Nano Carriers for Drug Transport across the Blood Brain Barrier

Xinming Li[1,2], John Tsibouklis[2], Tingting Weng[3], Buning Zhang[1], Guoqiang Yin[1], Guangzhu Feng[1], Yingde Cui[1], Irina N Savina[4], Lyuba I Mikhalovska[4], Susan R Sandeman[4], Carol A Howel[4], Sergey V Mikhalovsky[4,5]

1, School of Chemistry and Chemical Engineering, Zhongkai University of Agriculture and Engineering, Guangzhou 510225, China

2, School of Pharmacy and Biomedical Sciences, University of Portsmouth, White Swan Road, Portsmouth PO1 2DT, UK

3, Department of Chemical Engineering, Guangdong Petroleum and Chemical Technology Institute, Foshan 528225, China

4, School of Pharmacy and Biomolecular Science, University of Brighton, Lewes Road, Brighton BN2 4GJ, UK

5, School of Engineering, Nazarbayev Uiversity, Kabanbay Batyr Avenue, Astana, 010000, Kazakhstan

Correspondence to:

Xinming Li, lixinming@sina.com

Corresponding Address: School of Chemistry and Chemical Engineering, Zhongkai University of Agriculture and Engineering, No.24 Dongsha Street, Haizhu District, Guangzhou 510225, Guangdong, PR China

Tel: +86-20-8901 3955

Fax: +86-20-3417 2865

Abstract:

Effective therapy lies in achieving a therapeutic amount of drug to the proper site in the body and then maintaining the desired drug concentration for a sufficient time interval to be clinically effective for treatment. The blood brain barrier (BBB) hinders most drugs from entering the central nervous system (CNS) from the blood stream, leading to the difficulty of delivering drugsto the brain via the circulatory system for the treatment, diagnosis and prevention of brain diseases. Several brain drug delivery approaches have been developed such as intracerebral and intracerebroventricular administration, intranasal delivery, and blood-to-brain delivery as a result of transient BBB disruption induced by biological, chemical or physical stimuli such as zonula occludens toxin, mannitol, magnetic heating and ultrasound, but these approaches showed disadvantages of being dangerous, high cost as well as unsuitability for most brain diseases and drugs. The strategy of vector-mediated blood-to-brain delivery which involves improving BBB permeability of the drug-carrier conjugate can minimize such side effects; being submicrometre objects that behave as a whole unit in terms of their transport and properties, nanomaterials are promising carrier vehicles for direct drug transport across the intact BBB as a result of their potential to enter the brain capillary endothelial cells by means of normal endocytosis and transcytosis due to their small size, as well as their possibility of being functionalized with multiple copies of the drug molecule of interest. This review provids a concise discussion of nano carriers for drug transport across the intact BBB, various forms of nanomaterials including inorganic/solid lipid/polymeric nanoparticles, nanoemulsions, quantum dots, nanogels, liposomes, micelles, dendrimers, polymersomes and exosomes are critically evaluated, their mechanisms for drug transport across the BBB are reviewed, and the future directions of this area are fully discussed.

Keywords: Nanomaterials; Drug Delivery; Blood Brain Barrier (BBB)

Contents:

1. Introduction

The increasing incidence of cerebral diseases as a result of the worldwide aging population

demands the urgent development of therapeutic, diagnostic, and preventive agents. Whilst brain drug development has been limited, owing to the restrictive transport properties of the blood brain barrier (BBB), which is a unique regulatory system of brain capillaries that protects the brain environment by preventing most molecules in the blood stream from entering the central nervous system [1-6]. Thus, unlike other organs in the human body, more than 98% of small molecules and nearly 100% of large therapeutic molecules cannot reach brain via the circulatory system [7].

One strategy for brain drug delivery is bypassing the BBB using highly invasive methods such as intracerebral and intracerebroventricular administration [8-9], or non-invasive methods such as intranasal delivery. Another strategy is blood-to-brain delivery via circulatory system as a result of transient BBB disruption induced by biological, chemical or physical stimuli such as zonula occludens toxin, mannitol, magnetic heating and ultrasound [10-13]. These brain drug delivery strategies have not been widely used because they are risky, costly, or unsuitable for less localized brain diseases [14].

The strategy of blood-to-brain delivery which involves improving BBB permeability of drugs or drug-carrier conjugates under normal conditions can minimize the above mentioned side effects. One approach is to modify the chemical structure of drugs; so far only one drug has been successful: the conversion of morphine to heroin [15-16]. By coupling drugs with various brain nutrients or growth factors, such nutrients/growth factors-mediated delivery showed another promising approach to improve BBB permeability of brain drugs, as specific transporter proteins for brain nutrients/growth factors on the membrane of brain capillary endothelial cells can also facilitate the transfer of some nutrient/growth factor-drug conjugates by means of transcytosis [17-18]. Unfortunately, this approach is also of little value because only a small number of drugs can be converted to nutrient/growth factor-drug conjugates.

Nanomaterials are submicrometre objects that behave as a whole unit in terms of their transport and properties; it has been found that nanomaterials can internalize into most cells (including brain capillary endothelial cells) by means of normal endocytosis and transcytosis due to “nano effects” as a result of their small size [19]. Consider their possibility of being functionalized with multiple copies of the drug molecule of interest, widespread drug delivery to the brain via the transvascular route through the intact BBB by using nanomaterials as a carrier vehicle which can be dubbed ‘Molecular Trojan Horse’ is possible [20-23]. Nanomaterials have been widely investigated for

brain drug delivery; in this review paper we discuss: various forms of nanomaterials for blood-to-brain drug delivery through the intact BBB; the mechanisms of nanomaterials-mediated drug transport across the BBB; and the future directions of this innovative area of research.

2. Nano carriers for drug transport across BBB

Depending on the method of uploading, therapeutic, diagnostic or preventive molecules can be dissolved, entrapped, adsorbed, encapsulated or covalently attached to nanomaterials, to give nano sized drug-carrier conjugates in the forms of nanoparticles, liposomes, micelles, nanogels, nanoemulsions, quantum dots, dendrimers, exosomes, and polymersomes. These objects are in the nanometer (1-1000nm) size range differing markedly from items made of identical materials in the terms of properties and functions. With the minimal diameter of blood capillaries being 6-9 micrometers, nano sized drug-carrier conjugates hold the capability to reach organs via bloodstream. The average size of cells in the human body is 10-20 micrometers; thus, adsorption or uptake of the nano sized drug-carrier conjugates by cells is possible, providing an opportunity to deliver drugs into cells. With the possibility of being surfacely functionalized with targeting ligands, nano carriers offer the capability of transporting drugs across the BBB.

2.1 Inorganic nanoparticles

Nanoparticles from inorganic materials such as silica, carbon, metal or metal oxide are solid nano sized particulate objects which have been widely used in imaging techniques [24]. Inorganic nanoparticles are of stable size and form mono-disperse suspensions in body fluids; their surfaces can be functionalized to facilitate BBB penetration. With high surface area, large pore volume, good biocompatibility and ease of functionalization, mesoporous silica nanoparticles have been conjugated with poly(ethylene glycol) (PEG) for BBB penetration as a result of their improved hydrophilicity, the eliminated aggregation in the blood stream, as well as the minimized clearance by the reticuloendothelial system (RES) [25]. The fluorescein-doped magnetic silica nanoparticles (FMSNs) incorporating therapeutic molecules have been covalently conjugated with the second generation (G2) PAMAM (polyamidoamine) dendrimers through 3-(triethoxysilyl) propyl isocyanate (ICP) to yield PFMSNs, followed by the reaction with tresylated MPEG (Methoxypolyethylene glycol)-5000 to yield PEGylated PFMSNs. It has been found that PEGylated PFMSNs could penetrate the intact BBB through transcytosis of vascular endothelial

cells, with subsequent diffusion into the cerebral parenchyma and distribution in the neurons. In contrast, non-PEGylated FMSNs were not found to cross the BBB, which showed PEG modification on the surface of the silica nanoparticles may offer an opportunity to cross the BBB.

Just like PEG-modified silica nanoparticles, PEG-modified carbon nanotubes (CNTs) also exhibited the capability of crossing the BBB. CNTs have ultrahigh surface area that permits efficient loading of multiple molecules alongside the nanotube wall, and the supramolecular binding of aromatic molecules such as doxorubicin (DOX) can be easily achieved by p-p stacking of those molecules onto the polyaromatic surface of nanotubes. Oxidized multi-walled carbon nanotubes (O-MWNTs) uploaded with DOX have been conjugated with PEG and angiopep-2; the results of intracellular tracking *in vitro* and fluorescence imaging *in vivo* demonstrated that the combination of O-MWNTs-PEG and angiopep-2 constituted an ideal dual targeting drug delivery system for both BBB and glioma cells [26].

For some inorganic nanoparticles, lactoferrin (Lf) conjugation showed better efficiency in BBB penetration than PEG modification as a result of Lf-receptor-mediated transcytosis of cerebral endothelial cells. It has been found that the PEG coating favors the transfer of the underlying Fe_3O_4 nanoparticles across the intact BBB model, while Lf-conjugated Fe_3O_4 nanoparticles exhibited an enhanced ability to cross the BBB in comparison to the PEG-coated Fe_3O_4 nanoparticles [27]. Free cationic serum albumin can enter the brain via an adsorptive-transcytosis mechanism; and likewise it has shown that magnetic nanoparticles of $MnFe_2O_4$ surfacely coated with crosslinked serum albumin demonstrated the capability of BBB permeability without any breakdown [28]. Other surface coatings, such as poly(isobutylene-alt-1-tetradecene-maleic anhydride) (PMA), also facilitate BBB penetration of iron oxide nanoparticles uploaded with therapeutic molecules [29].

In addition to chemical modification, physical approaches such as magnetism can also be used for the enhancement of BBB penetration of Fe_3O_4 nanoparticles because of their capacity to be magnetized in the presence of a magnetic field. It has been found that superparamagnetic iron oxide nanoparticles (SPIONs) composed of magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) can pass through human brain microvascular endothelial cells facilitated by an external magnet, which showed the magnetic force-mediated dragging of SPIONs through the BBB may denote a novel

mechanism for the delivery of drugs to the brain [30].

Other inorganic nanoparticles, such as gold nanoparticles (GNPs), have also been investigated for drug transport across the intact BBB [31]. Although all research results showed inorganic nanoparticles are promising carrier vehicles to deliver drugs into brain, the issues of potential toxicity as a result of non-degradability and drug delivery efficiency still need to be addressed [32].

2.2 Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) are nano sized dispersions of biocompatible lipids such as triglycerides, fatty acids, or waxes, stabilized by surfactants possessing HLB (Hydrophile-Lipophile Balance) values lower than 12 [33-34]. With drugs that can be dissolved or dispersed in the hydrophobic core, such nano carriers possess the capability to deliver drugs into cells. A drug-loaded SLNs suspension consisted of 0.1% (w/w) camptothecin (the model drug), 2.0% (w/w) stearic acid, 1.5% (w/w) soybean lecithin and 0.5% (w/w) polyoxyethylene-polyoxypropylene copolymer (Poloxamer 188) with the average diameter of 196.8 nm and the Zeta potential of 269.3 mV exhibited higher drug concentration in the brain, heart and reticuloendothelial cells-containing organs after intravenous injection in comparison to free camptothecin, which indicated that it was possible for the SLNs to cross the BBB [35]. SLNs made from stearic acid stabilized with pluronic®F68 showed the possibility to effectively deliver atazanavir to human brain endothelial cells *in vitro* [36]; *In vivo* test in rats also showed that riluzole-uploaded SLNs exhibited a higher capability to carry the drug into the brain [37].

