branes, followed by the escape of ON from vesicles and translocation into nucleus. Our electron microscopy results are in line with data published earlier regarding the redirection of splicing with oligonucleotides delivered into cells by PFs.

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Live-cell imaging and single-particle tracking of polyplex internalization

Nadia Ruthardt*, Karla de Bruin, Kevin Braeckmans, Ernst Wagner, Christoph Bräuchle
Ludwig-Maximilians-Universität München, Department Chemie und Biochemie, Butenandtstr. 5-13, Haus E, 81377 München, Germany

*Corresponding author.
E-mail: Nadia.Ruthardt@cup.uni-muenchen.de (N. Ruthardt).

Systemic delivery of therapeutic genes for gene therapy or cancer gene therapy requires gene vectors that overcome several barriers. The vector has to enable tissue-selective delivery, internalize efficiently and finally release its cargo reliably within the target cell. Tissue specificity and enhanced internalization can be achieved by cell-specific ligands that bind to certain surface markers that are upregulated in, for example, solid cancers. Functionalization with pH-sensitive and redox-sensitive linkers or polymers allows the vector to 'sense' external stimuli that will trigger their activation in temporally and spatially controlled manner. We investigate the uptake of targeted and untargeted polymeric gene vectors (polyplexes) by highly sensitive fluorescence microscopic methods on a single cell level [1]. The epidermal growth factor receptor (EGFR) is overexpressed on a high percentage of human carcinomas and is therefore an attractive therapeutic target for tissue-specific targeting by non-viral vectors in cancer gene therapy. Comparing uptake kinetics and internalization dynamics, single particle tracking in combination with quenching experiments revealed typical three-phase dynamics of the uptake process independent of targeting. Phase I was characterized by slow, actin-cytoskeleton-mediated movement of the particles with drift and included the internalization process. During phase II, particles displayed increased velocities with confined and anomalous diffusion in the cytoplasm. Phase III was characterized by fast active transport along microtubules. Targeting of polyplexes for receptor-mediated endocytosis by the EGF receptor resulted in shortening of phase I and strongly accelerated internalization. Targeted as well as untargeted particles were transported in early endosomes marked by Rab5–GFP and accumulated in late endosomes marked by Rab9–GFP. The endosomal release dynamics of polyplexes consisting of DNA condensed with the cationic polymers linear polyethyleneimine (LPEI), poly-(L)-lysine (PLL) or poly-(D)-lysine (PDL) were studied by photochemical release in living cells [2]. Using double-labeled polyplexes, DNA and polymer were imaged simultaneously by dual-color fluorescence microscopy. Our results demonstrate that the characteristics of the cationic polymer significantly influence the release behavior of the polyplexes. For LPEI/DNA particles, LPEI quickly spread throughout the cytosol, whereas DNA was released slowly and remained immobile thereafter. In the case of PLL particles, both DNA and polymer showed quick release. PDL particles remained condensed upon photosensitizer activation.

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Vascular endothelium remodeling in human African trypanosomiasis

L. Sanderson, M. Dogruel, S.A. Thomas*
King's College London, Pharmaceutical Sciences Division, Hodgkin Building, Guy's Campus, London Bridge, London SE1 1UL, UK

*Corresponding author.
E-mail: sarah.thomas@kcl.ac.uk (S.A. Thomas).

Molecule movement into the central nervous system (CNS) is restricted by the blood–brain barrier (BBB) and the blood–cerebrospinal fluid (CSF) barrier. Human African trypanosomiasis (HAT) or sleeping sickness, caused by the parasites, *Trypanosoma brucei* (T.b.) gambiense or *T.b. rhodesiense*, is fatal if untreated. The first disease stage is associated with trypanosome proliferation in the periphery. The second stage is when the parasites reach the CNS. HAT treatment is stage specific with drugs, which are assumed to cross the BBB, used to treat CNS stage disease. Since the treatment of CNS-stage HAT is more toxic than that of early-stage, it is vital to stage HAT [1]. Staging requires a CSF sample. Lumbar puncture under field conditions is difficult and invasive. Improved tests for staging HAT are required [2]. Our studies have established that *T.b. brucei* crosses the murine blood–CNS interfaces at ~day 11 post-infection (p.i.) and the animals died at day 37.9 ± 1.23. At day 7, 14 and 21 p.i. no loss of barrier integrity was measurable using the inert tracer, [^{14}C]sucrose (342 Da; radius 4.6 Å), nor was there any endothelium remodeling (including transporter up/downregulation) as measured with eflornithine, pentamidine or nifurtimox [3,4]. BBB, but not choroid plexus, dysfunction, occurred at days 28 and 35 p.i. with resultant increases in [^{14}C]sucrose space [3,4]. Suramin (1429 Da) brain distribution increased at day 35 p.i., suggesting considerable BBB breakdown as this molecule is highly albumin (60 kDa; radius 35.5 Å) bound [4,5]. Furthermore, the increased [^{14}C]sucrose association with the endothelial cell at day 35 p.i. compared to the non-infected and other infected time groups suggested an increase in vesicular trafficking [3]. This loss of integrity may be a sign of terminal disease. However, perhaps there was an earlier loss of blood–CNS barrier integrity (possibly when the parasites entered the CNS) that was not measurable using [^{14}C]sucrose (an inert tracer with smaller molecular dimensions being needed) and/or this was a reversible process that was undetectable at the times studied. Furthermore, endocytosis may be a sensitive marker of endothelium remodeling. The characterization of vesicular expression in a murine model of HAT may be the first step towards vesicle targeted staging strategies. Overall understanding blood–CNS barriers breakdown in HAT could contribute to the development of therapeutics and therapeutic targets to control brain injury and to the characterization of biomarkers for safer staging of the disease.

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