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Nanotheragnostic Applications for Ischemic and Hemorrhagic Strokes: Improved Delivery for a Better Prognosis

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Abstract Stroke is the second leading cause of death worldwide and a major cause of long-term severe disability representing a global health burden and one of the highly researched medical conditions. Nanostructured material synthesis and engineering have been recently developed and have been largely integrated into many fields including medicine. Recent studies have shown that nanoparticles might be a valuable tool in stroke. Different types, shapes, and sizes of nanoparticles have been used for molecular/biomarker profiling and imaging to help in early diagnosis and prevention of stroke and for drug/RNA delivery for improved treatment and neuroprotection. However, these promising applications have limitations, including cytotoxicity, which hindered their adoption into clinical use. Future research is warranted to fully develop and effectively and safely translate nanoparticles for stroke diagnosis and treatment into the clinic. This work will discuss the emerging role of nanotheragnostics in stroke diagnosis and treatment applications.

Keywords Stroke · Nanoparticles · Neuroprotection · Drug delivery · Imaging · Biomarkers

Abbreviations

NP	Nanoparticle
SAH	Subarachnoid hemorrhage
CT	Computed tomography
MRI	Magnetic resonance imaging
DWI	Diffusion-weighted imaging
BBB	Blood-brain barrier
CNS	Central nervous system
SERS	Surface-enhanced Raman scattering
PTT	Photothermal therapy

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tPA	Tissue plasminogen activator
ROS	Reactive oxygen species
HHAuNPs	Hyaluronic acids (HA) immobilized on gold nanoparticles
MCAO	Middle cerebral artery occlusion
USPIO	Ultrasmall superparamagnetic particles of iron oxide
ELISA	Enzyme-linked immunosorbent assays
SR-PCT	Synchrotron radiation X-ray phase computed tomography
MPI	Magnetic particle imaging
RBCs	Red blood cells
HO@MNPs	Magnetic nanoparticles containing simple hydroxy groups
PtPFPP	Pt(II)-tetrakis(pentafluorophenyl) porphyrin
PA1	Dye
PA2	Poly(9,9-diheptylfluo-rene-alt-9,9-di-p-tolyl-9H-fluorene)
SPION	Superparamagnetic iron oxide nanoparticle
QD	Quantum dot
FA	Folic acid
SPIO	Superparamagnetic ion oxide
BrdU	Bromodeoxyuridine
NSC	Neural stem cell
PUE	Puerarin
PBCN	Poly(butylcyanoacrylate) nanoparticles
shRNA	Short hairpin RNA
PEG	Poly(ethylene-co-glycol)
DGL	Dermorphin-PEG-dendigraft poly-L-lysine
Ask1	Apoptosis signal-regulating kinase 1
RNAi	RNA interference
PLGA-b-PEG	Poly-(lactide-co-glycolide)-polyethyleneglycol nanoparticles
T3	Triiodothyronine
CBSA-PEG-TIIA-NPs	Cationic bovine serum albumin-conjugated tanshinone IIA PEGylated nanoparticles
TIIA	Tanshinone IIA
MPO	Myeloperoxidase
TNF-α	Tumor necrosis alpha
IL-1β	Interleukin 1 beta
IL-6	Interleukin 6
IL-10	Interleukin 10
TGF-β1	Transforming growth factor beta 1
iNOS	Inducible nitric oxide synthase
PPARγ	Enhanced peroxisome proliferator-activated receptor gamma
PLNs	PEGylated-lipid nanoparticles
MRgFUS	MRI-guided focused ultrasound
BNPs	Brain-penetrating nanoparticles
PNIPAM	Polymeric N-isopropyl acryl amide
DMC	Demethoxycurcumin
BDMC	Bisdemethoxycurcumin
SOD	Superoxide dismutase
H_2O_2	Hydrogen peroxide
QC	Quercetin
EGF	Epidermal growth factor
PCBs	Polychlorinated biphenyls
TLR4	Toll-like receptor 4
TRAF6	Tumor necrosis factor-associated factor 6

Introduction

Stroke represents a major health and socioeconomic problem throughout the world [1]. Every 45 s, someone in America has a stroke, and every 3 min, someone dies of a stroke [2]. Stroke also constitutes the second leading cause of death worldwide and is likely to continue as the most prevalent cause of disability [1].

Traditionally, stroke has been classified into two major categories: ischemic stroke (IS) (about 80 % in white populations) and hemorrhagic stroke (HS) (about 20 %). The latter, in turn, is divided in two subcategories: primary intracerebral hemorrhage (ICH) (about 15 %) and subarachnoid hemorrhage (SAH) (about 5 %) [3]. IS is the sudden cessation of blood circulation to one or more areas of the brain, leading to hypoxia, malnutrition, inflammation, edema, and the buildup of toxic substances. As a result, neuronal cell death and subsequent neurologic deficits occur in survivors [4]. ICH is characterized by sudden intracranial rupture and bleeding into the brain parenchyma that can be caused either by trauma or an underlying medical condition (e.g., hypertension). Regardless of the cause, the rupture of a blood vessel inside the brain causes a disruption of blood supply to the associated vascular territory. Continuous bleeding in hemorrhagic stroke also leads to the formation of a growing hematoma that can further cause mechanical damage, in addition to the one already caused by a dysfunctional circulation [5, 6]. SAH occurs when blood leaks into the subarachnoid space, and it is usually the result of ruptured aneurysms and vascular malformations. SAH is a medical emergency, and although it can be recognized and treated at an early stage, it is associated with a high mortality rate and severe disability [7, 8].

