Nanoparticles as a tool to deliver drugs to the retina and brain: an update

Qing You, Bernhard A. Sabel*

Over the past few years, different neuron-targeted nanoparticles (NPs) were designed to deliver drugs to enhance neuron protection and recovery, and much progress was made in our understanding of the uptake mechanism and the related physicochemical properties. Physicochemical properties attracting much attentions in NP's design and modification include particle size, surface hydrophobicity, and charge (Wohlfart et al., 2012). Despite many achievements *in vitro*, the *in vivo* efficacy of most NP modifications are still quite limited, especially in the central nervous system (CNS). In the CNS, the blood-brain barrier (BBB) shields neurons and non-neuronal cells in the brain tissue from being exposed to unwanted molecules through different mechanisms that regulate the exchange of molecules and ions. Because it prevents the entry of over 95% of small molecules and almost 100% of large molecules (Pardridge, 2007), the BBB is a key limitation for drug delivery to the brain. We have been studying for quite some time NPs' passage across the blood retina barrier (BRB) in living animals – a suitable surrogate model of the BBB. Here, the passage of fluorescent NP's across the BRB can be visualized in the living rat with a confocal laser scanning microscope using the *in vivo* confocal neuroimaging technique (Sabel et al., 1997). The retina is the only brain tissue available for non-invasive *in vivo* microscopic imaging of CNS neurons. Although the BRB is more permeable than the BBB for some compounds and the trans-endothelial electrical resistance of the BRB is lower than BBB *in vitro*, the BRB and BBB are similar regarding the expression of efflux proteins and the permeability for many drugs. Regarding passage of NPs into brain tissue, the preliminary data suggest that the results from our BRB model are also valid for the situation at the BBB (You et al., 2019). Unlike the CNS, the peripheral nervous system is not protected by the BBB, but there is still a long and complex route to trace the fate of NPs *in vivo*, including interaction with blood components and peripheral organs (**Figure 1**). Here, NPs may serve as a tool for sustained release of drugs which would otherwise be metabolized or filtered out too quickly. In this context it is important to consider the multiple *in vivo* interactions through physicochemical properties to advance our understanding of mechanism of action and NP design both for sustained release and passage across biological barriers.

Physicochemical properties of NPs and their interactions with blood components: The blood contains hundreds of different proteins which contribute to the recognition of foreign materials. When NPs come into contact with blood, proteins will immediately adsorb onto the surface and form a so-called protein corona. This protein corona changes the size, shape and surface chemistry, and the biological identity of the NPs may have quite different characteristics as compared to their state immediately after production. We studied different variations of the poloxamer 188-modified, DEAE-dextran-stabilized (PDD) polybutylcyanoacrylate (PBCA) NP (You et al., 2019) and observed how low- and high-charge NPs agglomerated after *in vitro* incubation with serum. Following intravenous injection, the low-charge NPs accumulated unevenly in blood vessel walls and formed agglomerates which attached to the surface of the vessel walls. Interestingly, the high-charge NPs preferentially accumulated in the small peripheral vessels and were found to be localized only in a small region along the vessels. It seems that NPs with the appropriate surface modification, medium-charge as in this case, have higher aggregation resistance property in the blood to avoid agglomeration and decrease the risk of blood clots. Surface charge and size also considerably influenced the degradation speed of the NPs in the blood. A small portion of the low-charge NPs was found to be decreased in size after 10 minutes of serum incubation, probably indicating degradation. Scanning electron microscopy imaging indicates that the high-charge NPs virtually disappeared after 10 minutes incubation in serum. One possible explanation is that this positive charge facilitates an interaction with negatively-charged serum-components like albumins which may facilitate solubilization.

