Nanoparticles and stem cells for drug delivery to the brain
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Chapter 1

Introduction and scope of the thesis
Chapter 1

The blood-brain barrier

The economic burden of brain diseases is enormous and expected to increase due to the aging of the population. In a recent study by the European Brain Council it was calculated that in 2010 the costs for screening and treatment of brain disorders in the European Union reached 798 billion euro ($ 1 trillion), which equals the costs made for heart diseases, cancer and diabetes together [1]. Effective treatments have been developed for mental disorders such as depression, epilepsy, schizophrenia and pain [2]. However, due to the increased life expectancy, particularly degenerative disorders such as Alzheimer’s disease and other dementias, Parkinson's disease and stroke are becoming more prevalent. Currently, the therapy for these disorders is limited to symptomatic medication. Specific therapy of the disorders presents a substantial challenge, since most of the potential drugs cannot reach their target site, i.e. the brain, because they cannot cross the blood-brain barrier.

In order to maintain a stable environment for synaptic transmission and information processing, the brain is separated from the periphery by the blood-brain barrier (BBB). The BBB is formed at the level of the brain capillaries and consists of a monolayer of endothelial cells supported by a basal lamina, pericytes and astrocytic end feet (Fig 1). Brain endothelial cells are highly polarized, which means that they express different sets of lipids, transport proteins, receptors and enzymes at their luminal (i.e., the apical surface, facing the blood) and abluminal (i.e., the basolateral surface, facing the brain) sides, respectively, which enables protection of the brain from foreign substances and at the same time allows essential metabolites such as glucose and amino acids to enter from the blood into the brain. Neighboring brain endothelial cells are connected by tight junctions that preclude paracellular transport. The pericytes give structural support to the capillary wall and together with the astrocytic end feet participate in maintenance of the BBB properties. It is thought that each neuron is fed by one capillary. In other words, once transported over the BBB intravenously administered drugs could reach literally every neuron in the brain.
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**Figure 1.** Diagram of the blood brain barrier in cross section. The BBB is composed of brain endothelial cells, surrounded by basement membrane, pericytes and astrocytic end feet.

**Drug delivery into the brain**

A molecule can pass the BBB by passive diffusion if it is lipophilic and smaller than 400 Da [3]. Most effective drugs, applied in brain therapy, fit this description. For example some of the top-selling central nervous system drugs are Zyprexa, an antipsychotic with a molecular weight of 312.4 Da; Paxil, an antidepressant with a molecular weight of 329.3 Da; and Stilnox, with a molecular weight of 307.4 Da, used for short-term treatment of insomnia [4]. Alternatively, some drugs enter the brain by exploiting endogenous transport proteins for glucose, amino acids, purine bases and other molecules that are present at the luminal side of the brain endothelium (Fig. 2). For example, L-DOPA for the treatment of Parkinson’s disease and Melphalan against brain cancer use the large neutral amino acid carrier system, while Abacivir, for the treatment of HIV and AIDS, uses the nucleoside carrier. Receptor-mediated and nonspecific adsorptive-mediated transcytosis have been described for transport of some peptides and proteins (Fig. 2). Receptor-mediated transcytosis (RMT) allows for transport of large molecules such as insulin and transferrin. This transport mechanism has been used for drug transport by attaching a drug to the receptor’s natural ligand or a receptor-specific antibody. Upon binding to the receptor the complex triggers its transcytosis. For example OX26 a murine monoclonal antibody directed against the rat transferrin receptor, which is highly expressed on the brain endothelial cells, has been coupled to VIPa, a vasoactive intestinal polypeptide analogue. Vasoactive intestinal polypeptide cannot cross the BBB, however systemic infusion of VIPa-OX26 complex increased cerebral blood flow in rats by 65% [5]. Adsorptive-mediated transcytosis (AMT) leads to transcytosis of molecules such as cationized albumin and wheat germ agglutinin. These molecules can cross the BBB and may thereby provide a means to carry a therapeutic peptide or protein into the brain. The process of AMT is mediated via nonspecific electrostatic interactions between the molecule and the luminal surface of the brain endothelial cell leading to vesicle formation and subsequent transcytosis.
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Since the interaction is nonspecific, internalization is not restricted to brain endothelial cells, and this approach is therefore not widely applied.