To date, brain imaging including, computed tomography (CT) and magnetic resonance imaging (MRI), is required for the reliable differentiation between cerebral ischemia and ICH in acute stroke [9]. CT, the most common imaging modality used, may not be sensitive enough to detect acute ischemia [10] with less than a third of patients with IS presenting characteristics from CT findings within 3 h of symptom onset [11]. As patients who present to the emergency room with stroke-like symptoms might have a number of other nonvascular diseases including epileptic seizures, mass lesions, and migraine, this represents a significant limitation for an accurate diagnosis and

the administration of thrombolysis. On the other hand, MRI, especially with diffusion-weighted imaging (DWI), offers advantages for the assessment of acute stroke [11–13]. However, the sensitivity of MRI to detect an IS within 3 h of symptom onset is around 70 % [11], and even with DWI, some infarcts do not appear for several days and some never become visible [10]. Furthermore, MRI cannot be used in patients with pacemakers and is also difficult for people with claustrophobia [14]. Therefore, the diagnosis of IS is still a challenge particularly in the critical early time period, and researchers have undertaken great efforts in investigating a way to increase the diagnostic accuracy of IS as this would improve management and facilitate a more reliable, timely delivery of stroke therapies.

Unlike other organs in the body, the brain is segregated from the rest of the body by the blood-brain barrier (BBB), which limits the delivery of diagnostic and therapeutic agents to the central nervous system (CNS), including imaging contrast agents, macromolecular compounds, nucleic acids, and proteins [15, 16]. The limitation in the BBB's selective permeability has a strong impact on the development of safe and efficacious diagnostic modalities and treatment. Thus, the need to deliver biologically active agents across the BBB safely and effectively remains to be the focus of research efforts.

Recently, the use of nanostructured material synthesis and engineering has been developed and largely integrated into many fields including nanoelectronics, biology, photonics, nanoproteomics, and medicine [17, 18]. In particular, nanomedicine is an application of nanotechnology which is wildly used for drug delivery, bioimaging, diagnosis, and therapy via nanoscale devices [19, 20]. Nanotechnology and serum biomarker testing have the potential to serve as valuable adjuncts to routine clinical examination and imaging data in the area of stroke research, via the development of nanoparticles (NPs). Nanoparticles have been investigated in the fields of biomarker identification and drug delivery, which later on contributed to the development of the field of nanotheragnostics (i.e., therapeutic and diagnostic abilities). Nanotheragnostics can be applied to noninvasively discover and target markers and deliver treatments based on biomarker distribution, thereby holding great promise for changes that will impact future management of stroke patients [21, 22].

The aim of this review is to provide an overview of the rapidly growing field of nanotheragnostics for the diagnosis and treatment of stroke. We focus on selected studies that appear to be of particular interest, providing a guide to the implementation of nanotechnologies in clinical practice. Furthermore, in this review, we provide an insight on the major types of NPs (metallic, magnetic, and polymeric) and discuss nanotheragnostic applications, including molecular profiling and biomarkers studies, targeted imaging, and drug delivery in different models of stroke. Moreover, a summary of the most relevant studies discussed in this review is found in Table 1.

Nanoparticles

Nanoparticles adopted in the field of biotechnology usually vary in size from around 1 nm to around 500 nm, rarely surpassing the 700 nm limit. Specific criteria, such as biological stability and selectivity to certain areas or cells, need to be met, before the nanoparticles are fit for use. To achieve this targeted effect, NPs can be conjugated to a specific ligand. They can also be loaded with therapeutic agents or imaging materials in order to achieve targeted therapy, enhanced imaging, or both. There is a variety of NPs including metallic, magnetic, and polymeric nanoparticles, which are discussed below.

Metallic and Magnetic Nanoparticles

Metallic NPs have been used as theragnostic tools due to their minute sizes that are usually below 100 nm. This characteristic facilitates their uptake by target cells and their passage across the BBB, making them excellent cargos for drug delivery and imaging [38, 39]. In addition, metallic NPs have been manipulated in order to gain neutrality in charge, making them practically invisible to the immune system [40]. These particles are also used in thermotherapy, a new modality for cancer treatment. This intervention exposes the body, or even the targeted area, to high temperatures causing death of cancer cells with minimal damage to the normal cells [41].

There are two types of metallic NPs; the first type is the magnetic NP, which is mainly established via iron oxides like magnetite or maghemite. These nanoparticles are widely accepted as safe and stable biomedical particles, which have a small size of 10 nm or less [39]. They exhibit superparamagnetic properties, which allow them to be magnetized in the presence of a magnetic field and neutral in its absence [39]. This feature is very important in reducing the risk of thrombosis that can be caused by these nanoparticles themselves, when injected intravenously.

Another type of widely discussed metallic nanoparticles is gold and silver nanoparticles, along with nanoshells and nanocages. Gold NPs possess properties that are useful in the fields of imaging and analysis such as plasmon resonance, light scattering, and absorption. Their surface plasmon scatters the light in both a radiative and a nonradiative manner as scattered light or heat, respectively [42]. They have an intense red color with sizes below 100 nm and a yellowish color with sizes exceeding 100 nm [42].

Applications of all NPs, even gold NPs, vary with their shape. Rod-shaped NPs have two oscillating resonances, one along the short axis and one along the long one resulting in

Table 1 A list of studies on application of nanoparticles in different models of stroke with a summary of their experimental procedure and results along with the characteristics of the nanoparticle they used and they type of injury they studied

