PERSPECTIVE

Superparamagnetic iron oxide nanoparticles: promote neuronal regenerative capacity?

Currently, we know that neuronal outgrowth during development and regeneration requires a complex interaction of intra- and extracellular molecules such as growth factors, neurotransmitters and extracellular matrix proteins (O'Donnell et al., 2009). Furthermore, the discovery of a broad spectrum of growth-promoting cues has led to novel concepts for therapeutic strategies. However, although our understanding of the molecular mechanisms underlying neuronal regeneration is constantly growing, there is still no therapy for the treatment of brain or spinal cord injuries (SCI) (Filli and Schwab, 2012). Recent attempts have been made to influence regeneration using nano-scaled particles that specifically act at the molecular level (Polak and Shefi, 2015).

In general, nanoparticles are defined as particles ranging in size from 1 nm with at least one dimension smaller than 100 nm (Win-Shwe and Fujimaki, 2011). The number of materials produced by means of nanotechnology is not only increasing in many areas of daily life such as pharmaceutical and food products, but also in biomedical applications as well. Given the great diversity of nanomaterials and respective fields of applications, we will focus on nanoparticles that are composed of iron oxide and have been specifically designed for their use as contrast agents in magnetic resonance imaging (MRI). In general, nanoparticles are organized in different categories according to their material they are made of, their hydrodynamic diameter, surface coating, electric charge, shape, and magnetic and optical properties (Polak and Shefi, 2015). The small size is responsible for the increased surface-to-volume ratio of nanoparticles and the resulting increased reactivity with organic substances such as proteins, lipids or carbohydrates. Iron-oxide nanoparticles are superparamagnetic and therefore ideally suited as contrast agents for MRI that have been intensively studied (Saini et al., 1987; Weinstein et al., 2010). The surface chemistry of SPIOs can be turned to optimize their pharmacokinetic properties and biocompatibility, *e.g.*, by PEG functionalization (Papastefanaki et al., 2015). Depending on their size, SPIOs show different organ distributions when injected intravenously. For instance, SPIOs with a hydrodynamic diameter of 50 nm are suitable for imaging tumors, *e.g.*, of the liver and spleen, whereas SPIOs with diameters of around 20 nm can be used for MR lymphography and angiography (Taupitz et al., 2004). In addition, in the field of neurooncology, SPIOs have not only been utilized for diverse diagnostic applications, but also for therapeutic purposes (Weinstein et al., 2010).

Furthermore, SPIOs have emerged as an effective tool for novel therapeutic strategies due to the fact that they can be modified by conjugation of their surface with reactive functional groups and loaded with cargos. At present, there is particular focus on the use of SPIOs to specifically influence neuronal regeneration after injury (Papastefanaki et al., 2015; Polak and Shefi, 2015). How can the use of nanoparticles significantly enhance the regenerative capacity of neurons after brain and spinal cord trauma?

The regeneration and growth potential of neurons within the adult brain is restricted (Pernet and Schwab, 2014). On the one hand, modulation of extracellular cues and receptors, including members of the netrins, ephrins and slits and their respective receptors DCC/UNC, Eph RTK, and Robos, influences neuronal outgrowth and regeneration. On the other hand, intrinsic properties of neurons are responsible for cytoskeletal dynamics and are thereby essentially involved in axonal and dendritic growth. Particularly for axon guidance, the growth cone - their distal tip - plays an important role. The intra- and extracellular environment communicates at the growth cone and thereby regulates molecular mechanisms for axon guidance (O'Donnell et al., 2009).

There is a growing body of evidence that SPIOs can be used to influence neurite outgrowth at different levels. Recently, it has been shown that SPIOs can accumulate at growth cones and axons can be subsequently remote guided using a magnetic force (Pita-Thomas et al., 2015). Here, SPIOs were functionalized with Cholera Toxin B and anti-Thy1 antibody and applied to primary retinal ganglion cells (RGCs) where they bind to growth cone membranes. After the application of local magnetic forces, membrane-bound, functionalized SPIOs were able to induce axon elongation up to 20 times higher compared to controls. Furthermore, the rate of elongation was controlled by reducing or increasing the voltage of the electromagnet or by changing the distance between the electromagnet tip and the growth cone-bound SPIOs (Pita-Thomas et al., 2015). This kind of controlled mechanism for directional axonal outgrowth could potentially be further developed to reestablish lamina, pathway and target specificity during regeneration processes in the adult brain. However, the fate, degradation, and potential cytotoxic effects of SPIOs have to be considered.

