PARPi BSI-201 is in phase I/II for newly diagnosed GBM in combination with TMZ to determine the MTD [78].

A recent development in the oncology field was the discovery that the combination of two cancer drugs could result in ‘synthetic lethality’. A PARPi was shown to be synthetically lethal in tumor cells with defective homologous recombination [79, 80]. Thus, breast or ovarian cancer patients with BRCA1 and BRCA2 mutations are particularly attractive groups to target for PARPi treatment. The highly specific ATMi, KU-60019, is currently being investigated as a potential radiosensitizer of glioma in an orthotopic mouse model [81]. In addition, combinations of ATMi with a variety of other DDR inhibitors, including Olaparib (AZD2262), are also expected to be synthetically lethal and are currently being tested.

With the aid of adaptable nanostructured carriers, the above described new strategies can be explored with the aid of nanotechnology combined with novel administration routes. Furthermore, the DIV-BBB can be engineered to mimic the BBB under normal or pathological conditions, thus serving as an efficient screening tool for accelerating development and optimization of brain-specific nanoparticulate delivery systems.

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Figure 1. Convection-enhanced delivery of a dye fast green into a mouse brain. Fast green was administered at a flow rate of $0.4 \mu\text{l}$ per min over 30 min. The brain was resected immediately after infusion. Unpublished data.

Figure 2. Amphiphilic block polymer-coated IONPs conjugated to the EGFRvIIIAb. Reproduced with permission from [43].

Figure 3. Representative histology from an animal euthanized immediately after third CED of GDL. H&E staining is indicated in the upper panel, and fluorescence in the lower panel. The left-hand panel shows the distribution in corona radiata infusion; right-hand panels show brainstem distribution. Reproduced with permission from [45].

Figure 4.

(A) Schematic representation of cell types involved in BBB signaling and potentially relevant for drug delivery. Note that intraluminal (white blood cells), abluminal (glia, neurons, pericytes), and exogenous (virus) elements are all relevant to BBB biology and neuropharmacology. (B) A double immunostaining of MDR1 in the human BBB. (C) Identification of CYP3A4 expression at the BBB of patients with drug-resistant epilepsy. Reproduced with permission from [69, 74].

Figure 5.

(A) The BBB established in this model is characterized by higher expression of tight junctions, appropriately segregated luminal/abluminal transporters (i.e. potassium, amino-acids, glucose GLUT-1) and a high transendothelial electrical resistance (TEER). (B) In the DIV-BBB, brain microvascular EC (animal or human; both primary cultures and cell lines) are cultured in the lumen of hollow microporous fibers in the presence of extraluminal astrocytes inside a sealed chamber. The endothelium lining the intraluminal space is exposed to a pulsatile flow, which induces and maintains the EC polarity. The major factor differentiating the DIV-BBB from others *in vitro* BBB models is that the EC cultured inside the capillaries are exposed to a luminal pulsatile flow. The shear stress generated by the intraluminal flow mimics the physiological condition of the BBB *in vivo*.