REVIEW

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Toxicity and health impact of nanoparticles. Basic biology and clinical perspective

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Abstract

Stroke has limited restorative treatment options. In search of new therapeutic strategies for the ischemic brain, cell-based therapies offered new hope, which has been, in the meanwhile, converted into a more realistic approach recognizing difficulties related to unfavorable environments causing low survival rates of transplanted neuronal precursors. Stem cell therapies are based on the transplantation of neuronal precursor cells (NPCs), adult stem cells propagated in cell culture or inducible pluripotent cells (iPSCs) obtained from patients and trans-differentiated into neural cells. Of these, autologous iPSCs have the advantage to be used in stroke patients because they do not raise ethical concerns and the risk of graft rejection is low. However, the use of stem cells for stroke therapy in humans has to take into account many factors including, dosage, route of administration, toxicity and side effects. For example, nanoparticles (NPs) may increase the efficacy of drugs and therapeutic cells delivery to the diseased brain. Medication dosages are generally determined by clinical trials done in relatively young, healthy people. However, *in vivo* and clinical data evaluating the toxic effects of NPs on neural cells are still scarce especially in the aged brain, which has a decreased homeostatic capacity and a reduced ability to cope with internal and environmental stress, as compared to the young brain. Previous studies in rodents indicate that aging along with neurodegenerative diseases may promote a proinflammatory state and leads to the development of gliosis in the aged brains. On the other hand, the nonspecific interaction between the shell of NPs and brain proteins leads to the adsorption of opsonins on their surface, forming the so-called "corona", thereby becoming ideal candidates to attract phagocytic microglia resulting in NPs engulfment and thus exacerbating neuronal death. Therefore, when designing NPs for clinical use, it should be considered that their systemic administration is associated with potential risks, especially in the aged subjects. Recently, NPs have been shown in recent years to play a crucial role in cell signaling processes involved in stroke recovery. Extracellular vesicles (EVs) are secreted by virtually all type of cells in the body and have been shown to reflect the physiological and metabolic status of the host cells. Thus, understanding the disease-specific contents of EVs would enable the discovery of novel predictive biomarkers.

Keywords: nanomaterials, carriers, brain diseases, therapies, toxicity.

Stroke therapy

Cerebrovascular infarct (stroke) represents the second leading cause of death worldwide. Stroke has not only a very high mortality rate, but also results in debilitating sequelae including cognitive decline, dementia, neurological impairments or physical disability in survivors, all associated with huge economic costs. However, except recanalization therapy during the acute phase, no restorative treatments exist. In search of new therapeutic strategies to stimulate restorative processes in the post-stroke brain, cell-based therapies offered new hope which has been in the meanwhile, replaced by a more realistic approach recognizing difficulties related to unfavorable environments causing low survival rates of transplanted neuronal precursors, especially in the aged brains. It has meanwhile converted into a more balanced view recognizing impediments related to unfavorable environments that are in part related to aging processes. It is greatly hoped that transplantation of neural progenitors will provide effective therapies for neurological disorders, including stroke. At molecular level but also cytologically, the aged brain responds differently to injuries, as compared to the young-adult brain. However, post-stroke therapies using both neuronal precursor cells (NPCs) and bone marrow-derived mesenchymal stem cells (BM-MSCs) suggest that the aged rat brain might initiates plasticity and remodeling in response to cell-based therapy albeit with different intensity and kinetics. For example, the aged brains develop a fulminant inflammatory reaction leading to an accelerated progression of brain infarction. Likewise, endogenous neurogenesis is impaired and functional recovery is severely delayed, too. The low

efficacy of transplanted cell survival and compromised brain remodeling may also be related to age-associated comorbidities, including diabetes or hyperlipidemia, which create unfavorable environments for cell-based therapies. All these factors should be taken into account when considering the clinical translation of restorative therapies [1].

In this review, we discuss two major topics: (*i*) nanosafety, toxicity mechanisms, and (*ii*) effects on human health of manufactured nanomaterials (MNMs), with a particular focus on the aged brain.

