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Nanoparticles-mediated emerging approaches for effective treatment of ischemic stroke

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ABSTRACT

Ischemic stroke leads to high disability and mortality. The limited delivery efficiency of most therapeutic substances is a major challenge for effective treatment of ischemic stroke. Inspired by the prominent merit of nanoscale particles in brain targeting and blood-brain barrier (BBB) penetration, various functional nanoparticles have been designed as promising drug delivery platforms that are expected to improve the therapeutic effect of ischemic stroke. Based on the complex pathological mechanisms of ischemic stroke, this review outline and summarize the rationally designed nanoparticles-mediated emerging approaches for effective treatment of ischemic stroke, including recanalization therapy, neuroprotection therapy, and combination therapy. On this bases, the potentials and challenges of nanoparticles in the treatment of ischemic stroke are revealed, and new thoughts and perspectives are proposed for the design of feasible nanoparticles for effective treatment of ischemic stroke.

1. Introduction

Ischemic stroke is a neurological disorder attributable to the cerebrovascular stenosis or occlusion. It is the second leading cause of death and the first leading cause of long-term disability in adults worldwide [1,2]. Moreover, as the global population ages, the incidence of ischemic stroke is on the rise [3]. Ischemia leads to the formation of irreversible injured infarct core and the surrounding viable penumbra in the ischemic hemisphere. Clinically, the gold standard of treatment is the early recanalization of the occluded cerebral arteries by intravenous thrombolysis or mechanical thrombectomy to reduce the infarcts while rescuing the penumbra. Lamentably, due to the narrow time window of intravenous thrombolysis (within 3–4.5 h from onset) and the lack of uniform standardization of mechanical thrombectomy techniques, the clinical treatment of ischemic stroke has many limitations and needs further improvement [4–7]. Intravenous thrombolysis or mechanical thrombectomy beyond the therapeutic time window may provoke hemorrhagic transformation or reperfusion injury, which is closely correlated with the high disability and mortality of ischemic stroke [8,9]. To overcome these limitations, neuroprotective agents, such as edaravone, have been recommended for clinical trials, since there is no strict time limit for their application [10,11]. Despite the positive effects, the failure of drugs to pass through the blood-brain barrier (BBB) and reach the ischemic penumbra leads to an inability to achieve the desired drug concentrations, further impeding the treatment process of ischemic stroke [12,13].

The brain has a rich vascular system and is the organ with the most blood perfusion in the human body [14]. The cerebral vessels not only provide nutrients and oxygen to the brain, but also protect the brain from neurotoxins. Therefore, the existence of the BBB is a necessity for maintaining the stability of the cerebral microenvironment and ensuring the normal functions of the central nervous system. Nevertheless, the existence of the BBB also impedes the intracerebral delivery of therapeutic agents [12]. It has been reported that approximately 98% of the small-molecule drugs and almost 100% of the large-molecule drugs cannot be administered to the brain through peripheral administration [15]. Although studies have shown that the occurrence of ischemic stroke can destroy and increase the permeability of the BBB, the BBB remains the main obstacle for drugs to overcome. In the past decades, compelling data have substantiated thatnano-delivery systems (NDS) can be promising vehicles for delivering therapeutic agents to the brain, including liposomes, polymer nanoparticles, amphiphilic polymer micelles, inorganic nanoparticles, and biomimetic nanoparticles [16–22]. Through intravenous injection, these nanoparticles can penetrate BBB

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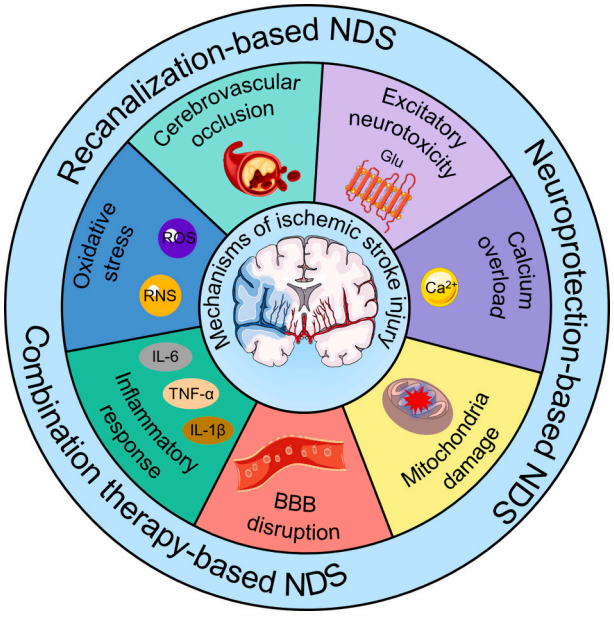
into the brain parenchyma by cell penetrating peptide-mediated, receptor-mediated, adsorption-mediated, biomimetic-mediated, or magnetic target-mediated manners, respectively [23]. Apart from the highly invasive intracerebral injection, the nanoparticles can also be directly delivered to the brain bypassing the BBB through intranasal, retro-orbital or intrathecal administration [24–26]. Unarguably, the advent of nanotechnology provides novel strategies for specifically delivering therapeutic substances to the brain.

To date, there are three major approaches for nanoparticles-mediated ischemic stroke therapy: recanalization, neuroprotection, and combination therapy. The benefits of utilizing nanoparticles lie in their increased bioavailability, enhanced therapeutic efficacy, and reduced unwanted toxicity due to their specific nanoscale, surface modifications, blood stability, and the ability to control the release of therapeutic agents in response to specific environmental stimuli such as pH, H_2O_2, or external magnetic fields. This review will outline and summarize the recent advances of nanoparticles-mediated emerging approaches to enhance the therapeutic effects of ischemic stroke (Scheme 1). The nanoparticles are summarized and discussed according to the three tactics mentioned above. On this basis, we anticipate to reveal the potentials and challenges of nanoparticles in the field of ischemic stroke, and provide new thoughts and perspectives for the design of nanoparticles targeting the cerebral ischemic regions for effective treatment of ischemic stroke.

2. The pathophysiology of ischemic stroke

The brain injury inflicted by ischemic stroke involves multiple complex pathological mechanisms. When an ischemic stroke occurs, neurons in the ischemic lesions cannot obtain sufficient blood and oxygen supply, lead-ing to ATP depletion, mitochondrial dysfunction and an imbalance between oxidation and anti-oxidation. Subsequently, the neurons in the ischemic hemisphere soon undergo necrosis and apoptosis, forming infarction and penumbra [27]. Afterwards, the intracerebral immune cells—microglia are activated and accumulate in the infarct area to participate in the immune response. The activated microglia are mainly divided into the "classically activated" M1-phenotype and the "selectively activated" M2-phenotype, which play distinct roles in brain damage. In a short period of time, the activated microglia are mainly M2-phenotype and then transform into M1-phenotype. A high M1/M2 ratio is closely related to the ischemic injury. The M1-phenotype microglia can secrete excitatory amino acids, pro-inflammatory factors, chemokines, and matrix metalloproteinases, which directly provoke inflammatory response in the brain and destroy the tight junctions of the BBB [28]. Furthermore, the secreted pro-inflammatory factors will then recruit a large number of peripheral immune cells to infiltrate into the brain through the compromised BBB [29]. The infiltrated peripheral immune cells release overwhelming inflammatory factors and interact with the activated microglia to cause the inflammatory cascade and further disrupt the BBB [30].