Surface modification of SLNs with targeting ligands could be an effective strategy for BBB delivery. Docetaxel-loaded SLNs have been prepared from monostearin, vitamin E and soya lecithin, followed by surface modification with stearylamine-betreliesoxybutyric acid (HBA, a ketone body and substrate for monocarboxylic acid transporter which is expressed on BBB) conjugate, for drug transport across the intact BBB [38]. The surface modification of docetaxel loaded SLNs with HBA resulted in enhanced brain uptake of docetaxel compared with un-modified docetaxel loaded SLNs and Taxotere®, which showed HBA present on the surface of SLNs caused the nanoparticles to be taken up by the monocarboxylic acid transporter as normal HBA and improved the brain uptake of docetaxel compared with un-modified SLNs. Similarly, Transferrin (Tf)-conjugated, quinine dihydrochloride-uploaded SLNs made from hydrogenated

soya phosphatidyl choline (HSPC), triolein, distearylphosphatidylethanolamine (DSPE) and cholesterol revealed enhanced uptake in brain tissue compared with un-conjugated SLNs [39]; it has also been demonstrated that poly(ethylene glycol)-grafted SLNs made from stearate using egg phosphatidyl choline and sodium glycocholate as surfactants uploaded with noscapine can improve drug accumulation in mice brain tissue [40].

Acting as a carrier vehicle for drug delivery, SLNs have shown the advantages of good biocompatibility, degradability as well as the capacity to be surfacely functionalized for brain targeting, while the issue of easy clearance by the RES as a result of their hydrophobicity needs to be addressed.

2.3 Polymeric nanoparticles

Polymeric nanoparticles are solid carrier vehicles ranging from 1 to 1,000 nm in diameter made from natural or synthetic polymers. According to the drug loading methods, therapeutic molecules-uploaded polymeric nanoparticles can form nanospheres (drugs are uploaded throughout or just onto the surface of the polymeric matrix) or nanocapsules (drugs are surrounded by a polymeric shell) which are thermodynamically stable [41]. As a result of the diversity of polymers, polymeric nanoparticles can be designed to achieve desired properties such as controlled and/or sustained drug release profile, as well as allowing drug release at the targeted site over a period of time [42]. For this reason, a variety of polymers have been investigated to form nanoparticles for brain drug delivery. *β*-cyclodextrin, which is composed of cyclic oligosaccharides with seven glucose unites that form a hydrophobic central cavity with the capacity to host therapeutic molecules, has been quarternized for the preparation of a series of quaternary ammonium *β*-cyclodextrin (QA-CD) nanoparticles. Uploaded with doxorubicin (DOX), QA-CD nanoparticles showed permeability across the *in vitro* BBB by means of endocytosis [43]. DOX-uploaded nanoparticles of *β*-cyclodextrin crosslinked with β-amino ester also showed biocompatibility and permeability in an *in vitro* BBB model without impairing the integrity of the barrier [44]. Another synthetic polymer, namely poly(MePEG2000cyanoacrylate-co-hexadecylcyanoacrylate) (PEG-PHDCA), demonstrated the capacity of their nanoparticles to reach the rat central nervous system after intravenous injection [45]. In contrast, polyhexadecylcyanoacrylate (PHDCA) nanoparticles did not penetrate across the BBB as a result of less adsorbed apolipoprotein E (ApoE) as well as apolipoprotein B-100

(ApoB-100) than PEG-PHDCA nanoparticles, which demonstrated that the involvement of apolipoproteins played an important role in the brain transport of PEG-PHDCA nanoparticles [46].

It has been found that some surfactants can enhance the permeability of polymeric nanoparticles across the BBB; poly(butylcyanoacrylate) (PBCA) nanoparticles coated with polysorbate 80 have been successfully used to transport drugs such as leu-enkephalin dalargin, met-enkephalin kyotorphin, rivastigmine and tacrine through the BBB after intravenous injection [47-51]. PBCA is a biodegradable and biocompatible polymer; PBCA nanoparticles showed hardly any cytotoxic or inflammatory effect at therapeutic concentrations and incubation times, and they did not induce nonspecific BBB disruption, but collaborate with plasma apolipoprotein E to facilitate BBB crossing [52-53].

Poly(lactic-co-glycolic acid) (PLGA) is a polymer with biodegradable and biocompatible nature which has been approved by the US FDA for human use. Research has shown that PLGA nanoparticle-delivered dopamine reduced its autoxidation-mediated toxicity in the brain, and reversed neurochemical and neurobehavioral deficits in parkinsonian rats [54]. When uploaded with a protein drug (tissue inhibitor of matrix metalloproteinases 1, TIMP-1), PLGA nanoparticles coated with polysorbate 80 had 11.21%±1.35% penetration, whereas TIMP-1 alone and PLGA nanoparticles without polysorbate 80 coating did not cross the endothelial monolayer. Due to no significant opening of the BBB caused by un-coated as well as polysorbate 80 coated PLGA nanoparticles, polysorbate 80 coating can be used to enhance protein delivery of PLGA nanoparticles across the BBB [55].

In addition to coating with surfactants, surface functionalization with certain peptides can also facilitate BBB penetration of polymeric nanoparticles. Surface-modified PLGA nanoparticles with octa-arginine (R8) showed a faster cell uptake compared with the control [56], while the fluorescent aminated polystyrene (PS) nanoparticles surfacely functionalized with gH625 (a viral fusion peptide derived from the glycoprotein gH of Herpes simplex virus type I) via a covalent binding procedure showed a greater uptake by brain endothelial cells than that of the PS nanoparticles without the peptide [57]. Modification with a motif of TGNYKALHPHNG (TGN) could facilitate BBB penetration of poly(ethylene glycol)-poly(DL-lactide-co-glycolide) (PEG-PLGA) nanoparticles, leading to significant higher cellular uptake and *in vivo* brain

accumulation [58].

As a result of receptor-mediated endocytosis being one of the mechanisms through which nano carriers cross the BBB, surface functionalization with targeting ligands represents another approach to facilitate BBB penetration of polymeric nanoparticles. Angiopep, a ligand targeting the low-density lipoprotein receptor-related protein (LRP) which is over-expressed on the BBB and glioma cells, has been conjugated with poly(ethylene glycol)-poly(ε-caprolactone) (PEG-PCL) nanoparticles [59]. Compared with non-targeting nanoparticles, a significantly higher amount of rhodamine isothiocyanate-labeled targeting nanoparticles were endocytosed by U87 MG cells. Tf conjugated nanoparticles of Poly(lactide)-D-α-Tocopheryl polyethylene glycol succinate (PLA-TPGS) diblock copolymer showed more efficiency than the bare PLA-TPGS nanoparticles in brain delivery [60], while human serum albumin (HSA) nanoparticles covalently coupled with Tf or transferrin receptor monoclonal antibodies (OX26 or R17217) also demonstrated the capacity to transport drugs across the intact BBB [61]. Lf, a mammalian cationic iron-binding glycoprotein belonging to the transferring family, consists of a polypeptide chain of about 690 amino acids folded into two globular lobes, when conjugated with poly(ethylene glycol)-poly(lactide) nanoparticles, significantly facilitates cell uptake of the nanoparticles [62]. After intravenous administration, near 3 folds of coumarin-6 were found in the mice brain carried by Lf-conjugated nanoparticles compared to that carried by the bare nanoparticles.

Campared with synthetic polymers, nanoparticles based on natural polymers showed advantages of low cost, less toxicity and biodegradability, which provide a potential candidate for brain drug delivery. A pluronic-based nano-carrier conjugated with both chitosan and a specific target peptide for brain (rabies virus glycoprotein; RVG29) was effective for brain targeting across the intact BBB [63]. Chitosan nanospheres conjugated with an anti-mouse transferrin receptor monoclonal antibody (TfRMAb) that selectively recognizes the TfR type 1 on the cerebral vasculature clearly demonstrated the effectiveness of bringing active peptides to the brain [64]. Chitosan nanospheres conjugated with poly(ethylene glycol) (PEG) bearing the OX26 monoclonal antibody whose affinity for the transferrin receptor (TfR) may trigger receptor-mediated transport across the BBB successfully translocated into the brain tissue after intravenous administration [65].

Due to hydrophobic molecules exhibiting better permeability in BBB penetration than hydrophilic

ones, chitosan has been chemically modified to improve hydrophobicity for permeability improvement of the nanoparticles in BBB penetration. Trimethylated chitosan (TMC) has been synthesized and covalently coupled to the surface of PLGA nanoparticles (PLGA-NP) via a carbodiimide-mediated link, the resulted nanoparticles exhibited negligible cytotoxicity and enhanced brain uptake following intravenous administration compared with PLGA nanoparticles without TMC conjugation [66]. A series of O-substituted alkylglyceryl chitosans with systematically varied alkyl chain length and degree of grafting has been prepared through synthetic steps that involved the protection of amino moieties via phthaloylation and employed for the formulation of aqueous nanoparticulate systems. During *in vitro* tests using a mouse-brain endothelial cell model, the efficient cellular uptake of these nanoparticles has demonstrated and identified butylglyceryl chitosan and butylglyceryl N,N,N-trimethyl chitosan as promising materials for the formulation of nanoparticles that could act as drug carriers into the brain [67].

With the possibility of achieving designed and tailored properties, polymeric nanoparticles have significant potential for brain drug delivery across the BBB. Biodegradable polymers exhibit the advantage of less accumulation in the body; however, before translation into clinical use, a good knowledge of degradation rates as well as catabolites, toxicity, and the possibility of causing adverse immunological responses is necessary.

2.4 Nanogels

Gels are physically or chemically crosslinked 3-dimensional polymeric networks that swell in certain liquids with compatible interfacial energies, and the swollen networks can retain a large amount of absorbed liquids [68]. Both aqueous and organic liquids can be supporting media of polymeric gels, leading to the formation of hydrogels and organic gels, respectively [69]. With good biocompatibility as a result of their relatively high water content, as well as the possibility of pharmaceutical molecules to diffuse into (drug loading) and out of (drug release) the swollen polymeric networks, hydrogels have been widely investigated for drug delivery [70-71].

Nanogels are nanoparticles of hydrogels and offer the prospect of drug transport across the intact BBB. It has been reported that nanogels with surface charge exhibited better internalization property on cell membrane than neutral ones; Gil and Lowe synthesized polysaccharide-based nanogels containing poly(β-amino ester) and β-cyclodextrin for transporting doxorubicin and insulin across the BBB, such cationic nanogels enhanced the permeability of insulin across the *in*

vitro BBB model by 20% [72].

It is known that lipophilic molecules are easier to penetrate the BBB than hydrophilic ones; so surface functionalization of nanogels has been introduced to accelerate encapsulated drug transport across the BBB. Azadia and co-workers prepared nanogels loaded with methotrexate (MTX) via an ionic gelation process using chitosan and sodium tripolyphosphate (TPP) as raw materials, the surfaces of the MTX-loaded nanogels were modified with polysorbate 80 to improve brain drug delivery [73]. The cumulative in vitro release profiles indicated non-Fickian diffusion kinetic, apparently governed by both diffusion of the drug out of the nanogels and swelling/disintegration of the polymeric networks for both surfactant coated and uncoated nanogels. After intravenous administration, remarkably higher brain concentrations of methotrexate were achieved with the nanogel formulations in comparison to the free drug, but there were no significant differences between the surface-coated and uncoated nanogels [74].

