UNIVERSITÉ DE GENÈVE

Archive ouverte UNIGE
https://archive-ouverte.unige.ch

Article scientifique | Article | 2010

Published version | Open Access

This is the published version of the publication, made available in accordance with the publisher's policy.

In vitro blood brain barrier models as a screening tool for colloidal drug delivery systems and other nanosystems

Garcel, Aude; Martel, Sophie; Carrupt, Pierre-Alain; Doelker, Eric; Delie Salmon, Florence

How to cite

GARCEL, Aude et al. In vitro blood brain barrier models as a screening tool for colloidal drug delivery systems and other nanosystems. In: International journal of biomedical nanoscience and nanotechnology, 2010, vol. 1, n° 2/3/4, p. 133–163. doi: 10.1504/IJBNN.2010.034650

This publication URL: https://archive-ouverte.unige.ch/unige:14543
Publication DOI: 10.1504/IJBNN.2010.034650

© This document is protected by copyright. Please refer to copyright holder(s) for terms of use.

In vitro blood brain barrier models as a screening tool for colloidal drug delivery systems and other nanosystems

Aude Garcel, Sophie Martel, Pierre Alain Carrupt, Eric Doelker and Florence Delie*

School of Pharmaceutical Sciences,
University of Geneva, University of Lausanne,
Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland
Fax: +41-223-796-567
E-mail: aude.garcel@igmm.cnrs.fr
E-mail: Sophie.Martel@unige.ch
E-mail: Pierre-Alain.Carrupt@unige.ch
E-mail: Eric.Doelker@unige.ch
E-mail: Florence.Delie@unige.ch
*Corresponding author

Abstract: The brain is one of the least accessible organs of the body due to the presence of the blood-brain barrier (BBB), thus making drug delivery to the brain quite a challenge. Various strategies have been explored to circumvent this physiological barrier, including the use of colloidal carriers. These carriers hold great promise as they may increase the delivery of drugs into the brain by protecting them from degradation and prolonging their circulation in the blood, as well as promoting their transport through the BBB. Moreover, functionalisation of these carriers with various ligands allows specific targeting of the central nervous system compartment. Additionally, various *in vitro* BBB models have been developed and are increasingly useful for screening of drug delivery systems, especially cell-based models that provide mechanistic information. In fact, this paper specifically reviews selected *in vitro* BBB models as a screening tool for drug delivery colloidal systems.

Keywords: drug delivery; colloidal systems; nanocarriers; nanoparticles; liposomes; micelles; blood-brain barrier; BBB; *in vitro* model.

Reference to this paper should be made as follows: Garcel, A., Martel, S., Carrupt, P.A., Doelker, E. and Delie, F. (2010) '*In vitro* blood brain barrier models as a screening tool for colloidal drug delivery systems and other nanosystems', *Int. J. Biomedical Nanoscience and Nanotechnology*, Vol. 1, Nos. 2/3/4, pp.133–163.

Biographical notes: Aude Garcel is a Pharmacist and received her PhD from Grenoble University in France. She worked on the neuropathogenesis of the vaccinia virus, studying the interaction of the virus with a blood-brain barrier cellular model. She then joined the Laboratory of Pharmaceutical Technology and Biopharmacy in Geneva (Switzerland) as a Postdoctoral Scientist working on the development of colloidal carriers for central nervous system drug delivery. She has adapted a cellular model of the blood-brain barrier to study the interaction of particles with this barrier.

Copyright © 2010 Inderscience Enterprises Ltd.

Sophie Martel graduated as a Chemist and obtained her PhD at the University of Marseille (France) at the end of 2002. Then, she spent one year as a post-doc with Prof. Bernard Testa and Prof. Pierre-Alain Carrupt in the Medicinal Chemistry Institute at the University of Lausanne (Switzerland) where she turned her interests in physicochemical properties and permeation profile of drug candidates as applied to drug design and molecular pharmacology. Since 2004, she works as Senior Scientist in the Pharmacochemistry Group with Prof. Pierre-Alain Carrupt at the School of Pharmacy Lausanne-Genève (Geneva, Switzerland). Her current research is focused on the development of *in vitro* physicochemical and pharmacokinetic filters devoted to the selection of validated hits.

Pierre-Alain Carrupt obtained his PhD in Synthetic Chemistry and Computational Chemistry Applied to Organic Physical Chemistry, in 1979 at the University of Lausanne under the direction of Prof. Pierre Vogel. Then, he worked as a Medicinal Chemist in molecular modelling and physicochemistry applied to drug design and molecular pharmacology. In 2004, he was appointed as a Full Professor of Pharmacochemistry at the University of Geneva. His current research is focused on in vitro and in silico physicochemical, permeation and pharmacokinetic filters able to select better hits in the field of multifunctional hits for aging-related neurodegenerative diseases such as Alzheimer or Parkinson diseases. He is the author or co-author of about 290 research papers and 45 chapters in scientific books.

Eric Doelker is Full Professor at the School of Pharmaceutical Sciences of the University of Geneva. He was a Postdoctoral Fellow at the School of Pharmacy, University of London. His main research interests are in the solid state (compression physics, polymorphism), polymer synthesis and characterisation, controlled release dosage forms, and drug targeting. He is the author or co-author of more than 200 publications and 12 book chapters, and the holder of several patents. He is a Consultant for many pharmaceutical companies and belongs to several journal editorial boards. He is a member of important scientific and professional organisations; among others, he is Chairman of the Swiss Association of Readers in Pharmacy, of the Cellulose Derivatives Committee of the European Pharmacopoeia, and of the Pharmaceutical Technology Committee of the Swiss Pharmacopoeia. He was the Dean of the Faculty of Sciences (1995–1998) and Vice-Rector of the University of Geneva (2000–2003).

Florence Delie is currently a Research Associate and Lecturer at the School of Pharmaceutical Sciences in the Laboratory of Pharmaceutical Technology and Biopharmacy in Geneva. She is a Pharmacist and received her PhD from the Paris Sud University. She then joined the Department of Pharmaceutical R&D – Drug Delivery Group at Genentech, Inc. (South San Francisco, CA) as a Visiting Scientist working on particle/cell interaction with the Caco-2 model. In Geneva, her research focuses on the development of polymeric nanoparticles for encapsulation of different therapeutic compounds such as photosensitisers, peptides, and anticancer derivatives. She has a special interest in the interaction of colloidal carriers with biological barriers (oral, BBB), cellular targeting, and intracellular trafficking of polymeric particles.

1 Introduction

The brain presents a challenging but important organ for drug delivery, considering that the number of central nervous system (CNS)-related disorders will increase with the

aging population. The most important factor limiting the development of drugs for the CNS is the blood brain barrier (BBB), which limits the brain penetration of most candidate treatments. Moreover, another goal of the pharmaceutical industry is to develop strategies to deliver drugs selectively to the CNS. In this way, the therapeutic index can be maximised to provide a beneficial effect and to limit adverse reactions observed upon systemic administration of the drug. Thus, lower doses can be administered, treatment costs are reduced, and patient compliance is increased. Invasive drug delivery strategies have been the most widely used and have been successful in circumventing the BBB. However, numerous limitations, such as cerebro-spinal fluid (CSF) volume increase and potential infectious risks must be considered. Designing drug delivery systems to cross the BBB and deliver therapeutics to the brain in a controlled and non-invasive manner is therefore a key goal. Nanocarriers have been proposed as drug delivery systems for transporting drugs to various targeted tissues, and some nanocarrier-based delivery systems have been commercialised (e.g. liposomal doxorubicin in Doxyl® or amphotericin B in Ambisome® and nanoparticulate formulation of paclitaxel associated with human albumin in Abraxane®); however, to date, there is no nanosystem currently used in the clinic for drug delivery to the CNS. Among these systems, colloidal drug delivery systems hold great promise. *In vivo* models have been predominantly used to test their ability to penetrate the brain and to provide reliable reference information. However, *in vitro* models are increasingly needed, especially to provide mechanistic information and to allow faster screening of possible drug delivery systems.

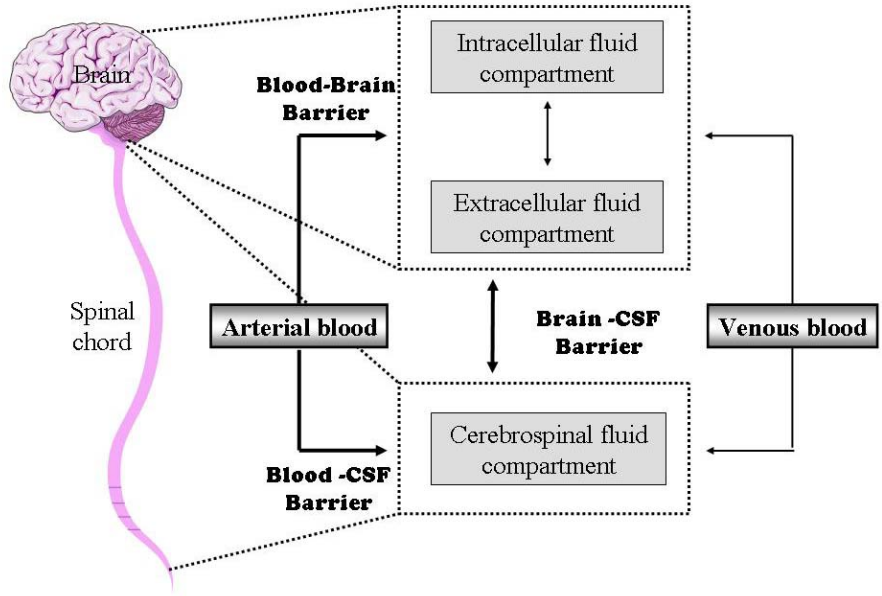
This manuscript reviews the current *in vitro* BBB models as screening tools for colloidal drug delivery systems. In order to understand the difficulties inherent in drug delivery to the CNS, the BBB structure and functions are briefly reviewed. Then, strategies developed for brain delivery are presented with a special interest applied to colloidal systems. Finally, the various *in vitro* BBB models are described along with their advantages and limitations, and a special section is devoted to studies performed on these models with colloidal carriers.

2 The blood-brain barrier

Two barriers, the blood-CSF barrier and the BBB, principally limit drug transport from the blood to the brain (Figure 1). The blood-CSF barrier is composed of the choroid plexus epithelial cells in cerebral ventricles and the arachnoid membrane. As the choroid plexus endothelia are extremely fenestrated and quite leaky, lacking tight-junctions between adjacent cells and P-glycoprotein (P-gp), so the blood-CSF barrier exists only at the level of the epithelial cells and acts as a minimal barrier for transport between the brain and the CSF (Rao et al., 1999; Zheng and Codobski, 2004). The BBB is a unique selective barrier comprised of brain capillary endothelial cells (BCECs) forming a continuous tubular cell layer separating blood from the brain; these are closely associated with astrocyte foot processes, perivascular neurons, and pericytes. With an estimated total length of 600 km and a total surface area of 20 m^2 in the human brain, this structure provides the major barrier preventing solutes from entering the brain. Fine structural differences exist between BCECs and peripheral endothelial cells. These include a lack of fenestration, a paucity of pinocytic vesicles, a greater number of mitochondria, expression of specific transport and efflux systems, and the presence of complex

tight-junctions in addition to the cell-cell adherens junctions that are responsible for the specific 'tightness' of the BCECs (Persidsky et al., 2006). Most of these properties are partly induced and maintained by the close association with astrocyte foot processes, which release environmental factors and cover close to 99% of the abluminal surface area of the capillary endothelium (Francis et al., 2003; Pardridge, 2005) (Figure 2).

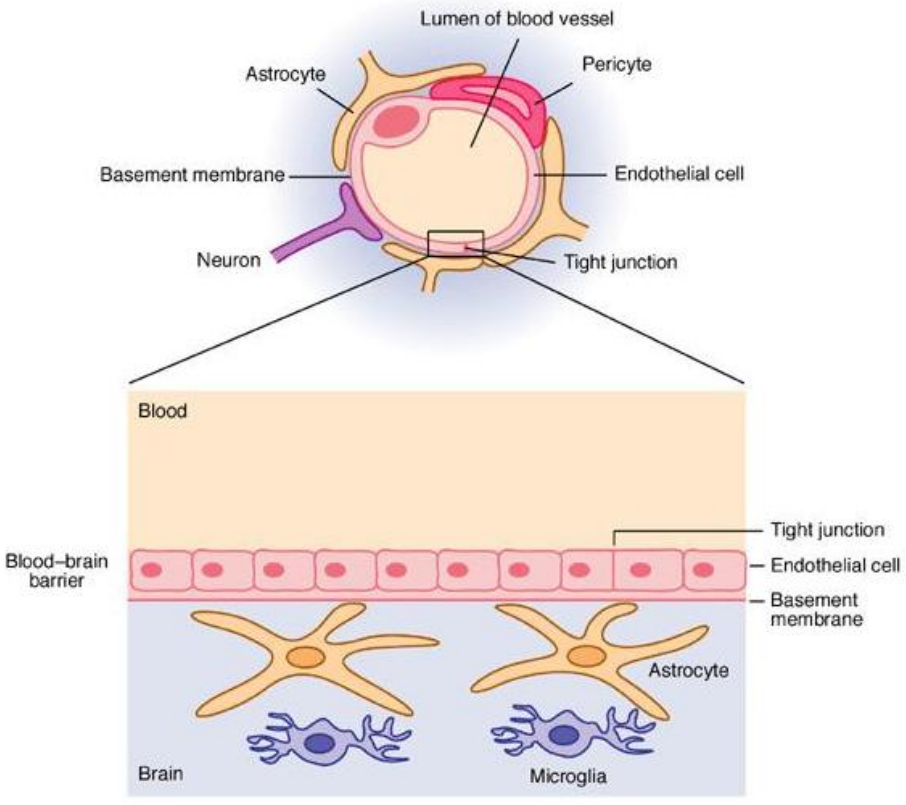
Figure 1 Schematic representation of the three barriers protecting the brain and their interconnection with the blood compartment (see online version for colours)



