Novel drug-delivery approaches to the blood-brain barrier

Xiaoqing Wang^{1,2,#}, Xiaowen Yu^{3,#}, William Vaughan⁴, Mingyuan Liu¹, Yangtai Guan¹

¹*Department of Neurology, Changhai Hospital,* ²*Stem Cells and Medical Research Center,* ³*Department of Gerontology, Changhai Hospital, Second Military Medical University, Shanghai 200433, China*

⁴*Department of Genetics, Yale University, New Haven, CT 06519, USA*

[#]These authors contributed equally to this review.

Corresponding authors: Yangtai Guan and Mingyuan Liu. E-mail: yangtaiguan@hotmail.com, liu.mingyuan@foxmail.com

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The blood-brain barrier (BBB) maintains homeostasis by blocking toxic molecules from the circulation, but drugs are blocked at the same time. When the dose is increased to enhance the drug concentration in the central nervous system, there are side-effects on peripheral organs. In recent years, genetic therapeutic agents and small molecules have been used in various strategies to penetrate the BBB while minimizing the damage to systemic organs. In this review, we describe several representative methods to circumvent or cross the BBB, including chemical and physical strategies.

Keywords: drug delivery; blood-brain barrier; nanoparticles; focused ultrasound

Introduction

Treatment of central nervous system (CNS) diseases is challenged by the difficulty in drug delivery through the blood-brain barrier (BBB). Although drug mechanism research has become quite sophisticated in recent years, the vast majority of drugs are blocked by the BBB and thus fail to reach the brain, making it difficult to treat intracranial disease^[1].

The BBB has the function of selective permeability which prevents bacteria and other pathogenic microorganisms from entering the brain while at the same time allowing oxygen and other vital compounds to traverse from the blood to the brain. However, it also fends off drugs, and so is an obstacle to treating brain diseases.

Methods for drug delivery to the brain can be divided into two types: invasive and non-invasive. The invasive methods can achieve a high local drug concentrations by direct injection or intracerebroventricular delivery but also has side-effects such as infection or trauma. Besides, the drug concentration decreases exponentially as a function of distance from the injection site. It is also hard to deliver

drugs repeatedly and patients hesitate to accept invasive treatments. For these reasons, we focus on the non-invasive drug-delivery strategies.

Structure of BBB

The BBB is a layer of endothelial cells on the basement membrane lining almost 99% of the brain capillary surface, continuously coupled with perivascular cells^[2], such as pericytes, smooth muscle cells, astrocytes, and microglia (Fig. 1)^[3]. Normally the BBB excludes ionic water-soluble drugs with a diameter >180 nm^[4].

Transplantation studies have shown that the properties of the endothelial cells that constitute the BBB are not innate^[5], but are induced in the special microenvironment of the CNS^[6]. The BBB is formed during embryogenesis when endothelial cells enter the CNS. One week before astrocyte formation, pericytes are recruited to the neonatal vessels and regulate the functions of the BBB, including the generation of tight junctions (TJs) and vesicle trafficking in brain microvascular endothelial cells (BMECs)^[7–9], a major component of the BBB^[10]. Pericytes are a prerequisite

Fig. 1. Diagram of BBB. The BBB is a layer of endothelial cells on the basement membrane lining almost 99% of the brain capillary surface, continuously coupled with perivascular cells, such as pericytes, smooth muscle cells, astrocytes, and microglia.

for the formation of the BBB and determine part of its permeability by inhibiting the expression of molecules that increase BBB permeability and immune cell infiltration. However, they do not induce BBB-specific gene expression in CNS endothelial cells^[11, 12]. Astrocytes induce BBB formation after birth because of the close spatial relation between astrocytes and BMECs. The timing of BBB formation has been controversial. Laboratory mice with null and hypomorphic *Pdgfrb* alleles that have defects in pericyte generation illustrated that the interactions between pericytes and BMECs are critical in regulating BBB permeability. This effect is caused by the inhibition of specific proteins that can increase the permeability of the BBB^[13–15].