Nanoparticle type	Nanoparticle zeta potential (mV)	Nanoparticle size (nm)	Injury type	Model	Experiment	Results	References
DGL-PEG-dermorphin/shRNA	10.31±2.29	104±12	Cerebral ischemia reperfusion	ICR mice	Nanoparticles injected into the tail vein accumulated in the brain and gene transfection of the brain was successful	Efficient neuroprotection was attained via Ask1 suppression, thus apoptosis and infarct suppression	An et al. [23•]
Gold	–	20	Ischemic stroke	MCAO rats	Hyaluronic acid-labeled nanoparticles were injected before MCAO and were found to accumulate in the ischemic regions	These nanoparticles provide a good strategy for detecting ROS production and monitoring their levels and thus identifying ischemic or infarcted regions	Hyun et al. [24•]
Liposome	0.7±0.7	100	Ischemic stroke	MCAO rats	Injected liposomes accumulated in ischemic cerebral regions and the penumbra of rats where perfusion was disrupted	Liposomes provide a fast and effective drug delivery system for patients with ischemic stroke	Ishii et al. [25]
Gold	34.48±1	20	Ischemic stroke	C57BL/6 mice	After forming a thrombus in the left distal common carotid artery, glycol chitosan gold nanoparticles were injected 30 min to 2 h later	The nanoparticles accumulated in the thrombus allowing CT monitoring of the presence, recurrences, and evolution of thrombi and the efficacy of thrombolytic therapy	Kim et al. [26•]
Iron oxide	–	40	Thromboplastin-induced pulmonary embolism	Mice	Following embolism formation, nanoparticles conjugated to thrombin-sensitive peptide was injected into mice	Once the nanoparticles encountered thrombin, it cleaved off the synthetic peptide and appeared in the host's urine and thus is a biomarker for the presence of thrombotic events in the host	Lin et al. [27•]
PEGylated lipid	19.6±1.4	118±14	Ischemic stroke	SD rats	Injected cationic bovine serum albumin-conjugated tanshinone IIA nanoparticles increased circulation time, plasma concentration, and BBB permeability of tanshinone IIA (treats cerebral ischemia)	Tanshinone IIA nanoparticles modulated the inflammatory cascade, thus decreasing severity of infarct spread, neurological deficit, and histopathology	Liu et al. [28]
PEGylated lipid	–	60.97±7.95	Ischemic stroke	MCAO mice	Nanoparticles conjugated to Fas ligand antibody and encapsulating 3-n-butylphthalide were i.v. injected and they accumulated in OX42-positive microglia in the ischemic part of the brain	Nanoparticles conjugated to Fas ligand antibody were more efficient than the nonconjugated, and there was improvement in brain injury and neurological deficit and they required a reduced dosage of 3-n-butylphthalide compared to normal administration of 3-n-butylphthalide	Lu et al. [29•]
USPIO	–	20–50	Ischemic stroke	Permanent MCAO mice	Following permanent MCAO, mice received 3 dosages of the antibiotic minocycline followed by USPIO administration 5 h later and MRI performed before and after 24 and 48 h of surgery	Phagocytic cells internalized USPIOs and these iron-labeled cells emitted strong MR signals and they showed a positive effect of minocycline in decreasing infarct size and BBB permeability	Marinescu et al. [30]
USPIO	–	20–50	Ischemic stroke	Permanent MCAO mice	Following permanent MCAO, mice were injected with different concentrations of USPIOs and imaged via MRI and SR-PCT	SR-PCT detected iron in nanomolar concentrations and was superior to MR imaging in detecting hyperintense areas and macrophages that do not appear on MRI	Marinescu et al. [31•]
PLGA-PEG	−6.51 and −1.70	50–100	Ischemic stroke	MCAO mice	Nanoparticles labeled with thyroid hormone (T3) were injected into the jugular veins following MCAO	There was a greater decrease in brain infarction and edema when using T3-labeled nanoparticles than normal drug delivery	Mdzinarishvili et al. [32]
SPIO	–	50–60	–	Crl:CD1 (ICR) mice	Mice received injections of SPIO-loaded RBCs followed by MPI and MRI 3 and 24 h post-injection	It was possible to track down SPIO-loaded RBCs in the blood pool of mice several hours after injection, and this could be	Rahmer et al. [33•]

Table 1 (continued)

Nanoparticle type	Nanoparticle zeta potential (mV)	Nanoparticle size (nm)	Injury type	Model	Experiment	Results	References
						used later for 3D vessel visualization and bleeding monitoring	
Superparamagnetic iron oxide (fluidMAG)	1±6	50–200	Vascular injury	New Zealand white rabbits	FluidMAG-labeled MSC were injected into injured femoral arteries of rabbits, and reendothelization was higher in the femoral artery of the leg that was surrounded by a magnet for 40 min	FluidMAG-labeled MSC decreases restenosis and increases reendothelization in vascular injury; this could be implicated in cerebral vascular injuries as well	Riegler et al. [34]
PLGA	–	800	Ischemic stroke	C57BL/6 mice	EGF-PEG in PLGA nanoparticles and EPO in biphasic microparticles were placed in hyaluronan methylcellulose hydrogel and injected 4 days postischemic stroke	The hydrogel attenuated the brain inflammatory response, while EGF and EPO stimulated the endogenous NSC and thus brain tissue regeneration	Wang et al. [35•]
Polymerosomes loaded with superparamagnetic iron oxide	+17.8	162±3	Acute cerebral infarction	SD rats	Neural stem cells labeled with polymerosomes were injected contralateral to the ischemic hemisphere and MR and fluorescence imaging was performed	Distribution and migration of grafted stem cells can be monitored for 6 weeks via MR and 4 weeks via fluorescence imaging	Wen et al. [36•]
Poly(butylcyanoacrylate)	−7.72	201.2	Cerebral ischemia reperfusion	Kunming mice and Wistar rats	After i.v. administration of puerarin-loaded nanoparticles, drug concentration was higher in the blood and brain compared to free drug administration	Infarct volume and neurological deficit decreased and a lower dose of puerarin was required which is better due to its toxicity	Zhao et al. [37]

two absorption peaks [43, 44]. Gold NP surfaces have strong affinity to proteins, oligonucleotides, and certain antibodies with functional groups like amines, thiols, mercaptans, and phosphines [45]. In imaging applications, gold NPs have been utilized in a number of applications such as dark field imaging, optical imaging for specific pathologies, and surface-enhanced Raman scattering (SERS) [46]. Furthermore, one characteristic of gold NPs is the ability to use them for photothermal therapy (PTT), which can be induced by infrared light, creating vibrational energy and heat which in turn kill unwanted cells. A limitation to these NPs is that they cannot be used with imaging methods of higher resolution, such as MRI, and the fact that the shapes of the nanorods cannot be reproduced [42]. Methods of production of gold NPs vary according to the desired size where reduction of gold salts produces particles ranging from 10 to 20 nm while seeding produces particles between 30 and 100 nm.