Stroke and nanotherapeutics

A major roadblock for the use of drugs and therapeutic cells to the clinic is the availability of appropriate carriers to pass the blood–brain barrier (BBB). Nanoparticle (NP)-loaded drugs hold great promise to fulfill this gap. However, *in vivo* and clinical data evaluating the toxic effects of NPs on neural tissue are still scarce, especially in the aged brain, which has both a decreased homeostatic capacity and a reduced ability to cope with environmental stressors as compared to the young brains. Although body homeostasis may be maintained during resting conditions, the reduction of functional reserve is responsible for an increased vulnerability to drug treatments especially in patients with systemic co-morbidities. As number of medications in older patients increases, age-related changes in pharmacokinetics and pharmacodynamics, together with comorbidities make elderly patients vulnerable to adverse reactions to drugs, a condition which is aggravated by a poor compliance due to cognitive decline or depression [2].

Medication dosages are generally determined by clinical trials done in relatively young, healthy people. However, for clinical applications of NPs tailored for treating neurological disease, it will be vital to perform baseline studies in appropriately aged, comorbid subjects. The ageing process is characterized by structural and functional changes influencing physiological reserve, drug bioavailability, and pharmacodynamic responses. Liver and kidney are the major organs coping with drug processing and detoxification. However, hepatic drug clearance of several drugs decreases with aging, mainly due to reduced blood flow and hepatocyte mass [3]. Kidney function also declines with increasing age, mainly due to sclerotic changes in the glomeruli [2, 4]. Since drug distribution depends largely on body composition, changes in hepatic and renal function are the major determinants for an increase in the volume of distribution of lipid soluble drugs, reduced clearance of lipid soluble and water soluble drugs, respectively. All these factors modulate drugs bioavailability and may cause a prolongation of the half-life of drugs in plasma. Significant pharmacodynamic changes also occur with increasing age, which, in general, tend to increase vulnerability to drugs' toxicity. A reduced homeostatic capacity itself also leads to an increase in vulnerability by impairing systemic compensatory mechanisms [5–7].

Superparamagnetic iron oxide NPs (SPIONs)

There is great interest to develop drug-loaded multifunctional NPs to cross the BBB and deliver the load to the diseased brain [8–10]. The need for diagnostics based on NPs further expands the importance of toxicology across several classes of MNMs [11].

Superparamagnetic iron oxide NPs (SPIONs) are useful as magnetic resonance imaging (MRI) contrast agents for the central nervous system (CNS) and residence times in the tissue after extravasation may be days to weeks [12]. Another promising application of SPIONs in both preclinical models and humans is labeled-cell tracking, using MRI for *in vivo* visualization of transplanted cells [13, 14]. A myriad of conjugates have appeared in the literature combining SPIONs with molecular imaging probes for optical, MRI, positron-emission tomography (PET), single-photon emission computed tomography (SPECT), and histology. However, the toxicity and brain delivery of synthesized SPIONs that have shown utility for the combined application of receptor-targeted delivery of drugs, contrast signal MRI, and fluorescence imaging has been not studied in detail [15].

Gold NPs

Gold (Au) NPs have unique features that are contributing to the development of new therapies for brain-related disorders [16, 17]. Chemical tuning of the NPs surface through conjugation with specific biomolecules has ensured biological compatibility and specificity to Au nanocarriers to reach brain regions. PEGylated (polyethylene glycol) Au nanorods conjugated with an arginine–glycine–aspartic acid (Arg–Gly–Asp) sequence, a peptide that specifically binds to $\alpha v\beta 3$ integrins, has shown an excellent tumor targeting ability in glioblastoma (GBM) models [18]. Several approaches have appeared in the literature and shown that suitably functionalized AuNPs are promising systems to deliver drugs to the CNS. For example, the delivery of small interfering ribonucleic acid (siRNA)-decorated AuNPs were able to knock down oncogenes overexpressed in GBM [19], while AuNPs conjugated to β-amyloid therapeutic peptides were used as drug carrier targeting transferring receptor [20]. We will compare the toxicity and brain delivery of AuNPs synthesized for the purpose of this project to provide information about influence of the new chemical functionalities on NPs surfaces and size/shape cores.

