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Pharmaceutical nanotechnology

Nanomedicine as a non-invasive strategy for drug delivery across the blood brain barrier

Vivienne H. Tam[a,*], Chris Sosa[a], Rui Liu[a], Nan Yao[b], Rodney D. Priestley[a]

[a] Chemical and Biological Engineering, Princeton University, Princeton, NJ 08544, USA
[b] Materials Science and Engineering, Princeton University, Princeton, NJ 08544, USA

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ABSTRACT

The blood brain barrier (BBB) is a major obstacle to drug delivery for diseases of the central nervous system (CNS). This brief review highlights the current invasive and non-invasive technologies available to address this problem. In particular, nanomedicine has shown much promise as a non-invasive strategy due to its drug loading capabilities, ease of targeting to the BBB, and small size. The versatility of this technology in terms of type of drug and imaging agent, carrier material, and targeting mechanism is highlighted in this review. The recent inclusion of imaging agents in the nanocarriers has important consequences for the field of theranostics.

Contents

* Corresponding author.
E-mail address: vtam@alumni.princeton.edu (V.H. Tam).

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1. Introduction

Neurological disorders remain one of the most unresolved questions in medical technology. Diseases in the central nervous system (CNS) affect 1.5 billion people worldwide and over 100 million people in the U.S. (Yang, 2010) As more than 50% of adults reaching the age of 70 are expected to develop degenerative CNS pathologies, such as Alzheimer's or Parkinson's (Blasi et al., 2007), the burden of these disorders on public health is only expected to grow exponentially with the rapidly aging population. Due to these trends coupled with the disturbing lack of any effective treatment, much research has been directed towards this field, resulting in the recent burgeoning of the CNS drug market into the largest of all therapeutic areas (IMS Health, 2004). However, the greatest constraint in drug delivery to the brain is not the absence of drugs to treat CNS diseases, but rather the mechanism to transport such drugs past the nearly impenetrable blood brain barrier (BBB). Nanomedicine has recently emerged as a promising field for innovative and effective approaches to cross the BBB and target deadly diseases such as glioblastoma (Kim et al., 2015). This review presents critical research in this field of nanomedicine as pertaining to CNS diseases. It will also delve into the more recent development of theranostics, the combination of imaging and drug delivery, as applied to the brain.

2. Structure of the BBB

The function of the BBB is to maintain the specific microenvironment required for the brain to operate as well as to protect it from any neurotoxic compounds that may present in a person's bloodstream. Thus, it is structured as a layer of endothelial cells surrounding the cerebral microvasculature, forming a tight barrier.

A more detailed description of BBB structure is available in the review by Azad et al. (2015) In short, tight junctions between each endothelial cell in the BBB capillaries, as shown in Fig. 1, prevent the passage of particles between the cells. In addition, efflux pumps such as the P-glycoprotein pumps quickly remove any foreign substance that bypasses the BBB (Patel et al., 2012; Barbu et al., 2009; Gabathuler 2010). As a result, only small, lipophilic compounds, such as O_2 or steroid hormones readily diffuse across the BBB facilitated by their concentration gradient.

Unfortunately, this poses great limitations on CNS therapeutics. Close to 100% of drugs do not cross the BBB because they are neither lipophilic or are larger than 500 Da, so they cannot diffuse across the membrane. This means that any brain disease which is not amenable to small-molecule drug therapy, including Alzheimer's disease, brain and spinal cord trauma, brain cancer, multiple sclerosis and stroke, cannot be treated by targeted drug delivery systems (Lawrence and Pardridge, 2002). Even among small molecule drugs, more than 98% are not transported across the BBB (Pardridge 2005).

3. Strategies for CNS drug delivery

Current strategies under development can be divided into two approaches: invasive and non-invasive. Invasive methods involve either disrupting the BBB to allow drugs to enter or directly injecting drugs into the CNS tissue, while non-invasive methods

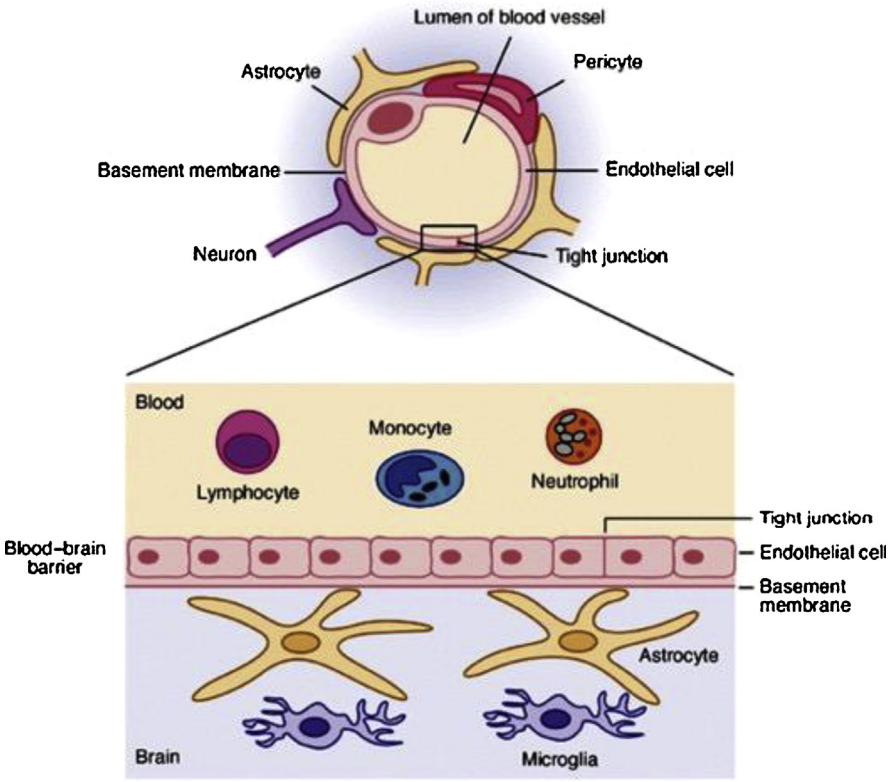

Fig. 1. A representative cross-section of a cerebral capillary of the BBB.
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rely on endogenous cellular mechanisms to facilitate drug transport.