Another method for promoting neurite outgrowth is to surface functionalize SPIOs with antibodies that activate neurotrophin receptors (Steketee et al., 2011). Neurotrophins are important mediators for neuronal survival and growth. Here, conjugated SPIOs were internalized by neurotrophin-activated signaling endosomes and transported between soma and growth cones of RGCs. While neurite growth on its own was not affected, as soon as magnetic force was applied, signaling endosome localization, growth cone motility, and neurite elongation and outgrowth were influenced. Furthermore, changes in endosome signaling pathways as well as gene expression associated with neurite growth were found. In addition, functionalized SPIOs enhanced the outgrowth of neurons in the same manner as the brain-derived neurotrophic factor (BDNF) that is critically involved in regeneration processes (Steketee et al., 2011).

The examples mentioned above already suggest the high potential of nanoparticles for therapeutic treatments relating to neuronal regeneration. In addition, it has also been shown that SPIOs alter cellular processes without the specific application of an external stimulus. One of these studies demonstrated the capability of SPIOs to enhance neurite outgrowth. When polyethylene glycol (PEG)-phospholipid-coated SPIOs were applied together with nerve growth factor to pheochromocytoma (PC)-12 cells, a neuronal tumor cell line, neurite outgrowth synergistically increased in a dose-dependent manner (Kim et al., 2011). However, immortalized cell lines only provide limited evidence about impacted cellular physiology or survival parameters because of their robust proliferative capacity. Quite recently, a rather clinically relevant study could show that intraspinal PEG-AuNPs administration can improve the recovery of hind limb motor function after SCI in mice (Papastefanaki et al., 2015). This is an essential *in vivo* study to demonstrate the potential of nanoparticle applications not only for diagnostics, but also for therapeutic strategies.

Nevertheless, in our recent study, we systematically analyzed the effects of four different types of clinically relevant SPIOs on

murine primary brain cells (Neubert et al., 2015). We applied the European Medicines Agency-approved ferucarbotran and the US Food and Drug Administration (FDA)-approved ferumoxytol. Carboxydextran-coated ferucarbotran is a contrast agent for liver imaging in humans that exhibits a hydrodynamic diameter of 60 nm. Ferucarbotran has been withdrawn by the marketing authorization holder in Europe, but is still available in pharmaceutical quality from Meito Sangyo in Japan, where the approval for clinical use is still valid. Carboxymethyldextran-coated ferumoxytol, with a hydrodynamic diameter of 30 nm, was originally developed for treating iron-deficiency anemia in patients with chronic kidney disease, and is currently used as a blood pool contrast agent for visualizing brain vascular malformations and creating cerebral blood volume maps with MRI. Furthermore, in our study we included two different types of novel, citrate-coated very small iron oxide particles (VSOP-R1, VSOP-R2) that have been tested in human Phase II clinical trials (Taupitz et al., 2004). These nanoparticles are of special interest because their small size of around 7 nm prolongs their blood half-life and facilitates their cellular incorporation, which could be beneficial for therapeutic interventions.

These four different SPIO types showed different effects on brain cells depending on: a) the SPIO's surface coating and charge, b) size, c) concentration, d) exposure time and e) cell culture condition (Neubert et al., 2015). Differences were found in cell survival, shape and neurite growth. Interestingly, primary hippocampal neurons very clearly degenerated following exposure to all SPIOs, whereas the vitality of primary microglia strongly decreased after exposure to VSOPs and ferucarbotran, but not ferumoxytol. This might be explained by the fact that ferumoxytol was not accumulated by microglia as compared to the other SPIOs. In contrast, cultivating both primary neurons and microglia in a neuron-glia coculture with SPIOs did not result in a coherent reduction of neurites, but rather enhanced neurite branching in a particle- and dose-dependent manner. For instance, lower concentrations of ferucarbotran and high concentrations of VSOP-R2 stimulated neurite outgrowth (Neubert et al., 2015).

In conclusion, based on our findings and those of other investigators, we postulate that there are a variety of possibilities for promoting neuronal regeneration using SPIOs. Compared to *in vivo* testing, our *in vitro* studies do have some limitations, *e.g.*, they do not permit the assessment of three-dimensional network interaction. However, *in vitro* SPIO studies are certainly necessary to clarify particle- and cell type-induced effects that exclude external stimuli. In addition, the benefits of nanoparticles must be weighed against their potential adverse effects. In particular, it is essential to refer more to physicochemical features including size, surface coating and SPIO charge as these strongly influence *e.g.,* their degradation, target specificity, and long-term storage. Furthermore, SPIOs may induce the release of mediators for inflammation, apoptosis and oxidative stress through microglial and astrocyte activation. The complex interaction of signaling molecules at both the inter- and intracellular levels is challenging for precisely determining SPIO-induced effects. We need a deeper understanding of the molecular mechanisms induced by SPIOs to develop safety guidelines for their clinical application. Nevertheless, nanoparticles have already proven to be powerful tools for basic research and offer many advantages. This trend is expected to expand in terms of their fields of application.