Silver NPs

Silver (Ag) NPs are well known for their antibacterial/antimicrobial properties and are a value-improving component of many consumer products, including clothing and coatings [21, 22]. Perhaps more importantly, Ag topical creams are used in wound/burn care and on catheters to limit infections. Future applications of Ag antimicrobials *in vivo* would include drug delivery (for sepsis), Ag coatings on implants, or *in vivo* sensors, and will likely be dependent on redox homeostasis in the host body affected by Ag^+ ions released from the metal. Characterization of AgNPs toxicity in the body is incompletely understood. Safety has been shown for topical application (skin, eyes) but effects on the brain could include oxidative stress resulting in misfolded proteins, apoptosis, to a degree that may differ between the young and aged brain.

Silica NPs

Silica NPs have received strong interest in nanomedicine because they are chemically inert, can be structured on

the nanoscale with tailored pores, are easily modified through chemistry, and have breakdown products that can be renally cleared (silicic acid). A wide range of silica MNMs have been generated for preclinical studies and an injectable product has recently entered clinical trials [23, 24]. Porous silicon (Si) is a promising MNM with a wide array of applications, from drug delivery (*e.g.*, in the eye) to integration as a biodegradable optical sensor. The size of the pores possible with this material is controllable, and is also larger than for mesoporous silica [25]. Si surface chemistry is similar to silica due to oxidation of Si in water. *In vitro* toxicity of Si has been reported as safe [26].

Clinical perspective

Synthetic materials that interact with biological systems to repair or treat tissues have complex performance requirements and must meet strict regulations. To meet these challenges, in the national and worldwide therapeutics level, a consolidated framework should exist between academic, government, and industry laboratories around the world. Our focus in this proposal is to forge a partnership between academic laboratories.

Many potential therapies have failed in recent years, in part due to problems with directing, moving and/or targeting materials within injury sites, and inholding the drug and releasing it over sufficient time to allow protection and/or tissue recovery. The long-term residence of the remnant material in cells and tissues, and the potential activation of stress pathways, is an important aspect for next-generation materials. Currently, the focus is on developing drug-loaded multifunctional NPs with the ability to cross the BBB and deliver the load to the diseased brain. Inside the brain, NPs may increase the efficacy of drugs or therapeutic cells by a controlled release of the load using of linkers sensitive to hypoxia/pH. However, *in vivo* and clinical data evaluating the toxic effects of NPs on neural cells are still scarce, especially in the aged brain, which has both a decreased homeostatic capacity and a reduced ability to cope with internal and environmental stress as compared to young brains.

Medication dosages are generally narrowed in clinical trials, which are done in relatively young, healthy people. However, for clinical applications of NPs tailored for treating neurological disease, it will be vital to perform baseline studies in appropriately aged subjects. Design and studies of the consortium will follow a clear stepwise approach with the goal to translate the results of the experimental studies into the clinical situation. The presented approach follows closely the recommendations of the *Stroke Academic and Industry Round Table* [27] for developing future stroke treatment. The rationale for these recommendations is to establish a maximum of preclinical evidence before any application undergoes further testing in the patient. This is particularly the case for potentially dangerous treatment approaches, such as cell therapy. Experiments using animal models shall focuses on a strict translational design with the goal to answer in the experiment questions, such as the beneficial role of NPs, subtoxic dose, underlying mechanisms time window, as well as robustness of the effect in several animal models as well on functional neurological outcome assessed by different laboratories. The perspective is clearly to build the basis for conduction of multicenter Phase II clinical trials, which then would focus on establishment of safety of the planned therapy in stroke patients.