4. Invasive strategies for CNS drug delivery

4.1. Disruption of the BBB

In order to increase the permeability of the BBB, techniques have been developed to temporarily disrupt the endothelial cells, allowing macromolecular drugs to leak into the CNS (Stockwell et al., 2014). One technique uses an osmotic shock to shrink the endothelial cells and disrupt the tight junctions (Bellavance et al., 2008) (Fig. 2). In a clinical study, the disruption followed by the subsequent administration of chemotherapeutic agents allowed sufficient drug molecules to cross the BBB in order to produce a therapeutic effect in brain cancer patients (Fortin et al., 2007). More recently, ultrasound-mediated drug delivery (USMD) that uses microbubbles that are 1–10 μm in diameter to mechanically disrupt the tight junctions, is showing potential for enhanced chemotherapy treatment especially in terms of spatial specificity, as ultrasound waves can be targeted within an area of a few millimeters (Hynynen 2008; Hynynen et al., 2001; Treat et al., 2007). Besides, vasoactive molecules such as bradykinin and cereport can selectively increase the permeability of brain tumor capillaries but not that of healthy ones. The administration of cereport with another chemotherapy drugs has been shown to successfully reduce the glioma tumor volume (Cloughesy et al., 1999).

USMD is of high clinical relevance as various chemotherapy drugs, such as doxorubicin, carmustine, trastuzumab, and temozolomide have been successfully transported across the BBB via this approach (Azad et al., 2015). As well, the delivery of anti-amyloid-beta antibodies for the treatment of Alzheimer's and other therapeutic agents such as PEG-coated gold nanoparticles, small interfering RNA and stem cells has been demonstrated, making this technique a versatile one (Liu et al., 2014).

Recent studies, such as the one by Downs et al., tested the technology on primates and found that the ultrasound-induced BBB opening in the basal ganglia did not result in any visual or motor deficits (Downs et al., 2015). However, although USMD shows promise in animal models, potential limitations still include the narrow sonication field and the targeting aberrations due to the skull; thus further studies need to be done to confirm its clinical potential in humans.

One such study was the clinical trial performed by Beccaria et al. (2016), demonstrating the effectiveness of USMD on BBB disruption and subsequent administration of the chemotherapy drug, carboplatin, for 28 out of 41 treatments without adverse effects. The pulsed ultrasound was administered at a resonance frequency of 1.06 MHz using a device called SonoCloud for 2 min and was tested up to an acoustic pressure of 1.1 MPa without toxicity (Beccaria et al., 2016). As the safety parameters are more precisely defined, USMD is becoming a feasible approach for BBB opening and subsequent drug delivery in humans.

4.2. Direct injection into CNS

Transcranial injections, either intracerebrally or intra-cerebroventricularly, offer the most direct delivery of drug to the tumor site, thus reducing possible negative effects on peripheral healthy tissue. Alternatively, a biodegradable chemotherapeutic impregnated wafer, such as the Gliadel wafer, can be implanted into the tumor resection cavity. Both of the above rely on diffusion to transport the drug into the brain parenchyma. However, as diffusion in the brain decreases exponentially with distance, this technology has significant limitation and requires very precise mapping of the injection or implantation site to achieve maximum targeting of the drug to the tumor (Gabathuler, 2010).

Convection-enhanced delivery (CED) attempts to overcome this challenge by using positive hydrostatic pressure to drive the drug farther into the tumor tissue (Bobo et al., 1994). However, in a clinical study, no difference in survival was shown in patients who underwent CED treatment and those with Gliadel wafers (Kunwar et al., 2010).

5. Major problems of invasive delivery

Unfortunately, as with any invasive technique, the methods outlined above are accompanied by a high neurosurgical cost and increased risk of infection as undesirable elements may enter the brain when the BBB is exposed. The brain could suffer as well from various traumatic injuries due to the mechanical nature of these approaches including parent vessel thrombosis or brain herniation (Bellavance et al., 2008). Thus, while highly invasive strategies can be used for well-defined tumors, they are undesirable for less localized diseases such as Alzheimer's or multiple sclerosis (Barbu et al., 2009).

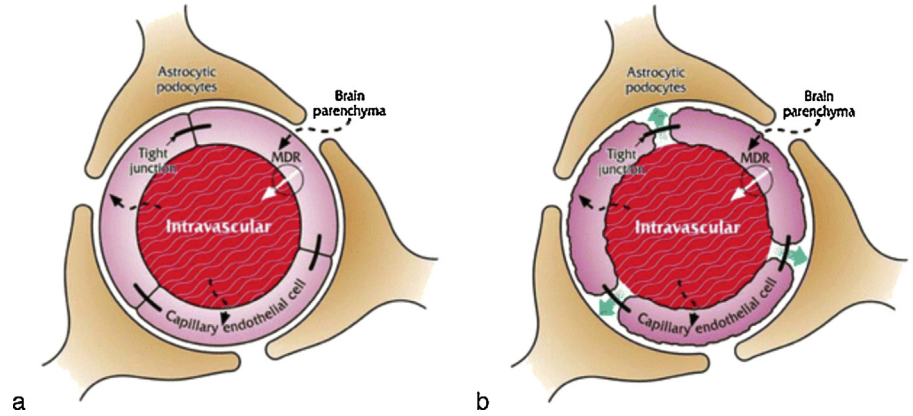

Fig. 2. Graphical sketch illustrating the hypothesis concerning the osmotic BBB modification. The tight junctions are shown as devoid of any anatomic space between the endothelial cells. Moreover, multi-drug resistance (MDR) gene products, such as the P-gp efflux pump, are also illustrated as they are integral to the mechanism of the barrier. The osmotic BBBD procedure induces a retraction in the cell membrane, and a physical opening **b** accompanied by a modification of the Ca^{2+} metabolism in the cell. Reprint from Bellavance et al., 2008 with permission

6. Non-invasive strategies for CNS drug delivery

Alternative pathways traversing the BBB have been investigated for their potential application in invasive drug delivery. In addition to the transcellular lipophilic pathway for small, lipophilic molecules, the other transport routes are: (1) the paracellular aqueous pathway for water-soluble agents (2) carrier-mediated transport that shuttles glucose and essential amino acids into the brain (3) receptor- mediated transport that relies on receptors for endogenous large molecules such as insulin and (4) absorptive-mediated transport that allows polycationic substances such as cationised albumin to attach to the negatively charged plasma membrane (Patel et al., 2012; Barbu et al., 2009; Chen and Liu, 2012).

7. Prodrugs

Drugs that are able to cross the BBB via passive diffusion have the following common characteristics: (1) small molecular size of less than 500 Da (2) high lipophilicity and (3) lack of ionization at physiological pH (Franc et al., 2001). Thus, there is much effort directed towards making water-soluble drugs lipid-soluble by reducing the number of its polar groups or by linking it to a lipid moiety. However, this engineering challenge is significant and until now, no drug modified in this manner has been able to cross the BBB in pharmacologically significant amounts, with the one exception being the acetylation of morphine to form heroin (Pardridge, 2007a,b; Rautio et al., 2008).