Scheme 1. Schematic illustration of the brain injury mechanisms in ischemic stroke and nanoparticles-mediated emerging approaches for effective treatment of ischemic stroke.

After reperfusion, the penumbra gets sufficient blood and oxygen supply. While the mitochondrial dysfunction prevents adequate aerobic respiration, resulting in oxygen surplus, activation of reactive oxygen-producing enzymes, and reduction of antioxidant enzymes. The balance between the production and scavenging of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is broken, eventually causing the accumulation of a large amount of ROS and RNS. The proliferation of ROS and RNS leads to oxidative stress injury, which further induces the activation and aggregation of immune cells, stimulates the secretion of inflammatory factors, and aggravates the inflammatory response. In addition, plenty of immune cells and platelets aggregate and adhere to the endothelium of cerebral capillaries, resulting in microvascular occlusion and "no reflow", which has become the major obstacle of drug delivery for ischemic stroke [31–38]. The relevant therapeutic substances against the pathological mechanisms such as antithrombotic agents, antioxidants, and anti-inflammatory agents have been proved effective in rescuing the infarct regions in preclinical trials. The purpose of these therapeutic agents focuses on establishing a collateral circulation in the ischemic area, so as to restore the blood and oxygen supply of the damaged brain tissues and repair the injured brain functions as much as possible [39].

3. Nanoparticles-mediated recanalization for effective treatment of ischemic stroke

For ischemic stroke patients, time is vital for saving the brain. Intravenous thrombolysis is preferred as the standard rescue measure to minimize the brain damage. Despite the strict time limitation for thrombolytic therapy, thrombolysis within the time window can effectively relieve the neurological damage of patients. Recanalization agents can dissolve or restrain the formation of blood clots and restore the blood perfusion in ischemic regions, thereby reducing the infarct size [40]. Recombinant tissue-type plasminogen activator (rt-PA) and other thrombolytic enzymes are the most appreciated thrombolytic substances in ischemic stroke. Besides, antiplatelet agents, anticoagulant drugs, and gas generating agents have also been reported to promote vasodilation and reperfusion of the microvascular [41,42]. The major defects of recanalization agents include thrombolysis failure and high risk of intracranial hemorrhage because of their thrombus off-targeting and nonspecific distribution in the body [43,44]. In view of these limitations, versatile recanalization-based nanoparticles, including thrombolytic agents, antiplatelet agents, and gas generating agents -based nanoparticles, have been delicately designed to overcome their thrombus targeting obstacles and enhance their circulation times for enhanced ischemic stroke therapy in preclinical trials. Table 1 summarizes the recanalization-based nanoparticles for effective treatment of ischemic stroke.

3.1. Thrombolytic agents-based nanoparticles

rt-PA is the only thrombolytic drug approved by the US Food and Drug Administration (FDA) for ischemic stroke therapy. This protein converts the endogenous plasminogen into plasmin, which lyses the fibrin in thrombus, thereby promoting reperfusion [59]. However, rt-PA has a short half-life (4–8 min) and poor thrombus affinity, which

Table 1
Summary of recanalization-based nanoparticles for effective treatment of ischemic stroke.

Design	Mechanism of action	Effectiveness	Administration route	Reference
Porous magnetic iron oxide (Fe_3O_4)-microrods incorporating rt-PA (tPA-MRs)	Thrombolysis	Lyse the blood clots through both the rt-PA and the rotating MRs	Intra-artery injection	[45]
Platelet-membrane camouflaged PLGA nanoparticles containing rt-PA	Thrombolysis	Improve the targeting delivery of rt-PA to the thrombus	Intravenous injection	[46]
Polymer nanoparticles functionalized with fucoidan and loaded with rt-PA	Thrombolysis	Improve the efficiency of rt-PA by targeting the activated platelets in the thrombus	Retro-orbital injection	[47]
Porous soft discoidal PLGA-PEG nanoconstructs containing rt-PA (tPA-DPNs)	Thrombolysis	Protect rt-PA from degradation in the blood circulation and reduce thrombus time	Retro-orbital injection	[48]
rtPA was conjugated to poly (ethylene glycol)-poly (ε-caprolactone) (PEG-PCL) nanoparticles (rtPA-NP)	Thrombolysis	Improve the fibrin-targeting ability and prolong the half-life of rt-PA	Intravenous injection	[49]
PEGylated PLGA nanoparticles containing rt-PA	Thrombolysis	Enhance the thrombolytic activity of tPA	Not applicable	[50]
RGD peptide conjugated liposomes containing streptokinase	Thrombolysis	Enhance the thrombus targeting and release streptokinase immediately after interacting with the activated platelets	Not applicable	[51]
Platelet microparticle-inspired nanovesicles (PMINs) for the targeting delivery of streptokinase	Thrombolysis	Prevent the off-target uptake of streptokinase, actively target the thrombus and enzymatic triggered release of streptokinase	Intravenous injection	[52]
pH-sensitive polyethylene glycol-conjugated urokinase nanogels	Thrombolysis	Suspend the urokinase release in the blood circulation and achieve pH-sensitive release of urokinase in the clots	Intravenous injection	[53]
Thrombus targeting aspirin polyconjugate nanoparticles	Antiplatelet	Specifically target fibrin-rich thrombus and displayed strong antithrombotic activity	Intravenous injection	[54]
Cilostazol nanodispersions	Antiplatelet	Effectively prevent the cerebral ischemia/reperfusion injury of ischemic stroke	Intravenous injection	[55]
3-n-Butylphthalide was encapsulated into Fas ligand antibody conjugated PEG-lipid nanoparticles	Anti-platelet	Efficiently penetrated the BBB and specifically bind to the microglia in the ischemic regions	Intravenous injection	[56]
Platelet membrane encapsulated with L-arginine and γ-Fe_2O_3 magnetic nanoparticles (PAMNs)	Antithrombosis	Rapidly target the intended stroke lesions and in situ generate NO with the help of an external magnetic field	Intravenous injection	[57]
Inert gas sulfur hexafluoride (SF_6) was encapsulated into platelet membrane vesicles to prepare biomimetic nanobubbles (PNBs)	Cavitation	Preferentially accumulate at the microvascular of the ischemic lesions and achieve recanalization of the occluded microvascular	Intravenous injection	[58]

requires continuous intravenous infusion. What's worse, delayed administration beyond the therapeutic window increases the risk of intracranial hemorrhage [60]. Nanoparticles provide an opportunity to prolong the half-life and enhance the specific delivery of rt-PA to the cerebral thrombus. Recently, rtPA-based nanoparticles have been developed for thrombolytic therapy of ischemic stroke [45–50]. For instance, Hu and coworkers incorporated rt-PA into the nanoporous, superparamagnetic-like Fe_3O_4–C microrods to form rtPA- Fe_3O_4–C microrods (rtPA-MRs) [45]. The targeted thrombolytic therapy with rtPA-MRs was applied for distal middle cerebral artery occlusion (dMCAO) mice through intra-arterial injection. The thrombus was lysed through both the rt-PA and the rotating MRs with an external rotating magnetic field. The well-designed rtPA-MRs could lyse the blood clots at about 77 times lower concentrations and about 3 times shorter than the

