Journal of Controlled Release 164 (2012) 145–155

Contents lists available at SciVerse ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Challenges in drug delivery to the brain: Nature is against us

Silke Krol *

Fondazione IRCCS Neurologic Institute "Carlo Besta", IFOM-IEO-campus, via Adamello 16, 20139 Milan, Italy

ARTICLE INFO

Article history:
Received 23 November 2011
Accepted 29 April 2012
Available online 17 May 2012

Keywords:
Brain drug delivery
Body physics
Brain physics
Crowding
Targeted delivery

ABSTRACT

Nanomedicine is a fast evolving field involving nanoparticles or nanostructures for medical applications. Especially in the underdeveloped field of drug delivery to the brain, there are high expectations for the ability of multifunctional nanoparticles (NPs) to cross the blood–brain barrier (BBB). In the present review the challenges nanoparticles face after injection into the body will be summarized. There is a broad range of biological, chemical and physical hurdles for NPs to reach the brain. Perhaps the most challenging task will be to design and develop nanoparticles that specifically target that right subset of diseased neurons without affecting other healthy neurons. This is of immense importance especially in the case of targeting toxic drugs to highly invasive brain tumors.

Already, without the additional obstacle in the form of the BBB, targeting nanoparticles against a small subset of cells in the body is a big challenge. While the permeability of the blood vessels in other tissues is comparably higher the brain microvasculature is highly restrictive. The reason for this is that uncontrolled invasion of nano-objects or molecules may lead to a pathological change in neurons responsible for memory, personality, senses and movement. With nanomedicine we have for the first time the possibility to design systems to meet requirements such as reduced side-effects, controlled release, targeted delivery as well as higher drug bioavailability at the target site. If the brain delivery of drugs for neurodegenerative disease or cerebral cancer is to be successful, a far better understanding of the complex processes taking place on the nanoparticles surface, as well as in cell–NP contact with the different transit organs and tissues, will be required.

1. Introduction

The application of nanotechnology to medical problems promises to change the future of medicine. There are increasingly high expectations for the delivery of drugs to brain-related diseases such neurodegeneration and cerebral tumors. After two decades of research, nanotechnology approaches to brain drug delivery remain underdeveloped. In the present review we will summarize the challenges that nanoparticle based brain drug delivery face, especially via the intravenous administration.

Three main reasons for low brain delivery of targeted drugs but also nanoparticles (NPs) can be identified. (I) post-administration modifications imposed on the NP surface by the body fluids and during transit cell compartments [1–4]; (II) physical parameters such as blood flow [5], residence time on target molecules, ratio of diseased to healthy cells; (III) and finally, the biological limitations in the form of lack of unique target biomarkers on the diseased cells [6], highly selective barriers [7,8], and limited exclusive transporters for specific tissues [6], or peculiarities such as e.g. differences in pH, the perineuronal nets surrounding specific neurons [9] and limiting the access to them.

The interaction between cells and nanoparticles is arguably the most important factor in determining both beneficial and adverse effects that NPs may introduce in the human body. In parallel to the development of new sophisticated nanoparticles and nanoparticle-composites, the research on the metabolic fate of nanoparticles after administration, targeting and after drug release in the target cells is a fast expanding field. Usually this research is performed in serum-free cell culture for a better control of the environmental conditions but this leads to an underestimation of post-administration modification the particles experience in vivo and its implication for biodistribution.

Targeted nanoparticulate drug delivery is defined by the nanoparticle surface and biomarkers specific for the surface of diseased cells allowing the particles to recognize and bind exclusively or at least to a higher extent to this subset of cells. Recent research has shown that in most cases the engineered nanoparticle surface is modified immediately after administration. As a consequence the intended biodistribution is determined by a corona of blood- or body fluid derived proteins rather than the designed surface [1–4,10–14]. This opsonization of the NPs and the resulting protein corona especially the complements usually initiate the recognition by the RES (reticular endothelial system) and hence fast blood clearance but as a consequence target moieties may become ineffective [15]. Moreover, for targeting, the residence time of targeting moieties on the NP and the corresponding cell surface biomarker on the target cell membrane must be long enough to allow binding. While biomarkers have a predictive value for the outcome of therapy and the

* Tel.: +39 02 574303708.
E-mail address: silke.krol@ifom-ieo-campus.it.

0168-3659/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jconrel.2012.04.044

prognosis of patients the pharmacodynamic biomarkers are more problematic as physical factors like blood flow, biomarker density on the target cell as well as the ratio of biomarker on diseased and hence target cells as compared to healthy cells plays an important role (e.g. folate receptor expression in tumors compared to kidneys [16]. Finally biological parameters such as regular uptake mechanisms by mediated endocytosis through the BBB (active delivery) versus membrane binding (nanoporation→adverse effects) to endothelial cells become evident.

With nanotechnology, for the first time, we have the possibility to design intelligent drug delivery systems that match the complexity of the tasks required. Success will strongly depend on our ability to translate the knowledge of biology into a multifunctional and stable designed nano-delivery system.

2. Nanoparticles and intracorporal challenges for successful brain drug delivery

2.1. Post-administration changes of the NP surface

Nanoparticles are defined by parameters such as their material, surface charge, curvature or size, and functional groups on the particle surface. In general it is now known that the NP surface and sometimes even the material are modified during the passage through different transit organs (lung, stomach, blood) and barriers (skin, lung surfactant, mucus etc.).

Binding of proteins to the NP surface influences surface charge, increases its total size and covers functional groups [15]. The stability of the NP attached protein corona is strongly time-dependent because the longer the NPs travel through the body and the more often the particles pass cell layers the more often the protein shell will be exchanged. In consequence this means that the originally bound targeting moieties and moderators for the barrier passage may be removed or covered by proteins [17].

So the first problem in brain-directed delivery is to predict or prevent the post-administration modifications which changes a well-designed NP surface into a hardly predictable one. Because the surface is “what the cells see” first when the NPs get into touch with them [1].

Recent in vitro studies has shown that not only the NP surface charge has an influence on the immobilized protein corona but also the size. The influence of different particle properties on protein binding was analyzed in detail by several research groups [1–3,10–14,18–20]. The initial protein shell of more abundant proteins was found to be substituted by proteins with a higher binding affinity for the NP material. This process is known as “Vroman Effect” [3,4]. Other groups studied the protein corona and its time evolution [11–13].

Some of the proteins detected frequently in the protein corona of NPs after serum incubation are listed in Table 1. For abundance [10,21] and pI [10,22] of the plasma proteins, controversial data were found. In Table 1 only the data from [21] and [22] were listed.

The work of Lacerda et al. [14] should be mentioned as it described not only the protein binding after serum incubation but the structural changes imposed onto frequently bound proteins from human blood by the binding to gold nanoparticles of different sizes.

The consequences of the protein corona on the in vivo biodistribution was described by Kreyling et al. [18] and Choi et al. [19]. They determined the route of inhaled or lung-instilled NPs with varying surface charges and sizes in rats at different times after administration. Moreover they tracked the NPs after extravasating from the lung and measured their secondary organ accumulation. Noteworthy is their observation that NPs extravasating through the lung surfactant layer tend to accumulate in higher concentrations into the brain compared to injected particles. This finding points toward the importance of the protein corona for passive targeting and undesired secondary organ distribution.

Table 1
Blood derived proteins and their binding to NPs.

Abundance in blood [21]	Protein	pI	Lit. for pI	Polymeric NPs	Fe_xO_y	Gold NP [2] (30 + 50 nm)	Liposomes/solid lipid NPs	SiNP [10]	CNT (single + double walled)	PEG-coating
1	Albumin	6.23*/4.9	[22,23]	+	+	+	+	+	+	+
2	IgG(4 subclasses)	6.4–9.0	[24]	+	+	n.d.	+	+	n.d.	+
3	Apolipoprotein A-I	5.59*/5.4	[22,25]	+	+	+ (30 nm)	+	+	+	+
4	Transferrin	7.06*/5.6	[22,23]	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
5	Apolipoprotein A-II	4.89/5.0	[26,27]	+	n.d.	n.d.	+	n.d.	+	+
6	α1-Antitrypsin	5.4*/4.0	[22,23]	n.d.	n.d.	+ (50 nm)	+	n.d.	n.d.	n.d.
7	α1-Acid glycoprotein	2.7	[23]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
8	Transthyretin (prealbumin; 4 subunits)	5.64*/5.7(CSF)5.4(blood)	[20,22,28]	n.d.	n.d.	n.d.	+	n.d.	n.d.	n.d.
9	Haptoglobin-1/2	6.58/6.57*	[22]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
10	Hemopexin	7.00*	[22]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
17	Fibrinogen	5.8	[20]	+	+	+	+	n.d.	+	+
12 for CIII	Apolipoproteins	B100: 6.62 CIII: 5.1 E: 5.5	[25]	+	n.d.	+ (apoD and E on 30 nm)	+ (exception: no apoE on solid lipid NPs with poloxamer 188)	+ (B100, E)	+	+
18	α1-Antichymotrypsin	4.1–4.45	[29]	n.d.	n.d.	+ (30 nm)	n.d.	n.d.	n.d.	n.d.
-	IgM	6.4–9.0	[30]	n.d.	+	n.d.	+	n.d.	n.d.	n.d.
13	α2-Macroglobulin	6.5	[31]	n.d.	n.d.	n.d.	+	+	n.d.	n.d.
26	Antithrombin III	4.7–5.2	[32]	+ (only on PLA NP)	n.d.	n.d.	n.d.	+	n.d.	n.d.
19 (C3)	Complements	5.7(C3)9.3 (C1q)	[33,34]	n.d.	+(C3b)	+	n.d.	+ (C3, H)	+ (C1q)	n.d.

*pI of the precursor protein found in Fountoulakis [22].n.d. = not detected, PLA = poly- (D,L)-lactic acid. If not indicated otherwise the protein corona data were found in Aggarwal [3].