Physicochemical properties and interactions of NPs with peripheral organs: During blood circulation there is a challenge in finding out how to prevent NPs from being removed by cells of the mononuclear phagocyte system. The mononuclear phagocyte system consists of dendritic cells, blood monocytes, macrophages in liver, spleen and lymph nodes, all of which are responsible for clearing, processing and degrading foreign objects from the body. It is reported that almost all NPs injected without a "stealth" strategy (i.e., surface modification) are cleared by the mononuclear phagocyte system from the blood circulation within a few hours (Moghimi et al., 2001). For the PDD PBCA NPs, we observed a clearance of NPs from the retina blood vessels within a relatively short time of 30 minutes, and this clearance rate is influenced by the size and surface properties of NPs (You et al., 2019). The small-size portion of medium-charge PDD PBCA NPs degraded relatively slowly when incubated with serum *in vitro*, but they were cleared from the blood rather quickly *in vivo* and accumulated significantly more in the kidney, liver and spleen and less in the brain. For all variations of NPs, over half of the dose accumulated in the liver, lung and spleen. The higher the surface charge, the more did the NPs accumulate in the liver. Thus, it seems that size and surface properties of NPs can influence their blood circulation time and their body distribution. Similarly, Ambruosi et al. (2005) found that coating PBCA NPs with a negatively-charged Tween 80 decreased the accumulation in these body organs and increased the accumulation in the brain, while loading with positively-charged doxorubicin had the opposite effect. However, with a long circulation time there is also the potential risk of accumulation in peripheral, non-target organs, and NPs could end up in places where they are not supposed to be, which increases the risk of unwanted side effects. Our PDD PBCA NPs are aimed at targeting the brain. In general, a higher blood concentration leads to a higher brain concentration, which means a longer circulation time or more sustained release facilitates the brain delivery. However, our data indicate that not only the surface characteristics influence the circulation time, but also their size: higher uptake levels of small-size NPs in peripheral organs have the effect of significantly decreasing their accumulation in the brain as compared to the NP fraction with larger NPs.

Interactions of NPs with the barriers and extracellular space: When NPs reach and pass the BBB or the BRB, they interact with them in multiple ways including transport proteins and tight junction, i.e., Tween 80-coated NPs pass the BBB by inhibition of P-glycoprotein and they reversible disrupt the BBB (Rempe et al., 2011). In our experiments (You et al., 2019), Tween 80 NPs accumulated more readily in both the bigger-sized main vessels and in the smaller peripheral vessels and their surrounding tissue, and they accumulated more readily in the retinal ganglion cells around. Compared to the PDD PBCA NPs, the uptake of the Tween 80 PBCA NPs by endothelial cells and retinal ganglion cells was efficient, but their movement ability in the extracellular space was not.

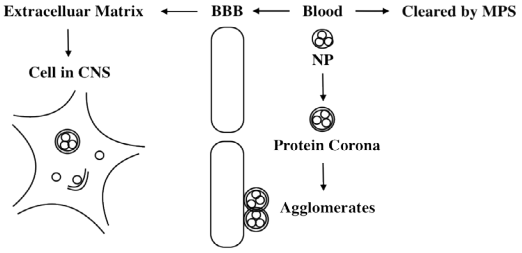

Figure 1 | **Long and complex route to trace the fate of NPs *in vivo*.**

NP physicochemical properties and neuronal targeting: Generally, the difficulties of designing neuron targeting NPs increase with the complexity of the administration method and targeted location. Single modification of the surface is usually enough to achieved satisfactory *in vitro* high-performance neuronal targeting of NPs. For example, Lopes et al. (2016) reported the use of a non-viral neurotropic poly(ethylene imine)-based NPs that are capable of mediating neuron-specific transfection following a subcutaneous injection *in vivo*. NPs were targeted to peripheral neurons by using the nontoxic carboxylic fragment of tetanus toxin, which, besides being neurotropic, is capable of being retrogradely transported from axon terminals to the cell bodies. However, when administrated *in vivo*, it would be better to consider the required NP design much more carefully. For example, Pereira Gomes et al. (2018) showed that the precise adjustment of the polyethylene glycol coverage-density presents a significant impact on the selectivity and bioactivity of the developed formulation, emphasizing the need for the fine-tuning of polyethylene glycol-modified NPs for the successful development of the next-generation nanomedicines. Similarly, we fine-tuned our PDD BPCA NPs so that a higher percentage of RGCs were co-localized with the medium-charge NPs as compared to the other NP variations, suggesting a higher uptake of these NPs by neurons (You et al., 2019).

No matter how the administration is done *in vivo*, it is always a long distance for a drug carrier to deliver its cargo to the final neuronal target with high efficiency and without unwanted, high accumulation in other organs of the body which carries risks of toxic side effects. And it is difficult to design a universal NP cargo because physicochemical properties change when different drugs are loaded, which can again influence almost all steps along NPs' fate. To find the key problem that limits the efficiency and solve it, a multifactorial, integrated strategy can provide better guidance for the design of promising carrier systems to target neurons *in vivo*. Apparently, despite many years of research since the early discovery by Kreuter (1983), there are no standard rules for the design of nanoparticulate carriers for neuron targeting; each nano-system requires its own design and optimization. However, the potential application of these carriers is broad, not only for single chemical drugs, but also for herbal or plant extracts that contain multiple active compounds. For example, Wang et al. (2018) performed cell experiments on Lycium barbarum polysaccharide encapsulated into electrospun nanofibers and found it could be a potential candidate for the tissue engineered scaffold for peripheral nerve regeneration. There is still much to learn about NPs mechanisms of action, and finding optimal fabrication procedures and applications is a challenging but worthwhile goal of drug delivery. Conjugating NPs with antibodies and peptides is another route to develop more specific targeting strategies to improve transport to the retina and the brain. Xu and Chau (2018) designed tetra peptide-modified NPs and observed enhanced uptake in Tropomyosin receptor kinase B-positive PC12 cells, which showed their potential to deliver drugs into neurons for neural regeneration. Yet, *in vivo* evidence is still missing. In any event, more research is worthwhile to further explore the feasibility and versatility of NPs as possible vehicles for targeted drug delivery to different body organs, with the CNS (retina and brain) being to most audacious goal to achieve neuroprotection, regeneration and restoration.