Apart from the passive diffusion route, other transport mechanisms, such as carrier-mediated or receptor-mediated transport have been exploited to a greater degree of success in trafficking pseudo-nutrient prodrugs across the BBB. One prominent example is the prodrug L-DOPA (L-3,4-dihydroxyphenylalanine) used to treat Parkinson's Disease (Rautio et al., 2008). It is designed to target the L-amino acid transporter, a carrier-mediated system with a high transport capacity for neutral amino acids (Gynther et al., 2008).

8. Intranasal drug delivery

Intranasal drug delivery is a non-invasive drug delivery technique that bypasses the BBB via the olfactory nerves (Misra et al., 2003). Recent studies have revealed its potential for possible treatment of autism spectrum disorder, as intranasal oxytocin improved social and emotional functioning in autistic individuals (Guastella et al., 2010). As well, Shingaki et al. found that an intranasal administration of methotrexate (MTX) to brain tumors reduced brain tumor weight by 80% (Shingaki et al., 2010). The ease and safety of administration makes this an attractive option for CNS drug delivery; however the major limitation is the small number of molecules capable of diffusing through the olfactory epithelium (Stockwell et al., 2014).

9. Nanomedicine for CNS drug delivery

In recent years, nanomedicine has received significant attention with regards to the management of CNS diseases (Wong et al., 2012) such as gliomas, Alzheimer's and Parkinson's (Saraiva et al., 2016), due to its potential for highly targeted drug delivery. The current systemic administration of therapeutics into the bloodstream delivers the drug throughout the body. Not only is this inefficient as very little of the drug actually bypasses the BBB, it also results in harmful side effects to tissues in unaffected areas (Chapman et al., 2013). Using BBB and tumor specific moieties on the surface, nanocarriers such as liposomes and nanoparticles are able to cross the BBB and deliver larger amounts of therapeutic agents to a specific site, thus reducing toxicity to surrounding tissues (Koo et al., 2006). The rapid advances in nanotechnology have enabled us to build functionalized particles that have optimized drug loading and release kinetics, stealth capabilities to avoid agglutination with plasma proteins in the blood or disposal by the reticoendothelial (RES) system, and grafted ligands that facilitate in-vivo imaging (Winer 2011; Kelkar and Reineke, 2011). As well, PEGylation of particles has been widely used to confer stealth capabilities to avoid agglutination with plasma proteins in the blood or disposal by the reticoendothelial (RES) system. However, recent studies by Dawson and co-workers have shown that PEGylation does not fully prevent the interaction of particles with plasma proteins, especially in biological mediums (Salvati et al., 2013; Hadjidemetriou et al., 2015). With the advantages of well-controlled size, surface functionalization, and chemical properties in various environments, nanocarriers present a non-invasive method of improving drug delivery and localization while enhancing solubility and protection of the drug across the BBB (Meyers et al., 2013). For example, when gadolinium was loaded into nanoparticles, 5.34% of injected NP-loaded gadolinium per gram of tissue remained in the brain compared with only 0.0009% of free gadolinium per gram of tissue, resulting in a several hundred fold increase of the desired compound in the brain (Koffie et al., 2011).

The design requirements for nanoparticles used in CNS drug delivery are biodegradability and nontoxicity, a particle diameter of less than 100 nm, prolonged blood circulation time with no aggregation, drug loading and releasing capabilities, and a BBB-targeting moiety (Tian et al., 2014). An example of a nanocarrier with the above characteristics is shown in Fig. 3.

Here, we provide an overview of nanocarriers with respects to (1) the type of nanocarrier (liposomes, polymeric, magnetic and solid lipid nanoparticles) (2) the BBB targeting mechanism via the four different routes of BBB bypass and (3) their applications in treating CNS diseases, and in particular, theranostics, which is the combination of imaging and therapy.

10. Types of nanocarriers

10.1. Liposomes

Liposomes are small vesicles comprised of one or more phospholipid bilayers enclosing an aqueous space. Their size, surface charge, lipid composition and cholesterol content can be manipulated to control drug delivery and tissue uptake (Samad et al., 2007); thus in addition to their low toxicity and ability to deliver both hydrophilic and lipophilic compounds, liposomes are probably the most well-studied and clinically recognized type of nanocarriers, especially for many types of cancers (Wong et al., 2012; Allen 1998).

The most common liposomal formulations in targeting the BBB are cationic, PEGylated and immunoliposomes (Garcia-Garcia et al., 2005). Yoshida and Mizuno (2003) used cationic liposomes to successfully deliver the gene interferon-β to malignant gliomas. Although the mechanism is not entirely understood, it is suggested that the cationic liposomes bind to the negatively charged endothelium of the brain and due to their small size (20 nm), they can then be transported via passive diffusion or phagocytosis (Zhao et al., 2012). PEG grafted on the surface of liposomes enables them to evade the RES system, therefore lengthening their blood circulation time and giving them time to slip past the BBB. Without PEG, liposomes tend to either be engulfed by phagocytes or exchange lipid materials with cell membranes (Franc et al., 2001). An in vivo experiment by Gaillard et al. using glutathione-coated PEGylated liposomes to deliver doxorubicin to human glioblastoma showed a strong inhibition of brain tumor growth with 2 out of

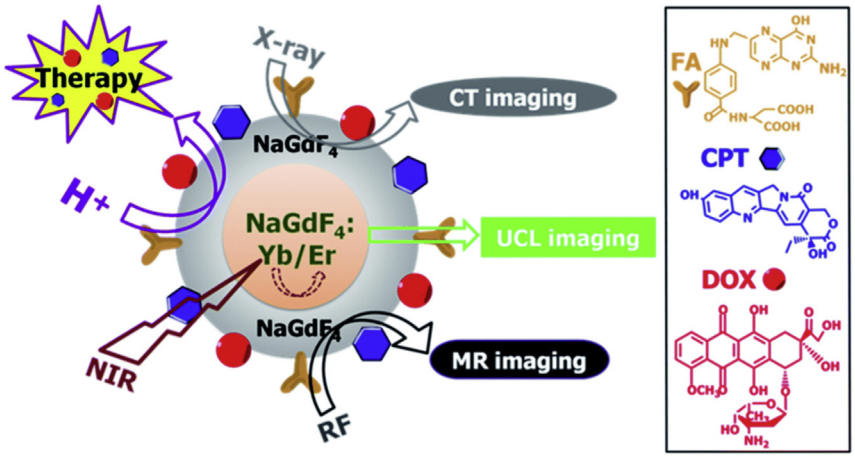

Fig. 3. Multifunctional nanoparticles with tri-modal imaging, targeted recognition and therapy properties. Reprint from Tian et al., 2014 with permission

9 animals showing a complete tumor regression (Gaillard et al., 2014).