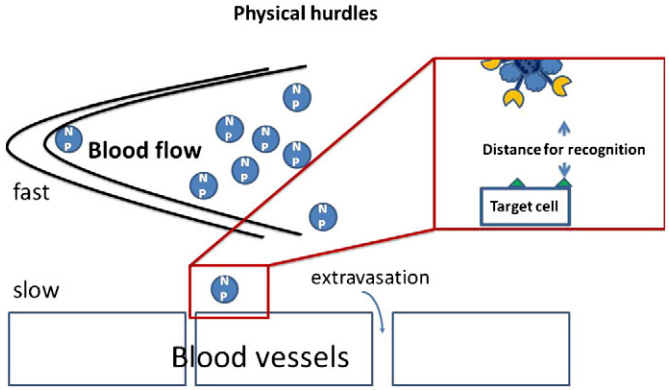

Fig. 1. Scheme of physical hurdles.

2.2. Physics comes into the game

Why is physics of importance? Successful nanoparticulate chemotherapy will strongly depend on two parameters: local high NP/drug concentration and NP residence time in contact with the target cells to allow sufficient drug release. Some of the effects influencing the drug delivery in vivo is summarized in Fig. 1.

Mostly direct administration to the target tissue is not possible or unwanted because it is too invasive. In consequence the first critical step of any NP administration into the human body is the dilution into 4.7–5 l of blood. The next step is that the NPs, once injected into the blood stream, move with a output speed of 0.3 m/s into the aorta coming from the heart and a velocity of 0.6 ± 0.4 mm/s in capillary vessels [35]. Initially injected in a bolus within the blood flow NPs become with time homogenously distributed over around 100,000 km of blood vessels and a total surface area of 8000 m^2 of vessel walls [5]. However with every blood cycle NPs or hydrophilic macromolecules extravasate into the tissue if their size is between 5 and 12 nm and in some exceptional cases even particles of 24 and 60 nm in diameter escape through large aqueous pores [7] and start to accumulate in different organs such as e.g. the liver. An exception is the brain where only particles or macromolecules with a size of 1 nm and smaller are able to extravasate freely [7] while the access for others are tightly regulated to the receptor-mediated endocytosis (e.g. insulin, glucose, transferrin, etc.) [36]. In brain microvessels, NPs are distributed on a surface area of 100 cm^2/g brain tissue and hence an overall surface of 13 m^2 in a brain of an average male subject (1.3–1.4 kg for an adult) with an average intercapillary distance of ~40 μm [8]. High vessel density and short intercapillary distance result in an almost immediate high concentration of all molecules which are able to pass the blood–brain barrier (BBB) in every part of the brain. This means that molecules do not travel far in the brain and distribute everywhere which is in agreement of recent findings of drug delivery into the brain [37]. In consequence brain drug delivery systems need to deliver the drug molecules or NPs exactly to the diseased brain area.

After successfully crossing the 20–30 nm thick and extremely tight (transendothelial electrical resistance: >1,500 Ω•cm^2) [38] endothelial cell layer of the BBB the NPs are diluted again, in 120–150 ml [39] of cerebrospinal fluid (CSF) and distributed into 1.5 l of total brain volume (average adult [40]). Cerebrospinal fluid or liquor is a clear filtrate of blood with a much lower protein load, no blood cells and a specific cut-off for the maximum molecular weight produced by the choroid plexuses (capillary networks) of the ventricles. It contains proteins with a molecular weight up to 2,200 kDa (β-lipoprotein) and a hydrodynamic radius of 12 nm [41]. However, the protein concentration in blood serum can be between 14 and 6000 times higher for single proteins than in liquor. The turnover of CSF is 3–4 times per day [42] and it can be considered as one mechanism of NP clearance from the brain.

Already the sheer number of cells building the brain makes targeted delivery to a small subset of diseased cells immensely challenging. The brain consist of 100,000,000,000 neurons [43], but astrocytes outnumber them by about 10-fold [44]. There is some controversy about the number of glial cells in brain as it is supposed to be 10–50 times the number of neurons. But recent research results suggest that the neuron-to-glial cell ratio in the grey matter may be much smaller, closer to 1:1 [45]. The length of myelinated nerve fibers which build the white matter of the brain is 150,000–180,000 km [46,47].

Calculating that a neuron can have 1,000–10,000 synapses the total number of synapses can be 1×10^{14} to 5×10^{14} in an adult or even 10^{15} in a newborn. A special type of GABAeric neurons, the Purkinje cells (15–26 million), mainly involved in massive signal-processing in the coordination of motor control in the cerebellum (cerebral cortex) additional to other functions, can have up to 200,000 synapses each. The synapses can be distinguished into two types of signaling: a chemical one in which the cleft is 20–40 nm wide and an electrical one where the cleft is only 3.5 nm bridged by gap junctions with a width of 1.2–2 nm [48,49]. Inhibitory as well as excitatory signal transfer across these clefts strongly depends on the membrane potential and its resulting change after delivery of the neurotransmitters.

2.3. Biological hurdles opposing proper delivery to the final target

After a long travel through the blood vessels some few NPs arrive finally in the cerebral microvessels, close to the target tissue. Up to this point NPs are diluted, modified by blood or retained or retarded in transit tissues such as liver, spleen, kidney or fat. In the brain nanoparticulate drug delivery faces another challenge in the form of one of the tightest barriers and the highest cell density per area. The ultimate goal is not only to overcome the barrier but to deliver the drug content only to a very low number of diseased cells e.g. cancerous cells and if possible spare all the other "brain cells" (Fig. 2).

What are these brain-related cells that NPs encounter, when they are washed into the flow of brain microvessels and overcome the blood–brain barrier? What is the NP fate in the brain? And what is already known about the NP-brain cell interaction? There are two research areas trying to answer these questions. One is brain drug delivery itself which aims to design targeted and functionalized NPs to transport drugs through the BBB, hence overcoming the limitations in accessibility to the brain and subsequent uptake by "brain cells" such as glial cells or neurons. The other one is nanotoxicology studying biodistribution of NPs in vivo and adverse effects resulting from NP-cell interaction.

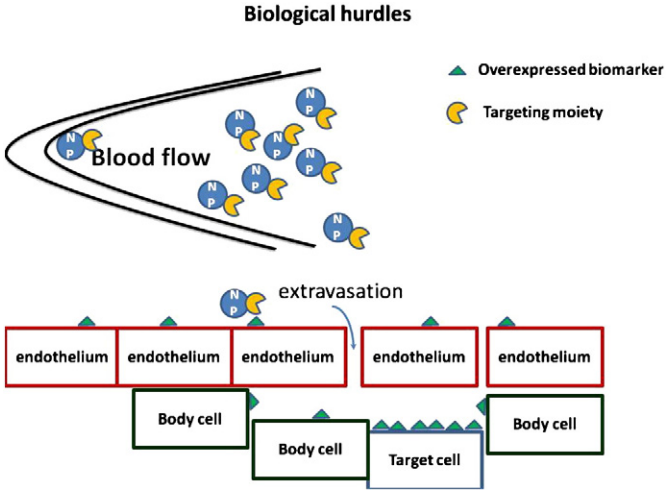

Fig. 2. Scheme of biological hurdles.

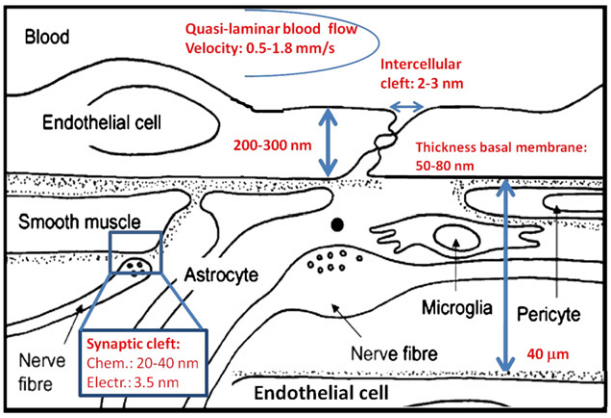

Fig. 3. Biological (data about from basal membrane [52]; tight junction [53]; synapses [42,49]; intercapillary [54]) and physical parameters (the data about the blood flow [55,56]) at the BBB and cells surrounding the target (modified from Abbott [57]).

In the brain the NPs are in contact with 3 different cell types (Fig. 3):

1) Blood–brain barrier (specialized endothelium) and/or blood liquor barrier (choriplexus endothelium between blood and CSF),
2) Glial cells or neuroglia (macroglia: astrocytes and oligodendrocytes; microglia: pericytes regulating BBB functionality [50] and precursors for macrophage-like cells [51]
3) Two general types of neurons (with (white matter) or without (gray matter) myelin sheath),

and the cerebrospinal fluid (CSF) or liquor embedding the "brain" itself. An additional hurdle for selected neurons is the perineuronal nets, a type of specialized extracellular matrix which can charge the direct neuronal environment either positively or negatively and limit the direct neuronal access by providing a specific cut-off [9].

2.3.1. Cell–nanoparticle interactions in general

Nanoparticle–cell interactions often lead to accidental toxic effects such as blocking of ion channels [58,59], disturbance of the membrane potential induced by nanoporation [60,61], or inhibition of signaling pathways or other essential cell functions such as nuclear chromatin decondensation [62].