The permeability of the BBB in drug delivery remains a problem, although many drugs have been developed in an attempt to combat it. Several available strategies for the safe and effective delivery of drugs are described below.

A Drug-Delivery Approach to Bypassing the BBB

Intranasal delivery of drugs is a potential strategy to bypass the BBB^[16]. The effectiveness of intranasal delivery is determined by administration factors and physicochemical properties, such as the patient's head position, dosing device, drug volume, pH value, osmotic pressure, and drug solubility. Intranasal delivery has been highly regarded

because it is noninvasive, safe, and simple. Since its early use by William Ewart^[17] for the treatment of diphtheria, intranasal delivery has been confirmed as a promising route of administration. On the other hand, its use is relatively limited. However, the method has been modified by various additions such as penetration enhancers, adhesion agents, and nanoparticles, which can significantly increase the efficiency of drug delivery. Wu *et al.*^[18] have successfully delivered stem cells using the intranasal approach as a therapy for experimental allergic encephalomyelitis in rats, an animal model of multiple sclerosis. Nasal glucagon-like peptide-1^[19] has already been used in patients. This is a promising development for patients with diabetes, and has the potential that insulin may be administered in a similar way. Future research is needed to further reveal the mechanisms of nasal drug delivery and at the same time improve the technology and solution preparation. This will achieve a better targeting, improved effectiveness, and higher drug concentrations.

New Drug-Delivery Approaches to Crossing the BBB

Relevant Carriers in Cerebral Microvascular Endothelia

Receptors on the surface membranes of cells can help drug delivery. Common carriers include medium-chain fatty-acid carriers, neutral amino-acid carriers, a monocarboxylic carrier, cation transporters, and the adenosine purine carrier.

Exosomes^[20] Scientists at the University of Oxford have used protein carriers called exosomes to transport drug molecules to the brain cells of laboratory mice. Exosomes are membrane vesicles released by a variety of cells such as dendritic cells^[21,22]. They transport material back and forth through the BBB. Exosomes are first extracted from mice. Then, a CNS-specific rabies viral glycoprotein is attached to the acetylcholine receptor, and fused to the exosomes. Finally, an siRNA is placed in the exosomes and the complex is intravenously injected into mice. Experiments have confirmed that the siRNA is delivered to the brain and binds to its receptors on brain cells. This results in a 60% decline of β -secretase 1 (BACE1) expression, a gene associated with Alzheimer's disease^[23].

Adenosine receptor Researchers at Cornell University found that adenine nucleotide can transport large molecules into the brain. When the adenine nucleoside receptors on cells are activated, a channel can be established through the BBB^[24]. In the experiments, the team succeeded in passing large molecules (a β -amyloid protein antibody) through the BBB of transgenic mice and reported the adhesion of the antibody to β -amyloid plaques (mice with genetically-modified plaques that have a lower risk of Alzheimer's disease). Furthermore, the selective A2A adenosine receptor agonist Lexiscan can temporarily open BBB channels.

Transferrin receptor^[25] The transferrin receptor (TfR) is a key cell-surface molecule that regulates the uptake of iron-bound transferrin^[26]. Plasma soluble TfR concentrations reflect the receptor density on cells and the number of cells expressing the receptor. Therefore, it is closely related to cellular iron demand and the erythroid proliferation rate. TfR is frequently overexpressed in cancer cells^[27]. Recently, transferrin-targeted conjugates have shown promise in reversing drug resistance in cancer cells, and transferrin immunotoxins with a diphtheria toxin mutant covalently bound to transferrin have shown promise for the treatment of glioblastoma in clinical trials^[28]. Thus, intracellular targeting by iron-saturated transferrin as a ligand for TfR-mediated endocytosis has become a focus of research. The natural receptor TfR has been used by Roche; therapeutic antibodies are attached to TfRs in a modified pattern, which they call a "Brain Shuttle Module". Monovalent binding to the TfR instead of bivalent binding, which causes lysosome sorting, can lead to a reduction of amyloid. This is a process of "receptor-mediated transcytosis".