As with many other nanoparticle formulations, liposomes can also be complexed with an antibody or ligand that will be recognized by a BBB receptor, inducing receptor-mediated endocytosis. One study by Huwyler et al. (1996) showed site-specific delivery of PEG liposomes conjugated with the OX26 monoclonal antibody (MAb) to the rat transferrin receptor which is abundant on the brain microvascular endothelium. PEGylated liposomes around 100 nm in diameter functionalized with transferrin and loaded with horseradish peroxidase were used in vitro to deliver drug to the lysosomes of brain endothelial cells (Visser et al., 2005). In another study, Ding et al. encapsulated magnetic nanoparticles 10 nm in diameter in PEGylated fluorescent liposomes to protect the drugs bounded on the particles. Transferrin was conjugated to the surface and the particles were then shown to transmigrate across an in vitro BBB model, with the presence of an external magnetic force improving the rate of transmigration by 25–30% between 12 and 24 h of experimental period (Ding et al., 2014).

In summary, liposomes have been used extensively for other cancers and show potential for CNS treatment (Medina et al., 2004); however their limitations include fast systemic elimination, possible instability after extended storage, and less control over drug release (Wong et al., 2012).

10.2. Polymeric nanoparticles

The most common polymers for controlled drug release applications today are poly(lactic acid) (PLA), poly(ε-caprolactone) (PCL), poly(aspartic acid), Poly(butylcyanoacrylate) (PBCA), poly (glycolic acid) (PGA), poly(D,L-lactide-*co*-glycolide) (PLGA) and poly (amino acids), with PLA, PGA and PLGA being the most extensively used in CNS drug delivery (Béduneau et al., 2007). A comprehensive review on the synthesis and design modalities of polymeric nanoparticles for cancer drug delivery was recently reported by Kamaly et al. (2012) (Fig. 4)

Kreuter et al. (1995) were the first to develop a polymeric system that delivered drugs to the CNS. PBCA nanoparticles loaded with the drug dalargin and coated with polysorbate 80 increased dalargin penetration by 3 folds when the drug was loaded into the nanoparticle. Although the exact modes for the particle internalization remains controversial, the polysorbate 80 coating is acknowledged to enhance drug BBB transport and has further been utilized for the drugs loperamide, and doxorubicin (Kreuter, 2001). Calvo et al. (2001) later showed that PEGylated poly(hexadecyl cyanoacrylate) (PHDCA) nanoparticles penetrated the brain to a greater extent than the p80 formulation and it was proposed that this occurred either by passive diffusion or intake by macrophages.

Due to their safe history in medicine, PLA, PGA and their copolymer PLGA have been extensively studied for CNS applications. These biodegradable polymers break down into lactic and glycolic acid, which feed into the Krebs Cycle (Béduneau et al., 2007). PEG is commonly grafted onto the particle's surface to prevent clearance by the RES system and confer long circulating properties (Jin et al., 2014). In one study by Vila et al., PLA-PEG particles were evaluated with different PEG coating densities for transport across the nasal mucosa. Results showed that particles with high PEG coating density and a small size had higher delivery efficiency. Subsequently, lectin-conjugated PEG-PLA nanoparticles were confirmed by a fluorescent marker to have entered the brain via intranasal delivery (Gao et al., 2006). Geldenhuys et al. (2011) created PLGA-PEG particles with conjugated glutathione, allowing paclitaxel which is normally removed by the P-glycoprotein efflux to bypass the BBB. Chitosan (CS) presents an interesting alternative material as a naturally occurring, biodegradable, and biocompatible polysaccharide. It can be either used as the polymer of choice as in the MTX-loaded CS-based nanoparticles coated with polysorbate 80 proposed by Trapani et al. (2011) or as a blend with other polymers (Parveen and Sahoo, 2011) to curb phagocytic effects and enhance longevity.

10.3. Solid lipid nanoparticles (SLNs)

SLNs comprise a solid hydrophobic core of lipids, such as mono-, di- and triglycerides or fatty acids with a monolayer of phospholipid coating (Kaur et al., 2008). Like polymeric nanoparticles, they are capable of controlled release of up to several weeks and can also be coated or grafted with ligands for drug targeting (Kaur et al., 2008). They are also stable, and biodegradable under physiological conditions (Barbu et al., 2009) with a high drug loading capacity for both hydrophilic and lipophilic drugs (Yadav et al., 2014). Thus, it is an attractive alternative to liposomes or polymeric nanocarriers. SLNs have been developed for delivering chemotherapeutic drugs to the brain with the first promising results being the delivery of the lipophilic antitumor drug, camptothecin (Yang et al., 1999). Further studies showed that the inclusion of camptothecin in lipid nanoparticles led to sustained drug release of a zero-order kinetic model, with ~45%

