Delivery of fluorescent nanoparticles to brain

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Abstract

Nanotechnology applications in neuroscience promises to deliver significant scientific and technological breakthroughs, providing answers to unresolved questions regarding the processes occurring in the brain. In this Perspective, we provide a short background on two distinct fluorescent nanoparticles and summarize several studies focussed on achieving delivery of these into the brain and their interaction with brain tissue. Furthermore, we discuss challenges and opportunities for further development of nanoparticle-based therapies for targeting delivery of drugs across the blood brain barrier.

Keywords

Fluorescent nanoparticles, nanodiamonds, upconversion nanoparticles, blood-brain barrier, multifunctional nanoparticles

Introduction

Over the past few decades, there has been a rapid growth in nanoparticles (NPs) discovery and their use for medical therapy and diagnostics (theranostics) (Lim et al. 2015), where many NPs have been applied for research of neurodegenerative diseases and clinical neurological disorders. However, the successful utilization of NPs for many central nervous system (CNS) applications requires an effective crossing of the blood-brain barrier (BBB) (Zhang et al. 2016). The BBB is a protective cellular barrier between the bloodstream and the brain extracellular fluid that prevents the passage of undesirable molecules/material into the cerebrospinal fluid (CSF) (Abbott 2013; Weiss et al. 2009). Over many years, various strategies have been proposed to increase BBB penetration efficiency, including chemical modification of compounds to facilitate their membrane permeability, osmotic or chemical opening of the BBB, carrier- or receptor-mediated transcytosis and invasive procedures via surgical intervention (Banks 2016). While some of the approaches are somewhat successful, the overall success in clinically verified trials is less than 1% (Liu et al. 2013).

Several factors need to take into consideration to achieve an effective delivery of species across the BBB: the BBB should not be damaged, the delivery method should be controllable, non-toxic, and targeted to achieve a desired concentration and action (Jain 2012). There are a few NP-based paradigms, such as lipid or polymeric NPs (Jain 2012), that have shown potential in addressing the above factors, but their lack of intrinsic fluorescence limits their versatile application. To visualize and efficiently track these NPs, external fluorescent probes, such as organic dyes or fluorescent proteins, need to be separately introduced; but these have well-acknowledged limitations of photobleaching, lack of site of specificity, compartmentalisation in cellular vesicles and a mild toxicity (Fu et al. 2007).

Owing to their exceptional photoluminescence and biophysical properties (high specificity and the ability to non-invasively visualise molecules in living cells in whole animals), fluorescent NPs have emerged as a promising platform for a variety of biomedical applications (Chinen et al. 2015). The most prominent NPs that possess these qualities are fluorescent nanodiamonds (FNDs) (Chang 2010; McGuinness et al. 2011; Mochalin et al. 2012) and upconversion nanoparticles (UCNPs) (Chen et al. 2014; Lu et al. 2014; Zhao et al. 2013).

In this Perspective, we provide a short background on these two distinct fluorescent NPs, and summarize several studies that focused on the delivery of these NPs into the brain and their subsequent interaction with brain tissue. Furthermore, we discuss challenges and opportunities for further development of NPs-based therapies for targeted delivery of drugs across the BBB and for studying cellular processes in the brain.

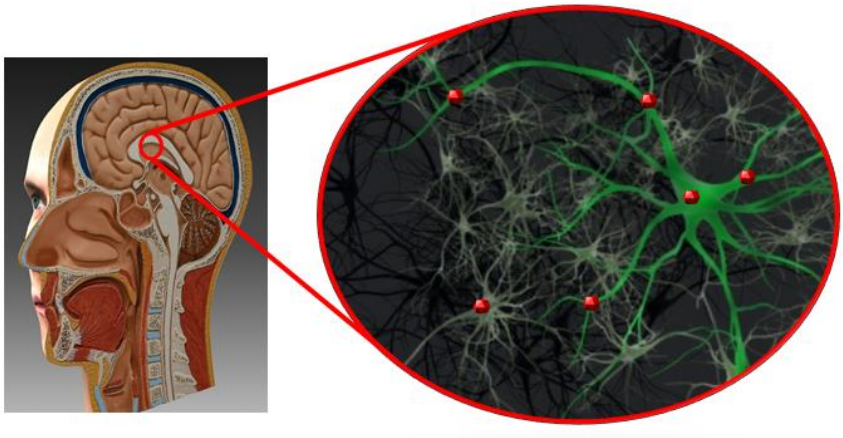


Figure 1. Delivery of fluorescent nanoparticles (red dots) to brain and neurons for studying cellular processes in the brain .