Nanoparticles^[29]

Nanoparticles make up solid colloids composed of polymers or lipid particles of 10–1000 nm (usually 50–300 nm). A drug can be embedded within a particle's substrate or attached to its surface^[30]. Drugs are transported in a controlled time period to a targeted location *in vivo*. During this process, certain principles should be followed: nanoparticles used as drug carriers should be non-toxic, biodegradable, and biocompatible; have a diameter <100 nm and no aggregation reaction in blood, as well as an efficient production process^[31].

Poly-nanoparticles such as PBCA-NPs^[32–35] (butylcyanoacrylate), PEG^[36–40] (polyethylene glycol), liposomes^[41–43], P-gp (P-glycoprotein), and even superparamagnetic iron oxide nanoparticles^[37] have been used for drug delivery.

To investigate the mechanisms behind nanoparticles, it must be recognized that materials on the nanoscale take on new biological and physical characteristics. For example, there may be a ubiquitous toxic effect on BMECs. A surfactant effect due to the solubilization of lipids in the endothelial cell membrane may lead to membrane fluidization and therefore enhanced drug permeability of the BBB.

Opening TJs between BMECs can allow drugs to pass through the BBB alone or with nanoparticles. Another option is receptor-mediated endocytosis followed by transcytosis into the CNS or drug release in endothelial cells^[31].

Adjustment of Tight Junctions^[44] between Endothelial Cells of the BBB

There are three means of barrier disruption (Table 1): osmotic, pharmacological, and mechanical (focused ultrasound (FUS) with microbubbles).

Many drugs are slow to exert an effect, as has been shown in *in vitro* studies^[51]. This is due to the low drug concentration caused by the BBB. The most direct way to increase drug permeability of the BBB is to open the TJs between endothelial cells. FUS can temporarily open the BBB and its efficiency is optimized when combined with microbubbles. FUS-induced BBB disruption occurs with sonication most of the time^[52]. Drug delivery by this method has been verified by extensive research^[53–57].

The mechanisms by which ultrasound opens the BBB rely on various physical characteristics and are closely associated with biological processes. Electron microscopy has confirmed that ultrasound causes enlarged biomembrane lacunae with no evident tissue damage both *in vivo* and *in vitro*. It has also been confirmed that after ultrasound irradiation, the capillary permeability increases, including endocytosis, opening of TJs, and free transportation through the endothelial lacunae^[58].

FUS is often used in oncotherapy, but it has not reached the same level of maturity in the field of BBB-opening. FUS is noninvasive and precise, causes only local damage, is time-efficient, and is secure and repeatable in operation. A high dose of ultrasound has mild direct

Table 1. Details of methods of barrier disruption

	Osmotic	Pharmacological	Mechanical
Device	/	/	Focused ultrasound
Appearance	1970s ^[45]	1980s ^[46]	1940s for noninvasive ablation in brain ^[47]
Reagent	Hypertonic solution of 25% mannitol	Bradykinin ^[48] RMP-7 ^[49]	Nano-microbubbles
Principal	Shrink endothelial cells and disrupt tight junctions between them	Bind to receptors, temporarily increase Ca^{2+} inflow, activate nitrogen oxidase, cytoskeletal contraction	Physical effects of ultrasound
Advantages	Effective in experimental and clinical applications	Used with antineoplastic drugs to amplify drug efficiency	Noninvasive; volume of drug, extent and degree of barrier disruption can be pre-established by parameter setting ^[50] ; drug-loaded microbubbles for better targeting.
Disadvantages	Risk of high-speed delivery into arterial circulation of brain	Mainly for brain-tumor barrier	Requires more trials on parameter setting.

cytotoxic effects. Injuries mostly occur in blood vessels and epithelial cells resulting in a targeted zone of oxygen deficiency^[59]. Research is focused on adjusting the parameters of FUS to make it effective in drug delivery^[60].

Tunneling Nanotubes

The tunneling nanotube (TNT)^[61–65] is a new general communication method between mammal cells. TNTs are somewhat similar to the protoplasmic connections in plants, but they differ in structure and function. TNTs have already been used to transport particles outside or inside the BBB^[66]. In particular, mitochondria are the most common particles transported from one cell to another through TNT (Fig. 2)^[67–71].