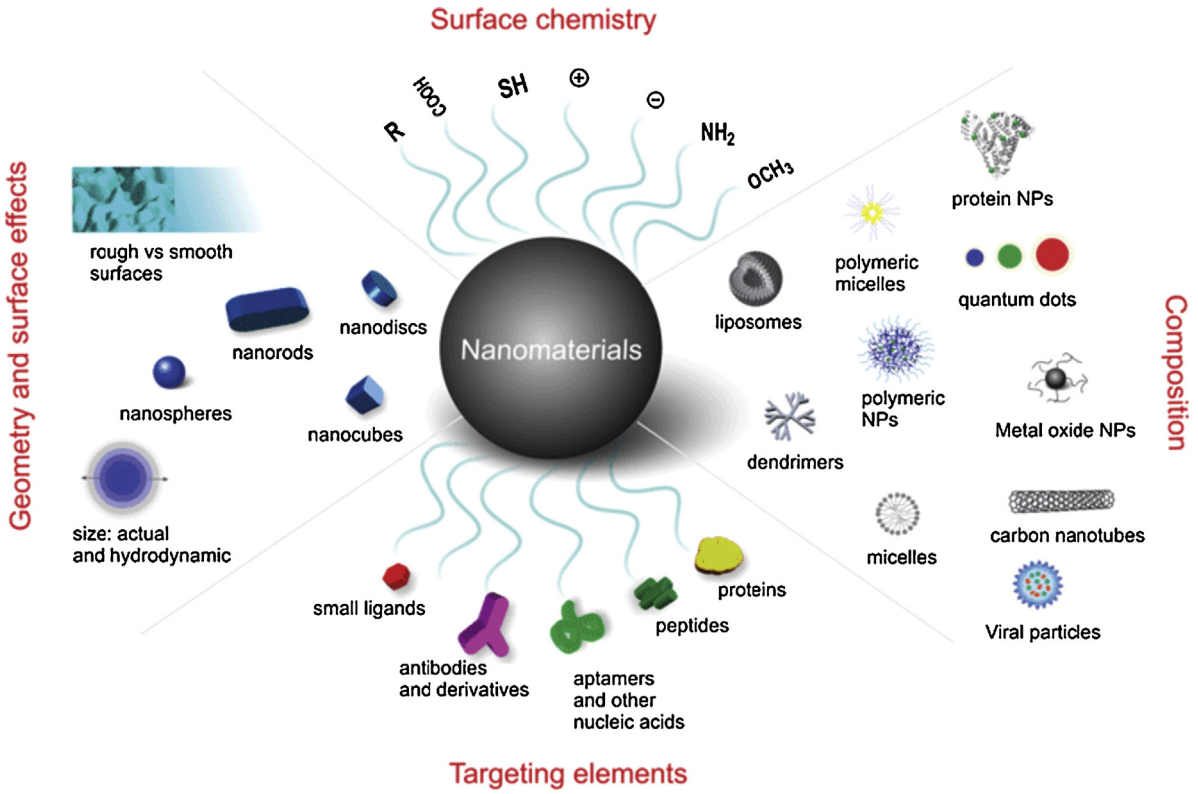

Fig. 4. NPs and their biophysicochemical characteristics which affect their performance both in vitro and in vivo. Reprint from Kamaly et al., 2012 with permission

of the drug released within 30 h. Furthermore, camptothecin-loaded SLNs showed stronger inhibition of melanoma cell proliferation than the free drug after a 24 h incubation period. It is hypothesized that SLNs are endocytosed by the cancer cells, leading to greater drug uptake and thus presenting SLNs as an attractive option for cancer therapy (Huang, 2008). When the coating of polysorbate-80 reported by Kreuter et al. (1995) and Kreuter (2001) was repeated on SLNs, brain targeting results were similar (Göppert and Müller, 2005). This technique is more suitable for lipophilic drugs as well as peptides and proteins (Ricci et al., 2006). For example, Bruun et al. (2015) encapsulated siRNA in cationic angiopep-functionalized SLNs with >95% efficiency for delivery to glioma cells (Bruun et al., 2015). In another study by Reis and coworkers, the chemically unstable resveratrol known to treat brain diseases such as Alzheimer's or epilepsy was encapsulated in SLNs coated with apolipoprotein E to enhance brain delivery. The encapsulation efficiency was high, with an average of 90%, and in vitro studies revealed that the cell monolayer maintained its integrity while resveratrol was transported across it (Neves et al., 2016).

10.4. Magnetic nanoparticles (MPs)

Much attention in recent years has been directed towards magnetic nanoparticles for their potential as dual usage in therapy and imaging, with the focus on superparamagnetic iron oxide nanoparticles, or SPIONS. Their application as an MRI contrast agent has been widely documented (Howes et al., 2010; Wang et al., 2012; Corr et al., 2008; Chertok et al., 2008), and unlike their gadolinium-based counterparts, SPIONS can be processed by a cell's iron metabolism pathway. With various surface modifications and stealth capabilities, SPIONS can be targeted to specific sites and help to delineate malignant tumors from healthy tissue. One clinical study demonstrated that SPIONS identified malignant lymph nodes in prostate cancer to a sensitivity of 90.5%, as compared to conventional MRI with a sensitivity of only 35.4% (Harisinghani et al., 2003). Furthermore, they can be directed to tumors via an external magnetic field, or even enhance radiation treatment of CNS tumors (Winer 2011). For example, SPIONS were used in an in vivo experiment to bind to endothelial vascular cell adhesion molecule-1 (VCAM-1), which is an indicator of established metastases in human brain tissue. The increased sensitivity of this detection method was found to have the potential to detect tumors as early as day 5 (Serres et al., 2012). As such, MPs will be critical for the intersection of diagnostics and treatment, otherwise known as theranostics. Theranostics will likely hold many answers for the effective treatment of CNS diseases and will be discussed at greater length later in this review.

11. Types of targeting mechanism

One essential part of the nanocarrier system is the conjugation of the appropriate BBB-targeting mechanism. Other than those with a polysorbate-80 coating or a small molecular size, most drug-carrying nanoparticles require the use of one of the native BBB transport routes for macromolecules, as illustrated in Fig. 5. Water-soluble agents dissolve through tight junctions via the paracellular aqueous pathway while lipid-soluble agents dissolve *trans*-cellularly through the lipid plasma membrane. However, for almost all other substances, the other three pathways, namely the carrier-mediated (CMT), receptor-mediated (RMT), and absorptive- mediated transport (AMT) systems are required. CMT relies on the conformational change of membrane transport proteins to move solutes such as glucose and amino acids along their concentration gradient. RMT, on the other hand, is triggered by a ligand-receptor interaction which induces endocytosis of the molecule into the brain, while AMT on electrostatic interaction. Via these pathways, the BBB shuttles metabolic compounds like