Application of nanodiamonds in brain

In the last decade, nanodiamonds (NDs) have attracted much interest from the biological community as the ultimate agent for biomedical applications, as their chemical inertness, biocompatibility, prolonged photo-stability and negligible toxicity, allow for potential biomarker, drug and gene delivery and biocatalyst applications (Aramesh et al. 2015b; Fu et al. 2007; Liu et al. 2007; Mochalin et al. 2012; Tong et al. 2014; Yu et al. 2005). The intrinsic fluorescence of NDs emanates from point defects (centres) that possess an unprecedented photo-stability, with emission at room temperature (Aharonovich et al. 2011; Yu et al. 2005). Moreover, colour centres emit light in red and infra-red spectra (wavelengths of more than 650 nm), avoiding spectral absorption in some cellular components to produce autofluorescence, and provide a large penetration depth through cells and tissues (Liu et al. 2007). An additional advantage of using NDs as a starting material is that they are made of carbon, which is an excellent candidate for surface modification for applications in biology or medicine (Aramesh et al. 2015a) as it can be readily modified with functional groups to attach biomolecules using standard organic chemistry procedures (Chang et al. 2010; Krueger and Lang 2012; Meinhardt et al. 2011; Vial et al. 2008). Therefore, NDs are a promising biomaterial platform as they unite a spectrum of unique physiochemical properties, enabling significantly improved capabilities in imaging and therapy.

In the context of the brain, FNDs have been extensively investigated in both *in vitro* and *in vivo* model systems. Specifically, FNDs can be internalized and exhibit no cytotoxicity in primary cortical neurons *in vitro* (Huang et al. 2014). Moreover, the intracranial injection of FNDs into rat hippocampi did not induce any behavioural changes in a standard memory task (the novel object recognition task) (Huang et al. 2014). Furthermore, FNDs have been tested with embryonic carcinoma stem (ECS) cells that are pluripotent and can be differentiated into neurons (Hsu et al. 2014). Researchers showed that FNDs can be successfully taken up into

ECS cells, had no toxicity and caused no disruption to cellular activity, such as cell growth, protein expression and neuronal differentiation, suggesting good biocompatibility. Additionally, due to the exceptional photostability of NDs it was possible to track NDs after seven days using confocal microscopy and flow cytometry.

To address targeting properties, NDs have been specifically and selectively targeted to glioma cells using a small peptide sequence (cRGD) (Slegerova et al. 2015) that specifically binds to the αvβ3 receptors overexpressed on glioma cells. The study proposed that the presence of the targeting moiety on the surface of NDs would facilitate their accumulation in solid glioblastoma due to the disrupted BBB in the area of brain tumour. Indeed, leveraging the background-free and stable fluorescence of FNDs, the researchers showed a high efficiency of NDs internalization into cells using confocal imaging.

The biodistribution of vaccine adjuvant (aluminum oxyhydroxide or alum) has been further investigated by conjugating with FNDs and then injecting them into the tibialis muscle of mice. This revealed that NPs were found within the brain 21 days post-injection (Eidi et al. 2015), demonstrating that FNDs allow for the long-term tracking and detection of the vaccine adjuvant in tissues even at very low concentrations. Furthermore, the presence of NDs in brain suggested that the particles are able to cross the BBB. Interestingly, the same group found that fluorescent polymer nanobeads could also be found in brain when injected into muscle, but were not present following intravenous injection (Khan et al. 2013). It was proposed that NPs accumulated in the brain via monocytes-assisted mechanisms, where monocytes become macrophages and dendritic cells at the site of injection and migrate to the draining lymph nodes that further distribute NPs across the body (Khan et al. 2013). This interesting finding advocates that one of the ways for NPs to passively cross the BBB is to be introduced via lymph nodes (Yokel 2016). Based on these findings, another study demonstrated a neuroprotective activity of NDs in a rodent model of Alzheimer's disease

(AD) (Alawdi et al. 2016). In this system, rats were fed NDs (15nm) together with aluminium ($AlCl_3$) to induce AD-like symptoms, and were compared to several control groups. After 6 weeks all the rats were sacrificed and histopathological changes in the brain were studied using multiple techniques. Importantly, rats that were supplied with NDs demonstrated a slowing in the progression of AD-like symptoms, likely driven by a reduced expression of the key AD-related proteins, BACE1, Aβ42, and phosphorylated-tau. The therapeutic effect of NDs was similar to that of memantine, an FDA-approved neuroprotective drug that has been shown to slow the progression of AD. Memantine has a similar structure to adamantine, the smallest structure of the diamonoids family that NDs belong too, and it has therapeutic effect *via* blocking immoderate stimulation of N-methyl-D-asparate (NMDA) receptors (Alawdi et al. 2016).