Tight junctions are composed of transmembrane proteins (occludin, claudins, and junction-associated membrane proteins), which are the main contributors that interact together to seal the paracellular pathway, as well as several cytoplasmic accessory proteins (zonula occludens (ZO) 1, 2, and cingulin) linked to the actin cytoskeleton to allow permeability modulation in response to stimuli (Hawkins and Davis, 2005). The permeability of tight junctions has been defined by the electrical resistance across the endothelium. The greater the resistance, the more restrictive the paracellular transport of ions and the tighter the junctions. The trans-endothelial electrical resistance (TEER) varies among the different cell types, and detailed data may be found in Deli et al. (2005). For example, the TEER is more than 1000 Ω cm^2 for BCECs, as compared to 5–10 Ω cm^2 for most other systemic capillary endothelia (Butt et al., 1990). These junctions effectively block an aqueous route of free diffusion and limit the passage of substances in both ways. Therefore, the BBB can carry out these two functions: neuroprotection and maintenance of a stable intracerebral extracellular fluid compartment (Begley, 2004). Nevertheless, the CNS must be supplied with essential nutrients and receive biological messengers. Indeed, due to the restricted paracellular pathway, molecular traffic between blood and brain is forced to take a transcellular route. Small and lipid-soluble agents can passively diffuse through the membranes, while BCECs must maintain a high level of expression of transport proteins for polar metabolites such

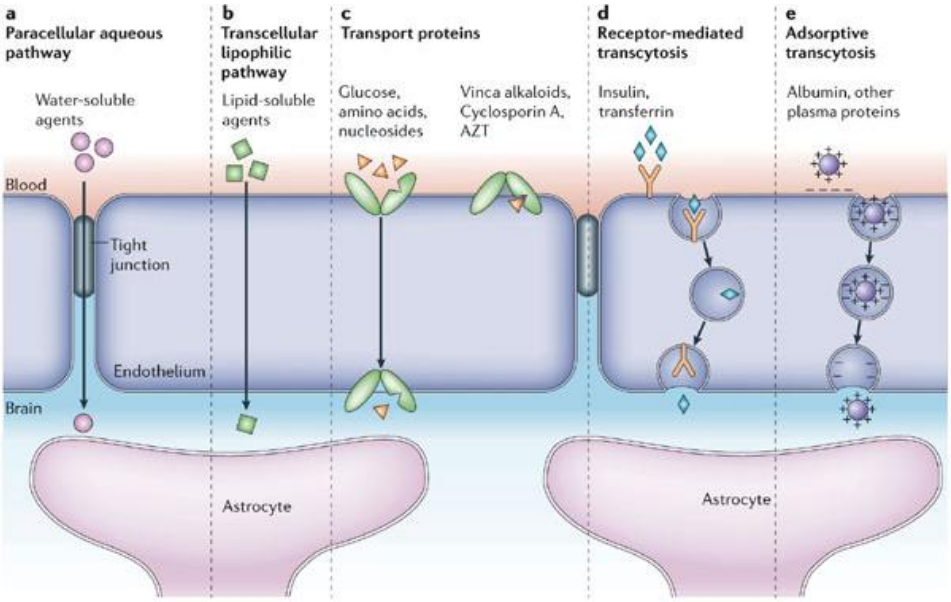
as glucose and amino acids to facilitate their entry into the brain. These transporters mostly originate from the ATP-binding cassette (ABC) protein superfamily and are uni- or bi-directional, but their expression is predominantly polarised, with some expressed exclusively in the luminal membrane of the BCECs and some in the abluminal side, while others are inserted into both membranes. Some, such as the P-glycoprotein and the multidrug resistance-associated protein family, are efflux carriers for waste products and exogenous compounds of potential toxicity (Persidsky et al., 2006). These transporters require substantially more energy, which results in a concentration in mitochondria that is four times higher than in other cells of the body. Moreover, molecules too large for carrier-mediated entry, such as peptides and proteins, must cross the endothelium via a vesicular route either by receptor-mediated transcytosis or nonspecific adsorptive-mediated transcytosis (Abbott et al., 2006; Begley, 2004). Potential routes across the BBB for solutes or drugs are sketched in Figure 3. BCECs also express several surface and intracellular enzymes, such as monoamine oxidase, γ-glutamyl transpeptidase, alkaline phosphatase, specific peptidases, nucleotidases, and several cytochrome P450 enzymes that form a metabolic barrier (El Bacha and Minn, 1999).

Figure 2 Schematic representation of the cellular basis of the BBB



Source: Modified from Francis et al. (2003). Reprinted with permission.

Figure 3 Pathways across the BBB



Source: Abbott et al. (2006). Reprinted with permission.

Finally, it is important to realise that if the protective function of the BBB is essential for physiological function within the brain microenvironment, it also results in an obstacle to the entry of drugs into the CNS.

3 Drug delivery to the brain

The diffusion of a drug from the blood into the brain is mainly dependent on its ability to enter the BCECs. This is correlated with physicochemical properties such as optimised lipophilicity, low molecular size, and a neutral or negative charge, or the appropriate domain for interaction with a receptor or a carrier required to cross the BBB. However, numerous potentially interesting molecules do not have these physicochemical characteristics. Therefore, several strategies have been developed to overcome this barrier. Three main approaches have been explored:

1 circumvent or disrupt the BBB

2 modify the drug characteristics or to attach specific ligands

3 use colloidal carriers (Patel et al., 2009).

Furthermore, the brain uptake of drugs depends not only on their ability to cross the BBB but also on their concentration in the blood. In fact, based on the pharmacokinetic rule where the dose taken up by the brain is proportional to the permeability-surface area and the area under the curve, brain uptake could be improved not only by an increase in the drug transport but also by increasing drug residence time in the blood.

3.1 Bypassing the BBB

To overcome the BBB, various invasive methods have been developed, such as intracerebral and intraventricular injections or infusions, where the drug is directly administered into the brain or into the CSF, respectively. When administered intracerebrally, 100% of the dose should reach the target. This approach may also be used to introduce a slow-release implant, even though drug diffusion decreases exponentially with the diffusion distance (Fung et al., 1996). Therefore, this strategy may only be applied to local treatment. Moreover, the brain's extracellular fluid turnover steadily drains the drug away from the injection site (Begley, 2004). The major drawback of this method is that it can damage the brain tissue. On the other hand, intraventricular administration should theoretically allow direct access for the drug to the brain by bypassing the BBB and the blood-CSF barrier. However, the efficacy of this method is limited by the presence of a functional CSF-brain barrier and by the high turnover of the CSF (Koziara et al., 2006). Because the diffusion from the ependymal cell surface of the CSF-brain barrier is slow and dependent on the molecular weight of the molecule, paradoxically, the drug distributes much better from the CSF to the blood than to the brain (Pardridge, 2007). Moreover, the intraventricular injection is responsible for an increase in CSF volume and pressure that could lead to side effects such as haemorrhage, CSF leakage, and neurotoxicity (Koziara et al., 2006).

Intranasal administration has been proposed as an alternative and non-invasive route for drug delivery to the brain. This method offers rapid assimilation into the blood without first-pass hepatic metabolism, but the drug must still pass through the BBB. However, direct transport from the olfactory cavity to the CNS has also been reported via the olfactory nerve (slow transport) or the olfactory epithelial pathways (rapid transport) (Bagger and Bechgaard, 2004). Major drawbacks of this method are the very low amounts transported to the brain and limited distribution (Koziara et al., 2006).

3.2 BBB disruption

Disruption of the BBB was one of the earliest techniques used for therapeutic purposes (Garcia-Garcia et al., 2005a). The BBB can be transiently disrupted by a variety of agents such as hyperosmotic solutions, vasoactive molecules, ultrasound and electromagnetic radiation, or efflux-pump inhibitors. Drug transport across the brain endothelium is thus increased via the paracellular or transcellular pathways.

Osmotic opening is performed by an intracarotid injection of a hypertonic solution of sugar, such as mannitol, and leads to a size-dependent entry into the brain, most likely due to tight-junction leakage (Begley, 2004). This approach also can cause undesired side effects, including physiological stress and intracranial pressure increase.

The BBB can also be permeabilised by vasoactive molecules such as bradykinin (BK), vascular endothelial growth factor (VEGF), serotonin, or histamine (Koziara et al., 2006). The BK approach seems to modulate the tight-junctions by elevating intracellular free calcium levels (Begley, 2004) and is responsible for a selective opening of the blood-brain tumour barrier, as compared to the normal BBB (Koziara et al., 2006). This selectivity is based on a divergence of γ-glutamyltranspeptidase and BK receptor type 2 expressions (Garcia-Garcia et al., 2005a). Alkylglycerols have also been shown to modify the BBB but the mechanism is not certain and it seems to act both on normal and tumour brain tissue (Erdlenbruch et al., 2003).

Recently, ultrasounds have been described as a BBB modulator by two major mechanisms, tight-junction opening and active transport by vacuoles (Hynynen, 2008). This disruption seems to be associated with minimal damage of the vasculature or the surrounding brain tissue. Concerning the effect of electromagnetic radiation, the effect on BBB permeability and the mechanism by which the BBB might be modulated are a matter of debate (Franke et al., 2005; Hossmann and Hermann, 2003). It is clear that, if they are accepted, these methods are attractive as they can be focused with precision to a specific brain region or to a tumour, and selectively modulate the BBB at a desired site and not globally throughout the brain.

Finally, the inhibition of efflux transporters of the BCECs could improve the brain delivery of substrates through these transporters (Koziara et al., 2006). Two strategies could be developed: co-administration of a specific inhibitor or design of a drug analogue that is still effective but does not interact with the efflux transporter. Nevertheless, blocking these pumps can be neurotoxic due to enhanced penetration of other toxic substrates and inefficient elimination of toxic metabolites.

The problem with BBB disruption for even a brief period of time is that it is not drug-specific. In fact, this approach allows the leakage of substances that circulate harmlessly through the peripheral bloodstream into the brain, such as albumin, which is toxic to astrocytes (Pardridge, 2007). Moreover, the brain is also vulnerable to the entry of infectious agents and toxins.

3.3 Drug modifications

It has been well established that the lipid solubility of a drug correlates with its ability to pass through the BBB (Levin, 1980). Thus, modification of a drug to optimise its hydrophobicity may result in enhancement of brain penetration. This could be done by masking and/or removing the hydrogen bonding groups, performing other structural changes, or by adding a lipophilic moiety. Nevertheless, increasing the lipid solubility of a molecule may be responsible for drawbacks such as a decrease in aqueous solubility and thus bioavailability, by an increase in plasma protein binding and hepatic retention, and by increased uptake by the reticuloendothelial system (Begley, 2004).

Regarding peptide drugs, glycosylation is also a strategy that leads to increased brain uptake (Egleton and Davis, 2005). This transport is not related to an interaction with the glucose transporter, but it is currently believed that it triggers adsorptive endocytosis. Moreover, glycosylation of small peptides leads to increase hydrophilicity, stability, and bioavailability.

The structure of a molecule could also be modified using a prodrug approach, which consists of the delivery of an inactive form that is then spontaneously or enzymatically converted to the active drug once in the brain. Ideally, the prodrug should be quite lipophilic in order to cross the BBB, while the active drug should be more polar so to remain into the brain (Begley, 2004). Bodor and colleagues have developed another prodrug concept that, in addition of providing access by increasing the lipophilicity, exploits the specific bidirectional properties of the BBB to 'lock-in' inactive drug precursors in the brain, preventing exit back across the BBB (Bodor and Buchwald, 2002, 2008; Tapfer et al., 2004). These chemical delivery systems (CDSs) are associated to a lipophilic targeting moiety allowing passive diffusion through the BBB, which is then modified in the brain by a metabolic conversion into a lipophobic molecule no longer able to exit.

Unlike previous drug modifications that rely on chemistry, conjugation with a ligand is a biological approach. As described in the first section, there are many carriers and receptors expressed at the luminal surface of the BCECs, and many of these can be targeted to achieve drug recognition and uptake. Thus, it is possible to attach a specific ligand or an antibody that interacts with the targeted transporter. Moreover, with knowledge of the stereochemical requirements for transport by these carriers, the drug can also be designed as a pseudosubstrate. De Boer and Gaillard (2007) have reviewed many possible receptors for brain targeting. Among them, the most common is the transferrin receptor, which has been extensively characterised. In this case, drug targeting is reached with either the transferrin ligand or an antibody against the receptor (OX-26). In addition to transferrin, insulin, LRP-1, and LRP-2 receptors can be cited, and more recently, the diphtheria toxin receptor has been identified as a novel applicable carrier protein. Antibodies present the major drawback of not being directly applicable to humans without humanisation. Moreover, they could be responsible for side effects as they reduce the receptor availability for endogenous ligands. Similar side effects can be observed with the use of ligands as they induce a down regulation of receptors. Another important concern is that several of the listed receptors are involved in cell signalling processes.

Drugs could also be conjugated to a cell-penetrating peptide, such as the HIV virus transactivating-transduction (TAT) peptide, which has the ability to translocate across the cellular membranes and gain access to the cell interior (Herve et al., 2008). However, its use is limited as it can penetrate many cell types, and specific targeted cell-penetrating peptide delivery strategies therefore need to be developed. Other problems are associated with its stability, toxicity, and immunogenicity.

3.4 Use of colloidal carriers

Colloidal carriers consist of lipidic or polymeric particles, with a size ranging from 1 to 1000 nm, in which the drug is adsorbed, entrapped, or encapsulated. These include micelles, liposomes, polymeric nanoparticles (nanospheres and nanocapsules), solid lipid nanoparticles and nanogels. For human application, they must respond to ideal characteristics of biocompatibility, biodegradability, nonimmunogenicity, physical stability in the blood (e.g., absence of aggregation), and reduced opsonisation (Beduneau et al., 2007). Specific interests in these carriers are multiple. Primarily, they mask the drug's physico-chemical properties. They also improve drug bioavailability by facilitating diffusion through biological membranes and by avoiding degradation by enzymes, but they are generally used to increase specificity towards cells or tissues (Garcia-Garcia et al., 2005a). Moreover, as relatively large amounts of drug can be incorporated into these structures and because their surface can be coated with ligands, they provide a key method for brain drug delivery. Among the different carriers, liposomes and nanoparticles have been the most largely studied for brain drug delivery. For a review of nanocarrier-based CNS delivery systems, see Tiwari and Amiji (2006).