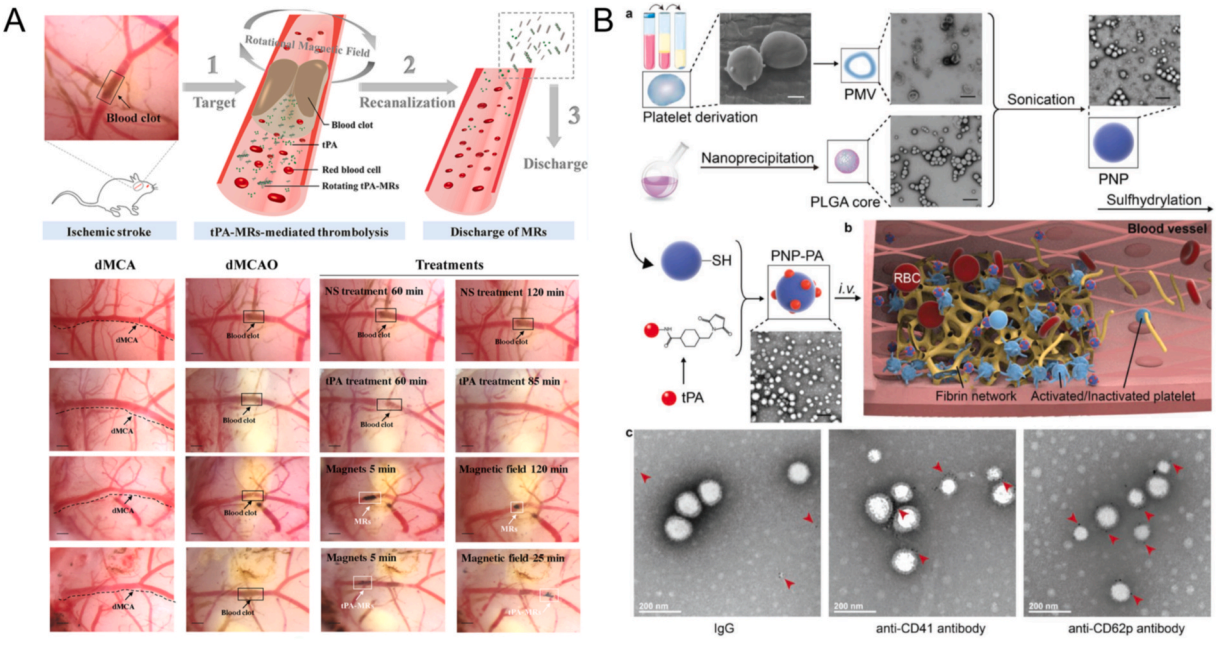


Fig. 1. Designing nanoparticles for targeting delivery of rt-PA to the thrombus sites. (A) rt-PA was incorporated into nanoporous, superparamagnetic-like Fe_3O_4–C microrods to form into rtPA-Fe_3O_4–C MRs (rtPA-MRs) for targeted ischemic stroke therapy. Reproduced with permission [45]. Copyright 2018, American Chemical Society. (B) rt-PA was chemically conjugated to the surface of platelet membrane-camouflaged PLGA polymeric nanoparticles to form into nanoplatelets for targeted delivery of rt-PA to the thrombus sites. Reproduced with permission [46]. Copyright 2019, Wiley-VCH.

rtPA alone. In addition, the infarct volumes were significantly decreased in the rtPA-MRs-treated dMCAO mice compared with the free rtPA group (Fig. 1A). Based on the clots targeting function of platelets, Xu and coworkers developed platelet membrane-camouflaged poly (lactic-*co*-glycolic acid) (PLGA) polymeric nanoparticles with a hydrodynamic diameter of about 167 nm for targeting delivery of rt-PA to the thrombus sites [46]. Through intravenous injection, the nanoparticles exhibited superior therapeutic efficacy and lower bleeding risk over free rt-PA in an ischemic stroke mouse model (Fig. 1B). In another research, Juenet and coworkers reported polysaccharide-poly (isobutylcyanoacrylate) polymer nanoparticles functionalized with fucoidan and loaded with rt-PA (Fuco-NPs) for retro-orbital injection. The Fuco-NPs exhibit a high affinity for the P-selectin over-expressed by activated platelets in the thrombus [47]. Inspired by the physicochemical properties of erythrocytes, Colasuonno and coworkers conjugated rt-PA to the mixture of poly (lactic-*co*-glycolic acid) (PLGA) and polyethylene glycol (PEG) to form discoidal polymeric nanoconstructs (tPA-DPNs) [48]. Through retro-orbital injection, the tPA-DPNs presented deformability and long blood circulating time similar to erythrocytes, which endowed them more effective and faster blood clot dissolution ability. Similarly, Deng and coworkers conjugated rt-PA to polyethylene glycol-polycaprolactone (PEG-PCL) nanoparticles (rtPA-NP) [49]. Through intravenous injection, the rtPA-NP showed fibrin-targeting, prolonged half-life (approximately 18 times longer than free rt-PA) and favorable fibrin clots dissolution properties. In another research, Zamanlu and coworkers encapsulated rt-PA into (polyethylene glycol)-poly (lactic-coglycolic acid) (PEG-PLGA) nanoparticles to prolong the blood circulation time and enhance the thrombolysis of rt-PA [50].

In addition to rt-PA, other thrombolytic drugs such as streptokinase and urokinase have also been reported for ischemic stroke therapy in pilot studies [61,62]. Nevertheless, they also raise systemic complications such as systemic fibrinogenolysis and hemorrhage. To improve the therapeutic effect and deal with the side effect of streptokinase, Vaidya et al. encapsulated streptokinase into arginine-glycine-aspartic acid (RGD) peptide-conjugated liposomes with a diameter of 100–120 nm [51]. The liposomes not only targeted the thrombus, but also released streptokinase immediately after interacting with the activated platelets embedded in the thrombus through a self-destructive mechanism. The clot lysis study demonstrated that compared with the streptokinase solution, the liposomes could dissolve the clot with less time and deeper degree. In another study, Pawlowski et al. designed platelet microparticle-inspired nanovesicles (PMINs) for the targeting delivery of streptokinase, which could prevent the off-target uptake of streptokinase, actively target the thrombus and enzymatic triggered release of streptokinase [52]. Cui et al. developed pH-sensitive polyethylene glycol-conjugated urokinase nanogels (PEG-UKs) [53]. Through intravenous injection, the PEG-UKs suspended the release of urokinase in blood circulation and achieve pH-sensitive release of urokinase in the clots, thereby realizing more effective thrombolytic therapy of ischemic stroke.