Interestingly, researchers at UCLA's Jonsson Comprehensive Cancer Center found that RNA can be transported into mitochondria, but little is known about the mechanism. They found that polynucleotide phosphorylase (PNPASE) protein^[72, 73] plays an important role in transporting RNA into mitochondria. When the expression of PNPASE is reduced, the amount of RNA entering mitochondria declines; PNPASE affects the RNA-encoding process of the mitochondrial genome and the synthesis of proteins necessary to sustain electron transfer. When PNPASE expression is reduced, mitochondrial

Fig. 2. Diagram of formation of tunneling nanotube between two mammal cells. Red dots: mitochondria.

RNA accumulates, unprocessed protein translation is suppressed, and energy generation is hampered, leading to the arrest or inhibition of cell growth. According to the research, PNPASE mediates the transport of cytoplasmic RNA for energy production by mitochondria. However, no experiment using current detection methods has been able to confirm this theory. If we could combine TNTs with PNPASE-dependent RNA, import them into mitochondria, and transport mitochondria into BMECs^[74] transcending the TJs between them, a direct route to brain would be available.

Conclusion

The BBB impedes the entry of many drugs into the CNS. Although these drugs are somewhat effective, they have not been used in clinical treatments due to the low solubility, chemical instability, low bioavailability, and harmful side-effects. These limitations restrict their clinical applications, leaving many CNS diseases poorly treated. Over the past 20 years, many experiments have been conducted to solve these problems. One main goal is to discover a way to deliver drugs across the BBB safely, effectively, and noninvasively.

Defects still exist in every drug-delivery strategy. For example, in nasal delivery, drug molecules can only stay in the nasal cavity for 15–20 min due to ciliary clearance, and are often not fully absorbed before clearance. In addition, nasal delivery may increase the circulating concentration through absorption by the respiratory or olfactory mucosa, causing decreased efficiency in brain targeting. Although the experiments described above are still in the initial stages and the data need to be verified, they inspire various possibilities for breakthroughs in the field of BBB permeability.

ACKNOWLEDGMENTS

This review was supported by the National Natural Science Foundations of China (81200924 and 81230027), the Natural Science Foundation of Shanghai Municipality, China (12ZR1427400), the Key Scientific Research Project of Shanghai Municipality, China (11411950300), the Youth Fund of the Shanghai Health Bureau, China (2011), and the Innovation Research Program, Second Military Medical University, China (SCMRC1403 and SCMRC1410).

Received date: 2014-06-23; Accepted date: 2014-07-21

REFERENCES

- [1] Pardridge WM. Why is the global CNS pharmaceutical market so under-penetrated? *Drug Discov Today* 2002, 7: 5–7.
- [2] Hosoya K, Tachikawa M. The inner blood-retinal barrier: molecular structure and transport biology. *Adv Exp Med Biol* 2012, 763: 85–104.
- [3] Choi YK, Kim KW. Blood-neural barrier: its diversity and coordinated cell-to-cell communication. *BMB Rep* 2008, 41: 345–352.
- [4] Zlokovic BV. The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron* 2008, 57: 178–201.
- [5] Daneman R, Zhou L, Kebede AA, Barres BA. Pericytes are required for blood-brain barrier integrity during embryogenesis. *Nature* 2010, 468: 562–566.
- [6] Dyrna F, Hanske S, Krueger M, Bechmann I. The blood-brain barrier. *J Neuroimmune Pharmacol* 2013, 8: 763–773.
- [7] Hawkins BT, Davis TP. The blood-brain barrier/neurovascular unit in health and disease. *Pharmacol Rev* 2005, 57: 173–185.
- [8] Persidsky Y, Ramirez SH, Haorah J, Kanmogne GD. Blood-brain barrier: structural components and function under physiologic and pathologic conditions. *J Neuroimmune Pharmacol* 2006, 1: 223–236.
- [9] Bauer HC, Bauer H, Lametschwandner A, Amberger A, Ruiz P, Steiner M. Neovascularization and the appearance of morphological characteristics of the blood-brain barrier in the embryonic mouse central nervous system. *Brain Res Dev Brain Res* 1993, 75: 269–278.
- [10] Lyck R RN, Moll AG, Steiner O, Cohen CD, Engelhardt B, et al. Culture-induced changes in blood-brain barrier transcriptome: implications for amino-acid transporters *in vivo*. *J Cereb Blood Flow Metab* 2009, 29: 1491–1502.
- [11] Butt AM, Jones HC, Abbott NJ. Electrical resistance across the blood-brain barrier in anaesthetized rats: a developmental study. *J Physiol* 1990, 429: 47–62.
- [12] Ek CJ, Dziegielewska KM, Stolp H, Saunders NR. Functional effectiveness of the blood-brain barrier to small water-soluble molecules in developing and adult opossum (*Monodelphis domestica*). *J Comp Neurol* 2006, 496: 13–26.
- [13] Hirase T, Staddon JM, Saitou M, Ando-Akatsuka Y, Itoh M, Furuse M, et al. Occludin as a possible determinant of tight junction permeability in endothelial cells. *J Cell Sci* 1997, 110 (Pt 14): 1603–1613.
- [14] Kiesel U, Risau W, Wolburg H. Development of blood-brain barrier tight junctions in the rat cortex. *Brain Res Dev Brain Res* 1996, 96: 229–240.