3.4.1 Liposomes

Liposomes are spherical vesicles formed by phospholipid bilayers in aqueous solutions. Depending on the selected lipids and the preparation protocol, liposomes can vary widely

in size, number, and position of lamellae, charge, and bilayer rigidity. Roughly, they can be divided into two groups: small unilamellar vesicles (SUV) with sizes below 100 nm and large multilamellar vesicles (MLV) ranging in size from 100 nm to several microns or even larger. They may be composed of various lipids: phospholipids from natural sources, modified natural phospholipids, and semi- and fully-synthetic phospholipids with nonnatural head groups. Along with phospholipids, cholesterol is commonly used to modulate fluidity, elasticity, and permeability. Liposomes can carry hydrophilic molecules in their cavities, and lipophilic compounds within their layers as well as amphiphilic ones. One major limitation is rapid clearance of the liposomes by the reticuloendothelial system (RES) after intravenous administration, with an accumulation particularly in the liver and spleen, with the MLVs accumulating more extensively and rapidly than the SUVs. To reduce this effect and prolong circulation time, liposome surface modifications based on hydrophylisation have been attempted (mannose, polysorbates, polyethylene glycols, and so on). Temperature-sensitive liposomes have also been reported in the literature using phospholipid compositions with an appropriate phase transition temperature (Kakinuma et al., 1996), and a more recent publication reported the use of magnetic liposomes (Jain et al., 2003).

3.4.2 Polymeric nanoparticles

Nanoparticles are solid colloidal particles ranging in size from 10 nm to 1 μm. Nanospheres consist of a matrix made up of a dense polymeric network, while nanocapsules consist of a thin polymeric envelope surrounding a lipophilic core. Drugs may be dissolved or suspended in the nanoparticle matrix, dissolved or suspended in the core, or adsorbed or attached to the surface. Several synthetic polymers are commonly used, such as poly(methyl methacrylate), poly(alkyl cyanoacrylate), poly(lactic acid), poly(lactic-co-glycolic acid), and acrylic copolymers, but natural proteins (albumin, gelatin) and polysaccharides (dextran, chitosan) can also be used. Use of nanoparticles provides several advantages; due to their small size, they circulate along the capillaries and are taken up within the cells and, moreover, the use of biodegradable material allows drug release over a long period after injection. Like liposomes, nanoparticles are rapidly cleared from the blood following intravenous administration, and several attempts to change their biodistribution have lead to coating with hydrophilic surfactants or to polymer hydrophilisation. Nanoparticles of various compositions have been used for drug delivery to the CNS and are precisely listed by Tiwari and Amiji (2006), but the most extensively studied have been poly(butyl) cyanoacrylate nanoparticles. Nanoparticles with polysorbate 80 as a surfactant have demonstrated the brain delivery capability of peptides and other drugs (Gulyaev et al., 1999; Kuo and Chen, 2006; Kuo and Su, 2007; Reimold et al., 2008; Weiss et al., 2008), especially those with a diameter under 100 nm (Gao and Jiang, 2006). Nevertheless, there are conflicting data with regards to their toxicity (Kreuter, 2001; Olivier et al., 1999). It has been reported that the use of apolipoproteins E and B or A-I coating of these nanoparticles in the blood compartment is essential for their transport across the BBB using LDL receptor-mediated transport or the scavenger receptor class B type I (Kreuter et al., 2002; Petri et al., 2007). Furthermore, several studies have highlighted the impact of nanoparticle surface charge with a preference for neutral or negatively charged ones as cationic nanoparticles have a toxic effect on the BBB (Fenart et al., 1999; Lockman et al., 2004).

3.4.3 Other types of colloidal carriers

Among the other types of colloidal carriers used for CNS drug delivery, solid lipid nanoparticles (SLN) are dispersions of lipids and waxes that have the particularity of remaining solid at physiological temperatures (triglycerides, cetyl palmitate) and are stabilised by emulsifiers (poloxamers, polysorbates, lecithins, and bile salts). Several characteristics and results obtained for drug delivery have shown these as promising systems (Blasi et al., 2007; Kaur et al., 2008; Tiwari and Amiji, 2006).

Polymeric micelles as drug delivery systems are formed by amphiphilic copolymers with a hydrophilic moiety making up the shell and a hydrophobic one acting as the core. Self-assembly occurs at the copolymer critical micelle concentration (CMC). Micelles are thermodynamically and kinetically stable in aqueous media and preserve their structure after dilution in the blood compartment. These carriers have been recently tested for antibiotic delivery across the BBB (Liu et al., 2008a, 2008b). Finally, a new type of carrier, called polymersomes, which are similar to liposomes but are made using amphiphilic synthetic block copolymers, have demonstrated some efficacy for brain delivery (Pang et al., 2008).

For all of these colloidal carriers, active targeting can be achieved by grafting a ligand or an antibody onto the surface to take advantage of carrier-mediated transport, receptor-mediated endocytosis, and adsorptive-mediated endocytosis systems, as explicitly described by Beduneau et al. (2007). The choice of ligand is based on its specificity, stability, and availability for selectivity on the target cells. Targeting ligands are attached to the carrier surface via covalent, ionic, or hydrophobic interaction.

Before clinical application, these colloidal carriers need to be further characterised for the influence of parameters such as size, polymer type, surface energy, and charge, and on targeting efficiency and cerebral uptake. It would also be very helpful to know more about the mechanism(s) of particle internalisation in the BCECs and/or the particles (or drug) transport from the BBB to the brain. To obtain this information, *in vitro* BBB models should be very useful for collection of data at the molecular level.

4 *In vitro* BBB models

Modelling of the BBB is currently a necessity for the pharmaceutical industry because it is important to determine the permeability of drug candidates early in the drug discovery process. High-throughput *in silico* models have been investigated as predictors of *in vivo* BBB permeability (Garg and Verma, 2006; Goodwin and Clark, 2005; Liu et al., 2004). Based on physical parameter properties such as octanol-water partition coefficient (logPoct), hydrogen-bonding potential (Δlog P), molecular polar surface area (PSA), and surface tension (Liu et al., 2004), they essentially predict passive transcellular diffusion. Even if *in silico* models do not account for metabolism, transporter-mediated processes, or any other drug-membrane or drug-protein interactions, they remain useful for rapid prediction of passive permeability in the early stages of drug discovery. On the other hand, *in vivo* approaches provide some of the most reliable reference information, but animal-based assays tend to be time-consuming and require bioanalytical input or access to radiolabelled compounds, and are therefore applied to a limited number of drug candidates. Moreover, care should be taken when interpreting results of correlations between different *in vivo* approaches, as discrepancies could result for many reasons. In

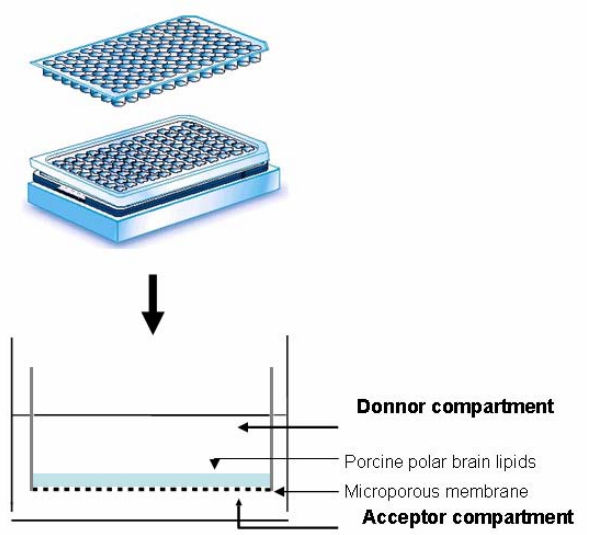
an intermediate stage, well-characterised *in vitro* BBB models that function as a moderate throughput screening tool would be highly useful in the discovery process. In comparison to animal-based evaluations, *in vitro* studies show several advantages: lower drug amounts are needed for evaluation, more compounds can be screened, most drugs can be assayed directly in buffer, and these methods both respond to ethical considerations and are also economical (Lundquist and Renftel, 2002). Generally, *in vitro* methods are based on non-cell-based models such as the parallel artificial membrane permeability assay (PAMPA), immobilised artificial membrane chromatography (IAMc), and capillary electrophoresis, or on cell-based models such as isolated brain capillaries or cultured cells.

4.1 Non cell-based models

IAMs are used as a liquid chromatographic interface with which the solute interaction is evaluated. IAM phases are commonly made of phosphatidylcholine residues covalently bound to silica propylamine in order to mimic a membrane lipid bilayer (Nicolazzo et al., 2006). A recent study using a set of 23 structurally unrelated drugs suggests that IAM chromatography may be useful to classify drugs as 'high brain penetration' (CNS+) and 'low brain penetration' (CNS-) drugs (Yoon et al., 2006).

The PAMPA technique has been extensively used to predict oral absorption (Kerns, 2001; Loftsson et al., 2006; Wohnsland and Faller, 2001) and skin permeation (Ottaviani et al., 2006, 2007) with better correlation than with octanol/water log D values (Faller, 2008). Recently, Di et al. (2003, 2009) developed a modified PAMPA model for BBB permeability prediction (PAMPA-BBB) using porcine polar brain lipids, and evaluated it with a set of 30 structurally diverse commercial drugs (Figure 4). This approach was shown to successfully predict passive diffusion through the BBB. Moreover, a cassette assay with six drugs in the same well was developed to enhance the throughput (Carrara et al., 2007).

Figure 4 PAMPA-BBB model in a 96-well plate and detail of a well (see online version for colours)



An alternative model based on liposome electro-kinetic chromatography (LEKC), previously reported to determine the lipophilicity of the solutes and to evaluate drug human absorption, has been tested for fast profiling of drugs for permeability across the BBB (Wang et al., 2007). LEKC has the advantages of low cost and no sample purity requirement as compared to PAMPA.

These models, based on membrane-mimic systems, offer the advantage of a shorter analysis time, experimental simplicity, and automation, but they are only relevant to passively permeating compounds.

4.2 Cell-based models

Although junction tightness of any relevant *in vitro* BBB model is an essential parameter, there are other aspects to consider. Apart from having the potential to account for the complex molecular interactions underlying BBB permeability, BBB cell-based models offer other advantages. Indeed, they allow the study of more complex mechanisms, such as active transport, structure-transport relationships, and molecular mechanisms. It is also possible to detect early signs of cell toxicity, and pathological conditions can be induced (Lundquist and Renftel, 2002). A range of *in vitro* BBB cell-based models have been developed, such as isolated brain capillaries, primary cultured/low passage brain capillary endothelial cells, immortalised brain endothelial cell lines, cell lines of non-cerebral origin, and the tri-dimensional model. These cell-based models must meet several criteria to closely mimic the *in vivo* condition; they must display a restrictive paracellular pathway, they should possess physiological endothelial cell properties, they should display functional expression of transport mechanisms, and they should be easy to culture (Gumbleton and Audus, 2001).

4.2.1 Isolated brain capillaries

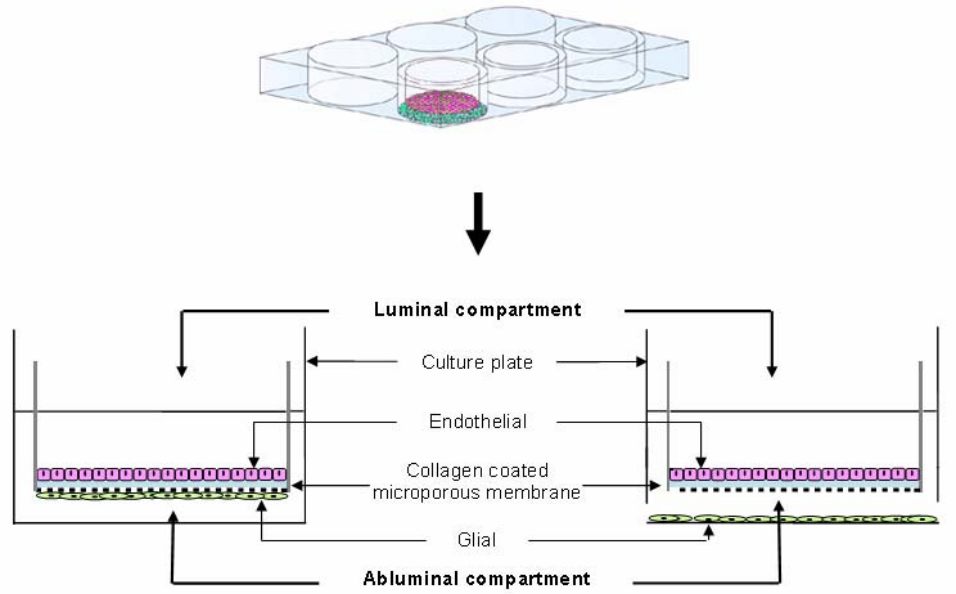
Brain capillary isolation from animal or human sources is possible. The major advantage for this system is a close resemblance to the *in vivo* situation because these capillaries remain metabolically active (Lasbennes and Gayet, 1984) and because BBB-specific endothelial receptors and carriers reflects are expressed. This type of model was first used to study the properties of the BBB (Joo, 1993), but it is not well suited for permeability measurements because of the difficulty of accessing the luminal surface and a low capacity of screening (Cecchelli et al., 2007). Recently, confocal microscopy was used to measure P-glycoprotein substrates concentrations at the luminal endothelial membrane of isolated microvessels (Miller et al., 2000).