3.2. Antiplatelet agents-based nanoparticles

Platelet adhesion and aggregation are not only closely related to thrombus formation, but also responsible for the “no-reflow” phenomenon of the microvascular after reperfusion [63,64]. Antiplatelet agents such as aspirin, cilostazol, and 3-n-Butylphthalide can restrain platelet aggregation, thereby preventing the cerebrovascular diseases caused by thrombosis [54,55]. However, long-term and high-dose use of antiplatelet agents by oral administration may increase the risk of gastrointestinal and intracranial hemorrhage. Therefore, antiplatelet agents-based nano-delivery systems, which reduce the off-target delivery of drugs, are desirable.

To further enhance the anti-platelet aggregation effect of aspirin, Lee et al. fabricated thrombus targeting aspirin poly-conjugate nanoparticles (T-APP) in which the aspirin poly-conjugate was co-assembled with thrombus targeting lipo-peptide (DSPE-PEG-Gly-Pro-Arg-Pro-Pro) and fluorescent IR780 [54]. The T-APP were able to be activated by an elevated level of H_2O_2 in the thrombosed vessels and exerted therapeutic effect for thrombosis. In tail bleeding mouse models, T-APP showed superior anti-platelet activity through intravenous injection. Compared with the ethyl salicylate (a major active ingredient of aspirin) group and the untreated group, T-APP significantly enhanced the bleeding time and bleeding volume. In carotid arterial thrombosis mouse models, T-APP could rapidly and specifically target the fibrin-rich thrombus and showed strong antithrombotic activity through suppressing the sCD40L and TNF-α expression and inhibiting the platelet adhesion and aggregation. In another study, using methylcellulose and docusate sodium salt, Nagai et al. designed a cilostazol nanoparticle injection formulation to effectively prevent the cerebral ischemia/reperfusion injury of ischemic stroke [55]. Recently, it was found that 3-n-Butylphthalide could inhibit platelet aggregation and reduce thrombus formation [65]. To enhance the target delivery of 3-n-Butylphthalide, Lu et al. encapsulated 3-n-Butylphthalide into Fas ligand antibody conjugated PEG-lipid nanoparticles (FL/NBP/PLNs), which could bind to the microglia in the ischemic regions [56]. Through intravenous injection, the FL/NBP/PLNs efficiently penetrated the BBB and specifically delivered 3-n-Butylphthalide to the cerebral ischemic regions. In addition, the in vivo treatment experiment showed that FL/NBP/PLNs remarkably mitigated the ischemia-reperfusion injury in tMCAO mice.

3.3. Gas generating agents-based nanoparticles

There are two major strategies for gas thrombolysis. One is bioactive gas, such as nitric oxide (NO). Endothelium-derived NO can regulate vasodilation and inhibit the aggregation of platelets and leukocytes, thereby maintaining the blood flow [66,67]. Soon after an ischemic stroke, the synthesis and release of endothelium-derived NO decreased. Consequently, actively delivering NO or NO donors to the stroke lesions would be beneficial for reperfusion [68,69]. The other is inert gas which is wrapped into proteins, lipids, or polymers to form into nanobubbles that produce cavitation effect to realize the recanalization of the blocked microvascular [58]. The nanobubbles do not even need to be combined with thrombolytic drugs to obtain satisfactory thrombolytic effects.

L-arginine is one of the NO donors and produces NO under the catalysis of nitric oxide synthase. Li et al. fabricated a biomimetic nanoparticle comprising a platelet membrane encapsulated with L-arginine and γ-Fe_2O_3 magnetic nanoparticles (PAMNs). The PAMNs were spherical with a hydrodynamic diameter of approximately 200 nm. By tail vein injection, the PAMNs could rapidly target the intended stroke lesions and generate NO in situ [57]. In in vitro platelet aggregation assays, PAMNs produced NO in bEnd.3 cells and reduced platelets aggregation. In in vivo T_2 magnetic resonance imaging (MRI), PAMNs themselves adhered to the injured vascular sites and accumulated at the ischemic stroke lesions. Moreover, an external static magnetic field gradient further enhanced the accumulation of PAMNs in the ischemic sites at 6 h post-injection. The pharmacodynamic experiments showed that compared with the saline group, significant recanalization of the ischemic regions occurred after injecting with PAMNs for 0.5–1 h, and the application of magnetic field gradient further promoted the revascularization rate and blood flow (Fig. 2).

Inert gas based-nanoparticles have also been reported for ischemic stroke therapy [58]. For instance, Li et al. encapsulated the inert gas sulfur hexafluoride (SF_6) into platelet membrane vesicles to prepare biomimetic nanobubbles (PNBs) that integrate diagnosis and treatment [58]. Based on the inherent inflammatory microvascular affinity of platelets, the PNBs with a mean diameter of 131.43 ± 19.84 nm preferentially accumulated at the microvascular of the ischemic lesions and achieve recanalization of the occluded microvascular through intravenous injection. In addition, by means of the contrast-enhanced ultrasound imaging technology, the well-designed PMBs could also be used to