There are two general ways for the NP to enter cells: (i) the active and foreseen uptake such as endocytosis, pinocytosis or membrane pores and (ii) a passive, often invasive one by direct plasma membrane penetration. Cells impose their own hurdles to a direct membrane penetration in the form of a protective glycocalyx. Another feature of the cell membrane is the lipid composition which can differ from cell to cell and between health and disease. The cell membranes may vary in fluidity, cell surface charge, membrane asymmetry and peculiarities such as lipid rafts leading to differences in cell permeability and NP toxicity. So far two mechanisms are described for forced NP uptake by membrane penetration: (i) nanoporation by positively charged NP and polymers leading to a disturbance of the membrane potential and apoptosis [61] and (ii) a non-invasive virus-like mechanism described by Verma et al. [63] for striped NPs. A review by Verma and Stellacci [61] describes the interaction between NP's surface charge and oppositely charged phospholipids leads to NPs internalization by membrane disruption. The lipid bilayer of most eukaryotic cells is asymmetric containing negative phospholipids, phosphatidylserine and -ethanolamine located on the inner leaflet, and the neutral phospholipids, (phosphatidylcholine) and sphingomyelin with choline (no net charge) or ethanolamine (negative net charge) head groups on the outer leaflet, accessible to direct contact to charged nanoparticles. It was found that a positive surface charge with a threshold percentage of 40–50% on nanoparticles is responsible for massive membrane disruption leading to nanoporation by transient holes, and hence lost of membrane integrity and membrane potential. Moreover, disturbance of the lipid asymmetry by an induced flip-flop of negative lipids from the inner to the outer leaflet also leads to apoptosis and macrophage activation [64,65]. Another study supporting that NP binding to the membrane was driven by electrostatic interactions was performed by Yu et al. [66]. They coated the surface of gold NPs with the pH sensitive cysteamine and were able to induce the NP binding to the cell membrane by changing the medium to a moderate acidic pH. Usually negative NP are considered non-toxic as the repulsive forces between the NPs and the net negatively charged outer leaflet of membrane prevent interaction. However Lin et al. [67] observed that even negative NPs can exhibit a minimal toxicity when they interact with membrane proteins or lipid raft structuring transmembrane proteins or channels. A disturbance of the membrane potential as well as of the membrane integrity, which are crucial for proper signaling in neuronal cells, may have a significant impact on the proper function of the CNS and hence seem to be the main reason for neurotoxicity observed for charged NPs.

A new generation of virus-like nanoparticles with negligible toxicity was introduced by Verma et al. [63]. They developed "striped" nanoparticles exhibiting a regular surface pattern of hydrophobic and hydrophilic (charged) molecules on the NP surface. These NPs were able penetrate directly the cell membrane without nanoporation and to enter the cytosol. This concept mimics the nature of cell penetrating proteins (CPP) found on virus capsids. The same concept was used by other authors to design cell-penetrating macromolecules such as an amphiphatic phospholipid polymer hybrid [68]. They confirmed that their material was entering the cell directly through membrane without causing significant cell toxicity.

But what happens once the NPs are inside cells? In the endocytotic–lysosomal pathway the protein corona can be digested by proteases or replaced by other enzymes. Once the protective coating is removed the NP material begin to play a role. The metal NP can corrode and release of potentially toxic ions e.g. cadmium from quantum dots (QD) or copper [69]. Polyamino acid NP can degrade and the high concentrations of amino acids can trigger or inhibit cellular pathways (e.g. [52]). Another possibility is that intact NPs escape into the cytosol and interfere with transport mechanisms, DNA condensation, mitochondrial functions or the cytoskeleton [60].

2.3.2. Blood–brain barrier (BBB)

The blood–brain barrier (BBB) is presented by the cerebrovascular endothelium sealed with tight junctions. Additional structures are supportive cells such as pericytes, astrocyte end-feet, and a discontinuous basal membrane (or basal lamina).

The basal lamina is situated between brain capillaries, and the supportive cells and consists of laminin, fibronectin, tenascin, collagens, and proteoglycan [70]. Its function is that of an extracellular matrix providing a scaffold for cell migration, mechanical support for cell attachment, and separation of adjacent tissue. In the context with NP entrance the most interesting feature is that it imposes a mesh with a certain cut-off and hence blocks the passage of macromolecules into the brain. Pakkenberg and Gundersen [46] found that small molecules of 43 kDa (electrical mobility diameter: ~6 nm) can pass easily through the basal membrane while 460 kDa (~10 nm) molecules are excluded. A very good review was published by Abbott et al. [71] describing the complex processes taking place between the BBB and the other brain-related cells.

The BBB presents the main obstacle for entrance of large or hydrophilic molecules, microorganisms or NPs into the brain. This true for accidental exposure to nanostructures (e.g. virus) which are successfully blocked but unfortunately also for intentional nanoparticulate drug delivery as treatment of a disease. As mentioned before the barrier function stems from specialized endothelial cells in the

cerebral microvessels. The restricted and highly controlled access to the brain is due to a combination of five different factors:

1) Tight junctions which are sealing the intercellular gap
2) Reduced rate of pinocytosis from the luminal side which prevents uncontrolled cell entrance
3) No fenestration which blocks the intercellular passage of the endothelium
4) An enzymatic barrier which presents the second line protection against unintentionally entered molecules, proteins or viruses
5) An efflux transporter system such as P-glycoproteins and others which removes small molecules from the endothelial cells before they reach the abluminal or basal side

However, the BBB has much more functions than only controlling brain access. It is involved in regulative activities by secretion of functional and informational molecules and their trafficking into brain or blood [6]. For this reason it contains many vesicular transport systems from the luminal (blood) site through the endothelial cells via receptor-mediated transport mechanisms (transcytosis) or back to the lumen of the microvessels (P-glycoprotein induced efflux).

In the past three major mechanisms were tested to overcome the BBB and deliver drugs into the brain: (I) the invasive disruption of the barrier, (II) transcellular diffusion and (III) active transport. The latter one and in particular the receptor-mediated transport has become the main strategy for non-invasive targeted brain drug delivery, especially for NPs. Usually the NP surface is functionalized with groups mimicking biological systems either by electrostatic or covalent binding. Some examples are lecithin NP coatings or liposomes, both incorporate blood-derived apolipoproteins and hence mimic LDL (low density lipoproteins) or HDL (high density lipoproteins), which can be taken up via LDL-receptor.

2.3.2.1. Transmembrane versus receptor-mediated delivery. Recently, several very good reviews [6,72,73] were published analyzing the different ways of nanoparticle-BBB interaction. Pardridge [73] defined two criteria for molecules able to pass the BBB by transcellular diffusion: (I) very small molecules (<400–500 Da) and (II) very hydrophobic substances (<8–10 hydrogen bonds with H_2O). However, recently also larger hydrophilic molecules such as e.g. the neutrophil attractant (CINC-1) with a MW of 7.8 kDa were found to be able to enter the brain, most probably with a receptor-mediated shuttle [6].

The lipid composition of BBB endothelial cells is an important parameter for transcellular diffusion of hydrophobic molecules or potential cellular toxicity of nanoparticles inducing plasma membrane disruption. However, recently Banks [6] showed that entrance into endothelial cells does not mean that the molecules are entering the brain. They found that especially small hydrophobic drug molecules present good substrates for P-glycoprotein induced efflux. This second line defense of BBB limits significantly their concentration in the brain parenchyma. Gabathuler [74] analyzed in his review several targeted nanoparticle systems. One was especially interesting in this context as it was able to by-pass the P-glycoprotein mediated efflux system. Pluronic P85 micelles with insulin or antibodies as targeting moieties, developed by Kabanov et al. [75], were found to accumulate in the brain. In previous works Kabanov and his coworkers [76,77] have shown that Pluronic NPs, initially developed to decrease the myocardial toxicity of doxorubicin in cancer treatment, were also successfully circumventing the multidrug resistance by bypassing and selectively inhibiting P-glycoprotein expression in tumor cells. A comparable mechanism for the BBB was found [75].

Banks [6] reviewed different invasive and non-invasive strategies to overcome the BBB and to deliver pharmaceutically relevant drug concentrations to the brain. He identifies some saturated transport systems as a possible gate for brain-directed drug delivery.

Another very good review was recently published by Gabathuler [74] discussing the pros and cons of different brain delivery techniques including pharmacologic and physiologic approaches. The author singled out three receptors in the BBB as main target for mediated nanoparticulate delivery: (I) transferrin (also highly expressed in liver, heart and most other cells), (II) insulin (also highly expressed in adipose tissue, liver and muscle cells) and (III) low-density lipoprotein (also highly expressed in liver). Nowadays several nanoparticle systems using the same receptors were described such as liposomes [78] or human serum albumin (HSA) – nanoparticles with transferrin or antibodies as targeting units [79,80]. HSA presents a special case as the protein itself may mediate transcytosis of NPs as it was described for cationized [81] and for unmodified HSA [82]. While the cationized HSA falls in the category of basic proteins described as target for adsorptive uptake [74], the results of the author's group [82] were surprising as albumin entrance into the brain is usually observed in pathologic conditions. The NPs accumulate mainly in the brain stem, which makes adsorptive mediated transcytosis as mechanism unlikely because in that case it a more homogenous distribution is expected.

The main drawback is, as stated by Gabathuler [74], that all three receptors are not exclusively expressed on the BBB, even so they are inducing transcytosis through it. In consequence this means that high concentrations of NPs with these target molecules were bound in other organs before they can reach the BBB. This problem is well known from tumor-targeting where only few “real” tumor-markers exist (e.g. prostate-specific membrane antigen which is highly expressed in the prostate tumor cells and tumor-induced neovasculature [83]).

Gabathuler [74] analyzed some novel approaches in search for more specific brain delivery concepts. Those use e.g. viral or toxin mediated targeting or a Llama single domain antibody in order to improve the specificity for the BBB. For example the antibody described in Gabathuler's review [74] was targeting a new BBB specific receptor with a yet unknown function. Llama single domain antibodies without light chains, so-called heavy chain antibodies [84] or nanobody have two advantages over the normal antibodies: (1) with their small size (13–14 kDa) they do not contribute too much to the total size of NPs; something which has to be taken into consideration for full size antibodies (approx. 10 nm) and very small NPs, (2) no immunogenicity was observed so far [85]; this is in contrast to complete antibodies.

Two independent research groups [86,87] who quantified the concentration of citrate-stabilized gold nanoparticles (AuNP) in different organs after intravenous injection showed that an amazingly high concentration especially of small AuNP (<30–50 nm) accumulate in the brain. This was in contrast to the paradigm that nanoparticles are not able to cross the BBB. From their studies it is unfortunately not clear if the particles remain in the endothelium or really enter brain parenchyma. Recently, Sousa et al. [82] determined the brain distribution of 15 nm gold nanoparticles functionalized with polyelectrolyte layers and human serum albumin (final hydrodynamic size of around 120 nm) and proved that the NPs were able to cross the BBB and accumulate in specific regions close to the brain stem in neuronal or glial cells.