NPs and their medical applications

Most of the medical applications of NPs are in the field of cancer therapy. NPs can be used as smart drugs to attack specific antigens present on cells that present a threat for the human body like cancer cells, by attaching immunotoxins on their surface using the antibody part to attach to proteins on cell membrane (Figure 1A). Magnetic particles have also been used to carry drugs to malignant cells using magnetic fields. Upon reaching the target tissue or therapeutic target, the drugs are released by enzymatic activity of the target cell (Figure 1B). A common application of nanocomposite hydrogel system has been used for drug delivery in a thermotherapeutic process involving drug release from AuNPs upon irradiation with infrared light (Figure 1C). Then, NPs can be used to detect cancer cells and viruses that threaten our health by covering the NPs with monoclonal antibodies directed against growth factor receptors that are commonly found on cancer cells. Colors are reflected when light of a specific wavelength hits the NPs (Figure 1D). Another imaging application of NPs is to detect angiogenesis in malignant tissues by tagging cellular proteins with AuNPs, injecting the AuNPs and detection of target tissue using computed tomography (CT). Clinical CT contrasting agents are often iodine-containing small molecules, but these usually suffer from short half-lives in the circulation and severe toxicity. One way to avoid these complications is to incorporate large atoms like iodine or Au into an NP structure that is more stable and has a longer lifetime in the circulation, which result in targeting a tumor site with an enhanced efficiency (Figure 1E).

NPs in diagnostics

Ischemic stroke is a leading cause of disability and death in adults, with 613 148 new events in Europe in 2015. Unfortunately, causative treatment [intravenous recombinant tissue plasminogen activator (rt-PA) and/or mechanical thrombectomy] is available only for 10% of patients. What is more, this treatment is effective only in 25% of them. For stroke survivals, secondary stroke prevention and long-term rehabilitation are the only available and scientifically proved interventions. Ischemic stroke causes several general and neurological chronic complications, among others physical disability in basic activities of daily living (26%), reduced mobility due to hemiparesis (50%), depression (30%), cognitive decline or dementia (50%) and finally, decreased quality of life in different aspects of daily living [28]. There is little knowledge on biomarkers that can predict different sequel of stroke outcome. Finding such biomarkers could predict individual course of the disease and introduce specific preventive or therapeutic strategies.