4.2.2 Primary or low passage brain capillary endothelial cell cultures

Primary or low passage brain capillary endothelial cell cultures provide the highest phenotypic and biochemical resemblance to the *in vivo* BBB phenotype (Lundquist and Renftel, 2002), although, once endothelial cells are isolated from brain capillaries, they quickly begin to lose their BBB typical features when cultured alone. Because there is strong evidence that glial cells dynamically interact with the BBB to regulate BBB cell properties, several groups have attempted to better recreate this brain environment. This goal has been achieved by co-culture of the endothelial cells together with either primary astrocytes or glial cells (Stanness et al., 1997; Zhang et al., 2006). Co-culture can be

performed by growing the astrocytes on the bottom of a well in which a cell culture insert supporting endothelial cells is placed (Cecchelli et al., 1999; Dehouck et al., 1990; Perriere et al., 2005), or by seeding astrocytes directly on the bottom of the insert membrane (Megard et al., 2002) (Figure 5). However, there is controversy regarding the need for direct contact between the two cell types for complete endothelial cell differentiation, or whether some astrocyte-secreted factors are sufficient, as some models have been developed using an astrocyte conditioned medium (Rubin et al., 1991; Wolburg et al., 1994). An alternative to the limitation of astrocyte isolation and culture is the use of C6 glioma cells, which are a continuous cell line cloned from a rat glial tumour, but their use is controversial as some groups report increased induction of BBB properties (Hurst and Fritz, 1996), while others have observed the opposite (Boveri et al., 2005). Another disadvantage of C6 glioma cells is that they may result in a tumour-like BBB rather than a healthy BBB (de Boer et al., 1999). Other groups have also shown an improvement in the BBB properties with the use of cAMP stimulants (Deli et al., 1995; Rubin et al., 1991), vasoactive peptides (Guillot and Audus, 1991), glucocorticoids such as hydrocortisone (Antonetti et al., 2002; Calabria et al., 2006; Hoheisel et al., 1998), and adrenergic agonists (Borges et al., 1994). In order to develop *in vitro* models that are more and more similar to the *in vivo* BBB, models have also been developed with a third cellular type, such as neurons (Schiera et al., 2005) or pericytes (Nakagawa et al., 2007). It has also been shown that the extracellular matrix on which the BCECs are grown exerts an inductive effect on BBB model permeability (Arthur et al., 1987). Studies have been performed to compare the influence of basement membrane proteins such as type IV collagen, fibronectin, and laminin (Tilling et al., 1998, 2002), and a recent model has been proposed using an innovative nanofabricated membrane (Ma et al., 2005).

Figure 5 Co-culture model of BCECs with or without direct contact with glial cells in a six-well plate and detail of a well



Procedures used for isolating and culturing BCECs involve mechanical or a combination of mechanical and enzymatic techniques. The most critical steps in the production of pure BCEC cultures are the filtration and separation of microvessels from the brain constituents (Lundquist and Renftel, 2002). Most laboratories use enzymatic digestion followed by subsequent centrifugation steps, but the use of homogenisation and filtration steps to obtain first a microvessel preparation is an alternative approach allowing cloned pure BCEC isolation from capillaries predominantly. When using this technique, pericyte contamination, that may influence TEER and transport through the monolayer, is avoided (Dehouck et al., 1997). The advantage of this technique is that the limitations of primary cultures may be avoided; the cells can be stored in liquid nitrogen for several months and then subcultured (Meresse et al., 1989).

Several sources of BCECs have been used. The original bovine brain endothelial cell (BBEC) culture model was developed by Audus and Borchardt (1986) and has been extensively used and characterised. Bovine tissue has received much attention because of the brain size and availability. Moreover, this tissue allows the production of pericyte-free clones that can be harvested with relative ease (Lundquist and Renftel, 2002). Experiments are typically conducted between day 9 and 16, after which the BBECs will begin to undergo noticeable morphological and functional changes, losing some of their BBB characteristics (Gumbleton and Audus, 2001). Although most research has focused on the development and characterisation of BBEC cultures, porcine brain endothelial cells (PBECs) are another convenient source with a sufficient number of BCECs to allow extensive testing. The use of primary cultures of PBECs as an *in vitro* permeability model of the BBB has been pioneered by Franke et al. (1999), and more recent studies have also used co-cultured PBECs with astrocytes in order to improve the restrictiveness of the culture system (Kido et al., 2002; Zhang et al., 2006). Rat brain endothelial cells (RBECs) have also been described and may be relevant to facilitate *in vitro*/*in vivo* correlations if the rat is primarily used to generate *in vivo* data (Demeuse et al., 2002; Perriere et al., 2007). Nevertheless, the use of RBECs has the disadvantage that capillary fragments from such small brains are obtained with low yields (Cecchelli et al., 2007). Moreover, pericytes and other contaminants are frequently observed and have a negative impact on the RBECs permeability (Lundquist and Renftel, 2002). The use of a puromycin-based purification method has been described to allow the production of rat BBB models (Perriere et al., 2005), and such models have also been developed to investigate nanoparticle diffusion (Garcia-Garcia et al., 2005b; Kim et al., 2007b). Likewise, mouse brain endothelial cell (MBECs) models have aroused increased interest since the number of disease models using mice and the number of molecular tools available is growing (Coisne et al., 2005). Finally, ethical concerns and constraints in obtaining tissue from human origin do not usually make human brain endothelial cells (HBECs) a feasible routine model. However, these may be the best option when there is a need to consider immunological aspects or when there are differences in transporter genotypes between human and other species (Cecchelli et al., 2007).

Primary cell systems used for BBB permeability screening within an industrial environment have limitations such as time and technical resources required to isolate cells, and intra-batch and inter-batch reproducibility in phenotypic properties that make them less amenable than continuous cell lines.

4.2.3 Immortalised brain endothelial cell lines

Due to the problems associated with harvesting and maintaining of primary cell cultures, various immortalised cell lines have been developed by transformation, transfection, and transduction. The RBE4 (rat brain endothelial cells transfected with the E1A adenovirus gene) system is probably the most extensively characterised cell line. This cell line functionally expresses a number of BBB transporters and endothelial markers (Begley et al., 1996). It has also been shown that even if monolayer tightness can be increased when exposed to glial factors, RBE4s are not able to generate the necessary restrictive paracellular barrier properties (Roux and Couraud, 2005). The other immortalised cell lines generated from rat endothelial cell lines are the RBEC1 cell line (Kido et al., 2000; Nagasawa et al., 2005), the GPNT cell line (Greenwood et al., 1996) and the TR-BBB13 cell line (Tetsuka et al., 2003). Human cell lines such as SV-HCEC (Muruganandam et al., 1997) or hCMEC/D3 (Poller et al., 2008) and murine TM-BBB4 (Asaba et al., 2000) and MBEC (Tatsuta et al., 1992) cell lines seem to suffer from similar inadequate barrier properties (Kannan et al., 2000; Weksler et al., 2005). Another murine brain endothelial cell line, the cEND cell line, has been shown to form tight monolayers when the occludin expression is upregulated by addition of hydrocortisone (Forster et al., 2005). Finally, none of the immortalised brain endothelial cell lines form monolayers with complete tight-junctions (Rist et al., 1997), making monolayers leakier than primary cells (Deli et al., 2005; Lauer et al., 2004) even if some models appear to express transporters at higher levels than in primary cultures (Terasaki et al., 2003). However, these cell lines may be suitable not for transendothelial permeability screening but for endothelial cell drug uptake/efflux studies (Gumbleton and Audus, 2001) and have proven useful in dissection of transport mechanisms and cell-cell interaction.

4.2.4 Continuous cell lines of non-cerebral origin

Because of the insufficient barrier properties of immortalised brain endothelial cell lines, even if the BBB and the intestinal mucosa are two fundamentally different biologic barriers (e.g., membrane lipids, enzymes, and transporters), some researchers have focused on using non-cerebral peripheral epithelial cell lines for which potential exists to meet at least some of the criteria supposedly appropriate for an *in vitro* BBB permeability model. These cell lines are epithelial cell lines, which are easily accessible. The MDCK cell line comprises different clones with different properties. One of them has been transfected with the human MDR-1 gene and over expresses the P-glycoprotein, which has been proven to be an important BBB efflux mechanism (Pastan et al., 1988). However, even if this cell line has sufficient restrictive paracellular transport (Garberg et al., 2005; Wang et al., 2005), other efflux proteins expressed in BCECs are lacking. Nevertheless, because the MDCK cell line shows low paracellular permeability and can grow easily, it has been proposed for use in screening of passively transported CNS compounds. Caco-2 cells have also been suggested to give accurate predictions of BBB transport. It has been demonstrated that culture conditions, passage number, and days in culture can influence the permeability and metabolism properties of this cell line (Delie and Rubas, 1997); a recent study has demonstrated that this cell line is a poor model compared to BBEC/astrocyte co-culture (Lundquist et al., 2002). An alternative cell line, the ECV304 cell line, is a bladder carcinoma cell line with epithelial and endothelial properties; it has been co-cultured with C6 glioma but was found to have poor

paracellular restrictive properties and a lack of P-glycoprotein expression (Hurst and Fritz, 1996).

In summary, noncerebral peripheral endothelial cell lines are easily accessible but present limited paracellular restriction as compared to *in vivo* BBB. Moreover, these cells are generally morphologically very different from BCECs and also differ with respect to transport properties, metabolism, and growth (Lundquist and Renftel, 2002).

4.2.5 Three-dimensional BBB models

In vivo endothelial cells are continuously exposed to blood flow, inducing a shear stress along their apical surface that is vital to support growth inhibition, cell differentiation, and metabolic changes (Cucullo et al., 2002). To account for this, efforts have been made to develop *in vitro* BBB models with a three-dimensional architecture. The first model was based on a pronectin-coated hollow fibre that enables the co-culture of BBECs intraluminally and astrocytes extraluminally (Cucullo et al., 2002; Stanness et al., 1997). This model is able to maintain long-term cell growth (five weeks), to respond to astrocyte inductive properties and to generate a restrictive paracellular pathway (Santaguida et al., 2006). An immortalised PBEC line has also been tested in co-culture with the C6 glioma cell line and showed increased longevity in the flow-based hollow-fibre model as compared to the classical two-dimensional co-culture model, allowing a more comfortable window of use (Neuhaus et al., 2006). Moreover, it can be successfully used to co-culture differentiated neurons in the presence of the BBB (Stanness et al., 1999). More recently, this model has been used to develop a humanised model (Cucullo et al., 2007, 2008). The technical requirements of this model prevent its use for screening; nevertheless, it represents an innovative development in the *in vitro* BBB model domain.

It should be noted that, in absence of a three-dimensional model, performing permeability studies under stirred conditions on a two-dimensional co-culture model (reducing the influence of the unstirred water layer) better mimics the *in vivo* BBB environment and provides an intermediate response to the shear stress (Zhang et al., 2006).

5 *In vitro* BBB studies involving colloidal carriers

To date, *in vitro* BBB models have enabled investigation of several colloidal carriers for uptake and translocation through the BBB in an experimental environment (Table 1). Non-cell-based models have been extensively used to evaluate passive diffusion of numerous drugs, but to our knowledge, only two permeation studies have been performed with polymeric micelles and liposomes using an enterocyte-mimicking PAMPA (KV et al., 2008; Mathot et al., 2007). The recent development of a PAMPA-BBB should now allow study of the interaction of colloidal carriers with the BBB lipid bilayer. In fact, this model will consist of a first approach to the BBB interface to allow screening, because it should be kept in mind that such non-cell based models do not support transporter, receptor, or efflux systems, which play a major role in the active transport through the BBB. In contrast, cell-based models have been generally used as a complement to *in vivo* BBB models in the exploration of the fundamental properties associated with nanoparticle and liposome targeting mechanisms. Polysorbate 80-coated poly(n-butyl cyanoacrylate) (PBCA) nanoparticles have largely been used to perform drug delivery to

the CNS. Since 1995, several studies have demonstrated *in vivo* that these nanoparticles could be used to enhance CNS entry and activity of some molecules such as dalargin, loperamide, tubocurarine, doxorubicin, and an N-methyl-D-aspartate (NMDA) receptor antagonist (Alyautdin et al., 1997, 1998; Friese et al., 2000; Gulyaev et al., 1999; Kreuter et al., 1995). Additionally, cell-based *in vitro* models have been used to investigate the mechanisms underlying nanoparticle entry into the brain and to study endothelial cell uptake. While Ramge et al. (2000) highlighted an increased uptake by BBECs and HBECs due to polysorbate 80 coating, Olivier et al. (1999) observed a nonspecific permeabilisation attested by an increase in sucrose and inulin paracellular diffusion through BBECs co-cultured with astrocytes. The toxic effect on the BBB that would be responsible for a disruption, followed by a diffusional drug entry into the brain, has then been contested by Kreuter et al. (2002), describing an apolipoprotein (apo-B and apo-E) adsorption that would induce receptor-mediated transcytosis (Kreuter et al., 2003). More recently, SV40-HBECs have been tested for polysorbate 80-coated PBCA nanoparticle internalisation, but BBB permeation has only been observed during *in vivo* experiments (Weiss et al., 2008). These observations indicate that, in some cases, *in vitro* models do not allow colloidal carrier trancytosis detection, most likely due to physical properties of the insert and the supported coating on which endothelial cells are seeded.