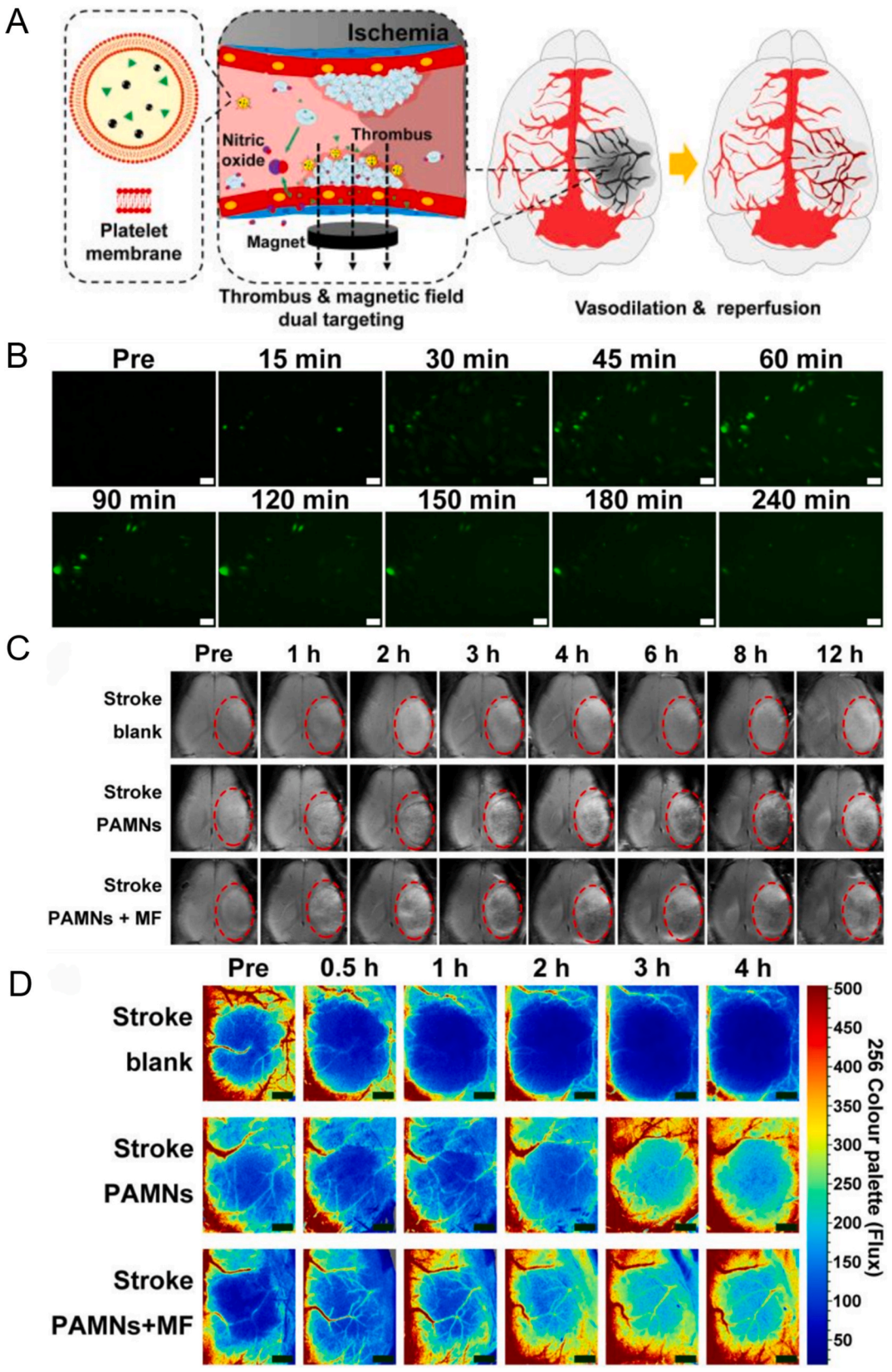


Fig. 2. Recanalization therapy of PAMNs for ischemic stroke. (A) Structure and in vivo targeting schematic diagram of PAMNs. (B) Photographs of NO production in bEnd.3 cells incubated with PAMNs and stained with DAF-FM DA; (C) In vivo T_2 MRI before (pre) and after PAMNs injection; (D) Color-coded laser speckle images of blood flow in the ischemic lesions before (pre) and after PAMNs injection. Reproduced with permission [57]. Copyright 2020, American Chemical Society. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

monitor the severity and development of stroke lesions in real time (Fig. 3).

As previously discussed, the recanalization-based nanoparticles have shown promising therapeutic results in preclinical trials, such as the prolonged circulation times and the enhanced thrombus targeting. Clinical trials have been carried out with lipid-based microbubbles combined with ultrasound and rtPA and showed encouraging results [70]. Moreover, RGD-modified urokinase liposomes present high clinical application potential owning to their simple components and hemocompatibility [51]. Yet, clinical translations of most recanalization-based nanoparticles have been hampered by the following issues: (i) the complex composition of the nanoparticles adds the complexity of the preparation process, resulting in poor consistency between batches. In addition, some non-clinically approved ingredients such as cell membranes may induce immune responses or other unknown side effects; (ii) the recanalization agents may be difficult to release in the ischemic regions, although some nanoparticles are designed to be pH or H_2O_2 responsive, the microenvironment of ischemic stroke is heterogeneous as the disease processes; (iii) the differences between the human patients and the rodent models must be considered in recanalization studies. In clinical practice, there are various types of thrombosis, such as venous thrombosis and arterial thrombosis. While most rodent models for recanalization treatment are $FeCl_3$-induced middle cerebral artery occlusion or photo-thrombosis models.

4. Nanoparticles-mediated neuroprotection for effective treatment of ischemic stroke

Although thrombolytic therapy is regarded as the standard treatment for ischemic stroke, neuronal damage occurs throughout the entire pathological process of ischemic stroke. Consequently, neuroprotection during or after thrombolysis is an indispensable issue. It is worth mentioning that, for some ischemic stroke patients who are not suitable for thrombolysis, the application of neuroprotective agents is conducive to improve the microenvironment for tissue regeneration and promote the establishment of collateral circulation. As mentioned above, the cerebral injury triggered by ischemic stroke mainly includes excitatory neurotoxicity, mitochondria damage, oxidative stress, and inflammatory response. Neuroprotective agents interfere with the pathophysiological process of ischemic stroke and reduce the functional damages of brain cells, which is now one of the popular topics in ischemic stroke research. Neuroprotective agents such as oxygen carriers, excitotoxicity inhibitors, antioxidants, anti-inflammation drugs, and stem cells and genes have been investigated to ameliorate the stroke microenvironment and repair the cerebral neurological functions in preclinical trials. Even though more than 100 neuroprotectants have been tested in clinical trials, none of them showed effects in randomized controlled trials [71]. Their failure may be attributed to the difficulty of crossing the BBB. To increase the solubility of neuroprotective agents and improve their brain targeting delivery, the related neuroprotection-based nanoparticles have been designed to obtain better outcomes over ischemic stroke. Table 2 summarizes the neuroprotection-based nanoparticles for effective treatment of ischemic stroke.

4.1. Oxygenation-based nanoparticles

Hypoxia plays an important role in the pathological progression of brain injury. Delivering sufficient oxygen to the penumbra in the early stage of ischemia not only prolongs the thrombolytic time window, but also improves the viability of neurons. Hemoglobin (Hb) is the most important component of red blood cells. Its reversible binding with oxygen and carbon dioxide enables red blood cells to function as oxygen carriers. The immunogenicity of Hb is weak, but its short half-life and vasoconstriction limit its clinical application [112,113]. In addition, hemoglobin can decompose into two toxic dimer peptide chains, causing hepatorenal toxicity and neurotoxicity, etc. [114–116] Encapsulating Hb into liposomes or polymer nanoparticles can not only prolong the half-life of Hb, but also avoid its direct contact with the body to reduce adverse reactions [117,118]. In some studies, liposome-encapsulated Hb has been demonstrated to reduce the BBB damage and brain edema in MCAO rats and mice. The mechanisms may attribute to the inhibition of neutrophils infiltration [72,119–121]. In addition to Hb, perfluorocarbons (PFCs) are another type of compound with high oxygen carrying capacity [122]. Studies have shown that early administration of PFC emulsion could decrease the brain damage in pMCAO rats [73,123]. Nevertheless, controversial studies have also reported that oxygen therapy does not prevent the brain damage from ischemic stroke, since excessive oxygen supply after reperfusion may exacerbate oxidative stress [124–126]. Consequently, the related mechanisms of oxygen therapy, the time window of administration, and the types of ischemic stroke suitable for oxygen therapy need to be further investigated.