2.3.2.2. Local BBB disruption versus transcellular delivery. That material properties strongly influence the nanoparticle-BBB interaction is shown by the example of apolipoprotein functionalized polysorbate 80 (Tween80) NPs developed by Kreuter et al. [88]. It was observed that polysorbate functionalized nanoparticles were able to enter the brain in significantly high concentrations. This was usually explained by the apolipoprotein mediated transcytosis. However as a possible alternative explanation Olivier [89] hypothesized that the polysorbate delivered by the NPs has a BBB opening effect. In the past polysorbate 80 solution is used for the invasive solvent-mediated BBB disruption in order to allow the passage of drugs [73]. Even if the total amount of polysorbate delivered by the NPs is low compared to the solution one has to consider that the local concentration on the NP surface can be as high or even higher than that in the injected the solution.

Summarizing the statements of the previously mentioned reviews one comes to the conclusion that the brain in- and efflux is a finely orchestrated gating system. A simple solution as the chemical modification of a drug into a prodrug and turning it into a "Trojan horse" will not be able to fulfill the multiple requirements such as targeting against the BBB, inducing transcytosis, targeting a specific subset of diseased cells, endocytosis, and controlled drug release. The complexity of BBB response calls for complex solutions in form of multifunctional NP. Additionally precaution has to be taken to prevent uncontrolled binding of blood derived proteins which can change the targeting moieties and NP biodistribution.

However, what happened if NPs really vanquish the BBB? Will the brain be flooded by a continuous flow of drug molecules? As already stated in the chapter about the physical hurdles the short intercapillary distance indicates that molecules do not travel far once they left the blood microvessel. On the abluminal side of the endothelium they will find a highly crowded environment with only small free interstitial space [52] (Fig. 4).

There are three possible pathways that can be followed once NPs overcome the BBB. (1) They are taken up by glial cells (astrocytes or pericytes) which are supporting the BBB and have macrophage-like functions in the brain. (2) They pass the semi-permeable basal membrane separating endothelial and glial cells [52]. (3) They are released from transcytosis in a part of the endothelium which is not covered by the discontinuous basal membrane and enter directly the CSF. From the CSF they can be transported also neighboring neurons.

2.3.3. Glial cells or neuroglia

There are two types of glial cells: (1) macroglia presented either by astrocytes or oligodendrocytes. Astrocytes surround tightly the cerebral microvasculature with their endfeet. Partially they are even directly connected to the endothelial cells via gap junctions. Oligodendrocytes, the second class of macroglial cells, wrap around neurons in the white matter being responsible for production and maintenance of the myelin sheath, an insulating multilayer stack. (2) The microglia contains pericytes, a type of cerebral macrophages. These cells may play a role in NPs clearance from the brain. In the following we will review briefly reported NP-cell interactions in the order that the cells contact penetrating NPs.

2.3.3.1. Astrocytes. The role of astrocytes in the brain is still unclear [90]. From results in cell culture and transplantation experiments it seems that they are necessary for the maintenance of barrier function and maturation of the cerebrovasculature. Accordingly they became the major player for neuronal tissue transplantation and neuron regeneration induced by ex vivo gene delivery. In this context the only reported NP-astrocyte interaction were between astrocytes and superparamagnetic iron oxide NPs (SPIONs), which were used for cell tracking in homing studies of transplanted cells.

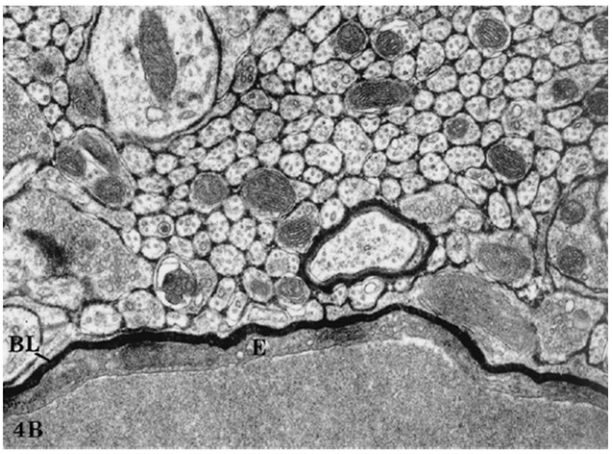

Fig. 4. TEM image of the interstitial space perfused by horseradish peroxidase (43 kDa = 6 nm). BL is the basal membrane and E indicates the endothelium (with permission from Brightman [52]).

Pickard et al. [91] found that SPIONs are taken up by astrocytes in high quantities and accumulate close to the perinuclear region. No statically relevant NP toxicity was detected. Quite the contrary, they observed differentiation of cells pre-incubated and doped with SPIONs which indicate that the cells prosper well. Au et al. [92] reported that a cell culture of astrocytes incubated with SPIONs showed only limited adverse effect in form of mitochondrial uncoupling [92] supporting the before mentioned results. It has to be noted that the NP-astrocyte interactions are studied mainly in vitro.

2.3.3.2. Pericytes. Pericytes are flat, undifferentiated, contractile connective tissue cells, surrounding the endothelial cells on the abluminal site. They seem to derive from microglia and have numerous functions. They are able to phagocytize exogenous protein from the central nervous system [93] and hence can act as cerebral macrophages [94]. However, their main function is to regulate proliferation, survival, migration, differentiation, and vascular branching of the endothelial cells [95,96] and regulation of the blood-flow. The required contractile behavior expressed by some but not all pericytes derives from an actin α-isoform which is typical of contractile smooth muscle cells. Pericytes expressing the protein are mainly located in the pre- and post-capillary regions while the pericytes on the actual microvasculature lack it [97,98]. The importance of pericytes for the endothelium stability as well as for the tightness of the BBB was demonstrated in vivo by Armulik et al. [50].

While the macrophage-like properties of pericytes [90,94] as immune response of the CNS may play a major role in NP contact and clearance the endothelium-maintaining functions may become interesting in case adverse NP effects causing BBB disruption.

In general it can be noted that the glial cell–nanoparticle interaction is not very well understood. One of the few studies focusing on the NP interaction with microglial cells in culture and in vivo comes from Hutter et al. [99]. She and her co-workers investigate the toxicity of AuNPs with different shapes (spheres, rods, urchins) and coatings (PEG-polyethyleneglycol, CTAB- cetyl trimethylammonium bromide) to primary microglia and neuronal cells and their immune activation. The coatings are interesting as PEG is frequently used to prevent or limit the opsonization of NP while CTAB is a surface-associated molecule remaining from the gold reduction process of nanorods. Gold nanorods are studied for the their superior optical properties for optical fluorescence imaging [100]. Hutter et al. [99] stated that excessive washing is required to eliminate loosely bound CTAB which in higher concentration causes a significant cytotoxicity. They observed in vitro the internalization of CTAB coated AuNPs and of PEG-coated urchins into primary microglial cells. Moreover they found in vivo an elevated immune activation (toll-like receptor 2 up-regulation) of the microglia after intranasal instillation into rats in order to circumvent the BBB. In vitro especially PEG-coated rods activate an immune response (Il-1a, granulocyte macrophage colony-stimulating factor) while a lower response to PEG-coated gold spheres is observed. This is in contrast to the results for PEGylated but much smaller quantum dots which do not induce immune response in astrocytes [101]. These findings indicate that shape, size and coating of NP have a significant influence on transient microglia activation.

Hutter's results [99] for microglia activation are in good agreement with those observed by Coomber and Stewart [93] in vitro for SiNP exposure. SiNPs have a relevance for nanomedicine as they are considered a vehicle for gene therapy or a novel contrast agent for gliomas in vivo [102–104]. While the viability of the primary microglial cells is not affected by SiNPs they caused a strong inflammatory response in the form of increased ROS (reactive oxygen species) and RNS (reactive nitrogen species) production, cytokine release and COX-2 overexpression

and TNF-α gene inhibition. Noteworthy was that especially low NP concentrations were found in the cytosol rather than in the endo-/exocytotic or lysosomal pathway. Clearance from the cytosol is more difficult and hence lead to a longer residence time of the NP in the cells. This may be relevant for in vivo brain drug delivery in a beneficial (better release) but also adverse way (toxicity) as low NP quantities are more likely to cross the BBB and reach the brain.

2.3.3.3. Oligodendrocytes. As mentioned before the main function of oligodendrocytes is production and maintenance of the myelin sheath wrapping and insulating neurons in the white matter of the brain. NP–oligodendrocyte interaction was mainly studied for iron oxide nanoparticles. Oligodendrocytes are the brain cells with the highest content of iron. This may make them more vulnerable to iron released from intracellular iron oxide nanoparticles degradation. A study by Hohnholt et al. [105] comparing oligodendrocyte exposure to different forms of iron as NP, or chelated iron supports that only nanoparticles are able to increase significantly the intracellular iron content as they are actively taken up and degraded in the endo-lysosomal pathway. However, recently Hohnholt et al. [106] found that no toxic effects for elevated iron levels were observed in oligodendrocytes as it is usually observed in others cells exposed to SPIONs [107]. Oligodendrocytes balance the additional iron by an increased expression of the iron-storage protein ferritin and prevent free iron ions and consequential formation of ROS.

Another but more uncommon NP effect on membranes was described by Boggs and Wang [108]. They studied a specific type of multilamellar vesicles consisting of the same compounds like the myelin sheath, the polyvalent carbohydrate, galactosylceramide and negatively charged cerebroside sulfate. They found a co-clustering of galactosylceramide and membrane proteins which are located in a signaling region of oligodendrocyte membranes. Interference with the signaling region of brain cells can have an significant impact on proper brain functioning. Moreover it was observed that the vesicles inhibit the proper build-up of an intact cytoskeleton in cells in culture. If this observation is of relevance in vivo is not yet known. However, the interaction between vesicles and signaling region of the oligodendrocytes seems to be electrostatically driven. If this is the case it is to be expected that other NPs with sulfate groups may cause the same clustering effect. Both vesicles and negatively charged NPs are usually considered to be biocompatible and hence less toxic to cells.