Along the same line, silver NPs vary in size from 1 to 100 nm and have been shown to be very useful in wound treatment [47–49]. Their surface plasmon makes them ideal for efficient scattering and use as molecular labeling agents [50]. Traditional production of these particles is also done by chemical reduction of silver salts, while newer techniques utilize β-D-glucose in the process of reduction [51].

Nanoshells and nanocages have been invented in order to overcome the limitations imposed by gold NPs. Silica-gold nanoshells' resonance falls in the range near that of infrared (800–1300 nm), which is important as this range is rarely absorbed by biomatter [52–54]. The uses of these newly developed nanoshells are many, such as PTT and complete blood immunoassays. Further development of the nanoshells resulted in the invention of magnetic gold nanoshells allowing MRI, PTT, and drug delivery. Gold nanocages, a further development of nanoshells, are being utilized in drug delivery and controlled release, as their hollow cores facilitate these functions. They have also been found to be able to host a NP inside their hollow core whether magnetic or metallic, thus, expanding their usability in the fields of imaging and therapy.

Polymeric Nanoparticles

A second category of NPs used in the field of theragnostics is the polymeric nanoparticles. These NPs range in size from 1 to 1000 nm [55]. Made from both biodegradable and biocompatible material, polymeric NPs are composed of two main categories of polymers. The first, natural hydrophilic polymeric, includes polymers such as proteins and polysaccharides. The second, synthetic hydrophobic, includes polymers such as polyesters and poly-cyanoacrylates. The method of preparation of these NPs yields two types of polymeric

nanoparticles: nanospheres and nanocapsules [56]. In nanospheres, drugs are distributed throughout the matrix, while in nanocapsules, they are localized either in the inner core or on the surface [56]. These particles can be prepared by an array of methods, most prominent of which are ionic gelation [57], nanoprecipitation [58, 59], emulsion cross-linking [60], spray drying [61], and salting out [62]. Drug loading procedures, however, include incorporation or incubation [63]. Limitations that arise with polymeric NPs include particle-to-particle aggregation, high cost, sophisticated skills required in the process of manufacturing, and low flexibility in dose adjustment.

Nanotheragnostic Applications for Stroke Diagnosis and Management

Differential Diagnosis of Intracerebral Hemorrhagic and Ischemic Stroke

Today, most clinicians base their actions and decisions regarding stroke management on the patient's past medical history and physical examination, followed by neuroimaging, usually a CT scan, which is the current standard method to assess patients with suspected stroke [12]. However, as discussed above, since the clot is usually of similar density to that of the surrounding blood, noncontrast CT is insufficiently sensitive for the diagnosis of acute ischemia. Therefore, it is difficult to discriminate patients experiencing an ischemic stroke from patients manifesting benign neurological symptoms mimicking ischemic stroke (e.g., migraine, hypoglycemia, brain neoplasms, and transient ischemic attack), stressing the need for a constant search for an ideal contrast.

Kim et al. were the first to investigate whether the administration of intravenous gold NPs can help in visualizing an acute thrombus using CT imaging [26•]. Gold NPs are considered to be biocompatible with a considerable level of stability (i.e., no aggregation) [64–68]. The experiment of Kim et al. on mice using micro CT scanning demonstrated that gold NPs gather inside the thrombi, making the visualization possible and allowing quantitative assessment and chronological monitoring. Additionally, tissue plasminogen activator (tPA)-mediated thrombolysis monitoring was enabled, giving a substantial advantage and, allowing a prognostic ability post-tPA treatment [26•]. This pioneer experiment supports the potential utility and advances in the use of nanoparticles applied to brain imaging that can help clinicians override the limitations of normal imaging techniques, reducing side effects and dramatically enhancing the detection of thrombi.

Molecular Profiling and Biomarkers

Apoptotic and/or necrotic cell death observed following brain damage in ischemic stroke is in part due to reactive oxygen species (ROS) secretion, which induces the release of pro-inflammatory cytokines [69, 70]. Therefore, early detection of ROS levels in the brain can be indicative of the severity and extent of the injury, making way for immediate intervention and stroke management [71]. Hyun et al. successfully used fluorescein-labeled hyaluronic acids (HA) immobilized on gold nanoparticles (HHAuNPs), to detect ROS in a middle cerebral artery occlusion (MCAO) rat model; the injections were done 1 h prior to MCAO or 6, 12, 24, and 36 h following MCAO [24•]. Indeed, under ischemia/reperfusion injury conditions, HAs are cleaved by ROS-dependent enzymes such as hyaluronidase and detached from the surface of the HHAuNPs [72]. The HHAuNPs emit strong fluorescent signals that can be detected and analyzed [73]. In the study by Hyun et al., the ROS-sensitive gold nanoprobes were locally injected into the focal ischemic brain, and they detected ROS levels for up to 41 h in postischemic rat brains [24•]. Therefore, HHAuNPs proved to be excellent in vivo markers for diagnostic detection of brain ischemic disease since they are sensitive to trace levels of ROS, found up to 41 h after ischemia.

In addition, thrombi are an important pathophysiological feature of stroke [74–76]. Recent approaches in stroke management and prevention have utilized blood biomarkers such as prothrombin fragment 1.2 and D-dimer [77] to detect thrombin activity and its role in the formation of blood clots [78]. However, the lack of right levels of specificity in these biomarkers led Lin et al. to engineer NPs capable of detecting thrombi and releasing reporters into the urine of a thromboplastin-induced mouse model of pulmonary embolism [27•]. These thrombin-sensitive synthetic biomarkers are composed of two parts: a thrombin substrate part and a ligand-encoded reporter. Lin et al. used fluorogenic assays and enzyme-linked immunosorbent assays (ELISA) to detect the level of thrombin activity and verify the release of urinary reporters, respectively [27•]. This successful approach shows great promise for clinical applications of both reaching an accurate diagnosis and devising an appropriate therapy.