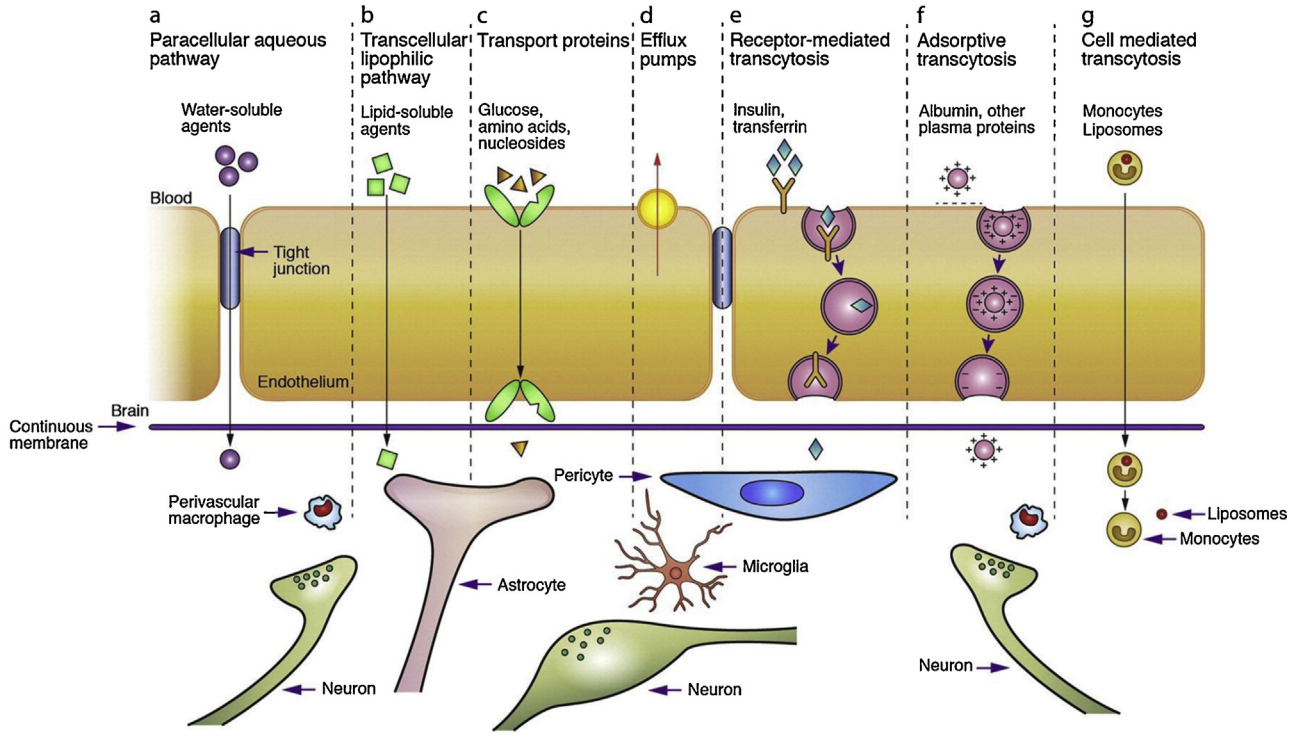

Fig. 5. Transport routes across the blood–brain barrier. Pathways "a" to "f" are commonly for solute molecules; and the route "g" involves monocytes, macrophages and other immune cells and can be used for any drugs or drugs incorporated liposomes or nanoparticles.
Reprint from Chen and Liu, 2012 with permission

insulin or low density lipoprotein (LDL) across the membrane and once modified, nanoparticles can take advantage of these systems to non-invasively deliver neuroactive drugs to the CNS (Gabathuler, 2010). As PEG is highly recommended for surface grafting in order to provide steric stabilization and decrease the rate of elimination from blood into the liver or spleen, ligands can be attached to the nanocarrier via the PEG chain so that they extend past the PEG corona for more effective targeting (Chen and Liu, 2012).

11.1. Carrier-mediated transport (CMT)

Nutrients such as glucose, lactose and neutral amino acids required for survival are brought into the brain using membrane proteins expressed at the surface of the BBB. One of such highly expressed proteins is the glucose transporter (GLUT1) that promotes the intake of D-glucose and glucose analogs from the blood into the brain (Béduneau et al., 2007). Drugs themselves can be chemically altered to resemble these nutrients as in the case of L-DOPA, or the substrates of the transporters could be conjugated to drug-loaded nanocarriers (Mora et al., 2002). The applications of these methods are limited however, as the drug/ligand must be very small and similar in structure to the nutrient. Furthermore, as the carrier protein is located at the membrane, the drug must still be moved via diffusion across the cell membranes at the BBB to penetrate into the brain itself (Gabathuler, 2010).

11.2. Receptor-mediated transport (RMT)

Unlike CMT, the RMT mechanism exists for the intake of larger molecules such as insulin or transferrin (Jones and Shusta, 2007). The contact of either the natural ligand or an artificial form (antibody) with the membrane receptor induces endocytosis of the macromolecule into an intracellular transport vesicle, which can then cross the endothelium lining of the BBB to be released into the brain (Jones and Shusta, 2007). The transferrin receptor (TfR) has been the most widely characterized RMT system for drug delivery, as it has proven to be an efficient cellular uptake pathway for anticancer drugs, while also being over-expressed in many tumors (Chang et al., 2009). Thus, much research has been focused on either attaching transferrin or an antibody against transferrin to one of the above nanocarriers to facilitate the transport of large drug molecules (Chen and Liu, 2012).

Transferrin-coated PLGA nanoparticles caused a 20-fold increase in the targeting of an in-vitro BBB model as compared to non-coated particles, as reported by Chang et al. (2009). It was confirmed via fluorescence microscopy that the particles entered by means of endocytosis. Similarly, lactoferrin, a protein of the transferrin family, also induced uptake in an in-vitro and in-vivo model, when attached to PEG-PLA nanoparticles (Xie et al., 2011). A major disadvantage of conjugating such proteins to the surface of particles is the consequent competition with endogenous transferrin for binding of the receptor, thus either inhibiting the brain's natural uptake of transferrin or discrediting the effectiveness of the particle (Georgieva et al., 2014).

The alternative to endogenous transferrin is the antibody that binds to an epitope located separate from the transferrin receptor. The nanoparticle would thus experience less competition from endogenous transferrin for receptor binding sites and also would not interfere with the brain's natural mechanism for transferrin intake (Chen and Liu, 2012). These receptor-specific MAbs are commonly known as molecular Trojan horses as they help to ferry large molecules across the BBB. Pardridge et al. have done extensive review on the details of this mechanism and the monoclonal antibodies that have proved effective (Pardridge, 2007a,b, 2003; Zhang and Pardridge, 2006). Briefly, murine OX26 is a common MAb that has been shown in many studies to induce RMT in rats, delivering molecules such as the anticaspase peptide

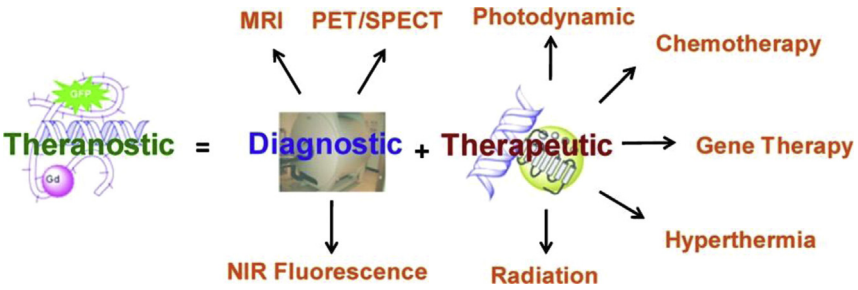

Fig. 6. Theranostics: combining imaging and therapy.
Reprint from Kelkar and Reineke, 2011 with permission

(Aktaş et al., 2005), vasoactive intestinal peptide analog (Wu and Pardridge, 1996), and human basic fibroblast growth factor (Wu et al., 2002). Other mAbs targeting the insulin, LDL, and diphtheria toxin receptors are described in more detail by Jones and Shusta (2007)

A unique receptor protein that has shown promising results is melanotransferrin (p97), which is an iron-binding protein that shows a 14-fold enrichment in the brain when compared to transferrin. It is preferentially transported from the blood into the brain tissue and has a lower plasma concentration of the endogenous protein, thus making it a more favorable target than transferrin. When covalently linked to chemotherapy drugs like paclitaxel, the total accumulation of the complex in the brain was 10 fold higher than that of the free drug (Karkan et al., 2008).