2.3.4. Neurons

The neuronal network in the brain are a quite complex assembly of associated cells with different functions. Neurons consist of the cell body, also called soma, and sprout usually only one axon and but several dendrites directly from the cell body. The axons in the white matter are surrounded by a myelin sheath responsible for insulation and high velocity electrical signal transfer along the axons. That the neuronal function crucially depends on the myelin sheath can be deduced from the grave pathologic consequences in multiple sclerosis, a myelin degenerating disease. The compact myelin sheath is produced by oligodendrocytes for the CNS (central nervous system) and Swann cells for PNS (peripheral nervous system). It is segmented by the so-called node of *Ranvier* (internodes) in which a high number of sodium channels is embedded. These internodes are shielded from the extracellular milieu by non-compacted myelin [109]. Myelin is a tight multistack of membranes which are composed of two glycosphingolipids, galactosylceramide and its sulfated form, galactosylceramide I(3)-sulfate in lipid rafts, and only a low amount of cytosol [110]. The lipid rafts are significantly stiffer and thicker than the "normal" fluidic cell membrane [111]. As the myelin sheath is preventing particle entrance due to its membrane stiffness and the absence of endocytosis large parts of the neurons are protected.

Neuronal signaling especially at the synapses strongly depend on action potentials and its transmission from neuron to neuron or to other cells such as muscles. This function is closely related to cell surface charges and membrane potentials. Consequently, a small disturbance of the membrane potential may have severe consequences on excitatory and inhibitory regulation of the neurons. The most vulnerable area for adverse charged NP interaction is the synaptic chemical clefts as it is slightly larger than the electrical cleft. Here small charged NPs can directly penetrate and disturb signal transmission. The postsynaptic membrane with its high density of receptors for neurotransmitter is a potential access gate for NPs. Another possibility of interference with the signaling is that the NPs locally change the pH or the ionic strength in or close to the cleft by binding counter ions. A pH increase can lead to an over-excitability to a point of convulsions (epilepsy) while a pH decrease can lead to a coma (diabetic coma) because of a decreased excitability [112].

There is some evidence that different metal oxide NPs have an influence on membrane potential and increase the neuronal firing rate by changing the response of potassium channels. Zhao et al. [113] reported that in vitro ZnO NP have this effect on hippocampal CA3 pyramidal neurons. A similar effect was observed for CuO NPs on CA1 hippocampal neurons [114]. That this can have a physiological impact in vivo was shown in rats by testing their spatial cognition capability [115]. Yang et al. [116] reviewed the interaction of metal and metal oxide with especially of hippocampal neuronal cells. They observed that the adverse effects are related to the release of metal ions from the nanoparticles rather than to the NPs themselves. However, they have to state that the current knowledge on neuron-NP interaction is still extremely limited and that the traditional research to determine neuro-nanotoxicology is too limited in complexity.

As mentioned in the section about microglial cells Hutter et al. [99] compared different surface coating (CTAB, PEG) on differently shaped gold NPs (rods, spheres, urchins) and their impact on microglial cells as well as on young primary neurons (3 week old animals). They found that gold rods (12 nm × 43 nm) independently of the coating are internalized into neurons, both in cell body and neurites. They stated that nanorod up-take is due to a matching shape of the nanoparticles and the dendrites. The urchins and spherical gold are not up-taken in neurons because they do not have phagocytosis like microglia cells which internalize also the other shaped NPs.

A detailed review of Cooper and Nadeau [117] discusses the interaction of different NPs ranging from quantum dots (QD) over carbons species to polymeric NP with neuronal (PNS and CNS) cells and their potential use for monitoring neuronal actions. Carbon nanotubes show promising results in terms of neuronal firing rate while quantum dots together with voltage-sensitive dyes were identified as useful tool for advanced fluorescence techniques on neurons to gain information about transport processes and action potentials.

3. Lessons learned

3.1. Different cell types do not behave comparably

The cell type strongly influences the interaction with NPs by the presence of surface receptors, lipid composition, and active up-take mechanisms. However if the in vitro observation of adverse NP effects have any significance for in vivo nanotoxicity remains to be determined. Some first results indicate in this direction as the immune activation in vitro in the macrophage-like pericytes was also reported in vivo in the microglia of nasal administrated NPs [99].

3.2. How much is enough?

It is still not clear if the concentration of NPs which finally arrives at the brain is high enough to cause any adverse or beneficial effects. For example little is known about the heavy metal ion concentration involved in the progress of Alzheimer's disease. It was reported that in amyloid plaques frequently high concentration of iron were found

[118]. From the in vitro iron oxide NP experiments with astrocytes [105,106] it can seen that NP may release a significant amount of metal ions into cells. Moreover some cells are better than others in regulating intracellular metal concentrations, hence preventing ROS-induced toxicity. We have to remember that if we succeed in targeted nanoparticulate deliver and controlled release the transported amount of drug of one NP must be sufficient to cure one single brain cell as we do not know if we will be able to deliver a second one. In general it is possible as the high surface area is the strongest feature of NP and allows for high drug payloads. One example is the observation that a high surface concentration of polysorbate on NPs locally may have the same effect as an injection to open the BBB [89].

That shape can play an important role at least for cell penetration can be concluded from the experiment with nanorods and carbon nanohorns up-take [99,117]. So far little is known how much influence single nanoparticles have on proper cell function e.g. when they attach to microtubules or the DNA.

3.3. Specific versus universal? Receptor-mediated targeting

Receptor-mediated transcytosis is a good strategy for non-invasive BBB passage. However, receptors which are not exclusively expressed on the BBB lead to high NP accumulation in organs other than the brain. This taken together with NP dilution in blood and physical parameters such as blood velocity and resulting short residence time of the target molecules on the receptor limits the maximal possible drug concentration. An optimal receptor binds with high affinity and a low dissociation constant to the target moiety on the NP to prevent fast detachment. Moreover, it is exclusively expressed in the diseased area of the brain or in close vicinity to it. This is because NPs and drugs do not move far once they entered the brain parenchyma.

The problem of low distribution volume due to short diffusion distance in the brain is well-known. Recent studies compare the volume from simple intracranial injection (bolus) with that of convection enhanced delivery (CED) of either drugs or nanoparticles [37]. CED is performed as a slow infusion creating a permanent concentration gradient which drives the NPs farther from the injection point without disturbance of the brain parenchyma. The results of this study confirms that CED allows for a broader distribution. If we transfer this knowledge to brain drug delivery it means the drug-loaded NPs need to be released directly or close to the target. The few NPs which succeed to arrive in the brain cannot create a concentration gradient as driving force. In consequence, this excludes shortcuts like nasal delivery or other routes by-passing the BBB as they are usually far from the tumor or the disease site (e.g. cerebral cortex, Alzheimer; substantia nigra, Parkinson).

3.4. First comes, first serves!

Physical factors like dilution and blood circulation time (protein corona, clearance, accumulation in non-target organs) play an important role in the NP concentration which arrives in the brain vessels. In order to keep the times short and the NP concentration high Maeda et al. [119,120] injected for treatment of brain and liver tumors intra-arterially rather than intravenous in limbs. They found an significant effect on delivery efficacy. In consequence, a injection into an artery closer to the head may improve the outcome of targeted brain delivery.

Brain drug delivery usually is studied research by testing permeation through the intact BBB, e.g. by using healthy animals or for in vitro experiments by testing nanoparticle penetration in BBB endothelial cell cultures with a high transendothelial resistance (TEER). An interesting finding in this context is that various diseases such as gliomas, multiple sclerosis, AIDS, ischemic stroke or Alzheimer lead to a reduced BBB integrity [121,122]. This pathologic effect, usually connected with severe co-morbidities such as inflammations and edema, may open a new and direct route to the diseased brain area. Perhaps an EPR (enhanced penetration and retention) effect, known from tumors, can lead to passive targeting and locally higher NP concentrations.

3.5. Opsonization – enemy or friend?

The protein corona or opsonization of NPs in the blood has an influence on targeting, biodistribution and fate of the NPs [2,3,18,19,123,124]. The attachment of the proteins to NPs is driven by surface charge, curvature, and circulation time [3,125] and strongly contributes to secondary organ accumulation [18,19]. There is some evidence that selected serum proteins bound on purpose to the NP surface [82] can trigger the facilitated passage through the BBB. The same was observed for a protein "corona" taken up from the lung surfactant during the transfer from the lung alveoli to the blood (Fig. 5).

If we analyze the protein corona of NPs found in the brain after intravenous injection and identify the proteins it should be possible to design protein shells on NPs triggering the BBB transcytosis. The main challenge is to analyze and identify such extremely low quantities of proteins. New techniques, for example that suggested by Melli et al. [126], may be able to solve this problem in the future. The new generations of blood-protein functionalized NPs will not unspecifically bind blood proteins as they equipped with them and will allow brain delivery perhaps via still unknown receptors.

Abbreviations

BBB	blood–brain barrier
CSF	cerebrospinal fluid
CNS	central nervous system
COX-2	cyclooxygenase-2
CTAB	cetyl trimethylammonium bromide
GABA	γ-aminobutyric acid
GM-CSF	granulocyte macrophage colony-stimulating factor

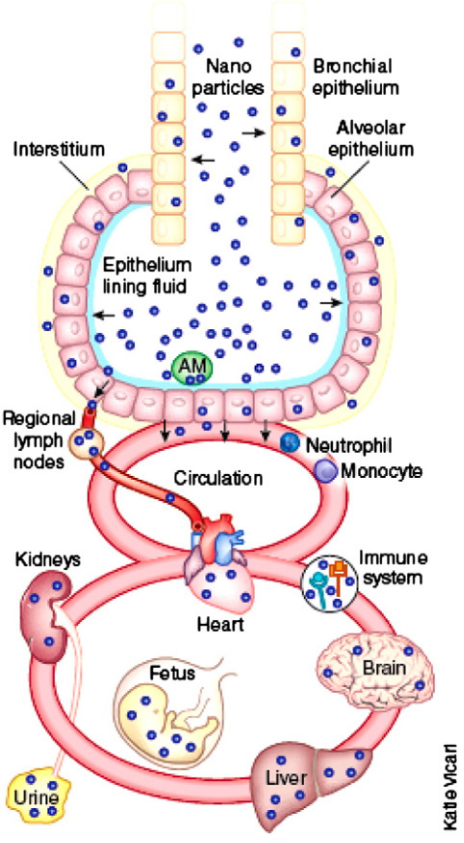

Fig. 5. Lung-secondary organ biodistribution (with permission from Kreyling [18]).