Table 1 Colloidal carrier evaluation in *in vitro* BBB models

Carrier	*Drug*	*BBB model*	*Reference*
Micelles			
		PAMPA	Mathot et al. (2007)
Liposomes			
		PAMPA	KV et al. (2008)
Nanoparticles			
TAT-PLA	Ritonavir	MDCK-MDR1	Rao et al. (2008)
Polysorbate 80-PBCA	-	SV40-HBECs	Weiss et al. (2008)
PBCA, MMA-SPM	Zidovidine(AZT), lamivudine(3TC)	BBECs	Kuo and Chen (2006)
Polysorbate 80-PMMA	-	BBECs	Borchard et al. (1994)
Emulsifying wax/Brij78	-	BBECs	Lockman et al. (2003)
Polysorbate 80/Brij72			
Polysorbate 80-PBCA	-	BBECs	Ramge et al. (2000)
		HBECs	

Notes: Abbreviations: TAT-PLA: HIV virus transactivating-transduction-conjugated poly(lactide), PBCA: poly(n-butyl cyanoacrylate), MMA-SPM: methylmethacrylate-sulfopropylmethacrylate copolymer, PMMA: poly(methylmethacrylic acid), CBSA-PEG-PLA: cationic bovine serum albumin conjugated poly(ethyleneglycol)-poly(lactide), PEG-PHDCA: poly(methoxypolyethyleneglygol cyanoacrylate-co-hexadecyl cyanoacrylate), SLN: solid lipid nanoparticles, SNV: smart nano-vehicles, PAMPA: parallel artificial membrane permeability assay, MDCK-MDR1: Madin-Darhy canine kidney cells transfected with the human MDR1 gene BBECs bovine brain endothelial cells, HBECs: human brain endothelial cells, PBECs: porcine brain endothelial cells, and RBECs: rat brain endothelial cells.

Table 1 Colloidal carrier evaluation in *in vitro* BBB models (continued)

Carrier	*Drug*	*BBB model*	*Reference*
Nanoparticles			
Apoliprotein A-I coated protamine-oligonucleotide		PBECs	Kratzer et al. (2007)
CBSA-PEG-PLA	-	RBECs	Lu et al. (2005b)
PEG-PHDCA	-	RBECs	Kim et al. (2007a, 2007b)
PBCA, MMA-SPM, SLN	Stavudine, delavirdine, saquinavir	HBECs	Kuo and Su (2007) Kuo and Kuo (2008)
Transferrin-quantum rods	-	HBECs	Xu et al. (2008)
Polysorbate 80-PBCA	Dalargin	BBECs + astrocytes	Olivier et al. (1999)
Polystyrene			
Polysorbate 80-PBCA	Dalargin	BBECs + astrocytes	Kreuter et al. (2003)
		RBE4	
Maltodextrin	-	BBECs + astrocytes	Fenart et al. (1999) Jallouli et al. (2007)
CBSA-PEG-PLA	-	RBECs + astrocytes	Lu et al. (2005a, 2007)
Quaternary ammonium β-cyclodextrin	Doxorubicin	BBECs	Gil et al. (2009)
Biosensor coated SNVs	-	BBECs	Agyare et al. (2008)
Nanogels			
Nano-PEG-*cross*-PEI ± insulin or transferin conjugation	Oligonucleotides	BBECs	Vinogradov et al. (2004)

Notes: Abbreviations: TAT-PLA: HIV virus transactivating-transduction-conjugated poly(lactide), PBCA: poly(n-butyl cyanoacrylate), MMA-SPM: methylmethacrylate-sulfopropylmethacrylate copolymer, PMMA: poly(methylmethacrylic acid), CBSA-PEG-PLA: cationic bovine serum albumin conjugated poly(ethyleneglycol)-poly(lactide), PEG-PHDCA: poly(methoxypolyethyleneglygol cyanoacrylate-co-hexadecyl cyanoacrylate), SLN: solid lipid nanoparticles, SNV: smart nano-vehicles, PAMPA: parallel artificial membrane permeability assay, MDCK-MDR1: Madin-Darhy canine kidney cells transfected with the human MDR1 gene BBECs bovine brain endothelial cells, HBECs: human brain endothelial cells, PBECs: porcine brain endothelial cells, and RBECs: rat brain endothelial cells.

Kuo and colleagues have described the use of PBCA, methylmethacrylate-sulfopropylmethacrylate (MMA-SPM) nanoparticles and SLN to facilitate anti-HIV agent penetration (Kuo and Chen, 2006; Kuo and Kuo, 2008; Kuo and Su, 2007). They proved that these carriers had little impact on HBECs viability and permeability using cytotoxicity assays and trans-endothelial electrical resistance (TEER) measurements. They also observed carrier uptake by HBECs and an increase of drug permeability across BBEC and HBEC BBB models.

Cationic bovine serum albumin (CBSA) conjugated poly(ethyleneglycol)-poly(lactide) (PEG-PLA) nanoparticles have been developed as novel drug carrier for brain delivery and the exploitation of an *in vitro* BBB model (RBECs cultured with or without astrocytes) revealed a little toxicity against BBB and an absorptive mediated transcytosis ability proportional to CSBA density (Lu et al., 2005a, 2005b, 2007).

In the same way, development of an *in vitro* rat BBB model based on BCECs co-cultured with astrocytes (Garcia-Garcia et al., 2005b) has allowed the description of the role of apolipoproteins in receptor-mediated brain endothelial cell endocytosis of poly(methoxypolyethyleneglygol cyanoacrylate-co-hexadecyl cyanoacrylate) (PEG-PHDCA) nanoparticles (Kim et al., 2007a, 2007b).

Numerous other nanoparticulate carriers have been tested on different *in vitro* BBB model drug CNS delivery, such as polysorbate 80-coated poly(methyl methacrylate) (PMMA) nanoparticles (Borchard et al., 1994), maltodextrin nanoparticles (Fenart et al., 1999; Jallouli et al., 2007), emulsifying wax/Brij 78, and polysorbate 80/Brij 72 nanoparticles (Lockman et al., 2003), quaternary ammonium β-cyclodextrin nanoparticles (Gil et al., 2009), biosensors coated smart nano-vehicles (SNVs) (Agyare et al., 2008) on BBEC-based models, apolipoprotein A-I coated protamine-oligonucleotide nanoparticles on a PBEC-based model (Kratzer et al., 2007), bioconjugated quantum rods on an HBEC-based model (Xu et al., 2008), and TAT-conjugated poly(L-lactide) nanoparticles on an MDCK-MDR1 model (Rao et al., 2008).

To avoid the low drug loading capacities of nanoparticles and the use of organic solvents for synthesis, Vinogradov and colleagues tested the use of poly(ethylene glycol)-polyethylenimine (nano-PEG-cross-PEI) nanogel with or without insulin or transferrin conjugation for oligonucleotides transport to the CNS (Vinogradov et al, 2004). Using a BBECs model, they demonstrated that oligonucleotides can effectively be transported across the BBB with a better efficacy after nanogel modification.

6 Conclusions

Different approaches have been developed to increase drug permeation into the brain. One of the strategies used to overcome the blood brain barrier is to use nanocarriers linking the drug with a polymer- or lipid-based system. Many formulation parameters may be changed to optimise the carrier, leading to a profusion of delivery systems to be tested for efficacy. The assessment and comparison of the suitability of these devices, as well as the understanding of the mechanisms behind efficacy, imply the use of a reliable model. *In vivo* evaluation is, of course, the best model; however, this approach may consume a tremendous number of animals. Therefore, *in vitro* models have been proposed. Ideally, a good model should be simple to use and to set up. It should also allow ready access to different types of data such as the permeability efficiency (of the drug and the carrier), and the possible ability to check the degradation of the carrier and the metabolism of both the drug and polymer. Finally, it should also give information regarding the potential toxicity of the system on cells with possible reversibility of this action. Different models have already been generated and characterised from the most convenient artificial membrane-mimicking systems to the most elaborated three-dimensional cell-based models; nevertheless, they are not all relevant. Indeed, non-cell based models offer experimental simplicity but are only valuable for passive permeation and do not allow precise investigation of the mechanism underlying the entry into

BCECs. Moreover, if continuous cell-lines from cerebral and non-cerebral origin meet some of the criteria appropriate for the BBB, they do not present all the properties of the primary or low passage BCECs. While brain capillary isolation is possible, the use of such a model is not well suited for permeability purposes due to difficult access to the luminal surface. Finally, the recent development of three-dimensional BCEC culture with the application of a shear stress is very innovative and promising, but to date, technical requirements prevent its easy use for screening. Taking all of the previous criteria into consideration, primary or low passage BCECs co-cultured with primary astrocytes or glial cells in order to obtain the most similar *in vitro* model to the *in vivo* BBB appear to be the better compromise between the results, reliability, and the ability to perform tests. With this model, colloidal carrier interaction, uptake, and transcytosis by brain endothelial cells could be evaluated by several methods, such as permeability assays, fluorescence, or electron microscopy. Mechanisms could be examined at a molecular level using inhibitors or substrates of specific cellular receptors, or efflux proteins and toxicity on BBB permeability could be evaluated by performing TEER measurements or paracellular diffusion of molecules of low BBB permeability. Nevertheless, because some details such as a collagen insert coating could prevent colloidal system detection in the abluminal compartment, this model needs to be improved and adapted to their evaluation. Concerning the use of colloidal carriers for brain drug delivery, permeation across the BBB is a critical parameter but is not the only one. In fact, kinetic parameters of carrier degradation influencing drug release should also been taken into consideration when evaluating a new model.

References

Abbott, N.J., Ronnback, L. and Hansson, E. (2006) 'Astrocyte-endothelial interactions at the blood-brain barrier', *Nat. Rev. Neurosci.*, Vol. 7, No. 1, pp.41–53.

Agyare, E.K., Curran, G.L., Ramakrishnan, M., Yu, C.C., Poduslo, J.F. and Kandimalla, K.K. (2008) 'Development of a smart nano-vehicle to target cerebrovascular amyloid deposits and brain parenchymal plaques observed in Alzheimer's disease and cerebral amyloid angiopathy', *Pharm Res.*, Vol. 25, No. 11, pp.2674–2784.

Alyautdin, R.N., Petrov, V.E., Langer, K., Berthold, A., Kharkevich, D.A. and Kreuter, J. (1997) 'Delivery of loperamide across the blood-brain barrier with polysorbate 80-coated polybutylcyanoacrylate nanoparticles', *Pharm. Res.*, Vol. 14, No. 3, pp.325–328.

Alyautdin, R.N., Tezikov, E.B., Ramge, P., Kharkevich, D.A., Begley, D.J. and Kreuter, J. (1998) 'Significant entry of tubocurarine into the brain of rats by adsorption to polysorbate 80-coated polybutylcyanoacrylate nanoparticles: an in situ brain perfusion study', *J. Microencapsul.*, Vol. 15, No. 1, pp.67–74.

Antonetti, D.A., Wolpert, E.B., DeMaio, L., Harhaj, N.S. and Scaduto, R.C., Jr. (2002) 'Hydrocortisone decreases retinal endothelial cell water and solute flux coincident with increased content and decreased phosphorylation of occludin', *J. Neurochem.*, Vol. 80, No. 4, pp.667–677.

Arthur, F.E., Shivers, R.R. and Bowman, P.D. (1987) 'Astrocyte-mediated induction of tight junctions in brain capillary endothelium: an efficient in vitro model', *Brain Res.*, Vol. 433, No. 1, pp.155–159.

Asaba, H., Hosoya, K., Takanaga, H., Ohtsuki, S., Tamura, E., Takizawa, T. and Terasaki, T. (2000) 'Blood-brain barrier is involved in the efflux transport of a neuroactive steroid, dehydroepiandrosterone sulfate, via organic anion transporting polypeptide 2', *J. Neurochem.*, Vol. 75, No. 5, pp.1907–1916.

Audus, K.L. and Borchardt, R.T. (1986) 'Characteristics of the large neutral amino acid transport system of bovine brain microvessel endothelial cell monolayers', *J. Neurochem.*, Vol. 47, No. 2, pp.484–488.

Bagger, M.A. and Bechgaard, E. (2004) 'The potential of nasal application for delivery to the central brain-a microdialysis study of fluorescein in rats', *Eur. J. Pharm. Sci.*, Vol. 21, Nos. 2–3, pp.235–242.

Beduneau, A., Saulnier, P. and Benoit, J.P. (2007) 'Active targeting of brain tumors using nanocarriers', *Biomaterials*, Vol. 28, No. 33, pp.4947–4967.

Begley, D.J. (2004) 'Delivery of therapeutic agents to the central nervous system: the problems and the possibilities', *Pharmacol. Ther.*, Vol. 104, No. 1, pp.29–45.

Begley, D.J., Lechardeur, D., Chen, Z.D., Rollinson, C., Bardoul, M., Roux, F., Scherman, D. and Abbott, N.J. (1996) 'Functional expression of P-glycoprotein in an immortalised cell line of rat brain endothelial cells, RBE4', *J. Neurochem.*, Vol. 67, No. 3, pp.988–995.

Blasi, P., Giovagnoli, S., Schoubben, A., Ricci, M. and Rossi, C. (2007) 'Solid lipid nanoparticles for targeted brain drug delivery', *Adv. Drug Deliv. Rev.*, Vol. 59, No. 6, pp.454-477.

Bodor, N. and Buchwald, P. (2002) 'Barriers to remember: brain-targeting chemical delivery systems and Alzheimer's disease', *Drug Discovery Today*, Vol. 7, No. 14, pp.766–774.

Bodor, N. and Buchwald, P. (2008) 'Retrometabolic drug design: principles and recent developments', *Pure Appl. Chem.*, Vol. 80, No. 8, pp.1669–1682.

Borchard, G., Audus, K.L., Shi, F.L. and Kreuter, J. (1994) 'Uptake of surfactant-coated poly(methyl methacrylate)-nanoparticles by bovine brain microvessel endothelial-cell monolayers', *Int. J. Pharm.*, Vol. 110, No. 1, pp.29–35.

Borges, N., Shi, F., Azevedo, I. and Audus, K.L. (1994) 'Changes in brain microvessel endothelial cell monolayer permeability induced by adrenergic drugs', *Eur. J. Pharmacol.*, Vol. 269, No. 2, pp.243–248.

Boveri, M., Berezowski, V., Price, A., Slupek, S., Lenfant, A.M., Benaud, C., Hartung, T., Cecchelli, R., Prieto, P. and Dehouck, M.P. (2005) 'Induction of blood-brain barrier properties in cultured brain capillary endothelial cells: comparison between primary glial cells and C6 cell line', *Glia*, Vol. 51, No. 3, pp.187–198.