Two proteins, connexin 43 (Cx43) and brain-specific anion transporter 1 (BSAT1), which are promising targeted antigens for drug delivery to gliomas, have been respectively conjugated with the cisplatin-loaded nanogels prepared from aqueous solutions of poly(ethylene glycol) (PEG)-block-poly(methyl methylacrylate) (PMAA) and $CaCl_2$ using 1,2-ethylenediamine and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) as crosslinkers, for the treatment of intracranial gliomas [75]. The median survival of rats treated with targeted nanogels conjugated with specific mAbs against extracellular loops of Cx43 and BSAT1 were 27 and 26.6 days longer than that in control group, respectively, which suggested the effectiveness of the ligands in promoting BBB-penetrating efficiency of the nanogels. Conjugation of insulin and Tf with the nanogels of crosslinked poly(ethylene glycol) and poly(ethylenimine) for the delivery of oligonucleotide in brain also showed similar results [76]. Being hydrophilic carriers, nanogels are favorable in uploading aqueously soluble drugs as well as proteins and nuclear acids, while it is unsuitable for hydrophobic drugs.

2.5 Micelles

Micelles are made from amphiphilic block copolymers which aggregate in aqueous solutions to form stable spheroidal nano structures with a hydrophobic core and hydrophilic surface [77]. As a result of the possibility of solubilizing poorly water-soluble, lipophilic compounds in the hydrophobic core region through hydrophobic interaction and/or hydrogen bonding for easy

administration, as well as the capacity to conjugate with certain targeting ligands, micelles are promising delivery vehicles for brain drugs.

A polymeric surfactant, poly(oxyethylene)-poly(oxypropylene) block copolymer (pluronic), has been used for the preparation of micelles [78]. Fluorescein isotbiocyanate (FITC) was solubilized in the micelles; it has been found that conjugation of FITC-containing micelles with insulin vector resulted in increase of FITC penetration in all tissues including the brain. By conjugating the micelles with antibodies to the antigen of brain glial cells (α_2-glycoprotein), the specific targeting of the solubilized FITC was observed in the brain, which showed that vector-containing pluronic micelles provide an effective transport of solubilized neuroleptics across the BBB.

TAT (49-57, YGRKKRRQRRR) peptide, the protein transduction domain from the transcriptional activator TAT protein of the human immunodeficiency virus type-1, has been anchored with cholesterol-terminated poly(ethylene glycol) (PEG) with a molecular weight of about 3 kDa (PEG-b-Chol), to form a functionalized TAT-PEG-b-Chol amphiphilic block copolymer [79]. FITC-loaded polymeric micelles self-assembled from the block copolymer exhibited the possibility of crossing the BBB and entering the brain, which showed that the TAT-conjugated micelles may in the future be used to deliver antibiotics across the BBB for the treatment of brain infections.

Several amphiphilic block copolymers have been synthesized and used for preparation of micelles; and several targeting ligands have been conjugated for the improved crossing of the intact BBB, such as poly(ethylene glycol)-block-poly-(L-glutamic acid), poly(ethylene glycol)-block-poly(D,L-lactide acid), cholesterol conjugated polyoxyethylene sorbitol oleate (CPSO), cyclic Arg-Gly-Asp (cRGD) ligand, and angiopep (a family of 19 amino acid peptides derived from the Kunitz domain) [80-82]. However, the efficiency of micelles delivering drugs across the intact BBB still needs further investigation.

2.6 Liposomes

Liposomes are nano sized vesicles with an aqueous inner core enclosed by unilamellar or multilamellar phospholipid bilayers. The common constituents that form part of phospholipid bilayer are naturally produced sphingomyelin, phosphatidylcholine, or glycerophospholipids [84]. With good biocompatibility, as well as the possibility of uploading drugs in the aqueous core, liposomes have been widely investigated for systemic delivery of therapeutics [85].

Several issues have impeded the drug delivery application of conventional liposomes; one is opsonization and rapid clearance by macrophages of the mononuclear phagocytic system (MPS) organs. By using surface coatings such as poly(ethylene glycol) (PEGylated liposomes), the circulation time of liposomes in the body can be prolonged [86]. Another is their low drug transport rate. By conjugating with targeting ligands, liposomes showed the possibility to transport drugs to certain organs including the brain. For example, the lipid bilayer of a 3/7 cholesterol/dipalmitoylphosphatidylcholine mixture was coated with n-alkyldimethylammoniumcyclodextrins which host adamantoylglucose molecules as a result of the adamantoyl moieties being included in the cyclodextrin cavities; such liposomes showed a 5-fold improved ability to enter the BBB endothelial cells compared with the non-coated samples [87]. The glucose moiety of the ligand provided special affinity of the liposome with BBB endothelial cells, leading to an improvement of the transport rate. Doxorubincin liposomes conjugated with both folate and transferrin (Tf) also showed effectiveness in penetrating the BBB and targeting brain tumors [88].

Cationization of the conjugated ligands is another method to improve BBB transport rate of liposomes. Liposomes made from cholest-5-en-3-ol-(3β)-[2-[[4-[(carboxymethyl) dithio]-1-iminobutyl]amino]ethyl] carbamate (CHETA5), a cholesterol derivative with a disulfide bond inside, were neutral or negatively charged at physiological pH. When they touched brain capillary endothelial cells with the help of a brain-targeting ligand, Lf, they were changed into cationic liposomes [89]. Such liposomes showed an improved performance in the uptake efficiency and cytotoxicity of the primary brain capillary endothelial cells. Liposomes conjugated with cationized bovine serum albumin showed similar results compared with sterically stabilized (PEGylated) liposomes without protein as well as liposomes conjugated with native bovine serum albumin [90].

Other issues limiting the brain delivery of liposomes are the poor stability, as well as the difficulty of binding ligands to the surface as a result of the small number of available surface groups and steric hindrance.

2.7 Dendrimers

Dendrimers are monodisperse symmetric macromolecules that comprise a series of branching units around an inner core [91]. With many reactive groups on the surface, the number of arms

and surface groups exponentially increase with each generation [92].

Being composed of repetitive units of branched molecules, dendrimers form a 3-dimensional spheroidal shape and radially crowded layers. As a result, the core is loosely packed in comparison to the periphery and is suitable for the entrapment of drugs. In addition, the presence of numerous surface groups allows for high drug payload and/or multifunctionality. Combined with their nanometer size range, dendrimers are attractive carrier vehicles for drug delivery [93].

The surface groups of dendrimers can be conjugated with ligands for transport across the BBB, as well as for targeting specific cells such as tumor cells. Thus, dendrimers are promising tailorable delivery systems for improved delivery of drugs to the brain. Li and co-workers have synthesized the fourth generation poly(amidoamine) (PAMAM) dendrimers with transferrin (Tf) conjugated on the exterior and tamoxifen (TAM) encapsulated in the interior [94]. It has been found that each dendrimer is a pH-sensitive drug carrier that can encapsulate 29 TAM molecules in the interior, and bond 7 doxorubicine (DOX) molecules, over 30 PEG1000 and PEG2000 chains and one Tf group on the periphery. The drug-dendrimer conjugate showed a fast drug release profile at weak acidic condition and a stable state at physiological environment, as well as a good BBB transportation ability with the transporting ratio of 6.06% in 3h. Angiopep, a ligand targeting to the low-density lipoprotein receptor-related protein-1 (LRP1), has been conjugated with PAMAM dendrimers for effective brain-targeting gene delivery [95]. Such a nano carrier was observed to be internalized by brain capillary endothelial cells (BCECs) through a clathrin- and caveolae-mediated energy-depending endocytosis, also partly through marcopinocytosis. Compared with the dendrimers without modification, angiopep-modified dendrimers showed higher efficiency in crossing the intact BBB, and more *in vivo* accumulation in the brain. Other research results also showed the capacity of targeting ligand conjugated dendrimers to cross the intact BBB [96-97].

Before translation of dendrimers into brain drug delivery use, it is necessary to address their biocompatibility issue. For example, PAMAM dendrimers have been shown to be haemolytic and cytotoxic [98]. Some research results also showed that biotinylated PAMAM dendrimers may prove to be more toxic compared to PAMAM dendrimers alone [99].

2.8 Nanoemulsions

Nanoemulsions are heterogenous dispersions of oil-in-water (O/W) or water-in-oil (W/O)

formulations stabilized with surface-active agents, where diameter of the inner phase is reduced to nanometer length scale [100]. The possibility of surface functionalization for targeting, as well as the ability to solubilize hydrophobic (O/W nanoemulsions) or hydrophilic (W/O nanoemulsions) drugs, could facilitate the uptake of nanoemulsions along with its encapsulated drugs through receptor-mediated endocytosis of cells, which makes nanoemulsions are promising carrier vehicles for drug transport across the BBB.

For biocompatibility purpose, nanoemulsions are usually made from edible oils such as flaxseed oil, pine-nut oil, hemp oil, fish oil as well as safflower oil and wheat-germ oil, biocompatible surfactants such as egg phosphatidylcholine which is one of the components of cell membrane lipids, deoxycholic acid, stearylamine, dioleoyltrimethylammoniumpropane (DOTAP), and water. The versatility of nanoemulsions is based on the different types of oils and surface modifiers that can be used [101]. The nanoemulsions of pine-nut oil with the oil droplet size of approximately 200 nm in diameter have been formulated for the delivery of paclitaxel (PTX) and C6-ceramide (CER) to the brain [102]; it has been found that the O/W nanoemulsions can improve the cytotoxic effect in brain tumor cells. As a result, pine-nut oil with high gamma linolenic acid content was expected to rapidly penetrate across the BBB and thus, incorporating paclitaxel in pine-nut oil-containing nanoemulsions was hypothesized to increase the drug availability in the brain.

In another study, O/W nanoemulsions containing saquinavir (SQV), an anti-HIV protease inhibitor, have been developed by dissolving SQV in different types of edible oils rich in essential polyunsaturated fatty acids (PUFA), followed by emulsifying in water containing surfactants Lipoid®-80 and deoxycholic acid [103]. When administered orally and intravenously to male Balb/c mice, the resultant flax-seed oil nanoemulsions with an average oil droplet size of 100-200 nm showed enhanced rate and extent of SQV absorption in the brain in contrast to aqueous suspension formulation.

Compared with other nano carriers, the advantages of nanoemulsions for overcoming the BBB are the ability to utilize safe oils, as well as possessing several beneficial biological properties as a result of essential omega-3 and omega-6 fatty acid in the oils. However, the thermodynamic instability is a shortcoming of nanoemulsions and requires further considersation.

2.9 Exosomes

Exosomes are a kind of nano sized membranous vesicle secreted by a number of cell types and

can be isolated from conditioned cell media or bodily fluids such as urine and plasma [104]. Once released from a cell, exosomes can fuse with membrane of another cell, transferring exosomal molecules from one cell to another [105]. This property provides exosomes with the chance to transport drugs into cells. The uptake mechanism of exosomes is clathrin mediated endocytosis followed by back fusion with the limiting membrane of the endosomes, allowing for the possibility of exosomes to cross the intact BBB [106].

Being secreted by living cells in the body, exosomes are natural delivery vehicles with advantages of non-toxicity, minimal immune responses, and better stability in the circulatory system in contrast to established formulations [107]. Alvarez-Erviti and co-workers used self-derived dendritic cells for exosome production; targeting peptides for muscle and brain were used to functionalize the exosomes [108]. Uploading exogenous siRNA onto the exosomes by electroporation, it has been found that the exosomes delivered siRNA specifically to neurons, microglia, and oligodendrocytes in the brain, resulting in a specific gene knockdown after intravenous injection. Yang and co-workers isolated four types of exosomes from brain cell culture media for drug delivery, and found that exosome-delivered anticancer drugs crossed the intact BBB and entered into the brain [109].

Proof of concept has been gained for exosome-based brain drug delivery systems; several issues should be addressed before clinical evaluation such as the choice of exosome donor cells, drug loading procedures, as well as the targeting peptides.