Il-1a	Interleukin-1a
NP	nanoparticle
PEG	polyethyene glycol
PNS	peripheral nervous system
quantum dots	QD
SPIONs	superparamagnetic iron oxide NPs
RNS	reactive nitrogen species
ROS	reactive oxygen species
TAT	transactivator of transcription
TNF-α	tumor necrosis factor

Acknowledgements

The author is grateful to Dr. F. Sousa for providing language help with the manuscript.

References

[1] D. Walczyk, F.B. Bombelli, M.P. Monopoli, I. Lynch, K.A. Dawson, What the cell "sees" in bionanoscience, J. Am. Chem. Soc. 132 (2010) 5761–5768.
[2] M.A. Dobrovolskaia, A.K. Patri, J. Zheng, J.D. Clogston, N. Ayub, P. Aggarwal, B.W. Neun, J.B. Hall, S.E. McNeil, Interaction of colloidal gold nanoparticles with human blood: effects on particle size and analysis of plasma protein binding profiles, Nanomedicine 5 (2009) 106–117.
[3] P. Aggarwal, J.B. Hall, C.B. McLeland, M. a Dobrovolskaia, S.E. McNeil, Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy, Adv. Drug Deliv. Rev. 61 (2009) 428–437.
[4] L. Vroman, A.L. Adams, G.C. Fischer, P.C. Munoz, Interaction of high molecular weight kininogen, factor XII, and fibrinogen in plasma at interfaces, Blood 55 (1980) 156–159.
[5] S. Vogel, R.A. Calvert, Vital circuits: on pumps, pipes, and the workings of circulatory systems, Oxford University Press, 1993.
[6] W. Banks, Characteristics of compounds that cross the blood–brain barrier, BMC Neurol. 9 (Suppl. 1) (2009) S3.
[7] H. Sarin, Physiologic upper limits of pore size of different blood capillary types and another perspective on the dual pore theory of microvascular permeability, J. Angiogenes. Res. 2 (2010) 14.
[8] H. Duvernoy, S. Delon, J.L. Vannson, The vascularization of the human cerebellar cortex, Brain Res. Bull. 11 (1983) 419–480.
[9] M. Karetko, J. Skangiel-Kramska, Diverse functions of perineuronal nets, Acta Neurobiol. Exp. 69 (2009) 564–577.
[10] S. Tenzer, D. Docter, S. Rosfa, A. Wlodarski, J. Kuharev, A. Rekik, S.K. Knauer, C. Bantz, T. Nawroth, C. Bier, J. Sirirattanapan, W. Mann, L. Treuel, R. Zellner, M. Maskos, H. Schild, R.H. Stauber, Nanoparticle size is a critical physicochemical determinant of the human blood plasma corona: a comprehensive quantitative proteomic analysis, ACS Nano 5 (2011) 7155–7167.
[11] E. Casals, T. Pfaller, A. Duschl, G.J. Oostingh, V. Puntes, Time evolution of the nanoparticle protein corona, ACS Nano 4 (2010) 3623–3632.
[12] D. Dell'Orco, M. Lundqvist, C. Oslakovic, T. Cedervall, S. Linse, Modeling the time evolution of the nanoparticle-protein corona in a body fluid, PLoS One 5 (2010) e10949.
[13] M.P. Monopoli, D. Walczyk, A. Campbell, G. Elia, I. Lynch, F.B. Bombelli, K.A. Dawson, Physical-chemical aspects of protein corona: relevance to in vitro and in vivo biological impacts of nanoparticles, J. Am. Chem. Soc. 133 (2011) 2525–2534.
[14] S.H.D.P. Lacerda, J.J. Park, C. Meuse, D. Pristinski, M.L. Becker, A. Karim, J.F. Douglas, Interaction of gold nanoparticles with common human blood proteins, ACS Nano 4 (2010) 365–379.
[15] M.S. Ehrenberg, A.E. Friedman, J.N. Finkelstein, G. Oberdörster, J.L. McGrath, The influence of protein adsorption on nanoparticle association with cultured endothelial cells, Biomaterials 30 (2009) 603–610.
[16] C. Müller, P.A. Schubiger, R. Schibli, In vitro and in vivo targeting of different folate receptor-positive cancer cell lines with a novel 99mTc-radiofolate tracer, Eur. J. Nucl. Med. Mol. Imaging 33 (2006) 1162–1170.
[17] R.F. Minchin, D.J. Martin, Nanoparticles for molecular imaging—an overview, Endocrinology 151 (2010) 474–481.
[18] W.G. Kreyling, S. Hirn, C. Schleh, Nanoparticles in the lung, Nat. Biotechnol. 28 (2010) 1275–1276.
[19] H.S. Choi, Y. Ashitate, J.H. Lee, S.H. Kim, A. Matsui, N. Insin, M.G. Bawendi, M. Semmler-Behnke, J.V. Frangioni, A. Tsuda, Rapid translocation of nanoparticles from the lung airspaces to the body, Nat. Biotechnol. 28 (2010) 1300–1303.
[20] T.S. Tsapikouni, S. Allen, Y.F. Missirlis, Measurement of interaction forces between fibrinogen coated probes and mica surface with the atomic force microscope: the pH and ionic strength effect, Biointerphases 3 (2008) 1–8.
[21] G.L. Hortin, The MALDI-TOF mass spectrometric view of the plasma proteome and peptidome, Clin. Chem. 52 (2006) 1223–1237.
[22] M. Fountoulakis, J.-F. Juranville, L. Jiang, D. Avila, D. Röder, P. Jakob, P. Berndt, S. Evers, H. Langen, Depletion of the high-abundance plasma proteins, Amino Acids 27 (2004) 249–259.
[23] T.M. Coimbra, M.R. Furtado, I.F. Carvalho, Crossed-immunoelectrophoresis analysis of the urinary excretion of alpha 1-acid glycoprotein, alpha 1-antitrypsin, albumin, and transferrin in normal subjects and in patients with renal disease, Braz. J. Med. Biol. Res. 17 (1984) 35–41.
[24] G. Li, R. Stewart, B. Conlan, A. Gilbert, P. Roeth, H. Nair, Purification of human immunoglobulin G: a new approach to plasma fractionation, Vox Sang. 83 (2002) 332–338.
[25] L.-Y. Ke, D.A. Engler, J. Lu, R.K. Matsunami, H.-C. Chan, G.-J. Wang, C.-Y. Yang, J.-G. Chang, C.-H. Chen, Chemical composition-oriented receptor selectivity of L5, a naturally occurring atherogenic low-density lipoprotein, Pure Appl. Chem. 83 (2011) 1731–1740.
[26] G. Schmitz, K. Ilsemann, B. Melnik, G. Assmann, Isoproteins of human apolipoprotein A-II: isolation and characterization, J. Lipid Res. 24 (1983) 1021–1029.
[27] A.M. Gotto, Apolipoproteins and metabolism in atherosclerosis, Trans. Am. Clin. Climatol. Assoc. 101 (1990) 46–57 [discussion 57–8].
[28] P. Davidsson, R. Ekman, K. Blennow, A new procedure for detecting brain-specific proteins in cerebrospinal fluid, J. Neural Transm. 104 (1997) 711–720 (Vienna, Austria: 1996).
[29] E. Gianazza, P. Arnaud, Isolectric patterns of human alpha1- antichymotrypsin (A1AChy) and A1AChy-protease complexes, Electrophoresis 2 (1981) 247–250.
[30] I. Nikolayenko, O. Galkin, N.I. Grabchenko, M.Y. Spivak, Preparation of highly purified human IgG, IgM, and IgA for immunization and immunoanalysis, Ukrainica 2 (2005) 3–11.
[31] S.A. Back, J.A. Alhadeff, Differential isoelectric focusing properties of crude and purified human α2-macroglobulin and α2-macroglobulin–proteinase complexes, J. Chromatogr. B Biomed. Sci. Appl. 278 (1983) 43–51.
[32] M. Daly, F. Hallinan, Analysis of antithrombin III microheterogeneity by isoelectric focusing in polyacrylamide gels and immunoblotting, Thromb. Res. 40 (1985) 207–214.
[33] R.H. McLean, Thin layer isoelectric focusing for final purification of human C3, J. Immunol. 116 (1976) 1741-b.
[34] H.P. Heinz, Biological functions of C1q expressed by conformational changes, Behring Inst. Mitt. (1989) 20–31.
[35] M. Weinbacher, B. Martina, P. Gasser, M. Köhler, T. Bart, Nail fold capillaroscopy and echocardiography in mild-to-moderate hypertension treated with cilazapril plus hydrochlorothiazide: first results, J. Cardiovasc. Pharmacol. 24 (Suppl. 3) (1994) S83–S85.
[36] M.W. Bradbury, The blood–brain barrier, Exp. Physiol. 78 (1993) 453–472.
[37] S. Vinchon-Petit, D. Jarnet, A. Paillard, J.-P. Benoit, E. Garcion, P. Menei, In vivo evaluation of intracellular drug-nanocarriers infused into intracranial tumours by convection-enhanced delivery: distribution and radiosensitisation efficacy, J. Neurooncol 97 (2010) 195–205.
[38] G.A. Grant, N.J. Abbott, D. Janigro, Understanding the physiology of the blood–brain barrier: in vitro models, News Physiol. Sci. 13 (1998) 287–293.
[39] R. Aghababian, E. Allison, E. Boyer, G. Braen, M. Manno, J. Moorhead, G.A. Volturo (Eds.), Essentials of Emergency Medicine, Jones and Bartlett Publishers, 2006.
[40] D.A. Drachman, Do we have brain to spare? Neurology 64 (2005) 2004–2005.
[41] K. Felgenhauer, Protein size and cerebrospinal fluid composition, Klin. Wochenschr. 52 (1974) 1158–1164.
[42] E.R. Kandel, J.H. Schwartz, T.M. Jessell (Eds.), Principles of Neural Science, 4th ed., McGraw-Hill, New York, 2000.
[43] A. Ndabahaliye, Number of neurons in a human brain, in: G. Elert (Ed.), The Physis Factbook, World Book Inc., 2002
[44] J. Choi, Q. Zheng, H.E. Katz, T.R. Guilarte, Silica-based nanoparticle uptake and cellular response by primary microglia, Environ. Health Perspect. 118 (2010) 589–595.
[45] F.A.C. Azevedo, L.R.B. Carvalho, L.T. Grinberg, J.M. Farfel, R.E.L. Ferretti, R.E.P. Leite, W. Jacob Filho, R. Lent, S. Herculano-Houzel, Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain, J. Comp. Neurol. 513 (2009) 532–541.
[46] B. Pakkenberg, H.J. Gundersen, Neocortical neuron number in humans: effect of sex and age, J. Comp. Neurol. 384 (1997) 312–320.
[47] B. Pakkenberg, D. Pelvig, L. Marner, M.J. Bundgaard, H.J.G. Gundersen, J.R. Nyengaard, L. Regeur, Aging and the human neocortex, Exp. Gerontol. 38 (2003) 95–99.
[48] M.V.L. Bennett, R.S. Zukin, Electrical coupling and neuronal synchronization in the Mammalian brain, Neuron 41 (2004) 495–511.
[49] S.G. Hormuzdi, M.A. Filippov, G. Mitropoulou, H. Monyer, R. Bruzzone, Electrical synapses: a dynamic signaling system that shapes the activity of neuronal networks, Biochim. Biophys. Acta 1662 (2004) 113–137.
[50] A. Armulik, G. Genové, M. Mäe, M.H. Nisancioglu, E. Wallgard, C. Niaudet, L. He, J. Norlin, P. Lindblom, K. Strittmatter, B.R. Johansson, C. Betsholtz, Pericytes regulate the blood–brain barrier, Nature 468 (2010) 557–561.
[51] K.K. Hirschi, P.A. D'Amore, Pericytes in the microvasculature, Cardiovasc. Res. 32 (1996) 687–698.
[52] M.W. Brightman, The brain's interstitial clefts and their glial walls, J. Neurocytol. 31 (2002) 595–603.
[53] M.W. Brightman, T.S. Reese, Junctions between intimately apposed cell membranes in the vertebrate brain, J. Cell Biol. 40 (1969) 648–677.
[54] W.M. Pardridge, Blood–brain barrier drug targeting: the future of brain drug development, Mol. Interv. 3 (2003) 90–105 [51].
[55] J. Seki, Y. Satomura, Y. Ooi, T. Yanagida, A. Seiyama, Velocity profiles in the rat cerebral microvessels measured by optical coherence tomography, Clin. Hemorheol. Microcirc. 34 (2006) 233–239.
[56] A.G. Hudetz, Blood flow in the cerebral capillary network: a review emphasizing observations with intravital microscopy, Microcirculation 4 (1997) 233–252 (New York, N.Y.: 1994).