Butt, A.M., Jones, H.C. and Abbott, N.J. (1990) 'Electrical resistance across the blood-brain barrier in anaesthetized rats: a developmental study', *J. Physiol*, Vol. 429, No. 1, pp.47-62.

Calabria, A.R., Weidenfeller, C., Jones, A.R., de Vries, H.E. and Shusta, E.V. (2006) 'Puromycin-purified rat brain microvascular endothelial cell cultures exhibit improved barrier properties in response to glucocorticoid induction', *J. Neurochem.*, Vol. 97, No. 4, pp.922–933.

Carrara, S., Reali, V., Misiano, P., Dondio, G. and Bigogno, C. (2007) 'Evaluation of in vitro brain penetration: optimized PAMPA and MDCKII-MDR1 assay comparison', *Int. J. Pharm.*, Vol. 345, Nos. 1–2, pp.125–133.

Cecchelli, R., Berezowski, V., Lundquist, S., Culot, M., Renftel, M., Dehouck, M.P. and Fenart, L. (2007) 'Modelling of the blood-brain barrier in drug discovery and development', *Nat. Rev. Drug Discov.*, Vol. 6, No. 8, pp.650–661.

Cecchelli, R., Dehouck, B., Descamps, L., Fenart, L., Buee-Scherrer, V., Duhem, C., Lundquist, S., Rentfel, M., Torpier, G. and Dehouck, M.P. (1999) 'In vitro model for evaluating drug transport across the blood-brain barrier', *Adv. Drug Deliv. Rev.*, Vol. 36, Nos. 2–3, pp.165–178.

Coisne, C., Dehouck, L., Faveeuw, C., Delplace, Y., Miller, F., Landry, C., Morissette, C., Fenart, L., Cecchelli, R., Tremblay, P. and Dehouck, B. (2005) 'Mouse syngenic in vitro blood-brain barrier model: a new tool to examine inflammatory events in cerebral endothelium', *Lab Invest*, Vol. 85, No. 6, pp.734–746.

Cucullo, L., Couraud, P.O., Weksler, B., Romero, I.A., Hossain, M., Rapp, E. and Janigro, D. (2008) 'Immortalized human brain endothelial cells and flow-based vascular modeling: a marriage of convenience for rational neurovascular studies', *J. Cereb. Blood Flow Metab*, Vol. 28, No. 2, pp.312–328.

Cucullo, L., Hossain, M., Rapp, E., Manders, T., Marchi, N. and Janigro, D. (2007) 'Development of a humanized in vitro blood-brain barrier model to screen for brain penetration of antiepileptic drugs', *Epilepsia*, Vol. 48, No. 3, pp.505–516.

Cucullo, L., McAllister, M.S., Kight, K., Krizanac-Bengez, L., Marroni, M., Mayberg, M.R., Stanness, K.A. and Janigro, D. (2002) 'A new dynamic in vitro model for the multidimensional study of astrocyte-endothelial cell interactions at the blood-brain barrier', *Brain Res.*, Vol. 951, No. 2, pp.243–254.

de Boer, A.G. and Gaillard, P.J. (2007) 'Drug targeting to the brain', *Annu. Rev. Pharmacol. Toxicol.*, Vol. 47, No. 1, pp.323–355.

de Boer, A.G., Gaillard, P.J. and Breimer, D.D. (1999) 'The transference of results between blood-brain barrier cell culture systems', *Eur. J. Pharm. Sci.*, Vol. 8, No. 1, pp.1–4.

Dehouck, B., Cecchelli, R. and Dehouck, M-P. (1997) *Drug Transport Across the Blood-brain Barrier. In Vitro and In Vivo Techniques*, Harwood Academic Publishers, Amsterdam.

Dehouck, M.P., Meresse, S., Delorme, P., Fruchart, J.C. and Cecchelli, R. (1990) 'An easier, reproducible, and mass-production method to study the blood-brain barrier in vitro', *J. Neurochem.*, Vol. 54, No. 5, pp.1798–1801.

Deli, M.A., Abraham, C.S., Kataoka, Y. and Niwa, M. (2005) 'Permeability studies on in vitro blood-brain barrier models: physiology, pathology, and pharmacology', *Cell Mol. Neurobiol.*, Vol. 25, No. 1, pp.59–127.

Deli, M.A., Dehouck, M.P., Abraham, C.S., Cecchelli, R. and Joo, F. (1995) 'Penetration of small molecular weight substances through cultured bovine brain capillary endothelial cell monolayers: the early effects of cyclic adenosine 3', 5'-monophosphate', *Exp. Physiol*, Vol. 80, No. 4, pp.675–678.

Delie, F. and Rubas, W. (1997) 'A human colonic cell line sharing similarities with enterocytes as a model to examine oral absorption. Advantages and limitations of the Caco-2 model', *Crit. Rev. Ther. Drug Carr. Syst.*, Vol. 14, No. 2, pp.221–286.

Demeuse, P., Kerkhofs, A., Struys-Ponsar, C., Knoops, B., Remacle, C. and van den Bosch de Aguilar, P. (2002) 'Compartmentalized coculture of rat brain endothelial cells and astrocytes: a syngenic model to study the blood-brain barrier', *J. Neurosci. Methods*, Vol. 121, No. 1, pp.21–31.

Di, L., Kerns, E.H., Bezar, I.F., Petusky, S.L. and Huang, Y. (2009) 'Comparison of blood-brain barrier permeability assays: in situ brain perfusion, MDR1-MDCKII and PAMPA-BBB', *J. Pharm. Sci.*, Vol. 98, No. 6, pp.1980–1991.

Di, L., Kerns, E.H., Fan, K., McConnell, O.J. and Carter, G.T. (2003) 'High throughput artificial membrane permeability assay for blood-brain barrier', *Eur. J. Med. Chem.*, Vol. 38, No. 3, pp.223–232.

Egleton, R.D. and Davis, T.P. (2005) 'Development of neuropeptide drugs that cross the blood-brain barrier', *NeuroRx.*, Vol. 2, No. 1, pp.44–53.

El Bacha, R.S. and Minn, A. (1999) 'Drug metabolizing enzymes in cerebrovascular endothelial cells afford a metabolic protection to the brain', *Cell Mol. Biol.*, Vol. 45, pp.15–23.

Erdlenbruch, B., Alipour, M., Fricker, G., Miller, D.S., Kugler, W., Eibl, H. and Lakomek, M. (2003) 'Alkylglycerol opening of the blood-brain barrier to small and large fluorescence markers in normal and C6 glioma-bearing rats and isolated rat brain capillaries', *Br. J. Pharmacol.*, Vol. 140, No. 7, pp.1201–1210.

Faller, B. (2008) 'Artificial membrane assays to assess permeability', *Curr. Drug Metab.*, Vol. 9, No. 9, pp.886–892.

Fenart, L., Casanova, A., Dehouck, B., Duhem, C., Slupek, S., Cecchelli, R. and Betbeder, D. (1999) 'Evaluation of effect of charge and lipid coating on ability of 60-nm nanoparticles to cross an in vitro model of the blood-brain barrier', *J. Pharmacol. Exper. Ther.*, Vol. 291, No. 3, pp.1017–1022.

Forster, C., Silwedel, C., Golenhofen, N., Burek, M., Kietz, S., Mankertz, J. and Drenckhahn, D. (2005) 'Occludin as direct target for glucocorticoid-induced improvement of blood-brain barrier properties in a murine in vitro system', *J. Physiol*, Vol. 565, Pt. 2, pp.475–486.

Francis, K., van Beek, J., Canova, C., Neal, J.W. and Gasque, P. (2003) 'Innate immunity and brain inflammation: the key role of complement', *Expert. Rev. Mol. Med.*, Vol. 5, No. 15, pp.1–19.

Franke, H., Galla, H.J. and Beuckmann, C.T. (1999) 'An improved low-permeability in vitro-model of the blood-brain barrier: transport studies on retinoids, sucrose, haloperidol, caffeine and mannitol', *Brain Res.*, Vol. 818, No. 1, pp.65–71.

Franke, H., Ringelstein, E.B. and Stogbauer, F. (2005) 'Electromagnetic fields (GSM 1800) do not alter blood-brain barrier permeability to sucrose in models in vitro with high barrier tightness', *Bioelectromagnetics*, Vol. 26, No. 7, pp.529–535.

Friese, A., Seiller, E., Quack, G., Lorenz, B. and Kreuter, J. (2000) 'Increase of the duration of the anticonvulsive activity of a novel NMDA receptor antagonist using poly(butylcyanoacrylate) nanoparticles as a parenteral controlled release system', *Eur. J. Pharm. Biopharm.*, Vol. 49, No. 2, pp.103–109.

Fung, L.K., Shin, M., Tyler, B., Brem, H. and Saltzman, W.M. (1996) 'Chemotherapeutic drugs released from polymers: distribution of 1,3-bis(2-chloroethyl)-1-nitrosourea in the rat brain', *Pharm Res*, Vol. 13, No. 5, pp.671–682.

Gao, K. and Jiang, X. (2006) 'Influence of particle size on transport of methotrexate across blood brain barrier by polysorbate 80-coated polybutylcyanoacrylate nanoparticles', *Int. J. Pharm.*, Vol. 310, Nos. 1–2, pp.213–219.

Garberg, P., Ball, M., Borg, N., Cecchelli, R., Fenart, L., Hurst, R.D., Lindmark, T., Mabondzo, A., Nilsson, J.E., Raub, T.J., Stanimirovic, D., Terasaki, T., Oberg, J.O. and Osterberg, T. (2005) 'In vitro models for the blood-brain barrier', *Toxicol. In Vitro*, Vol. 19, No. 3, pp.299–334.

Garcia-Garcia, E., Andrieux, K., Gil, S. and Couvreur, P. (2005a) 'Colloidal carriers and blood-brain barrier (BBB) translocation: a way to deliver drugs to the brain?', *Int. J. Pharm.*, Vol. 298, No. 2, pp.274–292.

Garcia-Garcia, E., Gil, S., Andrieux, K., Desmaele, D., Nicolas, V., Taran, F., Georgin, D., Andreux, J.P., Roux, F. and Couvreur, P. (2005b) 'A relevant in vitro rat model for the evaluation of blood-brain barrier translocation of nanoparticles', *Cell. Mol. Life Sci.*, Vol. 62, No. 12, pp.1400–1408.

Garg, P. and Verma, J. (2006) 'In silico prediction of blood brain barrier permeability: an artificial neural network model', *J. Chem. Inf. Model.*, Vol. 46, No. 1, pp.289–297.

Gil, E.S., Li, J., Xiao, H. and Lowe, T.L. (2009) 'Quaternary ammonium beta-cyclodextrin nanoparticles for enhancing doxorubicin permeability across the in vitro blood-brain barrier', *Biomacromolecules*, Vol. 10, No. 3, pp.505–516.

Goodwin, J.T. and Clark, D.E. (2005) 'In silico predictions of blood-brain barrier penetration: considerations to 'keep in mind'', *J. Pharmacol. Exp. Ther.*, Vol. 315, No. 2, pp.477–483.

Greenwood, J., Pryce, G., Devine, L., Male, D.K., dos Santos, W.L., Calder, V.L. and Adamson, P. (1996) 'SV40 large T immortalised cell lines of the rat blood-brain and blood-retinal barriers retain their phenotypic and immunological characteristics', *J. Neuroimmunol.*, Vol. 71, Nos. 1–2, pp.51–63.

Guillot, F.L. and Audus, K.L. (1991) 'Angiotensin peptide regulation of bovine brain microvessel endothelial cell monolayer permeability', *J. Cardiovasc. Pharmacol.*, Vol. 18, No. 2, pp.212–218.

Gulyaev, A.E., Gelperina, S.E., Skidan, I.N., Antropov, A.S., Kivman, G.Y. and Kreuter, J. (1999) 'Significant transport of doxorubicin into the brain with polysorbate 80-coated nanoparticles', *Pharm. Res.*, Vol. 16, No. 10, pp.1564–1569.

Gumbleton, M. and Audus, K.L. (2001) 'Progress and limitations in the use of in vitro cell cultures to serve as a permeability screen for the blood-brain barrier', *J. Pharm. Sci.*, Vol. 90, No. 11, pp.1681–1698.

Hawkins, B.T. and Davis, T.P. (2005) 'The blood-brain barrier/neurovascular unit in health and disease', *Pharmacol. Rev.*, Vol. 57, No. 2, pp.173–185.

Herve, F., Ghinea, N. and Scherrmann, J.M. (2008) 'CNS delivery via adsorptive transcytosis', *AAPS. J.*, Vol. 10, No. 3, pp.455–472.

Hoheisel, D., Nitz, T., Franke, H., Wegener, J., Hakvoort, A., Tilling, T. and Galla, H.J. (1998) 'Hydrocortisone reinforces the blood-brain barrier properties in a serum free cell culture system', *Biochem. Biophys. Res. Commun.*, Vol. 244, No. 1, pp.312–316.

Hossmann, K.A. and Hermann, D.M. (2003) 'Effects of electromagnetic radiation of mobile phones on the central nervous system', *Bioelectromagnetics*, Vol. 24, No. 1, pp.49–62.

Hurst, R.D. and Fritz, I.B. (1996) 'Properties of an immortalised vascular endothelial/glioma cell co-culture model of the blood-brain barrier', *J. Cell Physiol*, Vol. 167, No. 1, pp.81–88.

Hynynen, K. (2008) 'Ultrasound for drug and gene delivery to the brain', *Adv. Drug Deliv. Rev.*, Vol. 60, No. 10, pp.1209–1217.

Jain, S., Mishra, V., Singh, P., Dubey, P.K., Saraf, D.K. and Vyas, S.P. (2003) 'RGD-anchored magnetic liposomes for monocytes/neutrophils-mediated brain targeting', *Int. J. Pharm.*, Vol. 261, Nos. 1–2, pp.43–55.