The authors thank Andreas Wree for reading the paper and Kimberly Mason for her excellent help in polishing the paper as a native speaker.

Jenni Neubert, Anja U. Bräuer*

Institute of Cell Biology and Neurobiology, Center for Anatomy, Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117, Berlin, Germany (Neubert J)
Institute for Anatomy, Universitätsmedizin Rostock, Gertrudenstraße 9, 18057 Rostock, Germany (Bräuer AU)

***Correspondence to:** Anja U. Bräuer, Ph.D., anja.braeuer@med.uni-rostock.de.*
***Accepted:** 2015-08-06*
***doi:** 10.4103/1673-5374.165306* ***http**://www.nrronline.org/*
Neubert J, Bräuer AU (2015) Superparamagnetic iron oxide nanoparticles: promote neuronal regenerative capacity? Neural Regen Res 10(10):1568-1569.

References

Filli L, Schwab ME (2012) The rocky road to translation in spinal cord repair. Ann Neurol 72:491-501

Kim JA, Lee N, Kim BH, Rhee WJ, Yoon S, Hyeon T, Park TH (2011) Enhancement of neurite outgrowth in PC12 cells by iron oxide nanoparticles. Biomaterials 32:2871-2877.

Neubert J, Wagner S, Kiwit J, Bräuer AU, Glumm J (2015) New findings about iron oxide nanoparticles and their different effects on murine primary brain cells. Int J Nanomedicine 10:2033-2049.

O'Donnell M, Chance RK, Bashaw GJ (2009) Axon growth and guidance: receptor regulation and signal transduction. Annu Rev Neurosci 32:383-412.

Papastefanaki F, Jakovcevski I, Poulia N, Djogo N, Schulz F, Martinovic T, Ciric D, Loers G, Vossmeyer T, Weller H, Schachner M, Matsas R (2015) Intraspinal delivery of polyethylene glycol-coated gold nanoparticles promotes functional recovery after spinal cord injury. Mol Ther 23:993-1002.

Pernet V, Schwab ME (2014) Lost in the jungle: new hurdles for optic nerve axon regeneration. Trends Neurosci 37:381-387.

Pita-Thomas W, Steketee MB, Moysidis SN, Thakor K, Hampton B, Goldberg JL (2015) Promoting filopodial elongation in neurons by membrane-bound magnetic nanoparticles. Nanomedicine 11:559-567.

Polak P, Shefi O (2015) Nanometric agents in the service of neuroscience: manipulation of neuronal growth and activity using nanoparticles. Nanomedicine 11:1467-1479.

Saini S, Stark DD, Hahn PF, Wittenberg J, Brady TJ, Ferrucci JT Jr (1987) Ferrite particles: a superparamagnetic MR contrast agent for the reticuloendothelial system. Radiology 162:211-216.

Steketee MB, Moysidis SN, Jin X-L, Weinstein JE, Pita-Thomas W, Raju HB, Iqbal S, Goldberg JL (2011) Nanoparticle-mediated signaling endosome localization regulates growth cone motility and neurite growth. Proc Natl Acad Sci U S A 108:19042-19047.

Taupitz M, Wagner S, Schnorr J, Kravec I, Pilgrimm H, Bergmann-Fritsch H, Hamm B (2004) Phase I clinical evaluation of citrate-coated monocrystalline very small superparamagnetic iron oxide particles as a new contrast medium for magnetic resonance imaging. Invest Radiol 39:394-405.

Weinstein JS, Varallyay CG, Dosa E, Gahramanov S, Hamilton B, Rooney WD, Muldoon LL, Neuwelt EA (2010) Superparamagnetic iron oxide nanoparticles: diagnostic magnetic resonance imaging and potential therapeutic applications in neurooncology and central nervous system inflammatory pathologies, a review. J Cereb Blood Flow Metab 30:15-35.

Win-Shwe TT, Fujimaki H (2011) Nanoparticles and neurotoxicity. Int J Mol Sci 12:6267-6280.