2.10 Quantum dots

Quantum dots are a class of colloidal semi-conductor nano crystals composed of a metalloid crystalline core (such as cadmium selenium) and an intermediate unreactive metallic shell (such as zinc sulfide) that shields the core [110]. Such an inorganic nano material has high brightness, long-term photo-stability, and size-tunable narrow emission spectra, which makes it a revolutionary imaging technology for diagnostic purposes (fluorescent probes) [111]. With the possibility of being co-incorporated with a variety of diagnostic and therapeutic molecules for targeted therapy of CNS disorders as a result of their high surface area, quantum dots also showed the potential as a carrier vehicle for blood-to-brain drug delivery.

The outer coating of quantum dots can be chemically functionalized with bioactive molecules that promote aqueous solubility and desired bioactivity, enable targeting of specific molecules, as well

as carrying therapeutic molecules [112]. Captopril-conjugated CdSe/ZnS-core/shell-typed quantum dots (QDs-cap) have been synthesized by the hot soap method with tri-n-octyl phosphine oxide (TOPO) followed by the replacement of TOPO with captopril by a thiolexchange reaction. It has been found that intraperitoneally administered QDs-cap in mice were delivered through systemic blood circulation into the brain as well as into the liver, spleen and kidney, which showed the possibility of such quantum dots to penetrate the BBB [113].

It is widely known that the Tf receptor is a kind of specific BBB transporter that allows selected biomolecules to move across the BBB; lysine-coated CdSe/CdS/ZnS quantum dots have been synthesized followed by conjugation with Tf. It has been found that the migration rate of such Tf-conjugated QDs crossing the *in vitro* BBB model was concentration- and time-dependent after a systemic administration, which demonstrated a receptor-mediated transport mechanism [114].

TAT, a cell membrane translocation peptide, has been successfully used to internalize nanoparticles [115]. Research has shown that TAT-conjugated CdS:Mn/ZnS quantum dots can label the brain tissue within a few minutes after being intra-arterially delivered to a rat brain without manipulating the BBB; such TAT-conjugated quantum dots migrated beyond the endothelial cell line and reached the brain parenchyma [116-117]. Because the same quantum dots without TAT did not label the brain tissue, TAT peptide was necessary for the quantum dots to overcome the BBB.

2.11 Polymersomes

Polymersomes are a nanometer-sized vesicular system with a bi-layered membrane which is composed of amphiphilic block copolymers [118]. Such tiny hollow spheres can be formed from synthetic amphiphilic block copolymers using organic solvent, organic solvent/water systems, or aqueous media. Usually, amphiphilic block copolymers with a hydrophilic fraction of 35±10% will aggregate to form polymersomes [119-120].

Polymersomes enclose an aqueous solution in the core, which is useful for encapsulation and delivery of hydrophilic drugs, while the membrane of copolymer aggregates bi-layer can be loaded with small hydrophobic drugs [121-122]. Polymersomes are similar to liposome in structure; the main difference is that the external bi-layer is composed of amphiphilic copolymers with a molecular weight of up to 100 kDa, while the building block of liposome is in most cases naturally occurring phospholipid with a molecular weight below 1 kDa [123-124]. With regard to

the use of building blocks with high molecular weight, the membrane of polymersomes is thicker than that of liposome, providing a higher durable physical barrier that protects the enclosed drugs [125]. When used for drug delivery, the more robust and therefore less leaky membrane of polymersome can improve circulation time and prevent uncontrolled release of drugs [126-127].

Being a nano carrier vehicle, polymersomes have been functionalized with certain receptors for brain drug delivery across the BBB. Pang and co-workers synthesized biodegradable poly(ethyleneglycol)-poly(ε-caprolactone) (PEG-PCL) and used the block copolymer to prepare polymersomes (PO) by a nano precipitation method [128]. To facilitate brain targeting, the polymersomes were functionalized with Tf to form a conjugate Tf-PO. Using coumarin-6 as a model drug, fluorescent microscopy of brain coronal sections revealed more Tf-PO than PO accumulated in the brain after injection, and it has been evidenced that Tf-PO uptake by beta-End.3 cells occurred mainly through a clathrin-mediated, energy-dependent endocytosis and that both the Golgi apparatus and lysosomes were involved in intracellular transport of Tf-PO.

Lf also belongs to the transferring family and exists on the BBB involved in Lf transport across the BBB, which provides another chance for brain targeting of polymersomes. Gao and co-workers prepared polymersomes of poly(butadiene-b-ethylene oxide) (PBD-PEO), poly(ethylene glycol-b-lactic acid) (PEG-PLA) and maleimide-PEG-PLA (Mal-PEG-PLA) (the weight ratio of PBD-PEO:PEG-PLA:MAL-PEG-PLA was 7:2:1) using the film rehydration method [129], both Lf and Tf were used to functionalized the polymersomes (Lf-PO and Tf-PO) for brain targeting. It was found that cell uptake of both Lf-PO and Tf-PO were time-, temperature-, and concentration-dependent, which suggested a process of active endocytosis. Tf was more effective than Lf in facilitating cell uptake of the nano carriers, and Lf-PO was more easily identified and eliminated by cells of the mononuclear phagocytic system (MPS). Polymersomes prepared by self-assembly of methoxy-PEG-PLGA and maleimide-PEG-PLGA which were synthesized by the ring opening polymerization of D,L-lactide and glycolide showed similar results when conjugated with Tf [130].

Several polymersomes have been investigated for brain drug delivery; various receptors expressed on the BBB such as low-density lipoprotein receptor, insulin receptor, insulin-like growth factor receptor, diphtheria toxin receptor and nicotinic acetylcholine receptor provided many chances for the functionalization of polymersomes to mediate their penetration of the BBB. Poly(carboxyl

ethylene glycol-g-glutamate)-co-poly(distearin-g-glutamate) (CPEGGM-PDSGM) copolymer, poly(methyl ethylene glycol-g-glutamate)-copoly(distearin-g-glutamate) (mPEGGM-PDSGM) copolymer, des-octanoyl ghrelin-poly(ethylene glycol-gglutamate)-co-poly(distearin-g-glutamate) (des-octanoyl ghrelin-PEGGM-PDSGM) copolymer, poly(tert-butyl hydrazinecarboxylate ethylene glycol-g-glutamate)-co-poly(distearin-g-glutamate) (BPEGGM-PDSGM) copolymer, as well as folate-poly(ethylene glycol-g-glutamate)-co-poly(distearin-g-glutamate) (folate-PEGGM-PDSGM) copolymer have been prepared, different types of polymersomes based on such copolymers have been developed and conjugated with both des-octanoyl ghrelin and folate as a penetrating-targeting carrier for an enhanced drug transport through the BBB and glioma cells targeting [131]. It has been found that with bi-functional ligands on the surface of polymersomes, the BBB transport was greatly enhanced and the inhibition of glioma growth was significantly improved via a synergistic effect of two different endocytosis mechanisms by des-octanoyl ghrelin and folate.

Although several research results showed the possibility of receptor functionalization-mediated BBB penetration of polymersomes, the efficiency of drug transport was not ideal. In addition, the application of protein and antibody receptors is restricted by their instability and immunogenicity.

3. Mechanisms of nanomaterials transporting drugs across the BBB

Based on the biological characteristics of the BBB, the mechanisms of nano carriers transporting drugs across the BBB can be classified as follows: (1) transient BBB opening induced by the stimulus derived from “nano effects” or “nano toxicity” of the nano carriers, or the stimulus of surfactants coated on the surface of nano carriers, which results in diffusion of the drugs or drug-carrier conjugates into the brain parenchyma; (2) adsorption of the drug-carrier conjugates onto the surface of brain capillary endothelial cells, followed by drug release from the carrier vehicles on the surface, which increases drug concentration gradient facilitating diffusion of the drugs into the brain parenchyma; (3) transcytosis, endocytosis, and exocytosis of the drug-carrier conjugates by brain capillary endothelial cells, which results in direct penetration into the brain parenchyma or the cells [132-134].

It has been found that some chitosan-derived nanoparticles were capable of opening the tight junctions of the BBB [135-136], while high concentrations of some anionic nanoparticles and

cationic nanoparticles disrupted the BBB even though neutral nanoparticles and low concentrations of anionic nanoparticles have no effect on BBB integrity [137]. Sodium dodecylsulphate, a surfactant which is regarded as biocompatible, has also been found the capability to induce a breach in the blood brain barrier [138]. Transient BBB opening as a result of the stimulus derived from nano carriers or surfactants is dangerous as a result of the nano carrier's toxicity, as well as the possibility of other molecules in blood stream diffusing into the brain parenchyma at the same time.

Adsorption of the drug-carrier conjugates onto the surface of brain capillary endothelial cells depends on hydrophilicity, surface charge, and targeting ligands of the nano carrier. A lipophilic surface of the nano carrier facilitates the adsorption; and a positive surface charge of the nano carrier promotes electrostatic interactions with the negative charges of endothelial surfaces which also facilitate the adsorption [139-140]. Targeting ligands such as the apolipoproteins in plasma adsorbed on the surface of the nano carrier can target low density lipoprotein receptors on the microvessel endothelial cells, which facilitates the adsorption [141].

For the fate of drug-carrier conjugates adsorbed on the surface of brain capillary endothelial cells, one is desorption followed by re-entering the blood stream. In this case, the uploaded drug can release from the carrier vehicle on the surface of BBB during adsorption and then diffuse into the brain parenchyma through the BBB. Therefore, the possibility of drug penetrating into the brain parenchyma will be determined by characteristics of the drug. To promote drug transport across the BBB, it is necessary to modify the surface of the nano carriers for the minimization of clearance by the fixed macrophages of the mononuclear phagocytic system (MPS) and prolonged blood circulation enhancing exposure of the BBB. Improving hydrophilicity and increasing surface charge are good methods for nano carriers to mask themselves from the MPS. Another fate of the adsorbed drug-carrier conjugates is endocytosis by the cells, sometimes followed by exocytosis, leading to penetration of the nano carrier into the cells or brain parenchyma.

Endocytosis may occur by means of random uptake of soluble plasma molecules together with a bulk of plasma by caveolaes on the cells; such a process is independent of any interaction between the transported molecules and the caveolar vesicle membrane [142]. Because the brain capillary endothelial cells are characterized by few caveolaes while a high density of negatively charged and clathrin-coated pits through which drug-carrier conjugates can be be endocytosed, endocytosis

of drug-carrier conjugates is mainly based on the adsorption of drug-carrier conjugates to the clathrin-coated pit membrane [143].

Although it is a normal biological function of cells, endocytosis is energy-dependant which does not exhibit any preference for nano carriers conjugated with drugs, leading to a low efficiency of drug transport across the BBB. Several approaches have been proposed to promote endocytosis of nano carriers by brain capillary endothelial cells; one is coating the nano carriers with surfactants whose effectiveness has been testified. Three mechanisms have been proposed to explain surfactant-coating promoted endocytosis, namely [144-148]: (1) polysorbate 80 or poloxamers 188-induced adsorption of apolipoproteins A-I and/or E in plasma onto the surface of the nano carrier facilitating targeting low density lipoprotein receptors on the cell membrane; (2) surfactant-induced fluidity of the cell membrane contacted with the adsorbed drug-carrier conjugates facilitating deformation of the cell membrane and promoting endocytosis; and (3) surfactant-induced BBB opening.