Currently, RMT has shown much promise for the successful delivery of anticancer compounds to brain tumors. Multiple studies have reported an upregulation in expression of the transferrin receptor on metastatic and drug resistant tumors and a diphtheria toxin mutant covalently bound to transferrin (Tf-CRM107) is now being tested in human clinical trials for the treatment of glioblastoma (Tortorella and Karagiannis, 2014).

11.3. Absorptive-mediated transport (AMT)

The last of the transport routes explored for BBB drug delivery is the AMT system, which relies on the electrostatic interaction between a positively charged moiety and the negatively charged endothelial cell membrane (Béduneau et al., 2007). Originally, its application was isolated to cationized albumin nanoparticles as it was demonstrated by researchers such as Pardridge et al. that these particles were capable of delivering drugs and peptides to the cerebral parenchyma (Lu et al., 2007; Xu et al., 2009; Eisenberg and Pardridge, 1987).

Later, the concept of cell-penetrating peptides (CPPs) developed as positively charged peptides of length less than 30 amino acids that were able to penetrate cell membranes via AMT (Chen and Liu, 2012). One of the more prominent examples is the HIV-1 *trans*-activating transcriptor (TAT) peptide. When TAT-derived CPPs bind to the surface of the cell, they induce macropinocytosis, allowing unusually large molecules, including full-length fusion proteins (Schwarze et al., 1999), to make their way across the BBB. In a study by Liu et al., nanoparticles with a hydrophobic core containing the antibiotic ciprofloxacin and a hydrophilic shell of PEG with TAT molecules, crossed the BBB and was found in the cytoplasm of neurons, making it possible to decrease levels of brain infection (Liu et al., 2008).

However, as AMT only relies on electrostatic attraction, the lack of tissue specificity poses a challenge for both limiting drug concentration in non-target organs and achieving the desired therapeutic drug level in the brain. These issues have to be assessed before AMT can be implemented clinically (Allhenn et al., 2012).

12. Applications: theranostic nanoparticles for imaging and therapy

One of the greatest advantages of nanomedicine is its multi-modality. A nanoparticle can be functionalized with multiple ligands and probes, conferring almost any property a researcher desires. This characteristic proves extremely advantageous in meeting the important clinical need of combining therapeutics with diagnostics, a phenomenon coined by Funkhouser in 2002 as 'theranostics' (Kelkar and Reineke, 2011) (Fig. 6). Using nanoparticles as theranostic agents have thus garnered much attention as a means to improve treatment of deadly diseases like cancer.

Currently, available imaging techniques such as CT and MR imaging provide poor visual contrast between gliomas and normal tissue (Wan et al., 2010), making it difficult to give an accurate pathological diagnosis. One study found that 11% of patients with a diagnosis of metastatic brain tumor via CT scanning and MR imaging turned out not to have a brain tumorwhen examined surgically (Patchell et al., 1990). Given the non-specificity of conventional CT and MR imaging, and the limitations of gadolinium-based MR imaging such as high clearance and the large amounts needed for visualization (Remsen et al., 1996), an imaging modality that is both spatially-specific and biologically viable will meet a critical clinical need in pre-operative diagnosis, intra-operative guidance, and post-operative tracking of chemotherapy and radiation efficacy.

Since nanoparticles can be engineered to target brain tumors when delivering drugs, their ability to aggregate in tumors at higher concentrations than normal tissue make them a promising candidate in improving the sensitivity of these imaging techniques. If administered to a patient suspected of a malignant glioma, the contrast enhancement provided by the nanoparticles during MR imaging can help to determine the type of tumor and its localization in the brain (Remsen et al., 1996).

Functionalizing the nano-platform to sequentially bind to various cancer markers can even allow the molecular mapping of the entire tumor surface (Sumer and Gao, 2008). If the tumor is malignant and surgery is needed, the nanoprobes will offer an intra-operative tool for increased brain tumor delineation, therefore improving the chances of surgical success (Kircher et al., 2003). Often, surgery is then followed by rounds of chemotherapy and radiation treatment. With theranostic nanoparticles, drug delivery can be monitored in-vivo, giving real-time feedback on how the drug is released and the effect it is having on the tumor. In addition, if it is discovered via in-vivo imaging that one of the molecular targets has become unavailable and thus rendered the treatment ineffective, imaging could be used to map out alternate targets (Sumer and Gao, 2008). Finally, these particles can modulate the radiosensitivity of CNS tumors and so promote cell necrosis when radiation therapy is given (Winer, 2011).

SPIONS have been investigated most extensively for their role in combining imaging with therapy. They have been used widely as MRI contrast agents due to their induced magnetism with an external magnetic field and biocompatibility (Wang et al., 2012). A more extensive review on their physical properties and application in biomedicine has been done by Pankhurst et al. (2003). The contrast agents have a core of Fe_3O_4 crystals surrounded by a shell of organic material (Orringer et al., 2009).

Kircher et al. (2003) created a nanoprobe which could function both in the presurgical planning phase and during surgical resection. It consisted of an iron oxide core conjugated to a near-infrared fluorophore, Cy5.5. Rats were injected with these particles and then were examined with MRI imaging as well as in an intraoperative setting.

Subsequently, Sun et al. (2008) attached a chemotherapeutic agent, methotrexate (MTX) to the Cy5.5 iron oxide nanoparticle conjugate, as well as the brain tumor targeting peptide, chlorotoxin (CTX) through a PEG linker. Enhanced MRI contrast was shown in tumor cells for at least 2 weeks, which has important consequences for clinical applications such as when there are time delays between administration and surgery. The NP-MTX-CTX conjugate also induced cytotoxicity in glioma and medulloblastoma cancer cell lines (Pankhurst et al., 2003). Jain et al. (2008) were able to incorporate the drugs doxorubicin and paclitaxel into SPIONS coated with oleic acid and stabilized with pluronic F-127, thus demonstrating synergistic antiproliferative activity of these particles in cancer cells.

An exciting development in the field of theranostics is the work done by Reddy et al. (2006). By using F3 targeted nanoparticles that have both an imaging agent and a photosensitizer (Photofrin), they were able to make brain tumors sensitive to laser light irradiation such that when cancer cells were incubated for 4 h with the nanoparticles and targeted with laser light for 5 min, 90% of the cells were killed (Fig. 7). Therefore, during surgical resection of the tumor, not only will the tumor delineation be clearer but one could possibly use laser light to kill cancer cells.