Targeted Imaging

One main contributor of stroke outcome is neuroinflammation [79]. Previous studies aimed to use ultrasmall superparamagnetic particles of iron oxide (USPIO) as biomarkers of inflammatory disorders and to study their distribution via MRI [80]. Indeed, phagocytic cells internalize USPIOs and these iron-labeled cells start emitting strong MR signals that can be recorded and interpreted [30, 81]. Nevertheless, MRIs possess a very low resolution and can misrepresent the actual size/extent of the emitting cells [82].

Interestingly, Marinescu et al. developed a new method for detecting USPIOs in the brain of mouse models of cerebral ischemia, referred to as synchrotron radiation X-ray phase computed tomography (SR-PCT) [31•]. This method consists of using monochromatic X-ray beams to obtain 3D images of high resolution and sensitivity (5–10 μm) and accurately locate USPIOs [31•]. This novel phase-contrast X-ray CT is extremely valuable for cellular imaging of postischemic inflammation in intact brain.

Similarly, Rahmer et al. established a new magnetic particle imaging (MPI) approach that consists of encapsulating SPIOs directly into red blood cells (RBCs) in order to monitor bleeding after stroke [33•]. With this method, it was possible to track down SPIO-loaded RBCs in the blood pool of mice, several hours after the NPs' injection [83]. Thus, the trapped NPs are protected from the mononuclear phagocytic system and can circulate in the bloodstream for a longer period of time [84].

One article by Farr et al. describes the use of core shell silica superparamagnetic glyconanoparticles that target E and P selectin to track down early endothelial inflammation following stroke [85]. E and P selectin are pro-inflammatory particles that aggravate stroke outcome; thus, their detection further increases the accuracy of the stroke prognosis and can be used as potential target for imaging [86]. Farr et al. demonstrated that, although selectins' expression was induced in the entire brain, magnetic nanoparticles containing simple hydroxy groups (HO@MNPs) accumulated specifically in the injured blood vessels of a transient MCAO mouse model [85].

Another study by Dmitriev et al. highlighted the efficacy of anionic cell-permeable phosphorescent nanoparticles in imaging oxygen in neural cells as well as in tissue models [87]. Changes in oxygen levels correlate with the ongoing developmental processes occurring within a cell or an entire tissue following a pathological state such as stroke or hypoxia [88]. Dmitriev et al. developed two nanoparticle probes: one is a negatively charged poly-(methyl methacrylate-co-methacrylic acid)-based nanoparticle impregnated with a phosphorescent Pt(II)-tetrakis(pentafluorophenyl) porphyrin (PtPFPP) dye (PA1) and the other has an additional reference/antennae dye poly(9,9-diheptylfluo-rene-alt-9,9-di-p-tolyl-9H-fluorene) (PA2). The PA1 and PA2 probes were internalized by endocytosis which facilitated high-resolution imaging of oxygen upon examination in primary neurons, astrocytes, PC12 cells, and 3D models such as cell aggregates and brain tissue slices [87].

Obermeyer et al. developed bacteriophage MS2 capsids as biomolecule-based nanoparticles for in vitro fibrin imaging [89]. The identification of thrombi is crucial to improve detection and eventual treatment of ischemic syndromes such as stroke [90]. In this study, the MS2 capsids were modified by an oxidative coupling reaction and were conjugated to fibrin targeting peptides on the external shell of the protein. In particular, MS2 capsids, conjugated to the synthetic GPR (Gly-Pro-Arg) peptide, efficiently bound to fibrin—a major target in blood clots—and inhibited thrombin-mediated clotting [89]. In addition to using imaging techniques for diagnosing stroke, the above studies reveal that imaging is specifically important in assessing the severity of the stroke, which may aid in predicting future complications such as massive cerebral edema and neurological deficits.

Another application of NPs is to monitor administered treatments in order to assess their efficacy and the regression of stroke complications, thus guiding the management in an individualized way. Nowadays, stem cell therapy represents a promising method to treat acute ischemic stroke since it promotes endogenous neurogenesis [91]. However, one main obstacle is the ability to monitor stem cell transplants over time [92]. Hence, Wen et al. established a dual-modal MRI and optical imaging to longitudinally monitor transplanted stem cells in an acute ischemic stroke rat model [36•]. In this work, stem cells were labeled with cationic, superparamagnetic iron oxide nanoparticles (SPIONs) and quantum dot (QD)-loaded polymersomes. These positively charged polymeric vesicles were able to enter the cell through endocytosis or macropinocytosis and encapsulate a broad range of therapeutic agents [93]. In the study by Wen et al., labeled cells grafted into SD rats could be monitored for up to 6 weeks using MRI and up to 4 weeks using optical imaging [36•]. Interestingly, in a previous study by Lin et al., it was demonstrated that a daily supplementation of folic acid (FA) (0.8 mg/kg) to rats for 28 days prior to MCAO and 23 days post MCAO, along with transplantation of superparamagnetic ion oxide (SPIO)- and bromodeoxyuridine (BrdU)-labeled neural stem cells (NSCs) in the contralateral region, stimulates the proliferation and migration of the exogenous NSCs to the ischemic site [94]. Therefore, a combination of FA supplementation, SPION-labeled NSCs and (QD)-loaded polymersomes might represent an excellent and efficient method to deliver and monitor transplanted stem cells after experimental stroke.

Drug Delivery

Drug delivery is yet another application of NPs in medicine, which happens to be specifically useful in stroke management, as the BBB is impermeable to many molecules. This makes drug delivery via NPs a promising approach. However, the design of a successful nanoparticle formulation depends on an in-depth understanding of the biological environment, which permits the creation of various nanodrug delivery systems for brain disease treatment.