Promoting adsorption of drug-carrier conjugates to the brain capillary endothelial cells is another approach to prompt endocytosis; seeking help from specific carriers, transport proteins, or receptors on the membrane of cells has been employed. The relative high BBB permeability of acrylic nanoparticles has been attributed to the apolipoproteins in plasma adsorbed on the surface of the nano carrier targeting low density lipoprotein receptors on the microvessel endothelial cells by some researchers [149]. Many nano carriers have been surfacely functionalized with a series of functional peptides such as Tf and Lf, and both *in vitro* and *in vivo* tests have shown their effectiveness in promoting BBB penetration of the nano carriers. Because such specific carriers, transport proteins, or receptors expressed on the membrane of brain capillary endothelial cells are limited, the possibility of achieving a minimal effective drug concentration in the brain parenchyma by carrier-, transporter-, as well as receptor-mediated endocytosis of nano carriers is worthy of suspicion. For example, from the data available, the percentages of injected drug dose found in the brain after targeting with nano carriers was less than 1% [150].

4. Perspective

Brain drug delivery via the circulatory system is limited by the BBB, which hinders therapy, diagnosis and prevention of cerebral diseases. Being a non-invasive approach,

nanomaterials-mediated delivery transporting drugs across the intact BBB has shown the advantages of safety, low cost, as well as suitability for almost all drugs in contrast to other approaches of brain drug delivery. A variety of nanomaterials have been investigated for drug transport across the BBB; both *in vitro* and *in vivo* tests have testified the effectiveness of nanomaterials-mediated brain drug delivery. However, despite considerable advances that have resulted in improved brain drug delivery profiles over other brain delivery approaches, several issues have impeded the therapeutic application of nano carriers in brain drug delivery, namely: rapid opsonization, the fast and non-specifc clearance from the circulatory system by reticulo-endothelial system (RES) cells of nano carriers with hydrophobic/neutral surfaces; the metastable nature of some nano carriers; limited drug loading capacity; need to co-formulate with surfactants and co-solvents; aggregation or cluster formation in blood flow of neutral or hydrophobic nano carriers; and the most importantly, the low efficiency of crossing the intact BBB, leading to the difficulty of achieving effective local drug concentration in the brain parenchyma.

According to the various mechanisms of drug transport across BBB by nano carriers, only penetration of drug-carrier conjugates into the brain parenchyma via transcytosis or endocytosis exhibit suitability for all types of brain drugs. Therefore, to obtain a pratical delivery platform for brain drugs, it is suggested here that the development of a versatile delivery platform should focus on their capability as well as efficiency to be transcytosed or endocytosed.

In addition, the fate of drug carrier vehicles in the brain is a primary consideration. The first requirement for drug carrier vehicles is biocompatibility; compared with drug carrier vehicles targeting other organs, the accumulation of nano carriers in the brain parenchyma is a particular issue to be addressed in addition to toxicity and immunological response. Biodegradable nano carriers or nano carriers with the capability to penetrate out of the brain parenchyma across the intact BBB after drug release which can be removed by MPS are preferable. Unfourtunately, at present it is still unclear what effects of nano carrier’s accumulation in the brain parenchyma will be.

Lastly, to achieve the minimal effective drug concentration in brain parenchyma while the maximal safety drug concentration is not exceeded in other organs, the drug transport efficiency across the BBB by nano carriers should be elevated. From this viewpoint, it is necessary to explore new approaches facilitating endocytosis of nano carriers by the brain capillary endothelial

cells, although the efficiency of drug accumulation in the brain parenchyma is not only determined by crossing BBB, but also by drug uploading and release profile which are relatively easy to be engineered.

References

1 Giovanni Tosi, Luca Costantino, Barbara Ruozi, Flavio Forni, Maria Angela Vandelli. Polymeric nanoparticles for the drug delivery to the central nervous system. *Expert Opin. Drug Deliv.* 2008, 5(2): 155-174

2 Lipa Shah, Sunita Yadav, Mansoor Amiji. Nanotechnology for CNS delivery of bio-therapeutic agents. *Drug Deliv. and Transl. Res.*, 2013, 3: 336-351

3 Francesca Re, Maria Gregori, Massimo Masserini. Nanotechnology for neurodegenerative disorders. *Nanomedicine: Nanotechnology, Biology, and Medicine*, 2012, 8: S51-S58

4 Sonu Bhaskar, Furong Tian, Tobias Stoeger, et al. Multifunctional Nanocarriers for diagnostics, drug delivery and targeted treatment across blood-brain barrier: perspectives on tracking and neuroimaging. *Particle and Fibre Toxicology*, 2010, 7: 3

5 Ariel Gilert, Marcelle Machluf. Nano to micro delivery systems: targeting angiogenesis in brain tumors. *Journal of Angiogenesis Research*, 2010, 2: 20

6 Herve Hillaireau, Patrick Couvreur. Nanocarriers' entry into the cell: relevance to drug delivery. *Cell. Mol. Life Sci.*, 2009, 66: 2873-2896

7 Xinguo Jiang. Brain Drug Delivery Systems. *Pharm Res.*, 2013, 30: 2427-2428

8 Yan Chen, Lihong Liu. Modern methods for delivery of drugs across the blood-brain barrier. *Advanced Drug Delivery Reviews*, 2012, 64: 640-665

9 Seung Rim Hwang, Kwangmeyung Kim. Nano-enabled delivery systems across the blood-brain barrier. *Arch. Pharm. Res.*, 2014, 37: 24-30

10 Laura Biddlestone-Thorpe, Nicola Marchi, Kathy Guo, et al. Nanomaterial-mediated CNS delivery of diagnostic and therapeutic agents. *Advanced Drug Delivery Reviews*, 2012, 64: 605-613

11 Gerardo Leyva-Gomez, Hernan Cortes, Jonathan J. Magana, et al. Nanoparticle technology for treatment of Parkinson's disease: the role of surface phenomena in reaching the brain. *Drug Discovery Today*, 2015, 20(7): 824-837

12 Aditya Grover, Anjali Hirani, Vijaykumar Sutariya. Nanoparticle-Based Brain Targeted Delivery Systems. *Biomolecular Research & Therapeutics*, 2013, 2: e113

13 Rajesh Singh, James W. Lillard Jr. Nanoparticle-based targeted drug delivery. *Experimental and Molecular Pathology*, 2009, 86: 215-223

14 Eugen Barbu, Éva Molnàr, John Tsibouklis, Dariusz C Górecki. The potential for nanoparticle-based drug delivery to the brain: overcoming the blood-brain barrier. *Expert Opin. Drug Deliv.*, 2009, 6(6): 1-13

15 Massimo Masserini. Nanoparticles for Brain Drug Delivery. *ISRN Biochemistry*, 2013, e238428

16 David J. Mc Carthy, Meenakshi Malhotra, Aoife M. O'Mahony, John F. Cryan, Caitriona M. O'Driscoll. Nanoparticles and the Blood-Brain Barrier: Advancing from In-Vitro Models Towards Therapeutic Significance. *Pharm Res.*, 2015, 32: 1161-1185

17 Fang Zhang, Chun-Lei Xu, Chun-Mei Liu. Drug delivery strategies to enhance the permeability of the blood-brain barrier for treatment of glioma. *Drug Design, Development and Therapy*. 2015, 9: 2089-2100

18 Reddy LH, Arias JL, Nicolas J, Couvreur P. Magnetic nanoparticles: design and characterization, toxicity and biocompatibility, pharmaceutical and biomedical applications. *Chem Rev*., 2012, 112: 5818-5978

19 Vilella A, Tosi G, Grabrucker AM, et al. Insight on the fate of CNS-targeted nanoparticles. Part I: Rab5-dependent cell-specific uptake and distribution. *J Control Release*, 2014, 174: 195-201

20 Alyautdin R, Khalin I, Nafeeza MI, Haron MH, Kuznetsov D. Nanoscale drug delivery systems and the blood-brain barrier. *Int J Nanomedicine*, 2014, 9: 795-811

21 Krol S. Challenges in drug delivery to the brain: nature is against us. *J Control Release*, 2012, 164: 145-155

22 E. Garcia-Garcia, K. Andrieux, S. Gilb, P. Couvreur. Colloidal carriers and blood-brain barrier (BBB) translocation: A way to deliver drugs to the brain? *International Journal of Pharmaceutics*, 2005, 298: 274-292

23 Thomas M Barchet, Mansoor M Amiji. Challenges and opportunities in CNS delivery of therapeutics for neurodegenerative diseases. *Expert Opin. Drug Deliv.*, 2009, 6(3): 211-225

24 Hu Yang. Nanoparticle-Mediated Brain-Specific Drug Delivery, Imaging, and Diagnosis. *Pharm Res.*, 2010, 27: 1759-1771

25 Shuting Ku, Feng Yan, Ying Wang, Yilin Sun, Nan Yang, Ling Ye. The blood-brain barrier penetration and distribution of PEGylated fluorescein-doped magnetic silica nanoparticles in rat brain. *Biochemical and Biophysical Research Communications*, 2010, 394: 871-876

26 Jinfeng Ren, Shun Shen, Dangge Wang, Zhangjie Xi, Liangran Guo, Zhiqing Pang, Yong Qian, Xiyang Sun, Xinguo Jiang. The targeted delivery of anticancer drugs to brain glioma by PEGylated oxidized multi-walled carbon nanotubes modified with angiopep-2. *Biomaterials*, 2012, 33: 3324-3333

27 Ruirui Qiao, Qiaojuan Jia, Sabine Huwel, Rui Xia, Ting Liu, Fabao Gao, Hans-Joachim Galla, Mingyuan Gao. Receptor-Mediated Delivery of Magnetic Nanoparticles across the Blood_Brain Barrier. *ACS Nano*, 2012, 6(4): 3304-3310

28 Yeong Shin Yim, Jin-sil Choi, Gun Tae Kim, Chul Hoon Kim, Tae-Hyun Shin, Dong Goo Kim, Jinwoo Cheon. A facile approach for the delivery of inorganic nanoparticles into the brain by passing through the blood-brain barrier (BBB). *Chem. Commun.*, 2012, 48: 61-63

29 Luisa Fiandra, Miriam Colombo, Serena Mazzucchelli, Marta Truffi, Benedetta Santini, Raffaele Allevi, Manuela Nebuloni, Amedeo Capetti, Giuliano Rizzardini, Davide Prosperi, Fabio Corsi. Nanoformulation of antiretroviral drugs enhances their penetration across the blood brain barrier in mice. *Nanomedicine: Nanotechnology, Biology, and Medicine*, 2015, 11: 1387-1397

30 L. B. Thomsen, T. Linemann, K. M. Pondman, J. Lichota, K. S. Kim, R. J. Pieters, G. M. Visser, T. Moos. Uptake and Transport of Superparamagnetic Iron Oxide Nanoparticles through Human Brain Capillary Endothelial Cells. *ACS Chem. Neurosci.*, 2013, 4: 1352-1360

31 Jens Frigell, Isabel García, Vanessa Gómez-Vallejo, Jordi Llop, Soledad Penades. ^{68}Ga-Labeled Gold Glyconanoparticles for Exploring Blood-Brain Barrier Permeability: Preparation, Biodistribution Studies, and Improved Brain Uptake via Neuropeptide Conjugation. *J. Am. Chem. Soc.*, 2014, 136: 449-457

32 Zhong Yang, Yingge Zhang, Yanlian Yang, Lan Sun, Dong Han, Hong Li, Chen Wang. Pharmacological and toxicological target organelles and safe use of single-walled carbon nanotubes as drug carriers in treating Alzheimer disease. *Nanomedicine: Nanotechnology,*

Biology, and Medicine, 2010, 6: 427-441

33 Wolfgang Mehnert, Karsten Mader. Solid lipid nanoparticles: Production, characterization and applications. *Advanced Drug Delivery Reviews*, 2001, 47: 165-196

34 Paolo Blasi, Stefano Giovagnoli, Aurélie Schoubben, Maurizio Ricci, Carlo Rossi. Solid lipid nanoparticles for targeted brain drug delivery. *Advanced Drug Delivery Reviews*, 2007, 59: 454-477