4.2. Excitotoxicity inhibitors-based nanoparticles

After cerebral ischemia, the injured neurons abruptly release massive excitatory neurotransmitters, particularly glutamate and aspartate [127,128]. However, due to the insufficient energy supply, the re-uptake of

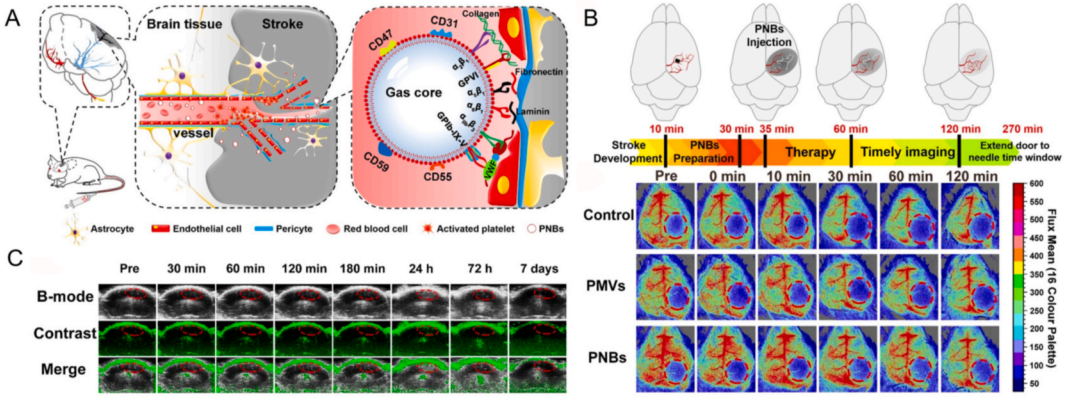


Fig. 3. Platelet bio-nanobubbles for theranostics of ischemic stroke. (A) Schematic illustration of biomimetic nanobubbles for diagnosis and treatment of ischemic stroke. (B) Representative color-coded laser speckle images of the blood flow in brain after injection of platelet-derived platelet membrane vesicles (PMVs) and platelet membrane-derived biomimetic nanobubbles (PNBs) at different time points. (C) Representative brain ultrasound images after injection of PNBs at different time points. Reproduced with permission [58]. Copyright 2018, Ivyspring international publisher. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Summary of neuroprotection-based nanoparticles for effective treatment of ischemic stroke.

Design	Mechanism of action	Effectiveness	Administration route	Reference
Hemoglobin was encapsulated into Liposome	Oxygen supply	Enhance the half-life of hemoglobin; Efficiently inhibit neutrophils infiltration	Intravenous injection	[72]
Perfluorocarbon emulsion	Oxygen supply	Significantly decrease the brain damage	Intravenous injection	[73]
Stroke homing peptide modified erythrocyte bioengineered ROS responsive nanoparticles loading NR2B9c polypeptide	Preventing the overproduction of nitric oxide	Enhance the targeting ability of NR2B9c; ROS responsive release of NR2B9c	Intravenous injection	[74]
Wheat germ agglutinin modified PEG-PLGA nanoparticles containing NR2B9c polypeptide	Preventing the overproduction of nitric oxide	Efficiently delivery NR2B9c to the cerebral ischemic neurons	Intranasal injection	[75]
T7 peptide and stroke homing peptide conjugated liposomes loading neuroprotectant ZL006	NMDAR inhibitor	Dual-peptide modified liposomes promote ZL006 cross the BBB and accumulate at the cerebral regions	Intravenous injection	[16]
Chitosan conjugated N-isopropylacrylamide nanoparticles coated with tween80 loading riluzole	NMDAR inhibitor	Enhance the BBB permeability of riluzole	Intraperitoneal injection	[76]
Ifenprodil liposomes	NMDAR inhibitor	Improve the ischemic lesion delivery of ifenprodil; Release ifenprodil under weak acid pH conditions	Intravenous injection	[77]
Engineered neuronal stem cell membranes coated PLGA nanoparticles comprising glyburide	Ca^{2+} inhibitor	Enhance the accumulation of PLGA NPs in the ischemic regions	Intravenous injection	[38]
pH-responsive chitosan nanoparticles loading nimodipine	Ca^{2+} inhibitor	Release nimodipine in pH-responsive manner in the cerebral ischemia	Brain surface incubation	[78]
PEG modified melanin nanoparticles	RONS scavenger	Show more potent and safer antioxidative effect	Intravenous injection	[79]
PGP-modified polymeric nanoparticles containing catalase	RONS scavenger	Enhance the delivery of catalase to the ischemic subregions and cerebral neurocytes	Intravenous injection	[80]
Cross-linked polyion complex (PEG-PLL) incorporating SOD1	RONS scavenger	Accumulate at the damaged cerebral vessels and exert satisfying antioxidant effect	Intravenous injection	[81]
PEGylated ceria nanoparticles	RONS scavenger	Improve the colloidal stability and prolong the circulation time	Intravenous injection	[82]
CeO_2@ZIF-8 nanoparticles	Scavenging of RONS and anti-inflammation	Enhance the catalytic and antioxidative activities; Improve stroke therapeutic effect	Intravenous injection	[19]
Hollow Prussian blue nanozymes	RONS scavenger	Exhibit outstanding RNOS scavenging property and neuroprotective effect	Intravenous injection	[83]
Molybdenum-based polyoxometalate nanoclusters for intrathecal injection	Scavenging of RONS and anti-inflammation	Bypass the BBB and quickly reach the cerebral ischemic regions	Intrathecal injection	[84]
Poly (ethylene glycolated) hydrophilic carbon clusters	RONS scavenger	Act as antioxidants similar to superoxide dismutase	Not applicable	[85]
Ultrasmall carbogenic nanozyme	RONS scavenger	Show better antioxidative activity than ascorbic acid; Behave effective for the traumatic brain injury treatment	Intravenous injection	[86]
Amine-modified single-walled carbon nanotubes	Scavenging of RONS and anti-inflammation	Efficiently protect neurons damage	Lateral cerebral ventricle injection	[87]
Mn_3O_4 was encapsulated into a T7 peptides attached natural erythrocyte	Free radical scavenger and oxygen regulator	Efficiently penetrate the BBB; Protect the neurocytes before and after thrombolysis	Intravenous injection	[88]
A novel core-shell nanoparticle containing Tempol	RONS scavenger	Prolong the half-life and reduce the side effect of Tempol	Intra-arterial injection	[89]
Edaravone-encapsulated agonistic micelles	RONS scavenger	Up-regulate the permeability of BBB; Facilitate the specific delivery of edaravone	Intravenous injection	[90]
cRGD peptide modified liposomes loaded with edaravone	RONS scavenger	Actively target the surface receptors of leukocytes to migrate into the BBB and effectively deliver edaravone to the injured neurocytes	Intravenous injection	[91]
4T1 cancer cell-inspired nanovehicle loaded with succinobucol	Scavenging of RONS and anti-inflammation	preferentially target the ischemic stroke lesions and effectively penetrate the BBB	Intravenous injection	[92]
Curcumin liposomes functionalized with mesenchymal stem cell membrane	Scavenging of RONS and anti-inflammation	Efficiently penetrate the BBB and accumulate in the ischemic regions; Enhance the survival rate of MCAO mice	Intravenous injection	[93]
c (RGDyK) peptide functionalized exosomes loading curcumin	Scavenging of RONS and anti-inflammation	Recognize the integrin $\alpha_v\beta_3$ on the reactive cerebral vascular endothelial cells and preferentially accumulate at the cerebral ischemic regions	Intravenous injection	[94]
mPEG-*b*-PLA nanoparticles encapsulating curcumin	Scavenging of RONS and anti-inflammation	Reduce oxidative stress; Protect the BBB; Inhibit M1 microglial activation	Intravenous injection	[95]
Platelet-mimetic nanoparticles containing piceatannol and superparamagnetic iron oxide	Anti-inflammation	Specifically recognize the adherent neutrophils; Enhance the inflammation alleviation effect	Intravenous injection	[96]
Neutrophil membrane-derived nanovesicles loading Resolvin D2	Anti-inflammation	Specifically adhere to the inflamed brain endothelium; Efficiently prevent the neurological damage	Intravenous injection	[20]
PEG/cRGD dual-modified liposomes loading 9-aminoacridine	Anti-inflammation	Reduce the cerebral infarct volume; Ameliorate the neurological function of tMCAO rats	Intraperitoneal injection	[97]
liposomal nanoparticles loading acetate	Anti-inflammation	Prolong the blood half-life and reduce the gastrointestinal tract irritation of acetate	Intraperitoneal injection	[98]
Cationic bovine serum albumin-conjugated PEGylated tanshinone IIA nanoparticles	Anti-inflammation	Significantly reduce the inflammatory cytokines and decrease the infarct volumes of tMCAO rats	Intravenous injection	[99]
Low density lipoprotein receptor and neutrophils receptor co-modified nano-carriers loading scutellarin	Anti-inflammation	Facilitate the penetration of scutellarin through the BBB; Significantly improve the neuroprotective effect of scutellarin	Intravenous injection	[100]