In a study conducted by Zhao et al., puerarin (PUE), a compound found in Chinese medicinal plants, was delivered to the brain while being encapsulated in NPs in order to cross the BBB and inhibit inflammatory responses, apoptosis, and neutrophil activation in a way to prevent cerebral ischemia [37]. Previous studies highlighted the ability of PUE to decrease the expression of Fas, inhibit tumor necrosis factor-

alpha (TNF-α) and activate caspase 3, and increase the expression of 70-kDa heat-shock protein, thus underlining the protective role of PUE in cerebral ischemia/reperfusion injury [95–97]. In the study of Zhao et al., PUE was contained in poly(butylcyanoacrylate) nanoparticles (PBCN) coated with polysorbate 80 (Ps 80), a hydrophilic layer, which protects the NP from phagocytosis and enhances the half-life of PUE [37, 98, 99]. As a result, PUE-PBCN delivery to mice brains proved to be a more effective system than intravenous administration of the free PUE drug. Moreover, vein injection of PUE-PBCN, in MCAO and reperfusion model rats, resulted in neuroprotective effects such as increased body weight, lower brain water content, reduced infarct volume, and reduced neurological deficit scores [37]. Hence, it can be concluded that encapsulation of PUE in PBCN is a useful delivery system, allowing the carriage of a smaller, nontoxic, and neuroprotective amount of PUE to the brain of an individual suffering from focal cerebral ischemic injury.

Furthermore, and in an attempt to control and reduce cell apoptosis in the brain postcerebral ischemia reperfusion injury, An et al. constructed dermorphin (a μ-opiate receptor agonist)-PEG (poly(ethylene-co-glycol))-dendigraft poly-L-lysine (DGL)/DNA NPs as a brain-targeting short hairpin RNA (shRNA) therapy system [23•]. An et al. used an in vitro and in vivo approach to demonstrate the successful brain-penetrating capacity of DGL-PEG-dermorphin/shRNA NPs [23•]. In addition, NPs that contained anti-apoptosis signal-regulating kinase 1 (Ask1) shRNA significantly reduced the expression of endogenous Ask1 [23•], a key player in the apoptotic pathway induced by cerebral ischemia reperfusion [100, 101]. Importantly, early injection of DGL-PEG-dermorphin/shRNA NPs did not only reduce apoptotic cell death, but it also reduced the infarct area in the brain of the rat subjected to cerebral ischemia reperfusion injury [23•]. Therefore, DGL-PEG-dermorphin/shRNA NPs proved to be a beneficial brain-targeting delivery system for RNA interference (RNAi) neuroprotection against cerebral ischemia-reperfusion injury [23•, 102].

In another study by Mdzinarishvili et al., the effectiveness of glutathione-coated poly-(lactide-co-glycolide)-polyethyleneglycol (PLGA-b-PEG) nanoparticles encapsulating triiodothyronine (T3) in an MCAO mouse model of ischemic stroke was demonstrated [32]. This delivery system successfully enhanced T3's drug permeability to the brain across the BBB and increased glutathione—an important antioxidant—levels in the brain. The encapsulation of the T3 hormone in PLGA-PEG NPs, coated with glutathione, showed a 58 % decrease in tissue infarction and a 75 % decrease in brain edema, in the MCAO stroke model [32].

Another study conducted by Liu et al. highlighted the efficacy of cationic bovine serum albumin-conjugated tanshinone IIA PEGylated nanoparticles (CBSA-PEG-TIIA-NPs) in a cerebral ischemia rat model [28]. Tanshinone IIA (TIIA), the main active component of *Salvia miltiorrhiza*, a medicinal plant, had been greatly used to treat cerebrovascular diseases, as it possesses both an antioxidant and anti-inflammatory role [103]. Noteworthy, in the study by Liu et al., CBSA-PEG-TIIA-NPs were efficiently delivered to the brain where they accumulated and played a neuroprotective effect by regulating neuronal signal pathways as well as inflammatory cascades [28]. In particular, TIIA-containing NPs reduced the levels of the pro-inflammatory cytokines myeloperoxidase (MPO), TNF-α, interleukin 1 beta (IL-1β), and interleukin 6 (IL-6); increased the expression of anti-inflammatory cytokines such as interleukin 10 (IL-10) and transforming growth factor beta 1 (TGF-β1); decreased mRNA and protein expression of the neuroinflammatory p38MAPK and inducible nitric oxide synthase (iNOS); and enhanced peroxisome proliferator-activated receptor gamma (PPARγ) expression [28, 104]. This, in turn, shows that CBSA-PEG-TIIA-NPs represent a promising delivery system to treat cerebral ischemia.

Another study by Lu et al. investigated the efficacy of PEGylated-lipid nanoparticles (PLNs), conjugated to a Fas ligand antibody, in targeting brain ischemia in a mouse model of focal cerebral ischemia-reperfusion injury [29•]. Fas ligands are known to recruit microglia to the injured region where they play a role in tissue repair and remodeling [105]. In this study, Fas ligand-conjugated PLNs mainly accumulated in activated microglial cells in the ipsilateral region of the ischemic brain. The successful delivery of these NPs was also followed by a decrease in the dephosphorylation of Akt (Ser473) and ERK (Thr202/Tyr204), which correlates with less BBB damage in the ischemic brain. Thus, Fas ligand-conjugated PLNs are an efficient neuroprotective drug delivery system for brain ischemia [29•].

A recent study by Nance et al. established a new drug delivery method that consists of transporting brain-penetrating NPs across the BBB in a noninvasive manner, using MRI-guided focused ultrasound (MRgFUS) [106]. The combination of MRgFUS and intravascular microtubules transiently disrupted tight junction complexes and induced the active transport of agents into the brain parenchyma via the reversibly disturbed BBB. This method allowed the safe, pressure-dependent delivery of 60 nm densely PEG-coated brain-penetrating nanoparticles (BNPs) to the rat brain parenchyma and represents a therapeutically relevant drug delivery system to treat CNS diseases such as stroke [106].