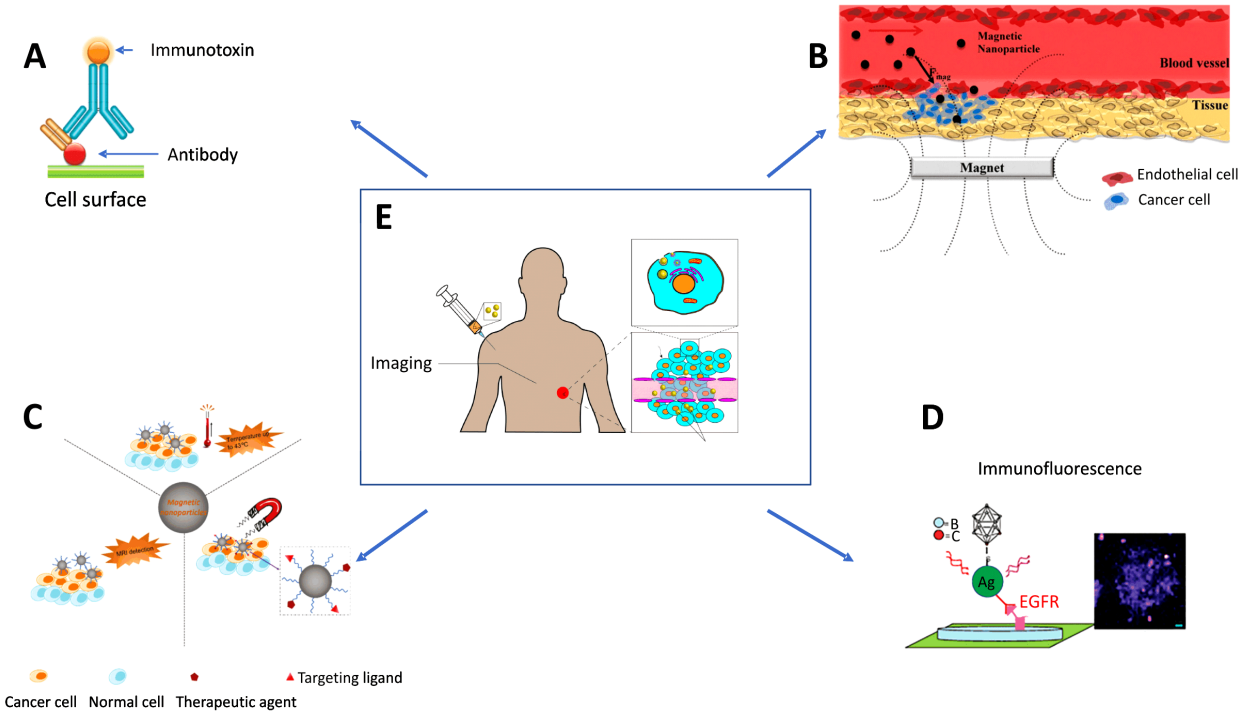

Figure 1 – ***(A–E) A schematic drawing showing biomedical applications of nanoparticles. Ag: Silver; EGFR: Epidermal growth factor receptor; F_{mag}: Magnetic force.***

Ischemic stroke is an acute disease, which often results in severe long-term consequences, such as physical disability, depression, cognitive decline or even dementia. To date, patients at risk for these late consequences of stroke are not duly diagnosed and treated due to the lack of reliable biomarkers. Previous work has shown that miR21 and miR223 are hypoxia inducible and secreted into extracellular vesicles (EVs) both in stroke models and stroke patients that were significantly associated with clinical outcome (measured by modified Rankin Scale – mRS) in post-stroke patients at 90 days [29, 30]. In addition, several *Genome Wide Association* studies have found genetic polymorphisms associated with stroke recovery [28, 31, 32].

EVs have been shown in recent years to play a crucial role in cell signaling processes involved in stroke recovery. EVs are 40–1000 nm nanovesicles that are released from many cell types into the extracellular space. EVs are widely distributed in various body fluids and can readily cross the BBB, a major target to drugs intended to reach the brain. The biogenesis of EV production starts from the plasma membrane invagination, which then further form inward buds forming so-called multivesicular bodies. During this process, molecules such as messenger RNAs (mRNAs), microRNAs (miRNAs), proteins and metabolites are selectively packed into the EVs. Indeed, there is active sorting mechanism of EV molecules, since the, *e.g.*, miRNA profiles of EVs may differ from those of the parent cells. Thus, EVs cannot only transfer biological messages between the cells in the body, but they also represent the physiological status of their parent cells, which makes EVs potential biomarker candidates. Indeed, published literature has described that neuronal derived EVs can be isolated from plasma samples of patient and harness specific disease-related proteins, such as β-amyloid and phosphorylated forms of *tau* in Alzheimer's disease patients.

NPs have been shown in recent years to play a crucial role in cell signaling processes involved in stroke recovery. EVs are secreted by virtually all type of cells in the body and have been shown to reflect the physiological and metabolic status of the host cells. EVs released from the brain cells entering the blood stream, have been shown to carry some disease-causing toxic proteins, such as specific miRNA upon ischemic stroke, however, detailed protein content of exosomes is completely unknown. In recent years, EVs containing miRNAs have been shown to be involved in cell signaling processes triggered by stroke [28, 33–35]. Thus, understanding the disease-specific contents of EVs would enable the discovery of novel predictive biomarkers.

The medical applications of NPs have been recently summarized by several publications [36–39].

Conclusions

NPs may increase the efficacy of drugs and therapeutic cells to the diseased brain. However, *in vivo* and clinical data evaluating the toxic effects of NPs on neural cells are still scarce especially in the aged brain, which has a decreased homeostatic capacity and a reduced ability to cope with internal and environmental stress as compared to the young brains. Moreover, the nonspecific interaction between the shell of NPs and brain proteins leads to the adsorption of opsonins on their surface, forming the so-called "corona", thereby becoming ideal candidates to attract phagocytic microglia leading to NPs engulfment and thus exacerbating neuronal death. Therefore, when designing NPs for clinical trials, it should be kept in mind that their systemic administration generates adverse reactions, in particular in the aged subjects. Recently,

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Conflict of interests

The authors have no conflict of interests to declare.

Authors' contribution

All authors have made substantial, direct and intellectual contribution to the work, and approved it for publication.

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