(*continued on next page*)

Table 2 (*continued*)

Design	Mechanism of action	Effectiveness	Administration route	Reference
Fas ligand antibody conjugated PEG-lipid nanoparticles encapsulated with 3-n-Butylphthalide	Anti-inflammation	Efficiently penetrate the BBB; Remarkably improve the brain injury	Intravenous injection	[56]
NSCs transfected with ROS responsive B-PDEA/BDNF plasmids polyplexes	Neuronal stem cell therapy and gene therapy	Enhance the BDNF expression in the mouse brain; Enhance the functional recovery of the ischemic brain	Intravenous injection	[101]
NSCs transfected with superparamagnetic iron oxide nanoparticles (SPIONs) and siRNA targeting NgR gene (siNgR)	Neuronal stem cell therapy and gene therapy	Exhibit the in vivo imaging of transfected NSCs; Silence the NgR gene; Enhance the differentiation of NSCs	Intracerebral injection	[102]
MSCs transfected with MFIONs-based gene complexes	Mesenchymal stem cell and gene therapy	Produce high gene transfer efficiency in MSCs; Improve the homing of MSCs in the cerebral ischemic regions; Significantly reduce the mortality of ischemic mice and recover the ischemic brain function	Intravenous injection	[103]
MSCs transfected with core-shell nanoparticles (CPMSN@^{125}I-SD)	Mesenchymal stem cell therapy	Realize real-time PA imaging of MSCs in the mouse brain and SPECT imaging of the ischemic mouse brain; Maintain superior therapeutic effect for ischemic stroke	Intracerebral injection	[104]
EPCs transfected with superparamagnetic iron oxide/siPHD2 complexes	Endothelial progenitor cell therapy and gene therapy	Enhance the expression CXCR4 and HIF-1α; Elevate the homing and survival ability of EPCs	Intracardiac injection	[105]
RGD peptides cationic polymer interact with HIF-1α plasmid DNA	Gene therapy	Effectively target the vascular endothelial cells; Significantly improved the recovery of tMCAO rats	Carotid injection	[106]
Cationic lipid assisted PEG-PLA nanoparticles encapsulated with C3-siRNA (NP_{siC3})	Gene therapy	Decrease the C3 expression in microglia; Reduce the microglial neurotoxicity	Intravenous injection	[107]
HO1-mRNA was delivered with DA-PEI2k to form into HO1-mRNA/DA-PEI2k complex	Gene therapy	Exhibit higher gene expression; Significantly reduce the infarct volume	Intracerebral injection	[108]
Self-assembled nanoparticles (HSAP-NP/pHO1) composed of hypoxia-specific anti-RAGE peptide (HSAP) and HO1 plasmid (pHO1)	Gene therapy	Increase the genes delivery and expression in the ischemic tissues	Intravenous injection	[109]
High mobility group box 1 (HMGB1) siRNA delivered by PAMAM dendrimer of e-PAM-R	Gene therapy	Efficiently realize the knockdown of HMGB1; Markedly suppress the infarct volume of MCAO rats	Intranasal administration	[110]
M2 microglia-derived exosomes	Gene therapy	Promote the neuronal function recovery by inhibiting neuronal inflammation	Intravenous injection	[111]

excitatory neurotransmitters is seriously impaired, leading to the accumulation of excitatory neurotransmitters in the synaptic. The accumulation of excitatory neurotransmitters triggers the depolarization of brain cells, which results in the influx of large amounts of Ca^{2+} and Na^{+}, and the outflow of K^{+}, ultimately leading to cerebral edema. Meanwhile, the influx of Ca^{2+} activates the calcium-dependent pathways, such as the activation of NO synthase and proteases, the destruction of mitochondria and cell nucleus, eventually leading to cells damage and death [129,130]. Glutamate receptor antagonists and calcium channel blockers have been shown to suppress excitotoxicity and exert neuroprotective effects for ischemic stroke [37,131,132].

4.2.1. *Glutamate receptor antagonists-based nanoparticles*

Glutamate is the principle neurotransmitter that triggers excitotoxicity. N-methyl-D-aspartate receptor (NMDAR) is one of the most important receptors of glutamate, and ischemia-reperfusion leads to the overstimulation of NMDAR. Glutamate binding NMDAR can stimulate the influx of Ca^{2+}, thereby leading to brain cells damage [133,134]. Recently, to enhance the solubility and BBB permeability of glutamate receptor antagonists, integrating glutamate receptor antagonists into nano-delivery systems have been expected to improve their therapeutic effects for ischemic stroke [16,74–77].