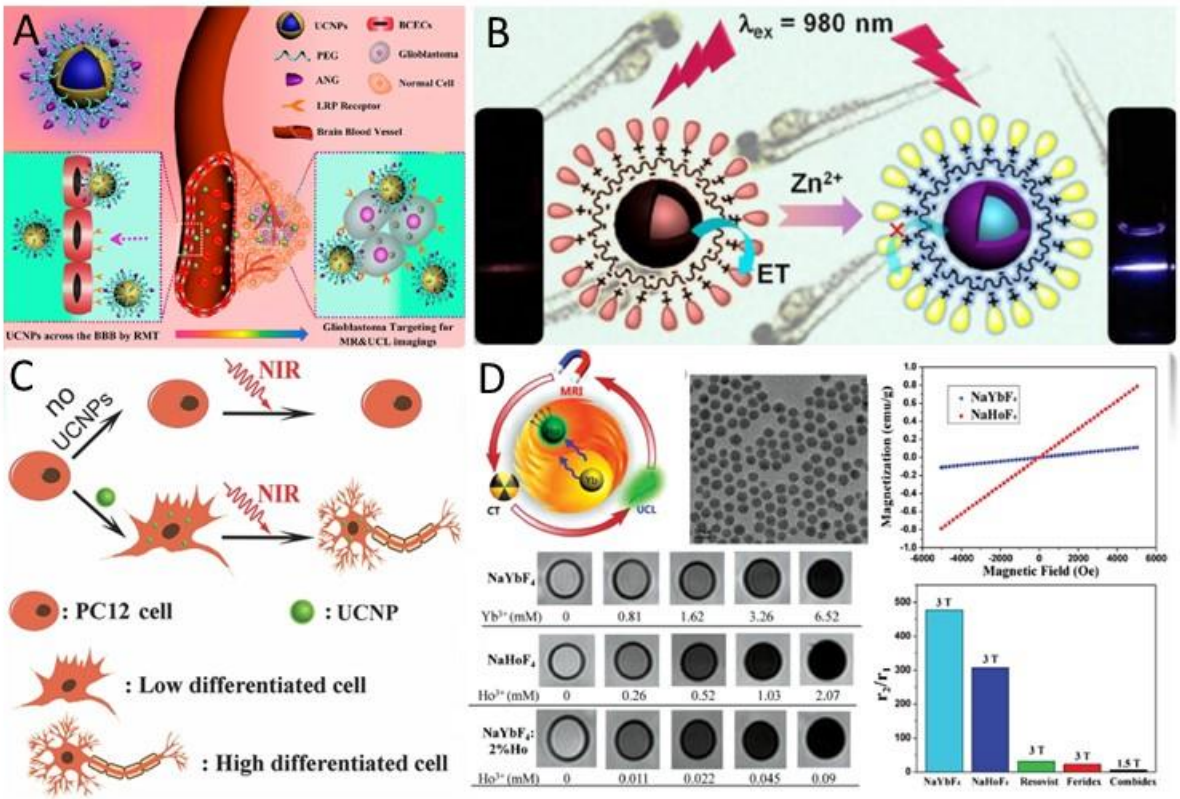
NDs have also been used to assist with drug delivery into brain tumours. Specifically, NDs have been conjugated with doxorubicin (DOX), a drug that is routinely used to treat various peripheral cancers but has not been used for brain cancer due to its inability to cross the BBB (Xi et al. 2013). Researchers directly introduced NDs-DOX conjugate into brain tumour via convection-enhanced delivery (CED) to surpass the BBB. Notably, they demonstrated that the efficacy of DOX was increased when paired with NDs as opposed to free DOX delivery via CED. Conjugation of DOX with NDs resulted in prolonged drug retention in the affected tissue site that led to a better cancer treatment.

Application of upconversion nanoparticles in brain

Upconversion nanoparticles (UCNPs) are the nanoscale crystals doped with trivalent lanthanide ions (Eu^{3+}, Tm^{3+}, Er^{3+}, etc) dispersed in dialectric host lattice. These unique nanocrystals feature in the capability of photon upconversion process, which relies on the unusual sequential absorption of two (or more) low-energy photons in near-infrared (NIR)

wavelength spectra to generate one high-energy photon in visible or ultra violet (UV) region. Importantly, UCNPs hold unique advantages, including tunable doping (different lanthanide ions) to achieve tunable emission, shape, size, lifetime and surfaces, which offers a promising toolbox to explore various biomedical applications in the CNS, such as enhancing brain uptake, diagnostics and multimodal therapy (Zhou et al. 2015).

Due to a unique magnetic resonance (MR) and upconversion luminescence (UCL) imaging feature of UCNPs, a new BBB and glioblastoma dual targeted brain nanoprobe has been successfully constructed by covalently coupling of targeting ligand Angiopep-2 (ANG) with the PEG-coated UCNPs (Ni et al. 2014b). Both cellular and animal experimental results demonstrated that the constructed nanosystem ANG/PEG-UCNPs can cross the BBB by receptor-mediated transcytosis and subsequently target glioblastoma efficiently. Moreover, the ANG/PEG-UCNPs show a great potential in pre-operative diagnosis and intraoperative positioning of the brain tumors by both MR and NIR-to-NIR fluorescence imaging (Fig. 2A).