- [15] Bolz S, Farrell CL, Dietz K, Wolburg H. Subcellular distribution of glucose transporter (GLUT-1) during development of the blood-brain barrier in rats. *Cell Tissue Res* 1996, 284: 355–365.
- [16] Gonda I. Systemic delivery of drugs to humans via inhalation. *J Aerosol Med* 2006, 19: 47–53.
- [17] William Ewart. The use of creosoted oil for the expulsion of tracheal false membranes after tracheotomy; and of intranasal injections of oil in various affection. *Br Med J* 1898, 1: 1381–1383.
- [18] Wu S. Intranasal Delivery of Neural Stem Cells: A CNS-specific, Non-invasive cell-based therapy for experimental autoimmune encephalomyelitis. *J Clin Cell Immunol* 2013, 4.
- [19] Ueno H, Mizuta M, Shiya T, Tsuchimochi W, Noma K, Nakashima N, *et al.* Exploratory trial of intranasal administration of glucagon-like Peptide-1 in Japanese patients with type 2 diabetes. *Diabetes Care* 2014, 37: 2024–2027.
- [20] Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat Biotechnol* 2011, 29: 341–345.
- [21] Sluijter JP, Verhage V, Deddens JC, van den Akker F, Doevendans PA. Microvesicles and exosomes for intracardiac communication. *Cardiovasc Res* 2014, 102: 302–311.
- [22] Johnstone R, Adam M, Hammond J, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). *J Biol Chem* 1987, 262: 9412–9420.
- [23] Moore DB, Gillentine MA, Botezatu NM, Wilson KA, Benson AE, Langeland JA. Asynchronous evolutionary origins of Abeta and BACE1. *Mol Biol Evol* 2014, 31: 696–702.
- [24] Carman AJ, Mills JH, Krenz A, Kim DG, Bynoe MS. Adenosine receptor signaling modulates permeability of the blood-brain barrier. *J Neurosci* 2011, 31: 13272–13280.
- [25] Niewoehner J, Bohrmann B, Collin L, Urich E, Sade H, Maier P, *et al.* Increased brain penetration and potency of a therapeutic antibody using a monovalent molecular shuttle. *Neuron* 2014, 81: 49–60.
- [26] Matsunaga N, Okazaki F, Koyanagi S, Ohdo S. Chrono-drug delivery system based on the circadian rhythm of transferrin receptor. *Nihon Rinsho* 2013, 71: 2200–2205.
- [27] Yang Y, Zhang X, Wang X, Zhao X, Ren T, Wang F, *et al.* Enhanced delivery of artemisinin and its analogues to cancer cells by their adducts with human serum transferrin. *Int J Pharm* 2014, 467: 113–122.
- [28] Tortorella S, Karagiannis TC. Transferrin receptor-mediated endocytosis: a useful target for cancer therapy. *J Membr Biol* 2014, 247: 291–307.
- [29] Rempe R, Cramer S, Qiao R, Galla HJ. Strategies to overcome the barrier: use of nanoparticles as carriers and modulators of barrier properties. *Cell Tissue Res* 2014, 355: 717–726.
- [30] Gao H, Pang Z, Jiang X. Targeted delivery of nano-therapeutics for major disorders of the central nervous system. *Pharm Res* 2013, 30: 2485–2498.
- [31] Kreuter J. Drug delivery to the central nervous system by polymeric nanoparticles: What do we know? *Adv Drug Deliv Rev* 2014, 71: 2–14.
- [32] Tan R, Niu M, Zhao J, Liu Y, Feng N. Preparation of vincristine sulfate-loaded poly (butylcyanoacrylate) nanoparticles modified with pluronic F127 and evaluation of their lymphatic tissue targeting. *J Drug Target* 2014, 22: 509–517.
- [33] Chung CY, Yang JT, Kuo YC. Polybutylcyanoacrylate nanoparticles for delivering hormone response element-conjugated neurotrophin-3 to the brain of intracerebral hemorrhagic rats. *Biomaterials* 2013, 34: 9717–9727.
- [34] Lin Y, Pan Y, Shi Y, Huang X, Jia N, Jiang JY. Delivery of large molecules via poly(butyl cyanoacrylate) nanoparticles into the injured rat brain. *Nanotechnology* 2012, 23: 165101.
- [35] Kuo YC, Chung CY. Transcytosis of CRM197-grafted polybutylcyanoacrylate nanoparticles for delivering zidovudine across human brain-microvascular endothelial cells. *Colloids Surf B Biointerfaces* 2012, 91: 242–249.
- [36] Huang JY, Lu YM, Wang H, Liu J, Liao MH, Hong LJ, *et al.* The effect of lipid nanoparticle PEGylation on neuroinflammatory response in mouse brain. *Biomaterials* 2013, 34: 7960–7970.
- [37] Thomsen LB, Linemann T, Pondman KM, Lichota J, Kim KS, Pieters RJ, *et al.* Uptake and transport of superparamagnetic iron oxide nanoparticles through human brain capillary endothelial cells. *ACS Chem Neurosci* 2013, 4: 1352–1360.
- [38] Liu Z, Gao X, Kang T, Jiang M, Miao D, Gu G, *et al.* B6 peptide-modified PEG-PLA nanoparticles for enhanced brain delivery of neuroprotective peptide. *Bioconjug Chem* 2013, 24: 997–1007.
- [39] Chen YC, Hsieh WY, Lee WF, Zeng DT. Effects of surface modification of PLGA-PEG-PLGA nanoparticles on loperamide delivery efficiency across the blood-brain barrier. *J Biomater Appl* 2013, 27: 909–922.
- [40] Liu X, An C, Jin P, Liu X, Wang L. Protective effects of cationic bovine serum albumin-conjugated PEGylated tanshinone IIA nanoparticles on cerebral ischemia. *Biomaterials* 2013, 34: 817–830.
- [41] Pinzon-Daza ML, Campia I, Kopecka J, Garzon R, Ghigo D, Riganti C. Nanoparticle- and liposome-carried drugs: new strategies for active targeting and drug delivery across blood-brain barrier. *Curr Drug Metab* 2013, 14: 625–640.
- [42] Ding H, Sagar V, Agudelo M, Pilakka-Kanthikeel S, Atluri