[57] N.J. Abbott, Dynamics of CNS barriers: evolution, differentiation, and modulation, Cell. Mol. Neurobiol. 25 (2005) 5–23.
[58] K.H. Park, M. Chhowalla, Z. Iqbal, F. Sesti, Single-walled carbon nanotubes are a new class of ion channel blockers, J. Biol. Chem. 278 (2003) 50212–50216.
[59] C. Chin, I.K. Kim, D.Y. Lim, K.S. Kim, H.A. Lee, E.J. Kim, Gold nanoparticle-choline complexes can block nicotinic acetylcholine receptors, Int. J. Nanomedicine 5 (2010) 315–321.
[60] S.K. Sohaebuddin, P.T. Thevenot, D. Baker, J.W. Eaton, L. Tang, Nanomaterial cytotoxicity is composition, size, and cell type dependent, Part. Fibre Toxicol. 7 (2010) 22.
[61] A. Verma, F. Stellacci, Effect of surface properties on nanoparticle–cell interactions, Small 6 (2010) 12–21.
[62] S.G. Skuridin, V.A. Dubinskaya, V.M. Rudoy, O.V. Dement'eva, S.T. Zakhidov, T.L. Marshak, V.A. Kuz'min, V.I. Popenko, Y.M. Evdokimov, Effect of gold nanoparticles on DNA package in model systems., Dokl. Biochem. Biophys. 432 (n.d.) 141–3.
[63] A. Verma, O. Uzun, Y. Hu, H.S. Han, N. Watson, S. Chen, D.J. Irvine, F. Stellacci, Surface-structure-regulated cell-membrane penetration by monolayer-protected nanoparticles, Nat. Mater. 7 (2008) 588–595.
[64] B. Fadeel, D. Xue, The ins and outs of phospholipid asymmetry in the plasma membrane: roles in health and disease, Crit. Rev. Biochem. Mol. Biol. 44 (2009) 264–277.
[65] S. Manno, Y. Takakuwa, N. Mohandas, Identification of a functional role for lipid asymmetry in biological membranes: phosphatidylserine-skeletal protein interactions modulate membrane stability, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 1943–1948.
[66] M. Yu, C. Zhou, J. Liu, J.D. Hankins, J. Zheng, Luminescent gold nanoparticles with pH-dependent membrane adsorption, J. Am. Chem. Soc. 133 (2011) 11014–11017.
[67] J. Lin, H. Zhang, Z. Chen, Y. Zheng, Penetration of lipid membranes by gold nanoparticles: insights into cellular uptake, cytotoxicity, and their relationship, ACS Nano 4 (2010) 5421–5429.
[68] T. Goda, Y. Goto, K. Ishihara, Cell-penetrating macromolecules: direct penetration of amphipathic phospholipid polymers across plasma membrane of living cells, Biomaterials 31 (2010) 2380–2387.
[69] H. Li, M. Li, W.Y. Shih, P.I. Lelkes, W.-H. Shih, Cytotoxicity tests of water soluble ZnS and CdS quantum dots, J. Nanosci. Nanotechnol. 11 (2011) 3543–3551.
[70] R.L. Heimark, Cell-cell adhesion of molecules the blood–brain barrier, in: W.M. Pardridge (Ed.), The Blood–Brain Barrier: Cellular and Molecular Biology, Lippincott-Raven, New York, 1993, pp. 88–106.
[71] N.J. Abbott, L. Rönnbäck, E. Hansson, Astrocyte-endothelial interactions at the blood–brain barrier, Nat. Rev. Neurosci. 7 (2006) 41–53.
[72] S. Bhaskar, F. Tian, T. Stoeger, W. Kreyling, J.M. de la Fuente, V. Grazú, P. Borm, G. Estrada, V. Ntziachristos, D. Razansky, Multifunctional Nanocarriers for diagnostics, drug delivery and targeted treatment across blood–brain barrier: perspectives on tracking and neuroimaging, Part. Fibre Toxicol. 7 (2010) 3.
[73] W.M. Pardridge, The blood–brain barrier: bottleneck in brain drug development, NeuroRx 2 (2005) 3–14.
[74] R. Gabathuler, Approaches to transport therapeutic drugs across the blood–brain barrier to treat brain diseases, Neurobiol. Dis. 37 (2010) 48–57.
[75] A.V. Kabanov, E.V. Batrakova, D.W. Miller, Pluronic block copolymers as modulators of drug efflux transporter activity in the blood–brain barrier, Adv. Drug Deliv. Rev. 55 (2003) 151–164.
[76] E.V. Batrakova, A.V. Kabanov, Pluronic block copolymers: evolution of drug delivery concept from inert nanocarriers to biological response modifiers, J. Control. Release 130 (2008) 98–106.
[77] A.V. Kabanov, E.V. Batrakova, S. Sriadibhatla, Z. Yang, D.L. Kelly, V.Y. Alakov, Polymer genomics: shifting the gene and drug delivery paradigms, J. Control. Release 101 (2005) 259–271.
[78] W.M. Pardridge, Blood–brain barrier delivery, Drug Discov. Today 12 (2007) 54–61.
[79] K. Ulbrich, T. Hekmatara, E. Herbert, J. Kreuter, Transferrin- and transferrin-receptor-antibody-modified nanoparticles enable drug delivery across the blood–brain barrier (BBB), Eur. J. Pharm. Biopharm. 71 (2009) 251–256.
[80] K. Ulbrich, T. Knobloch, J. Kreuter, Targeting the insulin receptor: nanoparticles for drug delivery across the blood–brain barrier (BBB), J. Drug Target. 19 (2011) 125–132.
[81] Y.S. Kang, W.M. Pardridge, Brain delivery of biotin bound to a conjugate of neutral avidin and cationized human albumin, Pharm. Res. 11 (1994) 1257–1264.
[82] F. Sousa, S. Mandal, C. Garrovo, A. Astolfo, A. Bonifacio, D. Latawiec, R.H. Menk, F. Arfelli, S. Huewel, G. Legname, H.J. Galla, S. Krol, Functionalized gold nanoparticles: a detailed in vivo multimodal microscopic brain distribution study, Nanoscale 2 (2010) 2826–2834.
[83] L.W. Chung, C.-L. Hsieh, A. Law, S.-Y. Sung, T. a Gardner, M. Egawa, S. Matsubara, H.E. Zhau, New targets for therapy in prostate cancer: modulation of stromal–epithelial interactions, Urology 62 (2003) 44–54.
[84] C. Hamers-Casterman, T. Atarhouch, S. Muyldermans, G. Robinson, C. Hamers, E.B. Songa, N. Bendahman, R. Hamers, Naturally occurring antibodies devoid of light chains, Nature 363 (1993) 446–448.
[85] I. Vaneycken, J. Govaert, C. Vincke, V. Caveliers, T. Lahoutte, P. De Baetselier, G. Raes, A. Bossuyt, S. Muyldermans, N. Devoogdt, In vitro analysis and in vivo tumor targeting of a humanized, grafted nanobody in mice using pinhole SPECT/micro-CT, J. Nucl. Med. 51 (2010) 099–106.
[86] W.H.D. Jong, M.C. Burger, M.a. Verheijen, R.E. Geertsma, Detection of the Presence of Gold Nanoparticles in Organs by Transmission Electron Microscopy, Materials 3 (2010) 4681–4694.
[87] G. Sonavane, K. Tomoda, K. Makino, Biodistribution of colloidal gold nanoparticles after intravenous administration: effect of particle size, Colloids Surf. B Biointerfaces 66 (2008) 274–280.
[88] J. Kreuter, D. Shamenkov, V. Petrov, P. Ramge, K. Cychutek, C. Koch-Brandt, R. Alyautdin, Apolipoprotein-mediated transport of nanoparticle-bound drugs across the blood–brain barrier, J. Drug Target. 10 (2002) 317–325.
[89] J.-C. Olivier, Drug transport to brain with targeted nanoparticles, NeuroRx 2 (2005) 108–119.
[90] P. Ballabh, A. Braun, M. Nedergaard, The blood–brain barrier: an overview: structure, regulation, and clinical implications, Neurobiol. Dis. 16 (2004) 1–13.
[91] M.R. Pickard, S.I. Jenkins, C.J. Koller, D.N. Furness, D.M. Chari, Magnetic nanoparticle labeling of astrocytes derived for neural transplantation, Tissue Eng. Part C Methods 17 (2010) 89–99.
[92] C. Au, L. Mutkus, A. Dobson, J. Riffle, J. Lalli, M. Aschner, Effects of nanoparticles on the adhesion and cell viability on astrocytes, Biol. Trace Elem. Res. 120 (2007) 248–256.
[93] B.L. Coomber, P.A. Stewart, Morphometric analysis of CNS microvascular endothelium, Microvasc. Res. 30 (1985) 99–115.
[94] H.K. Rucker, H.J. Wynder, W.E. Thomas, Cellular mechanisms of CNS pericytes, Brain Res. Bull. 51 (2000) 363–369.
[95] M. Hellström, H. Gerhardt, M. Kalén, X. Li, U. Eriksson, H. Wolburg, C. Betsholtz, Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis, J. Cell Biol. 153 (2001) 543–553.
[96] H. Wolburg, A. Lippoldt, Tight junctions of the blood–brain barrier: development, composition and regulation, Vascul. Pharmacol. 38 (2002) 323–337.
[97] R.J. Boado, W.M. Pardridge, Differential expression of alpha-actin mRNA and immunoreactive protein in brain microvascular pericytes and smooth muscle cells, J. Neurosci. Res. 39 (1994) 430–435.
[98] V. Nehls, D. Drenckhahn, Heterogeneity of microvascular pericytes for smooth muscle type alpha-actin, J. Cell Biol. 113 (1991) 147–154.
[99] E. Hutter, S. Boridy, S. Labrecque, M. Lalancette-Hébert, J. Kriz, F.M. Winnik, D. Maysinger, Microglial response to gold nanoparticles, ACS Nano 4 (2010) 2595–2606.
[100] P. Hao, Y. Wu, F. Li, Improved sensitivity of wavelength-modulated surface plasmon resonance biosensor using gold nanorods, Appl. Opt. 50 (2011) 5555–5558.
[101] D. Maysinger, M. Behrendt, M. Lalancette-Hébert, J. Kriz, Real-time imaging of astrocyte response to quantum dots: in vivo screening model system for biocompatibility of nanoparticles, Nano Lett. 7 (2007) 2513–2520.
[102] E. Ribot, A.-K. Bouzier-Sore, V. Bouchaud, S. Miraux, M.-H. Delville, J.-M. Franconi, P. Voisin, Microglia used as vehicles for both inducible thymidine kinase gene therapy and MRI contrast agents for glioma therapy, Cancer Gene Ther. 14 (2007) 724–737.
[103] M.F. Kircher, U. Mahmood, R.S. King, R. Weissleder, L. Josephson, A multimodal nanoparticle for preoperative magnetic resonance imaging and intraoperative optical brain tumor delineation, Cancer Res. 63 (2003) 8122–8125.
[104] E.J. Ribot, S. Miraux, J.P. Konsman, V. Bouchaud, L. Pourtau, M.-H. Delville, J.M. Franconi, E. Thiaudière, P.J. Voisin, In vivo MR tracking of therapeutic microglia to a human glioma model, NMR Biomed. 24 (2011) 1361–1368.
[105] M. Hohnholt, M. Geppert, R. Dringen, Effects of iron chelators, iron salts, and iron oxide nanoparticles on the proliferation and the iron content of oligodendroglial OLN-93 cells, Neurochem. Res. 35 (2010) 1259–1268.
[106] M.C. Hohnholt, M. Geppert, R. Dringen, Treatment with iron oxide nanoparticles induces ferritin synthesis but not oxidative stress in oligodendroglial cells, Acta Biomater. 7 (2011) 3946–3954.
[107] S. Naqvi, M. Samim, M. Abdin, F.J. Ahmed, A. Maitra, C. Prashant, A.K. Dinda, Concentration-dependent toxicity of iron oxide nanoparticles mediated by increased oxidative stress, Int. J. Nanomedicine 5 (2010) 983–989.
[108] J.M. Boggs, H. Wang, Co-clustering of galactosylceramide and membrane proteins in oligodendrocyte membranes on interaction with polyvalent carbohydrate and prevention by an intact cytoskeleton, J. Neurosci. Res. 76 (2004) 342–355.
[109] G. Saher, S. Quintes, K.-A. Nave, Cholesterol: a novel regulatory role in myelin formation, Neuroscientist 17 (2011) 79–93.
[110] J.M. Boggs, H. Wang, W. Gao, D.N. Arvanitis, Y. Gong, W. Min, A glycosynapse in myelin? Glycoconj. J. 21 (2004) 97–110.
[111] T.J. McIntosh, S. a Simon, Roles of bilayer material properties in function and distribution of membrane proteins, Annu. Rev. Biophys. Biomol. Struct. 35 (2006) 177–198.
[112] G.G. Somjen, Ion regulation in the brain: implications for pathophysiology, Neuroscientist 8 (2002) 254–267.
[113] J. Zhao, L. Xu, T. Zhang, G. Ren, Z. Yang, Influences of nanoparticle zinc oxide on acutely isolated rat hippocampal CA3 pyramidal neurons, Neurotoxicology 30 (2009) 220–230.
[114] L.-J. Xu, J.-X. Zhao, T. Zhang, G.-G. Ren, Z. Yang, In vitro study on influence of nano particles of CuO on CA1 pyramidal neurons of rat hippocampus potassium currents, Environ. Toxicol. 24 (2009) 211–217.
[115] D. Han, Y. Tian, T. Zhang, G. Ren, Z. Yang, Nano-zinc oxide damages spatial cognition capability via over-enhanced long-term potentiation in hippocampus of Wistar rats, Int. J. Nanomedicine 6 (2011) 1453–1461.
[116] Z. Yang, Z.W. Liu, R.P. Allaker, P. Reip, J. Oxford, Z. Ahmad, G. Ren, A review of nanoparticle functionality and toxicity on the central nervous system, J. R. Soc. Interface 7 (Suppl. 4) (2010) S411–S422.
[117] D.R. Cooper, J.L. Nadeau, Nanotechnology for in vitro neuroscience, Nanoscale 1 (2009) 183–200.
[118] A.C. Leskovjan, A. Kretlow, A. Lanzirotti, R. Barrea, S. Vogt, L.M. Miller, Increased brain iron coincides with early plaque formation in a mouse model of Alzheimer's disease, Neuroimage 55 (2011) 32–38.
[119] H. Kobayashi, H. Inoue, J. Shimada, T. Yano, T. Maeda, T. Oyama, S. Shinohara, Intra-arterial injection of adriamycin/mitomycin C lipiodol suspension in liver metastases., Acta Radiol. 28 (n.d.) 275–80.
[120] Y. Maeda, K. Matsumoto, S. Mizumatsu, T. Tamiya, T. Furuta, T. Ohmoto, Effect of intracarotid infusion of etoposide: modification of the permeability of the blood–brain barrier and the blood-tumor barrier in rat brain tumor model, Acta Med. Okayama 53 (1999) 5–11.