UCL can be effectively quenched by the chromophores on the surface of NPs via a

Figure 2. The application of upconversion nanoparticles in brain. A: Targeted UCNPs for BBB crossing via receptor-mediated transcytosis, MR and NIR imaging of glioblastoma (Guan et al. 2014); B: The detection of Zn^{2+} ions using FRET process on UCNPs that has been tested in zebrafish (Peng et al. 2015); C: UCNPs promote neurite outgrowth (Guan et al. 2014). D: Multimodal theranostical UCNPs-based toolbox that has been simultaneously used for UCL, MR and computer tomography (CT) imaging in single UCNPs (Ni et al. 2014a).

fluorescence resonant energy transfer (FRET) process and subsequently recovered upon the addition of Zn^{2+}, thus allowing for quantitative monitoring of Zn^{2+} ions. Based on this, a Zn^{2+} fluorescent-based probe was developed by assembling lanthanide-doped UCNPs with chromophores to detect Zn^{2+} in the amyloid plaque in AD brain (Fig. 2B), providing a new opportunity for disease diagnosis associated with Zn^{2+} in further clinical medicine (Peng et al. 2015). The new nanoprobe was successfully tested *in vivo* with zebrafish and *ex vivo* in the brain slices of the triple transgenic knock-in mice with AD.

Moreover, it was reported that positively charged UCNPs (decorated with positive charged polymer – polyethylenimine (PEI)) can cross the BBB and promote neurite outgrowth due to the high positive surface charges of the UCNPs and NIR activity of UCNPs to promote the neurite outgrowth (Fig.2C)(Guan et al. 2014).

The application of UCNPs for multimodal diagnostics in the brain has been explored *via* new functional lanthanide ions doping or surface modification. For example, T_2 – weighted MR imaging using UCNPs was successfully demonstrated via Ho^{3+} doping. The developed functional NPs could also be simultaneously used for UCL and computer tomography (CT) imaging, thus enabling high performance multimodal MR/UCL/CT imaging in single UCNPs (Ni et al. 2014a). As demonstrated in various *in vitro*, *in vivo* and *ex vivo* experiments, this provides a new strategy for acquiring T_2 -MR/optical imaging without fluorescence quenching with benefit for accurate MR diagnosis of brain tumors (Fig.2D) amongst other potential applications. In other studies, MR/UCNPs were developed for dual-modality imaging of glioblastoma before surgery in brain *via* labeling UCNPs with Gd^{3+}-DOTA and

RGD (Jin et al. 2013). These multi-functional UCNP-Gd-RGD nanosystems showed targeting towards U87MG cells through its specific binding to integrin αvβ3 receptors on the cell surface. More importantly, UCNP-Gd-RGD exhibits a capability in delineating U87MG tumor boundary by MRI together with high-contrast upconversion fluorescence towards precision diagnostics before surgery in brain.

Outlook and future challenges

One of the most intriguing questions is what are the exact processes happening in the brain? To date, the key aspects to understand how biological systems in brain work at the molecular level is to probe biomolecules individually and observe how they interact with each other directly *in vivo*. This has been achieved using organic dyes, fluorescent proteins, and more recently quantum dots. However, detrimental properties of these probes, such as photobleaching, photoblinking, and some cytotoxicity, predictably restrained any prolonged imaging *in vitro* and especially *in vivo* (Yu et al. 2005).

Recent advances in fabrication and utilisation of fluorescence NPs for biomedical applications have generated exciting platforms for bio-imaging and drug delivery therapies, and in this perspective we have discussed a number of paradigms that have utilised fluorescent NPs in the brain. However, the application of fluorescence NPs for the study of brain processes is still in its infancy.

Clearly, there is a need for multidisciplinary research to leverage pivotal discoveries in biolabeling and neuroscience. One such approach is to develop multi-functional NPs that will combine the properties of BBB penetration, with excellent biocompatibility, on-demand imaging and targeted delivery of compounds. Multi-functionality will assist with mapping of NPs distribution and tracking their entry pathways into the deep tissue of the living brain, where high background noise is typically generated by blood circulation. Moreover, further

systematic study of whether the size, shape, and surface of multifunctional NPs will affect BBB penetration in similar fashion to that observed in other cell types is required. Another fundamental challenge to address in the research of NPs is to how to avoid particle clearance by the immune system, and how to target the delivery of nanoparticles (and controlled release of drugs) to specific cells or tissues in sufficient quantities for therapeutic efficacy.

In summary, cutting-edge developments in nanobiotechnology hold an optimism to overcome the rising challenges in medical sciences by effective engineering of multifunctional NPs.

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