![Diagram showing drug delivery across the blood-brain barrier (BBB)](image)

**Figure 2.** The routes of drug delivery across the blood brain. The tight junctions block paracellular transport of hydrophilic compounds. Small lipophilic molecules can cross the BBB by passive diffusion. If mimic the structure of the substrate (glucose, amino acids, purine bases, nucleosides, choline) drug molecules can use transport proteins to enter the brain. Large peptides and proteins can use receptor mediated transcytosis by binding to a receptor (e.g. insulin and transferrin receptor) on the luminal side. Cationized peptides and proteins can enter the brain by adsorptive mediated transcytosis.

Frequently, promising drugs that showed in vitro activity, are not stable in blood, or are too big or hydrophilic to cross the BBB. These shortcomings thus clearly frustrate expression of their activity in vivo. Invasive methods to deliver an unstable and/or insoluble drug into the brain include administration by intracerebroventricular or intracerebral injection. However, the treatment volume in these cases is rather small. Therefore these procedures are suitable only for treatment of brain disorders that are confined to a specific localization, such as primary brain tumors, stroke or Parkinson’s disease.

In order to protect a drug from its environment and improve brain delivery efficiency, two promising strategies have been developed: incorporation of the drug into a small vehicle (nanocarrier) and the use of (neural) stem cells, engineered to produce the pharmaceutical agent. Both types of vehicles can be injected into the brain, as referred to above. Alternatively, they can be transported via a transvascular route, following intravenous administration. Although only a small portion of the injected engineered stem cells reaches the brain following i.v. injection, the method is of interest since it is much less invasive compared to cerebral injection. Insight into the *in vivo* migration behavior of stem cells, as obtained from (real time) *in vivo* tracking of stem cells may
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lead to improved protocols for stem cell (-mediated) delivery upon i.v. administration. Contrary to the intrinsic migratory capacity of (neural) stem cells into the brain, nanocarriers have to be decorated with targeting ligands, e.g. peptides or proteins, to accomplish effective bioavailability into the brain. Although a few brain drug delivery devices reached the stage of clinical application thus far, their success has been very limited. Therefore, further development of novel nanocarrier systems and brain targeting ligands is required in order to accomplish efficient delivery of drugs into the brain, and hence, specific and effective therapy of brain-related disorders.

Scope of the thesis

The work that is described in this thesis focuses on the development of novel brain drug delivery devices, and their potential is investigated at various experimental settings, both in vitro and in vivo, making use of various detection methods. Efficient methods to track the distribution and behavior of brain drug delivery devices will be of great help in the optimization of brain drug delivery to the point of clinical applications.

As the blood-brain barrier not only prevents the penetration of drugs into the brain, but also limits the access of many of the developed tracers, the options for both labeling and detection of brain drug delivery devices are limited compared to devices that target peripheral tissues. In Chapter 2 recent developments in the labeling of brain drug delivery devices, as well as invasive and noninvasive detection methods that are used to monitor their accumulation in the brain, are reviewed.

Interestingly, neural stem cells have an intrinsic capacity (tropism) to migrate towards areas within the brain that are affected by disease, even when they are intravenously administered, which implies that neural stem cells are capable of traversing the blood-brain barrier. However the potential of neural stem cells to cross the BBB and ‘find’ pathological areas within the brain has not been fully exploited yet. Many parameters, such as the number of cells, and the route and time of administration that are required for an effective disease treatment, are unknown. These parameters could be determined when it is possible to quantify cells at the target site, i.e. the brain, following their administration. Thus in Chapter 3, the labeling of C17.2 neural stem cells with radioactive tracers is explored together with their short term in vivo tracking in a rat tumor inflammation model, using positron emission tomography and planar scintigraphy.

In Chapter 4, the labeling of C17.2 neural stem cells with iron oxide particles for long term in vivo tracking by magnetic resonance imaging (MRI), is studied. In Chapter 5, using fluorescence microscopy, the migratory potential of C17.2 neural stem cells
across an *in vitro* model of the BBB is visualized and compared to that of plasma membrane vesicles that were reconstituted from C17.2 neural stem cells. Our hypothesis that, analogously as the intact C17.2 neural stem cells, these neural stem cell-derived vesicles might show affinity for the BBB and thus could display potential for drug delivery into the brain, is investigated both *in vitro* and *in vivo*.

In Chapter 6, the strong influence of the particle size of polymersomes on their blood circulation time is described.

In Chapter 7 the potential of polymersomes as nanocarriers is examined, employing surface-modified particles with peptides that target prion protein and GM1 ganglioside. Their ability to cross the BBB is investigated in an *in vitro* model. Moreover, the *in vivo* biodistribution of the peptide-targeted polymersomes and, specifically, their propensity to accumulate into brain, is measured.

Finally, in Chapter 8 the results are summarized and discussed and suggestions for future research are given.

References