This technique called photodynamic therapy (PDT) is one of the latest in treating malignant gliomas, especially those located in areas that cannot be resected. The idea behind PDT is that photosensitizers preferentially accumulate in tumor cells and upon photoactivation, release the singlet oxygen and other reactive oxygen species which then induce cytotoxicity in cancer cells. An excellent review on the clinical trials of PDT for malignant brain tumors up to 2015 was conducted by Quirk et al. (2015) In one trial conducted in 2011, Lyons et al. combined PDT with intra-operative radiotherapy (IORT). They divided 73 patients with glioblastoma into four groups: standard therapy (ST), ST + PDT, ST + IORT and ST + PDT + IORT. It was observed that the overall mean survival for the combined PDT groups was 14.5 months which was a significant improvement from the 4.6 months of the ST alone group. The addition of IORT increased the survival time to 18.2 months from the 9.2 months of the ST + PDT group. Thus, it was the PDT that provided the statistically significant survival advantage; however, the addition of IORT may prove to be promising in the future (Lyons et al., 2012).

PDT is comparable to the therapies developed by Franchini et al. (2010) and Maier-Hauff et al. (2011). The bovine serum albumin and PLGA-coated magnetic nanoparticles synthesized by Franchini provided higher image contrast as compared to the commercial contrast agent, Endorem, but also resulted in a 82% cell death when a high-frequency magnetic field was applied due to the hyperthermic effect induced on the surrounding cancer cells (Franchini et al., 2010). Similarly, Maier-Hauff et al. (2011) were able to magnetically induce thermotherapy via SPIONS in 59 glioblastoma multiforme patients, followed by SPION-enhanced radiation therapy, demonstrating significant increase in median survival

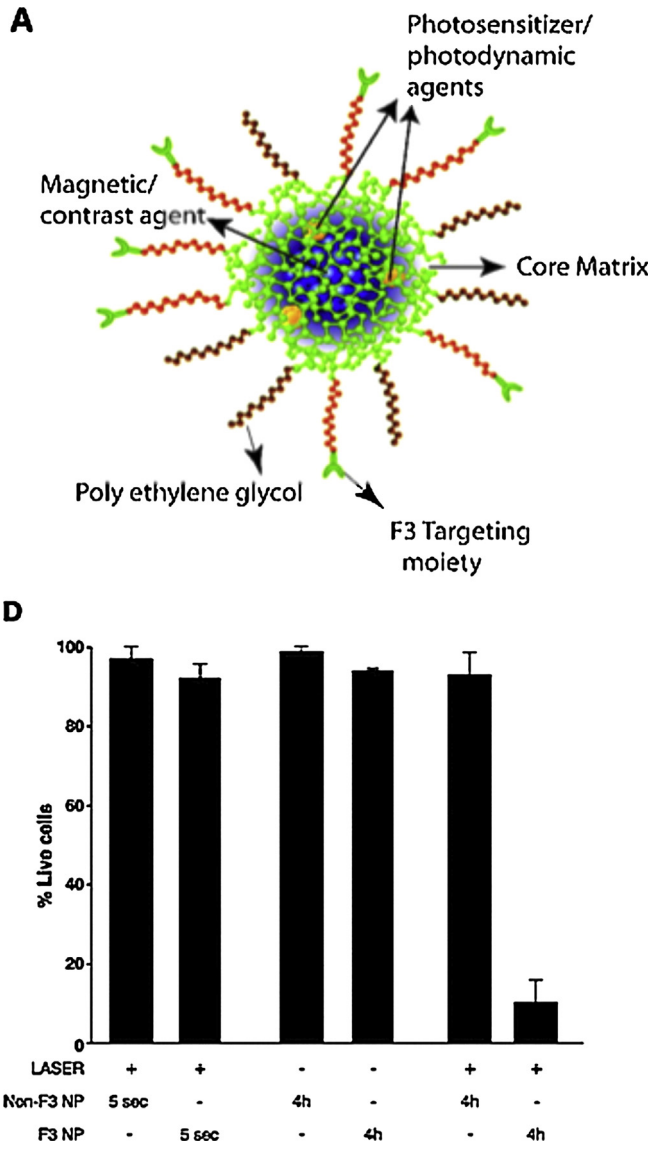

Fig. 7. The multimodal particle is functionalized with an F3 targeting moiety and Photofrin (a). Only when laser light is combined with incubation with the nanoparticles for 4 h does the cancer cell death reach 90% (b).
Adapted from Reddy et al. (2006) with permission

times from 6.2 to 13.4 months. Nanotherm is an example of intracranial thermotherapy currently on the market. Magnetic nanoparticles are injected directly into the tumor and then heated by an alternating magnetic field. The tumor cells are then either irreparably damaged or sensitized for additional chemotherapy or radiotherapy.

As for in vivo imaging and monitoring of drug delivery post-administratively, either the nanoprobes could be functionalized with magnetic and fluorescent modalities, or the drugs themselves could be engineered to exhibit these properties. In this way, one can track binding of the carrier to the tumor, possible clearance by the RES system, drug uptake, and overall efficacy of the treatment, making modifications in real-time if necessary. For example, Cheng et al. (2011) utilized a photodynamic drug, phthalocyanine, encapsulated in metallic NPs for in-vivo monitoring of the biodistribution of the carriers and the drug. Multimodal bioimaging that combined MR imaging with upconversion luminescence and CT imaging was demonstrated in Ho^{3+}-doped upconversion nanoparticles and found to induce high contrast of brain

tumor in both living cells and in vivo (Ho, 2014). Thus, in view of the anticipated move towards personalized medicine, the theranostic nanoparticle will likely be on the frontier of tailoring treatment to a patient's individual need. By intertwining therapy and imaging into one specifically-targeted non-invasive vehicle, patients will benefit from more accurate pre-operative diagnosis, clearer tumor delineation during intra-operative surgery, as well as monitored drug delivery and enhanced radiation therapy post-operatively.

13. Conclusions

Nanomedicine is clearly a powerful and versatile platform for delivering drugs to the CNS. As research into this area continues to progress, the wide variety of tools available for clinical researchers will diversify beyond the types of nanocarriers and targeting mechanisms described in this review. As the invasive methods described in the beginning of the review lose traction in effectively managing the anticipated rise in CNS diseases, we surmise that nanomedicine will give researchers new insight into innovative ways to non-invasively cross the BBB and deliver drugs to targeted pathological tissues. Furthermore, the newly developed field of theranostics will provide necessary answers to how we can better adapt therapies to the individual, a concept we believe will revolutionize medicine in the decades to come.

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Author's contributions

Vivienne Tam: Collected and analyzed literature, wrote the manuscript.

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Parts of this work has been included in Vivienne Tam's undergraduate senior thesis and has not been published elsewhere.

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