Jallouli, Y., Paillard, A., Chang, J., Sevin, E. and Betbeder, D. (2007) 'Influence of surface charge and inner composition of porous nanoparticles to cross blood-brain barrier in vitro', *Int. J. Pharm.*, Vol. 344, Nos. 1–2, pp.103-109.

Joo, F. (1993) 'The blood-brain barrier in vitro: the second decade', *Neurochem. Int.*, Vol. 23, No. 6, pp.499–521.

Kakinuma, K., Tanaka, R., Takahashi, H., Sekihara, Y., Watanabe, M. and Kuroki, M. (1996) 'Drug delivery to the brain using thermosensitive liposome and local hyperthermia', *Int. J. Hyperthermia*, Vol. 12, No. 1, pp.157–165.

Kannan, R., Chakrabarti, R., Tang, D., Kim, K.J. and Kaplowitz, N. (2000) 'GSH transport in human cerebrovascular endothelial cells and human astrocytes: evidence for luminal localization of Na+-dependent GSH transport in HCEC', *Brain Res.*, Vol. 852, No. 2, pp.374–382.

Kaur, I.P., Bhandari, R., Bhandari, S. and Kakkar, V. (2008) 'Potential of solid lipid nanoparticles in brain targeting', *J.f Control Release*, Vol. 127, No. 2, pp.97–109.

Kerns, E.H. (2001) 'High throughput physicochemical profiling for drug discovery', *J. Pharm. Sci.*, Vol. 90, No. 11, pp.1838–1858.

Kido, Y., Tamai, I., Nakanishi, T., Kagami, T., Hirosawa, I., Sai, Y. and Tsuji, A. (2002) 'Evaluation of blood-brain barrier transporters by co-culture of brain capillary endothelial cells with astrocytes', *Drug Metab Pharmacokinet*, Vol. 17, No. 1, pp.34–41.

Kido, Y., Tamai, I., Okamoto, M., Suzuki, F. and Tsuji, A. (2000) 'Functional clarification of MCT1-mediated transport of monocarboxylic acids at the blood-brain barrier using in vitro cultured cells and in vivo BUI studies', *Pharm. Res.*, Vol. 17, No. 1, pp.55–62.

Kim, H.R., Andrieux, K., Gil, S., Taverna, M., Chacun, H., Desmaele, D., Taran, F., Georgin, D. and Couvreur, P. (2007a) 'Translocation of poly(ethylene glycol-co-hexadecyl)cyanoacrylate nanoparticles into rat brain endothelial cells: role of apolipoproteins in receptor-mediated endocytosis', *Biomacromolecules*, Vol. 8, No. 3, pp.793–799.

Kim, H.R., Gil, S., Andrieux, K., Nicolas, V., Appel, M., Chacun, H., Desmaele, D., Taran, F., Georgin, D. and Couvreur, P. (2007b) 'Low-density lipoprotein receptor-mediated endocytosis of PEGylated nanoparticles in rat brain endothelial cells', *Cell Mol. Life Sci.*, Vol. 64, No. 3, pp.356–364.

Koziara, J.M., Lockman, P.R., Allen, D.D. and Mumper, R.J. (2006) 'The blood-brain barrier and brain drug delivery', *J. Nanosci. Nanotechnol.*, Vol. 6, Nos. 9–10, pp.2712–2735.

Kratzer, I., Wernig, K., Panzenboeck, U., Bernhart, E., Reicher, H., Wronski, R., Windisch, M., Hammer, A., Malle, E., Zimmer, A. and Sattler, W. (2007) 'Apolipoprotein A-I coating of protamine-oligonucleotide nanoparticles increases particle uptake and transcytosis in an in vitro model of the blood-brain barrier', *J. Control Release*, Vol. 117, No. 3, pp.301–311.

Kreuter, J. (2001) 'Nanoparticulate systems for brain delivery of drugs', *Adv. Drug Deliv. Rev.*, Vol. 47, No. 1, pp.65–81.

Kreuter, J., Alyautdin, R.N., Kharkevich, D.A. and Ivanov, A.A. (1995) 'Passage of peptides through the blood-brain barrier with colloidal polymer particles (nanoparticles)', *Brain Res.*, Vol. 674, No. 1, pp.171–174.

Kreuter, J., Ramge, P., Petrov, V., Hamm, S., Gelperina, S.E., Engelhardt, B., Alyautdin, R., von Briesen, H. and Begley, D.J. (2003) 'Direct evidence that polysorbate-80-coated poly(butylcyanoacrylate) nanoparticles deliver drugs to the CNS via specific mechanisms requiring prior binding of drug to the nanoparticles', *Pharm. Res.*, Vol. 20, No. 3, pp.409–416.

Kreuter, J., Shamenkov, D., Petrov, V., Ramge, P., Cychutek, K., Koch-Brandt, C. and Alyautdin, R. (2002) 'Apolipoprotein-mediated transport of nanoparticle-bound drugs across the blood-brain barrier', *J. Drug Target*, Vol. 10, No. 4, pp.317–325.

Kuo, Y.C. and Chen, H.H. (2006) 'Effect of nanoparticulate polybutylcyanoacrylate and methylmethacrylate-sulfopropylmethacrylate on the permeability of zidovudine and lamivudine across the in vitro blood-brain barrier', *Int. J. Pharm.*, Vol. 327, Nos. 1–2, pp.160–169.

Kuo, Y.C. and Kuo, C.Y. (2008) 'Electromagnetic interference in the permeability of saquinavir across the blood-brain barrier using nanoparticulate carriers', *Int. J. Pharm.*, Vol. 351, Nos. 1-2, pp.271–281.

Kuo, Y.C. and Su, F.L. (2007) 'Transport of stavudine, delavirdine, and saquinavir across the blood-brain barrier by polybutylcyanoacrylate, methylmethacrylate-sulfopropylmethacrylate, and solid lipid nanoparticles', *Int. J. Pharm.*, Vol. 340, Nos. 1–2, pp.143-152.

KV, S., Devi, G.S. and Mathew, S.T. (2008) 'Liposomal formulations of serratiopeptidase: in vitro studies using PAMPA and Caco-2 models', *Mol. Pharm.*, Vol. 5, No. 1, pp.92–97.

Lasbennes, F. and Gayet, J. (1984) 'Capacity for energy metabolism in microvessels isolated from rat brain', *Neurochem. Res.*, Vol. 9, No. 1, pp.1–10.

Lauer, R., Bauer, R., Linz, B., Pittner, F., Peschek, G.A., Ecker, G., Friedl, P. and Noe, C.R. (2004) 'Development of an in vitro blood-brain barrier model based on immortalized porcine brain microvascular endothelial cells', *Il Farmaco*, Vol. 59, No. 2, pp.133–137.

Levin, V.A. (1980) 'Relationship of octanol/water partition coefficient and molecular weight to rat brain capillary permeability', *J. Med. Chem.*, Vol. 23, No. 6, pp.682–684.

Liu, L., Guo, K., Lu, J., Venkatraman, S.S., Luo, D., Ng, K.C., Ling, E.A., Moochhala, S. and Yang, Y.Y. (2008a) 'Biologically active core/shell nanoparticles self-assembled from cholesterol-terminated PEG-TAT for drug delivery across the blood-brain barrier', *Biomaterials*, Vol. 29, No. 10, pp.1509–1517.

Liu, L., Venkatraman, S.S., Yang, Y.Y., Guo, K., Lu, J., He, B., Moochhala, S. and Kan, L. (2008b) 'Polymeric micelles anchored with TAT for delivery of antibiotics across the blood-brain barrier', *Biopolymers*, Vol. 90, No. 5, pp.617–623.

Liu, X., Tu, M., Kelly, R.S., Chen, C. and Smith, B.J. (2004) 'Development of a computational approach to predict blood-brain barrier permeability', *Drug Metab Dispos*., Vol. 32, No. 1, pp.132–139.

Lockman, P.R., Koziara, J., Roder, K.E., Paulson, J., Abbruscato, T.J., Mumper, R.J. and Allen, D.D. (2003) 'In vivo and in vitro assessment of baseline blood-brain barrier parameters in the presence of novel nanoparticles', *Pharm. Res.*, Vol. 20, No. 5, pp.705–713.

Lockman, P.R., Koziara, J.M., Mumper, R.J. and Allen, D.D. (2004) 'Nanoparticle surface charges alter blood-brain barrier integrity and permeability', *J. Drug Target*, Vol. 12, Nos. 9–10, pp.635–641.

Loftsson, T., Konradsdottir, F. and Masson, M. (2006) 'Development and evaluation of an artificial membrane for determination of drug availability', *Int. J. Pharm.*, Vol. 326, Nos. 1–2, pp.60–68.

Lu, W., Tan, Y.Z., Hu, K.L. and Jiang, X.G. (2005a) 'Cationic albumin conjugated pegylated nanoparticle with its transcytosis ability and little toxicity against blood-brain barrier', *Int. J. Pharm.*, Vol. 295, Nos. 1–2, pp.247–260.

Lu, W., Wan, J., She, Z. and Jiang, X. (2007) 'Brain delivery property and accelerated blood clearance of cationic albumin conjugated pegylated nanoparticle', *J. Control. Release*, Vol. 118, No. 1, pp.38–53.

Lu, W., Zhang, Y., Tan, Y.Z., Hu, K.L., Jiang, X.G. and Fu, S.K. (2005b) 'Cationic albumin-conjugated pegylated nanoparticles as novel drug carrier for brain delivery', *J. Control. Release*, Vol. 107, No. 3, pp.428–448.

Lundquist, S. and Renftel, M. (2002) 'The use of in vitro cell culture models for mechanistic studies and as permeability screens for the blood-brain barrier in the pharmaceutical industry – background and current status in the drug discovery process', *Vascul. Pharmacol.*, Vol. 38, No. 6, pp.355–364.

Lundquist, S., Renftel, M., Brillault, J., Fenart, L., Cecchelli, R. and Dehouck, M.P. (2002) 'Prediction of drug transport through the blood-brain barrier in vivo: a comparison between two in vitro cell models', *Pharm. Res.*, Vol. 19, No. 7, pp.976–981.

Ma, S.H., Lepak, L.A., Hussain, R.J., Shain, W. and Shuler, M.L. (2005) 'An endothelial and astrocyte co-culture model of the blood-brain barrier utilizing an ultra-thin, nanofabricated silicon nitride membrane', *Lab Chip.*, Vol. 5, No. 1, pp.74–85.

Mathot, F., Schanck, A., Van Bambeke, F., Arien, A., Noppe, M., Brewster, M. and Préat, V. (2007) 'Passive diffusion of polymeric surfactants across lipid bilayers', *J. Control. Release*, Vol. 120, Nos. 1–2, pp.79–87.

Megard, I., Garrigues, A., Orlowski, S., Jorajuria, S., Clayette, P., Ezan, E. and Mabondzo, A. (2002) 'A co-culture-based model of human blood-brain barrier: application to active transport of indinavir and in vivo-in vitro correlation', *Brain Res.*, Vol. 927, No. 2, pp.153–167.

Meresse, S., Dehouck, M.P., Delorme, P., Bensaid, M., Tauber, J.P., Delbart, C., Fruchart, J.C. and Cecchelli, R. (1989) 'Bovine brain endothelial cells express tight junctions and monoamine oxidase activity in long-term culture', *J. Neurochem.*, Vol. 53, No. 5, pp.1363–1371.

Miller, D.S., Nobmann, S.N., Gutmann, H., Toeroek, M., Drewe, J. and Fricker, G. (2000) 'Xenobiotic transport across isolated brain microvessels studied by confocal microscopy', *Mol. Pharmacol.*, Vol. 58, No. 6, pp.1357–1367.

Muruganandam, A., Herx, L.M., Monette, R., Durkin, J.P. and Stanimirovic, D.B. (1997) 'Development of immortalized human cerebromicrovascular endothelial cell line as an in vitro model of the human blood-brain barrier', *FASEB J.*, Vol. 11, No. 13, pp.1187–1197.

Nagasawa, K., Ito, S., Kakuda, T., Nagai, K., Tamai, I., Tsuji, A. and Fujimoto, S. (2005) 'Transport mechanism for aluminum citrate at the blood-brain barrier: kinetic evidence implies involvement of system Xc- in immortalized rat brain endothelial cells', *Toxicol. Lett.*, Vol. 155, No. 2, pp.289–296.

Nakagawa, S., Deli, M.A., Nakao, S., Honda, M., Hayashi, K., Nakaoke, R., Kataoka, Y. and Niwa, M. (2007) 'Pericytes from brain microvessels strengthen the barrier integrity in primary cultures of rat brain endothelial cells', *Cell Mol. Neurobiol.*, Vol. 27, No. 6, pp.687–694.

Neuhaus, W., Lauer, R., Oelzant, S., Fringeli, U.P., Ecker, G.F. and Noe, C.R. (2006) 'A novel flow based hollow-fiber blood-brain barrier in vitro model with immortalised cell line PBMEC/C1-2', *J. Biotechnol.*, Vol. 125, No. 1, pp.127–141.

Nicolazzo, J.A., Charman, S.A. and Charman, W.N. (2006) 'Methods to assess drug permeability across the blood-brain barrier', *J. Pharm. Pharmacol.*, Vol. 58, No. 3, pp.281–293.

Olivier, J.C., Fenart, L., Chauvet, R., Pariat, C., Cecchelli, R. and Couet, W. (1999) 'Indirect evidence that drug brain targeting using polysorbate 80-coated polybutylcyanoacrylate nanoparticles is related to toxicity', *Pharm. Res.*, Vol. 16, No. 12, pp.1836–1842.

Ottaviani, G., Martel, S. and Carrupt, P.A. (2006) 'Parallel artificial membrane permeability assay: a new membrane for the fast prediction of passive human skin permeability', *J. Med. Chem.*, Vol. 49, No. 13, pp.3948–3954.