Of importance, curcumin is an anti-inflammatory, anti-thrombotic, antioxidative, and bioavailable molecule, capable of crossing the BBB and exerting neuroprotective effects during cerebral ischemic stroke [107–109]. As a consequence, the delivery of curcumin-encapsulated exosomes proved to be highly beneficial to treat brain inflammatory diseases such as stroke [110]. Tiwari et al. reported the neuroregenerative capacity of curcumin when delivered via NPs [111]. Sun et al.

assessed the anti-inflammatory role of curcumin-encapsulated exosomes along with their high solubility and stability in the blood [112]. Finally, Kalani et al. proposed the use of curcumin-primed exosomes, which were observed to improve the permeability of endothelial cells and the recovery of junction proteins in an attempt to reduce stroke pathology [113]. Similarly, Ahmad et al. used curcumin to treat MCAO-induced focal cerebral ischemia in rats; however, in this study, curcumin (100 μg/kg body weight) was encapsulated in polymeric N-isopropyl acryl amide (PNIPAM) nanoparticles and was administered intranasally [114]. Curcumin-loaded PNIPAM NPs proved to reduce lipid peroxidation and, thus, protect against oxidative stress in a more powerful manner than demethoxycurcumin (DMC)- and bisdemethoxycurcumin (BDMC)-loaded PNIPAM NPs [114]. Thus, intranasal administration of PNIPAM-loaded curcumin NPs ensured the efficient delivery of the drug to the brain, by bypassing the BBB, and allowed the drug to play its neuroprotective/antioxidant effect on an experimental stroke rat model [115].

Another study by Singhal et al. explains the possibility to treat neurological disorders by using catalase-loaded, poly(lactic co-glycolic acid) nanoparticles in primary human neurons [116]. The idea behind this concept was to develop a permeable capsule capable of crossing the BBB, protecting the therapeutic drug—in this case superoxide dismutase (SOD) enzyme—from degradation, and delivering it to the affected region of the brain [117]. The concept of antioxidant delivery, such as catalase, in the brain of a patient suffering from a neurodegenerative disease is crucial to degrade hydrogen peroxide (H_2O_2) and reduce its leakage from the mitochondria [118], in an attempt to control oxidative stress damage [119, 120]. In the study of Singhal et al., the delivery of catalase-loaded NPs successfully reduced DNA damage, apoptosis events, H_2O_2-induced protein oxidation and restored neuronal structure [116]. This approach not only reduces the progression of the ischemic lesion but also offers a wider window for therapeutic interventions.

As mentioned previously, the delivery of antioxidants in the brain is necessary to protect neuronal cells from oxidative damage observed in cerebral stroke [121, 122]. Consequently, Ghosh et al. studied the effects of nanoencapsulated quercetin (QC), a bioflavonoid polyphenolic antioxidant [123], in ischemia/reperfusion induced young and aged rats [124]. PLGA-encapsulated QC (2.7 mg/kg b wt) was administered via oral gavage and resulted in reduction of iNOS and caspase-3 activity [125] along with reduction in the loss of pyramidal neurons from the hippocampal CA1 and CA3 regions. Thus, oral treatment with nanoencapsulated QC plays a neuroprotective role against ischemic oxidative attack [124].

Finally, another new delivery system was developed by Wang et al., which consists of releasing growth factors directly into the brain, via NPs, to stimulate tissue repair in a mouse stroke model [35•]. In particular, epidermal growth factor (EGF) was encapsulated in PLGA nanoparticles [126], while erythropoietin was enclosed in biphasic NPs composed of a PLGA core and a poly(sebacic acid) coating [127]. These polymeric particles were then confined within a hyaluronan methylcellulose hydrogel to reduce possible inflammatory reactions [128]. The delivery of these vehicles stimulated neural stem/progenitor cells that replenished the tissues lost during stroke [129]. Furthermore, this new delivery strategy bypassed the BBB, minimized the damage usually observed in intracerebroventricular infusion, a catheter/minipump system [130, 131], and allowed the release of growth factors with effective neuroregenerative capacity. All the above studies describing different applications of NPs in the field of stroke are depicted in Fig. 1.

Limitations and Future Directions

Nanoparticles have provided novel methods for neuroprotection, yet some limitations still need to be addressed. Even though NPs have greatly improved the half-life and the bioavailability of the delivered drugs, their clearance from the body (especially via macrophages) is still uncontrolled, and thus, one has to give multiple injections of NPs into the host in order to maintain an adequate amount in the bloodstream. That is why there are studies on modulating the surface markers of NPs to inhibit their opsonization and clearance by the reticuloendothelial system [132–134]. However, the surface markers themselves (or any of the other constituents of NPs) may cause cytotoxicity, as well as particle size, zeta potential, pH, electronic properties, and time of exposure may influence cell viability and activate cellular pathways such as the p53, TGF-β1, Wnt, and cadherin pathways that regulate cytotoxicity [135].