The project "Nanoparticle Functionalization for Blood-Brain Barrier Passage" was funded by German Research Foundation (SA433/28-1). QY was supported by China Scholarship Council (201406370149).

Qing You, Bernhard A. Sabel*
Institute of Medical Psychology, Otto-von-Guericke University of Magdeburg, Medical Faculty, Magdeburg, Germany
***Correspondence to:** Bernhard A. Sabel, PhD, bernhard.sabel@med.ovgu.de.
https://orcid.org/0000-0002-4472-5543 (Bernhard A. Sabel)
Received: March 1, 2020
Peer review started: March 5, 2020
Accepted: April 20, 2020
Published online: August 24, 2020

https://doi.org/10.4103/1673-5374.290886
How to cite this article: *You Q, Sabel BA (2021) Nanoparticles as a tool to deliver drugs to the retina and brain: an update. Neural Regen Res 16(2):283-284.*

References

Ambruosi A, Yamamoto H, Kreuter J (2005) Body distribution of polysorbate-80 and doxorubicin-loaded [14C]poly(butyl cyanoacrylate) nanoparticles after i.v. administration in rats. J Drug Target 13:535-542.

Kreuter J (1983) Evaluation of nanoparticles as drug-delivery systems. III: materials, stability, toxicity, possibilities of targeting, and use. Pharm Acta Helv 58:242-50.

Lopes CD, Oliveira H, Estevão I, Pires LR, Pêgo AP (2016) In vivo targeted gene delivery to peripheral neurons mediated by neurotropic poly(ethylene imine)-based nanoparticles. Int J Nanomedicine 11:2675-2683.

Moghimi SM, Hunter AC, Murray JC (2001) Long-circulating and target specific nanoparticles: theory to practice. Pharmacol Rev 53:283-318.

Pardridge WM (2007) Drug targeting to the brain. Pharm Res 24:1733-1744.

Pereira Gomes C, Leiro V, Ferreira Lopes CD, Spencer AP, Pêgo AP (2018) Fine tuning neuronal targeting of nanoparticles by adjusting the ligand grafting density and combining PEG spacers of different length. Acta Biomater 78:247-259.

Rempe R, Cramer S, Hüwel S, Galla HJ (2011) Transport of Poly(n-butylcyano-acrylate) nanoparticles across the blood-brain barrier in vitro and their influence on barrier integrity. Biochem Biophys Res Commun 406:64-69.

Sabel BA, Engelmann R, Humphrey MF (1997) In vivo confocal neuroimaging (ICON) of CNS neurons. Nat Med 3:244-247.

Wang J, Tian L, He L, Chen N, Ramakrishna S, So KF, Mo X (2018) Lycium barbarum polysaccharide encapsulated Poly lactic-co-glycolic acid Nanofibers: cost effective herbal medicine for potential application in peripheral nerve tissue engineering. Sci Rep 8:8669.

Wohlfart S, Gelperina S, Kreuter J (2012) Transport of drugs across the blood-brain barrier by nanoparticles. J Control Release 161:264-273.

Xu J, Chau Y (2018) Polymeric nanoparticles decorated with BDNF-derived peptide for neuron-targeted delivery of PTEN inhibitor. Eur J Pharm Sci 124:37-45.

You Q, Hopf T, Hintz W, Rannabauer S, Voigt N, van Wachem B, Henrich-Noack P, Sabel BA (2019) Major effects on blood-retina barrier passage by minor alterations in design of polybutylcyanoacrylate nanoparticles. J Drug Target 27:338-346.

You Q, Sokolov M, Grigartzik L, Hintz W, van Wachem B, Henrich-Noack P, Sabel BA (2019) How nanoparticle physicochemical parameters affect drug delivery to cells in the retina via systemic interactions. Mol Pharm 16:5068-5075.

C-Editors: Zhao M, Li JY; T-Editor: Jia Y