Ottaviani, G., Martel, S. and Carrupt, P.A. (2007) 'In silico and in vitro filters for the fast estimation of skin permeation and distribution of new chemical entities', *J. Med. Chem.*, Vol. 50, No. 4, pp.742–748.

Pang, Z., Lu, W., Gao, H., Hu, K., Chen, J., Zhang, C., Gao, X., Jiang, X. and Zhu, C. (2008) 'Preparation and brain delivery property of biodegradable polymersomes conjugated with OX26', *J. Control. Release*, Vol. 128, No. 2, pp.120–127.

Pardridge, W.M. (2005) 'Molecular biology of the blood-brain barrier', *Mol. Biotechnol.*, Vol. 30, No. 1, pp.57–70.

Pardridge, W.M. (2007) 'Blood-brain barrier delivery', *Drug Discov. Today*, Vol. 12, Nos. 1–2, pp.54–61.

Pastan, I., Gottesman, M.M., Ueda, K., Lovelace, E., Rutherford, A.V. and Willingham, M.C. (1988) 'A retrovirus carrying an MDR1 cDNA confers multidrug resistance and polarized expression of P-glycoprotein in MDCK cells', *Proc. Natl. Acad. Sci. U.S.A*, Vol. 85, No. 12, pp.4486–4490.

Patel, M.M., Goyal, B.R., Bhadada, S.V., Bhatt, J.S. and Amin, A.F. (2009) 'Getting into the brain: approaches to enhance brain drug delivery', *CNS. Drugs*, Vol. 23, No. 1, pp.35–58.

Perriere, N., Demeuse, P., Garcia, E., Regina, A., Debray, M., Andreux, J.P., Couvreur, P., Scherrmann, J.M., Temsamani, J., Couraud, P.O., Deli, M.A. and Roux, F. (2005) Puromycin-based purification of rat brain capillary endothelial cell cultures. Effect on the expression of blood-brain barrier-specific properties', *J. Neurochem.*, Vol. 93, No. 2, pp.279–289.

Perriere, N., Yousif, S., Cazaubon, S., Chaverot, N., Bourasset, F., Cisternino, S., Decleves, X., Hori, S., Terasaki, T., Deli, M., Scherrmann, J.M., Temsamani, J., Roux, F. and Couraud, P.O. (2007) 'A functional in vitro model of rat blood-brain barrier for molecular analysis of efflux transporters', *Brain Res.*, Vol. 1150, No. 1, pp.1–13.

Persidsky, Y., Ramirez, S.H., Haorah, J. and Kanmogne, G.D. (2006) 'Blood-brain barrier: structural components and function under physiologic and pathologic conditions', *J. Neuroimmune. Pharmacol.*, Vol. 1, No. 3, pp.223–236.

Petri, B., Bootz, A., Khalansky, A., Hekmatara, T., Muller, R., Uhl, R., Kreuter, J. and Gelperina, S. (2007) 'Chemotherapy of brain tumour using doxorubicin bound to surfactant-coated poly(butyl cyanoacrylate) nanoparticles: revisiting the role of surfactants', *J. Control. Release*, Vol. 117, No. 1, pp.51–58.

Poller, B., Gutmann, H., Krahenbuhl, S., Weksler, B., Romero, I., Couraud, P.O., Tuffin, G., Drewe, J. and Huwyler, J. (2008) 'The human brain endothelial cell line hCMEC/D3 as a human blood-brain barrier model for drug transport studies', *J. Neurochem.*, Vol. 107, No. 5, pp.1358–1368.

Ramge, P., Unger, R.E., Oltrogge, J.B., Zenker, D., Begley, D., Kreuter, J. and von Briesen, H. (2000) 'Polysorbate-80 coating enhances uptake of polybutylcyanoacrylate (PBCA)-nanoparticles by human and bovine primary brain capillary endothelial cells', *Eur. J. Neurosci.*, Vol. 12, No. 6, pp.1931–1940.

Rao, K.S., Reddy, M.K., Horning, J.L. and Labhasetwar, V. (2008) 'TAT-conjugated nanoparticles for the CNS delivery of anti-HIV drugs', *Biomaterials*, Vol. 29, No. 33, pp.4429–4438.

Rao, V.V., Dahlheimer, J.L., Bardgett, M.E., Snyder, A.Z., Finch, R.A., Sartorelli, A.C. and Piwnica-Worms, D. (1999) 'Choroid plexus epithelial expression of MDR1 P glycoprotein and multidrug resistance-associated protein contribute to the blood-cerebrospinal-fluid drug-permeability barrier', *Proc. Natl. Acad. Sci.*, Vol. 96, No. 7, pp.3900–3905.

Reimold, I., Domke, D., Bender, J., Seyfried, C.A., Radunz, H.E. and Fricker, G. (2008) 'Delivery of nanoparticles to the brain detected by fluorescence microscopy', *Eur. J. Pharm. Biopharm.*, Vol. 70, No. 2, pp.627–632.

Rist, R.J., Romero, I.A., Chan, M.W., Couraud, P.O., Roux, F. and Abbott, N.J. (1997) 'F-actin cytoskeleton and sucrose permeability of immortalised rat brain microvascular endothelial cell monolayers: effects of cyclic AMP and astrocytic factors', *Brain Res.*, Vol. 768, Nos. 1–2, pp.10–18.

Roux, F. and Couraud, P.O. (2005) 'Rat brain endothelial cell lines for the study of blood-brain barrier permeability and transport functions', *Cell Mol. Neurobiol.*, Vol. 25, No. 1, pp.41–58.

Rubin, L.L., Hall, D.E., Porter, S., Barbu, K., Cannon, C., Horner, H.C., Janatpour, M., Liaw, C.W., Manning, K. and Morales, J. (1991) 'A cell culture model of the blood-brain barrier', *J. Cell Biol.*, Vol. 115, No. 6, pp.1725–1735.

Santaguida, S., Janigro, D., Hossain, M., Oby, E., Rapp, E. and Cucullo, L. (2006) 'Side by side comparison between dynamic versus static models of blood-brain barrier in vitro: a permeability study', *Brain Res.*, Vol. 1109, No. 1, pp.1–13.

Schiera, G., Sala, S., Gallo, A., Raffa, M.P., Pitarresi, G.L., Savettieri, G. and L.iegro, I.D. (2005) 'Permeability properties of a three-cell type in vitro model of blood-brain barrier', *J. Cell Mol. Med.*, Vol. 9, No. 2, pp.373–379.

Stanness, K.A., Neumaier, J.F., Sexton, T.J., Grant, G.A., Emmi, A., Maris, D.O. and Janigro, D. (1999) 'A new model of the blood--brain barrier: co-culture of neuronal, endothelial and glial cells under dynamic conditions', *Neuroreport*, Vol. 10, No. 18, pp.3725–3731.

Stanness, K.A., Westrum, L.E., Fornaciari, E., Mascagni, P., Nelson, J.A., Stenglein, S.G., Myers, T. and Janigro, D. (1997) 'Morphological and functional characterization of an in vitro blood-brain barrier model', *Brain Res.*, Vol. 771, No. 2, pp.329–342.

Tapfer, M.K, Sebestyen, L., Kurucz, I., Horvath, K., Szelenyi, I. and Bodor, N. (2004) 'New evidence for the selective, long-lasting central effects of the brain-targeted estradiol, Estredox', *Pharm. Biochem. Behavior*, Vol. 77, No. 3, pp.423–429.

Tatsuta, T., Naito, M., Oh-hara, T., Sugawara, I. and Tsuruo, T. (1992) 'Functional involvement of P-glycoprotein in blood-brain barrier', *J. Biol. Chem.*, Vol. 267, No. 28, pp.20383–20391.

Terasaki, T., Ohtsuki, S., Hori, S., Takanaga, H., Nakashima, E. and Hosoya, K. (2003) 'New approaches to in vitro models of blood-brain barrier drug transport', *Drug Discov. Today*, Vol. 8, No. 20, pp.944–954.

Tetsuka, K., Takanaga, H., Ohtsuki, S., Hosoya, K. and Terasaki, T. (2003) 'The l-isomer-selective transport of aspartic acid is mediated by ASCT2 at the blood-brain barrier', *J. Neurochem.*, Vol. 87, No. 4, pp.891–901.

Tilling, T., Engelbertz, C., Decker, S., Korte, D., Huwel, S. and Galla, H.J. (2002) 'Expression and adhesive properties of basement membrane proteins in cerebral capillary endothelial cell cultures', *Cell Tissue Res.*, Vol. 310, No. 1, pp.19–29.

Tilling, T., Korte, D., Hoheisel, D. and Galla, H.J. (1998) 'Basement membrane proteins influence brain capillary endothelial barrier function in vitro', *J. Neurochem.*, Vol. 71, No. 3, pp.1151–1157.

Tiwari, S.B. and Amiji, M.M. (2006) 'A review of nanocarrier-based CNS delivery systems', *Curr. Drug Deliv.*, Vol. 3, No. 2, pp.219–232.

Vinogradov, S.V., Batrakova, E.V. and Kabanov, A.V. (2004) 'Nanogels for oligonucleotide delivery to the brain', *Bioconjug Chem.*, Vol. 15, No. 1, pp.50–60.

Wang, Q., Rager, J.D., Weinstein, K., Kardos, P.S., Dobson, G.L., Li, J. and Hidalgo, I.J. (2005) 'Evaluation of the MDR-MDCK cell line as a permeability screen for the blood-brain barrier', *Int. J. Pharm.*, Vol. 288, No. 2, pp.349–359.

Wang, Y., Sun, J., Liu, H. and He, Z. (2007) 'Rapidly profiling blood-brain barrier penetration with liposome EKC', *Electrophoresis*, Vol. 28, No. 14, pp.2391–2395.

Weiss, C.K., Kohnle, M.V., Landfester, K., Hauk, T., Fischer, D., Schmitz-Wienke, J. and Mailander, V. (2008) 'The first step into the brain: uptake of NIO-PBCA nanoparticles by endothelial cells in vitro and in vivo, and direct evidence for their blood-brain barrier permeation', *Chem. Med. Chem.*, Vol. 3, No. 9, pp.1395–1403.

Weksler, B.B., Subileau, E.A., Perriere, N., Charneau, P., Holloway, K., Leveque, M., Tricoire-Leignel, H., Nicotra, A., Bourdoulous, S., Turowski, P., Male, D.K., Roux, F., Greenwood, J., Romero, I.A. and Couraud, P.O. (2005) 'Blood-brain barrier-specific properties of a human adult brain endothelial cell line', *FASEB J.*, Vol. 19, No. 13, pp.1872–1874.

Wohnsland, F. and Faller, B. (2001) 'High-throughput permeability pH profile and high-throughput alkane/water log P with artificial membranes', *J. Med. Chem.*, Vol. 44, No. 6, pp.923–930.

Wolburg, H., Neuhaus, J., Kniesel, U., Krauss, B., Schmid, E.M., Ocalan, M., Farrell, C. and Risau, W. (1994) 'Modulation of tight junction structure in blood-brain barrier endothelial cells. Effects of tissue culture, second messengers and cocultured astrocytes', *J. Cell Sci.*, Vol. 107, No. 1, pp.1347–1357.

Xu, G., Yong, K.T., Roy, I., Mahajan, S.D., Ding, H., Schwartz, S.A. and Prasad, P.N. (2008) 'Bioconjugated quantum rods as targeted probes for efficient transmigration across an in vitro blood-brain barrier', *Bioconjug. Chem.*, Vol. 19, No. 6, pp.1179–1185.

Yoon, C.H., Kim, S.J., Shin, B.S., Lee, K.C. and Yoo, S.D. (2006) 'Rapid screening of blood-brain barrier penetration of drugs using the immobilized artificial membrane phosphatidylcholine column chromatography', *J. Biomol. Screen.*, Vol. 11, No. 1, pp.13–20.

Zhang, Y., Li, C.S., Ye, Y., Johnson, K., Poe, J., Johnson, S., Bobrowski, W., Garrido, R. and Madhu, C. (2006) 'Porcine brain microvessel endothelial cells as an in vitro model to predict in vivo blood-brain barrier permeability', *Drug Metab Dispos*, Vol. 34, No. 11, pp.1935–1943.

Zheng, W. and Codobski, A. (2004) *The Blood-Cerebrospinal Fluid Barrier*, CRC press, Boca Raton, Florida, USA.

List of abbreviations

ABC	ATP-binding cassette
BBB	blood-brain barrier
BBECs	bovine brain endothelial cells
BCECs	brain capillary endothelial cells
BK	bradykinin
CBSA-PEG-PLA	cationic bovine serum albumin conjugated poly(ethyleneglycol)-poly(lactide)
CMC	critical micelle concentration
CNS	central nervous system
CBSA	cationic bovine serum albumin
CSF	cerebro-spinal fluid
HBECs	human brain endothelial cells
IAMc	immobilised artificial membrane chromatography
LDL	low density lipoprotein
LEKC	liposome electro-kinetic chromatography
MBECs	mouse brain endothelial cells

MLV	multilamellar vesicles
MMA-SPM	methylmethacrylate-sulfopropylmethacrylate copolymer
NMDA	N-methyl-D-aspartate
PAMPA	parallel artificial membrane permeability assay
PBCA	poly(n-butyl cyanoacrylate)
PBECs	porcine brain endothelial cells
PEG-PHDCA	poly(methoxypolyethyleneglygol cyanoacrylate-co-hexadecyl cyanoacrylate)
PEG-PLA	poly(ethyleneglycol)-poly(lactide)
P-gp	P-glycoprotein
PMMA	poly(methyl methacrylate)
PSA	polar surface area
RBECs	Rat brain endothelial cells
RES	reticuloendothelial system
SLN	solid lipid nanoparticles
SNV	smart nano-vehicles
SUV	small unilamellar vesicles
TAT-PLA	HIV virus transactivating-transduction-conjugated poly(lactide)
TEER	trans-endothelial electrical resistance
VEGF	vascular endothelial growth factor.