- VS, Raymond A, *et al.* Enhanced blood-brain barrier transmigration using a novel transferrin embedded fluorescent magneto-liposome nanoformulation. *Nanotechnology* 2014, 25: 055101.
- [43] Arnold RD, Mager DE, Slack JE, Straubinger RM. Effect of repetitive administration of Doxorubicin-containing liposomes on plasma pharmacokinetics and drug biodistribution in a rat brain tumor model. *Clin Cancer Res* 2005, 11: 8856–8865.
- [44] Artus C, Glacial F, Ganeshamoorthy K, Ziegler N, Godet M, Guilbert T, *et al.* The Wnt/planar cell polarity signaling pathway contributes to the integrity of tight junctions in brain endothelial cells. *J Cereb Blood Flow Metab* 2014, 34: 433–440.
- [45] Rapoport SI. Effect of concentrated solutions on blood-brain barrier. *Am J Physiol* 1970, 219: 270–274.
- [46] Raymond JJ, Robertson DM, Dinsdale HB. Pharmacological modification of bradykinin induced breakdown of the blood-brain barrier. *Can J Neurol Sci* 1986, 13: 214–220.
- [47] Hynynen K, Clement GT, McDannold N, Vykhodtseva N, King R, White PJ, *et al.* 500-element ultrasound phased array system for noninvasive focal surgery of the brain: a preliminary rabbit study with *ex vivo* human skulls. *Magn Reson Med* 2004, 52: 100–107.
- [48] Bartus RT, Elliott PJ, Dean RL, Hayward NJ, Nagle TL, Huff MR, *et al.* Controlled modulation of BBB permeability using the bradykinin agonist, RMP-7. *Exp Neurol* 1996, 142: 14–28.
- [49] Thomas HD, Lind MJ, Ford J, Bleehen N, Calvert AH, Boddy AV. Pharmacokinetics of carboplatin administered in combination with the bradykinin agonist Cereport (RMP-7) for the treatment of brain tumours. *Cancer Chemother Pharmacol* 2000, 45: 284–290.
- [50] Chen H, Konofagou EE. The size of blood-brain barrier opening induced by focused ultrasound is dictated by the acoustic pressure. *J Cereb Blood Flow Metab* 2014, 34: 1197–1204.
- [51] Aryal M, Arvanitis CD, Alexander PM, McDannold N. Ultrasound-mediated blood-brain barrier disruption for targeted drug delivery in the central nervous system. *Adv Drug Deliv Rev* 2014, 72: 94–109.
- [52] Cho EE, Drazic J, Ganguly M, Stefanovic B, Hynynen K. Two-photon fluorescence microscopy study of cerebrovascular dynamics in ultrasound-induced blood-brain barrier opening. *J Cereb Blood Flow Metab* 2011, 31: 1852–1862.
- [53] Fan CH, Ting CY, Lin HJ, Wang CH, Liu HL, Yen TC, *et al.* SPIO-conjugated, doxorubicin-loaded microbubbles for concurrent MRI and focused-ultrasound enhanced brain-tumor drug delivery. *Biomaterials* 2013, 34: 3706–3715.
- [54] Ting CY, Fan CH, Liu HL, Huang CY, Hsieh HY, Yen TC, *et al.* Concurrent blood-brain barrier opening and local drug delivery using drug-carrying microbubbles and focused ultrasound for brain glioma treatment. *Biomaterials* 2012, 33: 704–712.