[121] H.E. de Vries, J. Kuiper, a G. de Boer, T.J. Van Berkel, D.D. Breimer, The blood–brain barrier in neuroinflammatory diseases, Pharmacol. Rev. 49 (1997) 143–155.
[122] P. Grammas, J. Martinez, B. Miller, Cerebral microvascular endothelium and the pathogenesis of neurodegenerative diseases, Expert Rev. Mol. Med. 13 (2011) e19.
[123] J. Sund, H. Alenius, M. Vippola, K. Savolainen, A. Puustinen, Proteomic characterization of engineered nanomaterial-protein interactions in relation to surface reactivity, ACS Nano 5 (2011) 4300–4309.
[124] J. Lipka, M. Semmler-Behnke, R. a Sperling, A. Wenk, S. Takenaka, C. Schleh, T. Kissel, W.J. Parak, W.G. Kreyling, Biodistribution of PEG-modified gold nanoparticles following intratracheal instillation and intravenous injection, Biomaterials 31 (2010) 6574–6581.
[125] S. Hirn, M. Semmler-Behnke, C. Schleh, A. Wenk, J. Lipka, M. Schäffler, S. Takenaka, W. Möller, G. Schmid, U. Simon, W.G. Kreyling, Particle size-dependent and surface charge-dependent biodistribution of gold nanoparticles after intravenous administration, Eur. J. Pharm. Biopharm. 77 (2011) 407–416.
[126] M. Melli, G. Scoles, M. Lazzarino, Fast detection of biomolecules in diffusion-limited regime using micromechanical pillars, ACS Nano 5 (2011) 7928–7935.