Furthermore, polychlorinated biphenyls (PCBs) are one of the most widely spread organic pollutants that accumulate in our bodies due to environmental toxicity, and since they are able to assemble onto injected NPs, it has been found in one study that PCB153 could increase cerebrovascular toxicity mediated through the toll-like receptor 4 (TLR4) and tumor necrosis factor-associated factor 6 (TRAF6) [136]. NPs (specifically silver NPs) were also found to enter embryonic cells (cellular nuclei, mitochondria, cytoplasm, and lysosomes) and regulate hundreds of genes pertaining to energy metabolism, oxygen transport, enzyme activities, molecular binding, and inflammatory cascades [137]. Moreover, NPs could disrupt the body's homeostasis as they can affect levels of albumin, cholesterol, triglycerides, total protein, urea, alkaline phosphatase, and aspartate aminotransferase [138]. Such changes

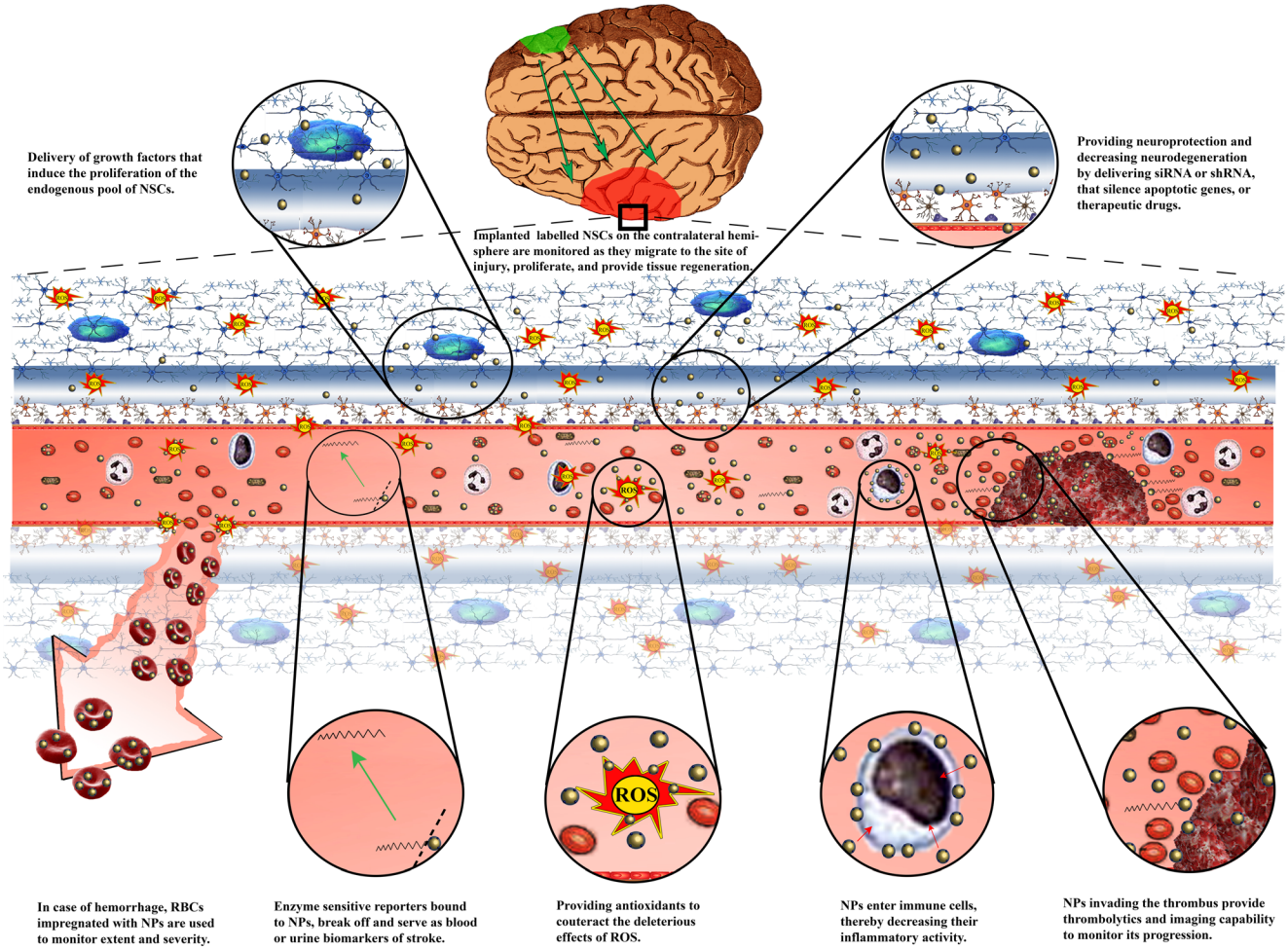

Fig. 1 A schematic depicting the different uses of nanoparticles: imaging ischemic and hemorrhagic regions and thrombus progression, monitoring the migration and proliferation of neural stem cells, detecting biomarkers in the blood or urine, and delivering drugs or siRNA or shRNA for neuroprotection, thrombolysis, neurogenesis, and anti-inflammation

could predispose an individual to enhanced plaque and thrombus formation, which in turn are risk factors for stroke.

Unfortunately, there are also some types and sizes of particles that are incapable of invading immune cells that infiltrate the brain, and thus, these NPs lack the privilege of a fast and efficient drug delivery or imaging capability [139]. Therefore, there still exist a handful of limitations that one should overcome through designing enhanced versions of NPs that do not pose any cytotoxic effects. And in order to do so, further studies should be pursued to assess the limitations and side effects of NPs.

Conclusion

The rapid onset of strokes and their devastating and fatal consequences make this medical condition one of the most challenging conditions to prevent and diagnose via imaging and molecular profiling, and treat and undo via targeted delivery. The field of nanoparticles has recently emerged and invaded various areas of medicine and has shown promising results. The ability to administer drugs more efficiently is the highlight of using NPs, through which they have been proven to be more neuroprotective and efficiently thrombolytic as compared to traditional drug administration methods. When combined with NP imaging, these efficient and targeted therapies will be better guided and assessed through monitoring the effectiveness of drug/RNA delivering NPs. The realm of NPs is still new and there are many limitations and unwanted side effects yet to be discovered as we delve further into this field and manipulate it into our own advantage.

Compliance with Ethics Guidelines

Conflict of Interest Tarek H. Mouhieddine, Muhieddine M. Itani, Amaly Nokkari, Changhong Ren, Georges Daoud, Asad Zeidan, Stefania Mondello, and Firas H. Kobeissy declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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