- [55] Wang F, Shi Y, Lu L, Liu L, Cai Y, Zheng H, *et al.* Targeted delivery of GDNF through the blood-brain barrier by MRI-guided focused ultrasound. *PLoS One* 2012, 7: e52925.
- [56] Huang Q, Deng J, Wang F, Chen S, Liu Y, Wang Z, *et al.* Targeted gene delivery to the mouse brain by MRI-guided focused ultrasound-induced blood-brain barrier disruption. *Exp Neurol* 2012, 233: 350–356.
- [57] Fan CH, Ting CY, Liu HL, Huang CY, Hsieh HY, Yen TC, *et al.* Antiangiogenic-targeting drug-loaded microbubbles combined with focused ultrasound for glioma treatment. *Biomaterials* 2013, 34: 2142–2155.
- [58] Sheikov N, McDannold N, Vykhodtseva N, Jolesz F, Hynynen K. Cellular mechanisms of the blood-brain barrier opening induced by ultrasound in presence of microbubbles. *Ultrasound Med Biol* 2004, 30: 979–989.
- [59] Carstensen EL, Gracewski S, Dalecki D. The search for cavitation *in vivo*. *Ultrasound Med Biol* 2000, 26: 1377–1385.
- [60] Hou GY, Marquet F, Wang S, Konofagou EE. Multi-parametric monitoring and assessment of high-intensity focused ultrasound (HIFU) boiling by harmonic motion imaging for focused ultrasound (HMIFU): an *ex vivo* feasibility study. *Phys Med Biol* 2014, 59: 1121–1145.
- [61] Figeac F, Lesault PF, Coz OL, Damy T, Souktani R, Trebeau C, *et al.* Nanotubular crosstalk with distressed cardiomyocytes stimulates the paracrine repair function of mesenchymal stem cells. *Stem Cells* 2014, 32: 216–230.
- [62] Gerdes HH, Bukoreshtliev NV, Barroso JF. Tunneling nanotubes: a new route for the exchange of components between animal cells. *FEBS Lett* 2007, 581: 2194–2201.
- [63] Yasuda K, Khandare A, Burianovskyy L, Maruyama S, Zhang F, Nasjletti A, *et al.* Tunneling nanotubes mediate rescue of prematurely senescent endothelial cells by endothelial progenitors: exchange of lysosomal pool. *Aging (Albany NY)* 2011, 3: 597–608.
- [64] Lokar M, Kabaso D, Resnik N, Sepcic K, Kralj-Iglic V, Veranic P, *et al.* The role of cholesterol-sphingomyelin membrane nanodomains in the stability of intercellular membrane nanotubes. *Int J Nanomed* 2012, 7: 1891–1902.
- [65] Pasquier J, Guerrouahen BS, Al Thawadi H, Ghiabi P, Maleki M, Abu-Kaoud N, *et al.* Preferential transfer of mitochondria from endothelial to cancer cells through tunneling nanotubes modulates chemoresistance. *J Transl Med* 2013, 11: 94.
- [66] Tosi G, Vilella A, Chhabra R, Schmeisser MJ, Boeckers TM, Ruozi B, *et al.* Insight on the fate of CNS-targeted nanoparticles. Part II: Intercellular neuronal cell-to-cell transport. *J Control Release* 2014, 177: 96–107.
- [67] Bukoreshtliev NV, Wang X, Hodneland E, Gurke S, Barroso JF, Gerdes HH. Selective block of tunneling nanotube (TNT)