35 Shicheng Yang, Lifang Lu, Ying Cai, Jiabi Zhu, Bing Wen Liang, Changzheng Yang. Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. *Journal of Controlled Release*, 1999, 59: 299-307

36 Niladri Chattopadhyay, Jason Zastre, Ho-Lun Wong, Xiao Yu Wu, Reina Bendayan. Solid Lipid Nanoparticles Enhance the Delivery of the HIV Protease Inhibitor, Atazanavir, by a Human Brain Endothelial Cell Line. *Pharmaceutical Research*, 2008, 25(10): 2262-2271

37 Maria Luisa Bondì, Emanuela Fabiola Craparo, Gaetano Giammona, Filippo Drago. Brain-targeted solid lipid nanoparticles containing riluzole: preparation, characterization and biodistribution. *Nanomedicine*, 2010, 5(1): 25-32

38 Vinay Kumar Venishetty, Ramakrishna Samala, Rojarani Komuravelli, Madhusudana Kuncha, Ramakrishna Sistla, Prakash V. Diwan. β-Hydroxybutyric acid grafted solid lipid nanoparticles: A novel strategy to improve drug delivery to brain. *Nanomedicine: Nanotechnology, Biology, and Medicine*, 2013, 9: 388-397

39 Yashwant Gupta, Anekant Jain, Sanjay K. Jain. Transferrin-conjugated solid lipid nanoparticles for enhanced delivery of quinine dihydrochloride to the brain. *Journal of Pharmacy and Pharmacology*, 2007, 59: 935-940

40 Jitender Madan, Ravi Shankar Pandey, Vikas Jain, Om Prakash Katare, Ramesh Chandra, Anju Katyal. Poly (ethylene)-glycol conjugated solid lipid nanoparticles of noscapine improve biological half-life, brain delivery and efficacy in glioblastoma cells. *Nanomedicine: Nanotechnology, Biology, and Medicine*, 2013, 9: 492-503

41 Toral Patel, Jiangbing Zhou, Joseph M. Piepmeier, W. Mark Saltzman. Polymeric nanoparticles for drug delivery to the central nervous system. *Advanced Drug Delivery Reviews*, 2012, 64: 701-705

42 Jörg Kreuter. Drug delivery to the central nervous system by polymeric nanoparticles: What

do we know? *Advanced Drug Delivery Reviews*, 2014, 71: 2-14

43 Eun Seok Gil, Jianshu Li, Huining Xiao, and Tao Lu Lowe. Quaternary Ammonium *β*-Cyclodextrin Nanoparticles for Enhancing Doxorubicin Permeability across the In Vitro Blood-Brain Barrier. *Biomacromolecules*, 2009, 10: 505-516

44 Eun Seok Gil, Linfeng Wu, Lichong Xu, Tao Lu Lowe. β‑Cyclodextrin-poly(β-Amino Ester) Nanoparticles for Sustained Drug Delivery across the Blood-Brain Barrier. *Biomacromolecules*, 2012, 13: 3533-3541

45 E. Garcia-Garcia, S. Gil, K. Andrieux, D. Desmaële, V. Nicolas, F.Tarane, D. Georgin, J. P. Andreux, F. Roux, P. Couvreur. A relevant in vitro rat model for the evaluation of blood-brain barrier translocation of nanoparticles. *Cell. Mol. Life Sci.*, 2005, 62: 1400-1408

46 Hyun R Kim, Karine Andrieux, Sophie Gil, et al. Translocation of Poly(ethylene glycol-co-hexadecyl)cyanoacrylate Nanoparticles into Rat Brain Endothelial Cells: Role of Apolipoproteins in Receptor-Mediated Endocytosis. *Biomacromolecules*, 2007, 8: 793-799

47 Renad N. Alyautdin, Valery E. Petrov, Klaus Langer, Achim Berthold, Dimitry A. Kharkevich, Jorg Kreuter. Delivery of Loperamide Across the Blood-Brain Barrier with Polysorbate 80-Coated Polybutylcyanoacrylate Nanoparticles. *Pharmaceutical Research*, 1997, 14(3): 325-328

48 Ulrike Schroder, Bernhard A. Sabel. Nanoparticles, a drug carrier system to pass the blood-brain barrier, permit central analgesic effects of i.v. dalargin injections. *Brain Research*, 1996, 710: 121-124

49 Ulrike Schroeder, Petra Sommerfeld, Sven Ulrich, Bernhard A. Sabel. Nanoparticle Technology for Delivery of Drugs Across the Blood-Brain Barrier. *Journal of Pharmaceutical Sciences*, 1998, 87(11): 1305-1307

50 Barnabas Wilson, Malay Kumar Samanta, Kumaraswamy Santhi, et al. Poly(n-butylcyanoacrylate) nanoparticles coated with polysorbate 80 for the targeted delivery of rivastigmine into the brain to treat Alzheimer's disease. *Brain Research*, 2008, 1200: 159-168

51 Barnabas Wilson, Malay Kumar Samanta, Kumaraswamy Santhi, et al. Targeted delivery of tacrine into the brain with polysorbate 80-coated poly(n-butylcyanoacrylate) nanoparticles. *European Journal of Pharmaceutics and Biopharmaceutics*, 2008, 70: 75-84

52 Marise Kolter, Melanie Ott, Christian Hauer, Isolde Reimold, Gert Fricker. Nanotoxicity of poly(n-butylcyano-acrylate) nanoparticles at the blood-brain barrier, in human whole blood and in vivo. *Journal of Controlled Release*, 2015, 197: 165-179

53 Robert M. Koffie, Christian T. Farrar, Laiq-Jan Saidi, Christopher M. William, Bradley T. Hyman, Tara L. Spires-Jones. Nanoparticles enhance brain delivery of blood-brain barrier-impermeable probes for in vivo optical and magnetic resonance imaging. *PNAS*, 2011, 108(46): 18837-18842

54 Richa Pahuja, Kavita Seth, Anshi Shukla, et al. Trans-Blood Brain Barrier Delivery of Dopamine-Loaded Nanoparticles Reverses Functional Deficits in Parkinsonian Rats. *ACS Nano*, 2015, 9(5): 4850-4871

55 Mayank Chaturvedi, Yves Molino, Bojja Sreedhar, Michel Khrestchatisky, Leszek Kaczmarek. Tissue inhibitor of matrix metalloproteinases-1 loaded poly(lactic-co-glycolic acid) nanoparticles for delivery across the blood-brain barrier. *International Journal of Nanomedicine*, 2014, 9: 575-588

56 Aisling O'Donnell, Azeema Moollan, Samantha Baneham, Melike Ozgul, Ritesh M. Pabari, Dermot Cox, Brian P. Kirby, Zebunnissa Ramtoola. Intranasal and intravenous administration of octa-arginine modified poly(lactic-co-glycolic acid) nanoparticles facilitates central nervous system delivery of loperamide. *Journal of Pharmacy and Pharmacology*, 2014, 67: 525-536

57 Daniela Guarnieri, Annarita Falanga, Ornella Muscetti, Rossella Tarallo, Sabato Fusco, Massimiliano Galdiero, Stefania Galdiero, Paolo A. Netti. Shuttle-Mediated Nanoparticle Delivery to the Blood-Brain Barrier. *Small*, 2013, 9(6): 853-862

58 Jingwei Li, Chi Zhang, Jing Li, Li Fan, Xinguo Jiang, Jun Chen, Zhiqing Pang, Qizhi Zhang. Brain Delivery of NAP with PEG-PLGA Nanoparticles Modified with Phage Display Peptides. *Pharm Res*, 2013, 30: 1813-1823

59 Hongliang Xin, Xinyi Jiang, Jijin Gu, Xianyi Sha, Liangcen Chen, Kitki Law, Yanzuo Chen, Xiao Wang, Ye Jiang, Xiaoling Fang. Angiopep-conjugated poly(ethylene glycol)-co-poly(ε-caprolactone) nanoparticles as dual-targeting drug delivery system for brain glioma. *Biomaterials*, 2011, 32: 4293-4305

60 Chee Wee Gan, Si-Shen Feng. Transferrin-conjugated nanoparticles of

Poly(lactide)-D-α-Tocopheryl polyethylene glycol succinate diblock copolymer for targeted drug delivery across the blood-brain barrier. *Biomaterials*, 2010, 31: 7748-7757

61 Karsten Ulbrich, Telli Hekmatara, Elisabeth Herbert, Jorg Kreuter. Transferrin- and transferring-receptor-antibody-modified nanoparticles enable drug delivery across the blood-brain barrier (BBB). *European Journal of Pharmaceutics and Biopharmaceutics*, 2009, 71: 251-256

62 Kaili Hu, Jingwei Li, Yehong Shen, Wei Lu, Xiaoling Gao, Qizhi Zhang, Xinguo Jiang. Lactoferrin-conjugated PEG-PLA nanoparticles with improved brain delivery: In vitro and in vivo evaluations. *Journal of Controlled Release*, 2009, 134: 55-61

63 Ja-Young Kim, Won Il Choi, Young Ha Kim, Giyoong Tae. Brain-targeted delivery of protein using chitosan- and RVG peptide-conjugated, pluronic-based nano-carrier. *Biomaterials*, 2013, 34: 1170-1178

64 Hulya Karatas, Yesim Aktas, Yasemin Gursoy-Ozdemir, et al. A Nanomedicine Transports a Peptide Caspase-3 Inhibitor across the Blood-Brain Barrier and Provides Neuroprotection. *J. Neurosci.*, 2009, 29(44): 13761-13769

65 Yesüim Aktasü, Muge Yemisci, Karine Andrieux, et al. Development and Brain Delivery of Chitosan-PEG Nanoparticles Functionalized with the Monoclonal Antibody OX26. *Bioconjugate Chem.*, 2005, 16: 1503-1511

66 Zhao H. Wang, Zhan Y. Wang, Chang S. Sun, Chun Y. Wang, Tong Y. Jiang, Si L. Wang. Trimethylated chitosan-conjugated PLGA nanoparticles for the delivery of drugs to the brain. *Biomaterials*, 2010, 31: 908-915

67 Eva Molnar, Eugen Barbu, Chun-Fu Lien, Dariusz C. Gorecki, John Tsibouklis. Toward Drug Delivery into the Brain: Synthesis, Characterization, and Preliminary In Vitro Assessment of Alkylglyceryl-Functionalized Chitosan Nanoparticles. *Biomacromolecules*, 2010, 11: 2880-2889

68 Xuejiao Yang, Yanxiong Fang, Xinming Li, et al. Synthesis of two AMPS-based polymerizable room temperature ionic liquids and swelling difference between their co-polymeric gels with HEMA. *e-Polymers*, 2014, 14(5): 335-343

69 Tingting Weng, Jianwei Guo, Xinming Li, et al. Synthesis of the polymerizable room temperature ionic liquid AMPS-TEA and superabsorbency for organic liquids of its

copolymeric gels with acrylamide. *Designed Monomers and Polymers*, 2014, 17(2): 140-146

70 Tingting Weng, Jianwei Guo, Xinming Li, et al. Synthesis, Chloramphenicol Uptake, and In Vitro Release of Poly (AMPS–TEA-Co-AAm) Gels with Affinity for Both Water and Alcohols. *International Journal of Polymeric Materials and Polymeric Biomaterials*, 2014, 63(2): 73-79

71 Xinming Li, Yingde Cui. Study on Synthesis and Chloramphenicol Release of Poly (2-hydroxyethylmethacrylate-co-acrylamide) Hydrogels. *Chinese Journal of Chemical Engineering*, 2008, 16(4): 640-645

72 Eun Seok Gil, Tao Lu Lowe. Invention of polysaccharide-based nanoparticles for enhancing drug permeability across the blood brain barrier. *NSTI-Nanotech*, 2008, 2: 379-381