- formation inhibits intercellular organelle transfer between PC12 cells. *FEBS Lett* 2009, 583: 1481–1488.
- [68] Callan-Jones A, Sorre B, Bassereau P. Curvature-driven lipid sorting in biomembranes. *Cold Spring Harb Perspect Biol* 2011, 3.
- [69] Rustom A, Saffrich R, Markovic I, Walther P, Gerdes HH. Nanotubular highways for intercellular organelle transport. *Science* 2004, 303: 1007–1010.
- [70] Sowinski S, Jolly C, Berninghausen O, Purbhoo MA, Chauveau A, Kohler K, *et al.* Membrane nanotubes physically connect T cells over long distances presenting a novel route for HIV-1 transmission. *Nat Cell Biol* 2008, 10: 211–219.
- [71] Tangl E. Ueber offene communicationen zwischen den Zellen des Endosperms einiger Samen. *Jahrb Wiss Botanik* 1880, 12: 170–190.
- [72] Wang G, Shimada E, Koehler CM, Teitell MA. PNPASE and RNA trafficking into mitochondria. *Biochim Biophys Acta* 2012, 1819: 998–1007.
- [73] Wang G, Chen HW, Oktay Y, Zhang J, Allen EL, Smith GM, *et al.* PNPASE regulates RNA import into mitochondria. *Cell* 2010, 142: 456–467.
- [74] Dong HJ, Shang CZ, Peng DW, Xu J, Xu PX, Zhan L, *et al.* Curcumin attenuates ischemia-like injury induced IL-1 β elevation in brain microvascular endothelial cells *via* inhibiting MAPK pathways and nuclear factor- κ B activation. *Neurol Sci* 2014, 35: 1387–1392.