73 Amir Azadia, Mehrdad Hamidib, Mohammad-Reza Khoshayandc, Mohsen Aminid, Mohammad-Reza Rouini. Preparation and optimization of surface-treated methotrexate-loaded nanogels intended for brain delivery. *Carbohydrate Polymers*, 2012, 90: 462-471

74 Amir Azadi, Mehrdad Hamidi, Mohammad-Rez Rouini. Methotrexate-loaded chitosan nanogels as 'Trojan Horses' for drugdelivery to brain: Preparation and in vitro/in vivo characterization. *International Journal of Biological Macromolecules*, 2013, 62: 523-530

75 Vladimir P. Baklaushev, Natalia N. Nukolova, Alexander S. Khalansky, Olga I. Gurina, Gaukhar M. Yusubalieva, Nadejhda Ph. Grinenko, Ilya L. Gubskiy, Pavel A. Melnikov, Karina Sh. Kardashova, Alexander V. Kabanov, and Vladimir P. Chekhonin. Treatment of glioma by cisplatin-loaded nanogels conjugated with monoclonal antibodies against Cx43 and BSAT1. *Drug Delivery*, 2015, 22(3): 276-285

76 Serguei V. Vinogradov, Elena V. Batrakova, and Alexander V. Kabanov. Nanogels for Oligonucleotide Delivery to the Brain. *Bioconjugate Chem.*, 2004, 15: 50-60

77 Pengcheng Zhang, Luojuan Hu, Qi Yin, Linyin Feng, Yaping Li. Transferrin-Modified c[RGDfK]-Paclitaxel Loaded Hybrid Micelle for Sequential Blood-Brain Barrier Penetration and Glioma Targeting Therapy. *Mol. Pharmaceutics*, 2012, 9: 1590-1598

78 Alexander V. Kabanov, Elena V. Batrakova, Nikolai S. Melik-Nubarov, Nikolai A. Fedoseev, Tatiyana Yu. Dorodnich, Valery Yu. Alakhov, Vladimir P. Chekhonin, Irina R. Nazarova, Victor A. Kabanov. A new class of drug carriers: micelles of

poly(oxyethylene)-poly(oxypropylene) block copolymers as microcontainers for drug targeting from blood in brain. *Journal of Controlled Release*, 1992, 22(2): 141-157

79 Lihong Liu, Subbu S.Venkatraman, Yiyan Yang, Kun Guo, Jia Lu, Beiping He, Shabbir Moochhala, Lijing Kan. Polymeric Micelles Anchored With TAT for Delivery of Antibiotics Across the Blood-Brain Barrier. *Biopolymers PeptideScience*, 90(5): 617-623

80 Yutaka Miura, Tomoya Takenaka, Kazuko Toh, ShourongWu, Hiroshi Nishihara, Mitsunobu R. Kano, Yasushi Ino, Takahiro Nomoto, Yu Matsumoto, Hiroyuki Koyama, Horacio Cabral, Nobuhiro Nishiyama, Kazunori Kataoka. Cyclic RGD-Linked Polymeric Micelles for Targeted Delivery of Platinum Anticancer Drugs to Glioblastoma through the Blood Brain Tumor Barrier. *ACS Nano*, 2013, 7(10): 8583-8592

81 Chang Li, Shasha Li, Taojian Tu, Xingxing Qi, Yerong Xiong, Shuang Du, Yan Shen, Jiasheng Tu, Chunmeng Sun. Paclitaxel-loaded cholesterol-conjugated polyoxyethylene sorbitol oleate polymeric micelles for glioblastoma therapy across the blood-brain barrier. *Polym. Chem.*, 2015, 6: 2740-2751

82 Jie Shen, Changyou Zhan, Cao Xie, Qinggang Meng, Bing Gu, Chong Li, Yingkai Zhang, Weiyue Lu. Poly(ethylene glycol)-block-poly(D,L-lactide acid) micelles anchored with angiopep-2 for brain-targeting delivery. *Journal of Drug Targeting*, 2011, 19(3): 197-203

83 Matthias Schnurr, Karl Sydow, Honor May Rose, Margitta Dathe, and Leif Schröder. Brain Endothelial Cell Targeting Via a Peptide-Functionalized Liposomal Carrier for Xenon Hyper-CEST MRI. *Adv. Healthcare Mater.*, 2015, 4(1): 40-45

84 Jing Qin, Dawei Chen, Haiyang Hu, Qiao Cui, Mingxi Qiao, Baoyu Chen. Surface Modification of RGD-Liposomes for Selective Drug Delivery to Monocytes/Neutrophils in Brain. *Chem. Pharm. Bull.*, 2007, 55(8): 1192-1197

85 Chih-Jen Wen, Li-Wen Zhang, Saleh A Al-Suwayeh, Tzu-Chen Yen, Jia-You Fang. Theranostic liposomes loaded with quantum dots and apomorphine for brain targeting and bioimaging. *International Journal of Nanomedicine*, 2012, 7: 1599-1611

86 Cecile Machut-Binkowski, Frederic Hapiot, Rome´o Cecchelli, Patrick Martin, Eric Monflier. A versatile liposome/cyclodextrin supramolecular carrier for drug delivery through the blood-brain barrier. *J Incl Phenom Macrocycl Chem*, 2007, 57: 567-572

87 Jianqing Gao, Qing Lv, Liming Li, Xinjiang Tang, Fanzhu Li, Yulan Hua, Min Han. Glioma

targeting and bloodebrain barrier penetration by dual-targeting doxorubincin liposomes. *Biomaterials*, 2013, 34: 5628-5639

88 Huali Chen, Lei Tang, Yao Qin, Yujia Yin, Jie Tang, Wenwei Tang, Xun Sun, Zhirong Zhang, Ji Liuc, Qin He. Lactoferrin-modified procationic liposomes as a novel drug carrier for brain delivery. *European Journal of Pharmaceutical Sciences*, 2010, 40: 94-102

89 Frieder Helm, Gert Fricker. Liposomal Conjugates for Drug Delivery to the Central Nervous System. *Pharmaceutics*, 2015, 7: 27-42

90 Zainulabedin M Saiyed. Nimisha H Gandhi. Madhavan PN Nair. Magnetic nanoformulation of azidothymidine 5'-triphosphate for targeted delivery across the blood-brain barrier. *International Journal of Nanomedicine*, 2010, 5: 157-166

91 Huihui Yan, Jiyao Wang, Peiwei Yi, Hao Lei, Changyou Zhan, Cao Xie, Linglin Feng, Jun Qian, Jianhua Zhu, Weiyue Lu, Cong Li. Imaging brain tumor by dendrimer-based optical/paramagnetic nanoprobe across the blood-brain barrier. *Chem. Commun.*, 2011, 47: 8130-8132

92 Hai He, Yan Li, Xinru Jia, Ju Du, Xue Ying, Wanliang Lu, Jinning Lou, Yan Wei. PEGylated Poly(amidoamine) dendrimer-based dual-targeting carrier for treating brain tumors. *Biomaterials*, 2011, 32: 478-487

93 Renu Singh Dhanikula, Anteneh Argaw, Jean-Francois Bouchard, Patrice Hildgen. Methotrexate Loaded Polyether-Copolyester Dendrimers for the Treatment of Gliomas: Enhanced Efficacy and Intratumoral Transport Capability. *Molecular Pharmaceutics*, 2008, 5(1): 105-116

94 Yan Li, Hai He, Xinru Jia, Wanliang Lu, Jinning Lou, Yen Wei. A dual-targeting nanocarrier based on poly(amidoamine) dendrimers conjugated with transferrin and tamoxifen for treating brain gliomas. *Biomaterials*, 2012, 33: 3899-3908

95 Weilun Ke, Kun Shao, Rongqin Huang, Liang Han, Yang Liu, Jianfeng Li, Yuyang Kuang, Liya Ye, Jinning Lou, Chen Jiang. Gene delivery targeted to the brain using an Angiopep-conjugated polyethyleneglycol-modified polyamidoamine dendrimer. *Biomaterials*, 2009, 30: 6976-6985

96 A. Janaszewska, B. Ziemba, K. Ciepluch, D. Appelhans, B. Voit, B. Klajnert, M. Bryszewska. The biodistribution of maltotriose modified poly(propylene imine) (PPI) dendrimers

conjugated with fluorescein-proofs of crossing blood-brain-barrier. *New J. Chem.*, 2012, 36: 350-353

97 Alice Bertero, Adriano Boni, Mauro Gemmi, Mariacristina Gagliardi, Angelo Bifone, Giuseppe Bardi. Surface functionalisation regulates polyamidoamine dendrimer toxicity on blood-brain barrier cells and the modulation of key inflammatory receptors on microglia. *Nanotoxicology*, 2014, 8(2): 158-168

98 Renu Singh Dhanikula, Taha Hammady, Patrice Hildgen. On the Mechanism and Dynamics of Uptake and Permeation of Polyether-Copolyester Dendrimers Across an In Vitro Blood-Brain Barrier Model. *Journal of Pharmaceutical Science*, 2009, 98(10): 3748-3760

99 Heather A. Bullen, Ruth Hemmer, Anthony Haskamp, Chevelle Cason, Stephen Wall, Robert Spaulding, Brett Rossow, Michael Hester, Megan Caroway, Kristi L. Haik. Evaluation of Biotinylated PAMAM Dendrimer Toxicity in Models of the Blood Brain Barrier: A Biophysical and Cellular Approach. *Journal of Biomaterials and Nanobiotechnology*, 2011, 2: 485-493

100 Ganta S, Amiji M. Coadministration of Paclitaxel and curcumin in nanoemulsion formulations to overcome multidrug resistance in tumor cells. *Mol. Pharm.*, 2009, 6: 928-939

101 Srinivas Ganta, Dipti Deshpande, Anisha Korde. A review of multifunctional nanoemulsion systems to overcome oral and CNS drug delivery barriers. *Molecular Membrane Biology*, 2010, 27(7): 260-273

102 Ankita Desai, Tushar Vyas, Mansoor Amiji. Cytotoxicity and Apoptosis Enhancement in Brain Tumor Cells Upon Coadministration of Paclitaxel and Ceramide in Nanoemulsion Formulations. *J Pharm Sci*, 2008, 97: 2745-2756

103 Tushar K. Vyas, Aliasgar Shahiwala, Mansoor M. Amiji. Improved oral bioavailability and brain transport of Saquinavir upon administration in novel nanoemulsion formulations. *International Journal of Pharmaceutics*, 2008, 347: 93-101

104 Laura J. Vella, Robyn A. Sharples, Rebecca M. Nisbet, Roberto Cappai, Andrew F. Hill. The role of exosomes in the processing of proteins associated with neurodegenerative diseases. *Eur Biophys J*, 2008, 37: 323-332

105 Samir EL Andaloussi, Samira Lakhal, Imre Mäger, Matthew J. A. Wood. Exosomes for targeted siRNA delivery across biological barriers. *Advanced Drug Delivery Reviews*, 2013,

65: 391-397

106 Samira Lakhal, Matthew J.A. Wood. Exosome nanotechnology: An emerging paradigm shift in drug delivery. *Bioessays*, 2011, 33: 737-741

107 Matthew J. Haney, Natalia L. Klyachko, Yuling Zhao, Richa Gupta, Evgeniya G. Plotnikova, Zhijian He, Tejash Patel, Aleksandr Piroyan, Marina Sokolsky, Alexander V. Kabanov, Elena V. Batrakova. Exosomes as drug delivery vehicles for Parkinson's disease therapy. *Journal of Controlled Release*, 2015, 207: 18-30

108 Lydia Alvarez-Erviti, Yiqi Seow, HaiFang Yin, Corinne Betts, Samira Lakhal, Matthew J A Wood. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nature Biotechnology*, 2011, 29(4): 341-347

109 Tianzhi Yang, Paige Martin, Brittany Fogarty, Alison Brown, Kayla Schurman, Roger Phipps, Viravuth P. Yin, Paul Lockman, Shuhua Ba. Exosome Delivered Anticancer Drugs Across the Blood-Brain Barrier for Brain Cancer Therapy in Danio Rerio. *Pharm. Res.*, 2015, 32: 2003-2014

110 Shirin Ghaderi1, Bala Ramesh1, Alexander M. Seifalian. Fluorescence nanoparticles "quantum dots" as drug delivery system and their toxicity: a review. *Journal of Drug Targeting*, 2011; 19(7): 475-486

111 Jinhao Gao, Kai Chen, Renguo Xie, Jin Xie, Yongjun Yan, Zhen Cheng, Xiaogang Peng, Xiaoyuan Chen. In Vivo Tumor-Targeted Fluorescence Imaging Using Near-Infrared Non-Cadmium Quantum Dots. *Bioconjugate Chem.* 2010, 21: 604-609

112 Xiaoling Gao, Jun Chen, Jiyao Chen, Bingxian Wu, Hongzhuan Chen, Xinguo Jiang. Quantum Dots Bearing Lectin-Functionalized Nanoparticles as a Platform for *In Vi*W*o* Brain Imaging. *Bioconjugate Chem.*, 2008, 19: 2189-2195

113 Shingo Kato, Kyoko Itoh, Takeshi Yaoi, Takenori Tozawa, Yutaka Yoshikawa, Hiroyuki Yasui, Narisato Kanamura, Akiyoshi Hoshino, Noriyoshi Manabe, Kenji Yamamoto, Shinji Fushiki. Organ distribution of quantum dots after intraperitoneal administration, with special reference to area-specific distribution in the brain. *Nanotechnology*, 2010, 21: 1-7

114 Gaixia Xu, Ken-Tye Yong, Indrajit Roy, Supriya D. Mahajan, Hong Ding, Stanley A. Schwartz, Paras N. Prasad. Bioconjugated Quantum Rods as Targeted Probes for Efficient Transmigration Across an in Vitro Blood-Brain Barrier. *Bioconjugate Chem.*, 2008, 19:

1179-1185

115 Lihong Liu, Kun Guo, Jia Lu, Subbu S. Venkatraman, Dan Luo, Kian Chye Ng, Eng-Ang Ling, Shabbir Moochhala, Yi-Yan Yang. Biologically active core/shell nanoparticles self-assembled from cholesterol-terminated PEG-TAT for drug delivery across the blood-brain barrier. *Biomaterials*, 2008, 29(10): 1509-1517

116 Swadeshmukul Santra, Heesun Yang, Jessie T. Stanley, Paul H. Holloway, Brij M. Moudgil, Glenn Walterd, Robert A. Mericlee. Rapid and effective labeling of brain tissue using TAT-conjugated CdS:Mn/ZnS quantum dots. *Chem. Commun.*, 2005, 3144-3146

117 Swadeshmukul Santra, Heesun Yang, Paul H. Holloway, Jessie T. Stanley, Robert A. Mericle. Synthesis of Water-Dispersible Fluorescent, Radio-Opaque, and Paramagnetic CdS:Mn/ZnS Quantum Dots: A Multifunctional Probe for Bioimaging. *J. Am. Chem. Soc.*, 2005, 127: 1656-1657

118 Discher BM, Won YY, Ege DS, Lee JC, Bates FS, Discher DE, et al. Polymersomes: tough vesicles made from diblock copolymers. *Science*, 1999, 284: 1143-1146

119 Huile Gao, Zhiqing Pang, Xinguo Jiang. Targeted Delivery of Nano-Therapeutics for Major Disorders of the Central Nervous System. *Pharm Res*, 2013, 30: 2485-2498

120 Rajesh Singh, James W. Lillard Jr. Nanoparticle-based targeted drug delivery. *Experimental and Molecular Pathology*, 2009, 86: 215-223

121 Mariacristina Gagliardi, Giuseppe Bardi, Angelo Bifone. Polymeric nanocarriers for controlled and enhanced delivery of therapeutic agents to the CNS. *Therapeutic Delivery*, 2012, 3(7): 875-887

122 Pardridge WM. Molecular Trojan horses for blood-brain barrier drug delivery. *Curr Opin Pharmacol.*, 2006, 6: 494-500

123 Zhiqing Pang, Wei Lu, Huile Gao, Kaili Hu, Jun Chen, Chaolin Zhang, Xiaoling Gao, Xinguo Jiang, Cuiqing Zhu. Preparation and brain delivery property of biodegradable polymersomes conjugated with OX26. *Journal of Controlled Release*, 2008, 128(2): 120-127

124 James C-M. Lee, Harry Bermudez, Bohdana M. Discher, et al. Preparation, stability, and in vitro performance of vesicles made with diblock copolymers. *Biotechnol. Bioeng.*, 2001, 73: 135-145

125 Lomas H, Canton I, MacNeil S, Du J, Armes SP, Ryan AJ, et al. Biomimetic pH sensitive

polymersomes for efficient DNA encapsulation and delivery. *Adv. Mater.*, 2007, 19: 4238-4243

126 Dalia Hope Levine, P. Peter Ghoroghchian, Jaclyn Freudenberg, et al. Polymersomes: A new multi-functional tool for cancer diagnosis and therapy. *Methods*, 2008, 46(1): 25-32

127 Kevin Letchford, Helen Burt. A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes. *European Journal of Pharmaceutics and Biopharmaceutics*, 2007, 65: 259-269

128 Zhiqing Pang, Huile Gao, Yuan Yu, Jun Chen, Liangran Guo, Jinfeng Ren, Ziyi Wen, Jinghan Sub, Xinguo Jiang. Brain delivery and cellular internalization mechanisms for transferrin conjugated biodegradable polymersomes. *International Journal of Pharmaceutics*, 2011, 415: 284-292

129 Huile Gao, Zhiqing Pang, Li Fan, Kaili Hu, Bingxian Wu, Xinguo Jiang. Effect of lactoferrin- and transferrin-conjugated polymersomes in brain targeting: *in vitro* and *in vivo* evaluations. *Acta Pharmacologica Sinica*, 2010, 31: 237-243

130 Yuan Yu, Zhiqing Pang, Wei Lu, Qi Yin, Huile Gao, Xinguo Jiang. Self-Assembled Polymersomes Conjugated with Lactoferrin as Novel Drug Carrier for Brain Delivery. *Pharm. Res.*, 2012, 29: 83-96

131 YungChu Chen, ChiFeng Chiang, LiFang Chen, PoChin Liang, Wen-Yuan Hsieh, Win-Li Lin. Polymersomes conjugated with des-octanoyl ghrelin and folate as a BBB-penetrating cancer cell-targeting delivery system. *Biomaterials*, 2014, 35: 4066-4081

132 Jörg Kreuter. Mechanism of polymeric nanoparticle-based drug transport across the blood-brain barrier (BBB). *Journal of Microencapsulation*, 2013, 30(1): 49-54

133 Sulin Zhang, Ju Li, George Lykotrafitis, Gang Bao, Subra Suresh. Size-Dependent Endocytosis of Nanoparticles. *Adv. Mater.*, 2009, 21: 419-424

134 Khin Yin Wina, Si-Shen Feng. Effects of particle size and surface coating on cellular uptake of polymeric nanoparticles for oral delivery of anticancer drugs. *Biomaterials*, 2005, 26: 2713-2722

135 Shirui Mao, Wei Sun, Thomas Kissel. Chitosan-based formulations for delivery of DNA and siRNA. *Advanced Drug Delivery Reviews*, 2010, 62: 12-27

136 Driton Vllasaliua, Ruth Exposito-Harris, Angeles Heras, Luca Casettari, Martin Garnett, Lisbeth Illum, Snow Stolnik. Tight junction modulation by chitosan nanoparticles: Comparison with chitosan solution. *International Journal of Pharmaceutics*, 2010, 400: 183-193

137 Paul R. Lockman, Joanna M. Koziara, Russell J. Mumper, David D. Allen. Nanoparticle Surface Charges Alter Blood-Brain Barrier Integrity and Permeability. *Journal of Drug Targeting*, 2004, 12(9-10): 635-641

138 D Kobiler, S Lustig, Y Gozes, D Ben-Nathan, Y Akov. Sodium dodecylsulphate induces a breach in the blood-brain barrier and enables a West Nile virus variant to penetrate into mouse brain. *Brain Res.*, 1989, 496: 314-316

139 Eleni Markoutsa, Georgios Pampalakis, Anna Niarakis, et al. Uptake and permeability studies of BBB-targeting immunoliposomes using the hCMEC/D3 cell line. *European Journal of Pharmaceutics and Biopharmaceutics*, 2011, 77: 265-274

140 Chun-Fu Lien, Éva Molnár, Petr Toman, John Tsibouklis, Geoffrey J. Pilkington, Dariusz C. Górecki, Eugen Barbu. In Vitro Assessment of Alkylglyceryl-Functionalized Chitosan Nanoparticles as Permeating Vectors for the Blood-Brain Barrier. *Biomacromolecules*, 2012, 13: 1067-1073

141 Hyun Ryoung Kim, Karine Andrieux, Claudine Delomenie, Héléne Chacun, Martine Appel, Didier Desma, Fréderic Taran, Dominique Georgin, Patrick Couvreur, Myriam Taverna. Analysis of plasma protein adsorption onto PEGylated nanoparticles by complementary methods: 2-DE, CE and Protein Lab-on-chip® system. *Electrophoresis*, 2007, 28: 2252-2261

142 M. Simionescu, N. Simionescu. Endothelial transport of macromolecules: transcytosis and endocytosis. A look from cell biology. *Cell. Biol. Rev.*, 1991, 25: 1-78

143 Françoise Hervé, Nicolae Ghinea, Jean-Michel Scherrmann. CNS Delivery via Adsorptive Transcytosis. *The AAPS Journal*, 2008, 10(3): 455-472

144 Dimple Chopra, Monica Gulati, Vikrant Saluja, Purnima Pathak, Parikshit Bansal. Brain Permeable Nanoparticles. *Recent Patents on CNS Drug Discovery*, 2008, 3: 216-225

145 Jörg Kreuter. Nanoparticulate systems for brain delivery of drugs. *Advanced Drug Delivery Reviews*, 2001, 47: 65-81

146 Ho Lun Wong, Xiao Yu Wu, Reina Bendayan. Nanotechnological advances for the delivery

of CNS therapeutics. *Advanced Drug Delivery Reviews*, 2012, 64: 686-700

147 Sibel Bozdağ Pehlivan. Nanotechnology-Based Drug Delivery Systems for Targeting, Imaging and Diagnosis of Neurodegenerative Diseases. *Pharm Res.*, 2013, 30: 2499-2511

148 Lucienne Juillerat-Jeanneret. The targeted delivery of cancer drugs across the blood-brain barrier: chemical modifications of drugs or drug-nanoparticles? *Drug Discovery Today*, 2008, 13(23/24): 1099-1106

149 Torsten M. Göppert, Rainer H. Müller. Polysorbate-stabilized solid lipid nanoparticles as colloidal carriers for intravenous targeting of drugs to the brain: Comparison of plasma protein adsorption patterns. *Journal of Drug Targeting*, 2005, 13(3): 179-187

150 Luca Costantino, Diana Boraschi. Is there a clinical future for polymeric nanoparticles as brain-targeting drug delivery agents? *Drug Discovery Today*, 2012, 17(7-8): 367-378