NO has been shown to inhibit platelet aggregation, improve blood flow perfusion, and reduce ischemic injury [66,135]. Nevertheless, excessive NO induced by ischemic stroke may provoke excitatory neurotoxicity [136]. NR2B9c polypeptide (KLSSIESDV) can disrupt the interaction between NMDAR and postsynaptic density protein-95 (psd-95) to prevent the overproduction of NO, thereby blocking the

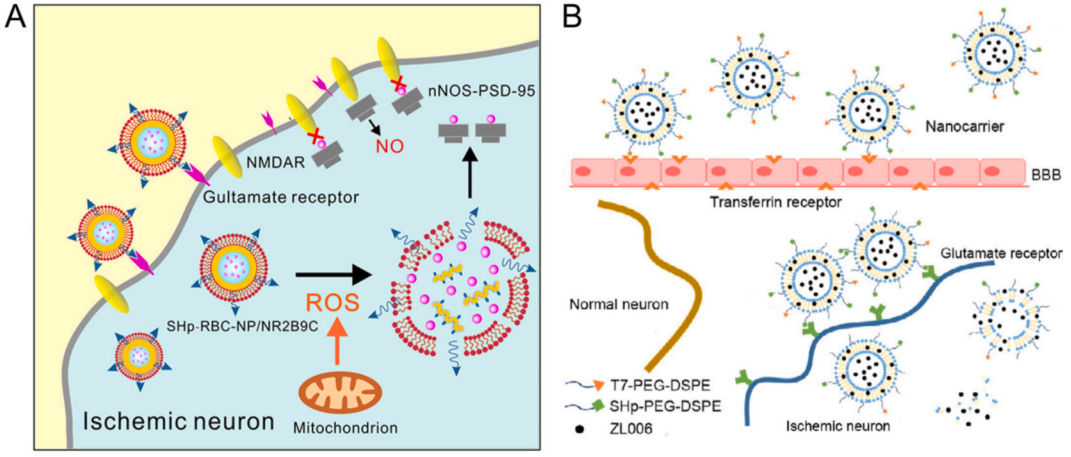


Fig. 4. Glutamate receptor antagonists-based nanoparticles for ischemic stroke therapy. (A) Schematic design of stroke homing peptide modified erythrocyte bioengineered ROS responsive nanoparticles for ischemic stroke therapy. Reproduced with permission [74]. Copyright 2018, American Chemical Society. (B) Dual targeting peptides modified liposomes loading ZL006 for ischemic stroke therapy. Reproduced with permission [16]. Copyright 2016, Elsevier.

downstream neurotoxic signaling pathways [136,137]. However, it is difficult for free NR2B9c polypeptide to pass through the BBB. To achieve better therapeutic effects, Lv et al. designed a ROS-responsive nanoparticle with an average hydrodynamic diameter of 194.6 ± 8.5 nm for specific delivery of NR2B9c to the ischemic brain tissues (Fig. 4A) [74]. The nanoparticle consists of a boronic ester modified dextran polymer core and an erythrocyte membrane shell inserted with stroke homing peptide (SHp). Aside from enhancing the targeting ability of NR2B9c with the help of erythrocyte membrane and stroke homing peptide, the smart designed nanoparticles could also control the release of NR2B9c in the ischemic neurons due to the high intracellular ROS level. Through intravenous injection, the nanoparticles showed superior therapeutic effect in temporary middle cerebral artery occlusion (tMCAO) rat models over free NR2B9c. In another research, Li et al. designed an intranasal route of wheat germ agglutinin (WGA) modified PEG-PLGA nanoparticles containing NR2B9c polypeptide (NR2B9c-WGA-NPs), which efficiently delivered NR2B9c to the ischemic cerebral neurons since WGA recognizes and binds to the sialic acid residues n-acetyl-D-glucosamine (GlcNAc) that are abundant in the nasal epithelium [75]. Through intranasal administration, the NR2B9c-WGA-NPs can bypass the BBB and prevent the ischemic neurons from excitotoxicity.

ZL006 is a novel neuroprotectant that disrupts the interaction between neuronal NO synthase (nNOS) and psd-95, thereby inhibiting the activity of NMDAR and preventing glutamate induced excitotoxicity. To enhance the BBB penetration ability and deal with the nonspecific distribution of ZL006, Zhao et al. designed ZL006 loaded liposomes conjugated with T7 peptide and stroke homing peptide (T7&SHp-P-LPs/ZL006) with mean particle size of 96.24 ± 1.13 nm (Fig. 4B) [16]. The T7&SHp-P-LPs/ZL006 effectively delivered ZL006 across the BBB and specifically target the ischemic lesions through tail vein injection. The in vivo experiments demonstrated that the T7&SHp-P-LPs/ZL006 efficiently penetrated the BBB and significantly reduced the excitotoxicity of MCAO rats than the non-peptide modified liposomes or single peptide (T7-or SHp-) modified liposomes.

Apart from NR2B9c and ZL006, riluzole and ifenprodil have also been reported as NMDAR antagonists to prevent glutamate transmission and overcome the excitotoxicity [138–140]. To enhance the aqueous solubility and prolong the half-life of riluzole, Verma et al. encapsulated riluzole into tween80 coated nanoparticles composed of chitosan conjugated N-isopropylacrylamide [76]. By intraperitoneal injection, the nanoparticles efficiently penetrated the BBB and showed significant neuroprotection effect at very low concentration in tMCAO rat models. In another research, to improve the ischemic lesion delivery efficacy of ifenprodil, Kikuchi et al. encapsulated ifenprodil into PEG-modified liposomes (Ifen-Lip) [77]. By intravenous injection, the Ifen-Lip significantly alleviated the ischemic stroke injury than free Ifenprodil.

4.2.2. Calcium blockers-based nanoparticles

Calcium channel blockers not only reduce the excitatory amino acids release and intracellular Ca^{2+} overload by inhibiting excessive Ca^{2+} influx, but also increase the cerebral blood flow by dilating the cerebral blood vessels [141]. In recently years, glyburide has been found effective in treating central nervous system injuries. Glyburide inhibits Ca^{2+} influx by inhibiting the sulfonylurea receptor 1-regulated non-selective cation channel Trpm4 (Sur1-Trpm4, Sur1-NC_{Ca}-ATP) and mitigates cerebral edema [142]. To improve the target delivery of glyburide, Ma et al. loaded glyburide into (lactic-co-glycolic) acid (PLGA) nanoparticles and coated the nanoparticles with engineered neuronal stem cell membranes (Gly-CMNPs) [38]. The engineered neuronal stem cell (NSCs) that overexpressed CXCR4 enhanced the tropism of PLGA nanoparticles through the chemotactic interaction of CXCR4 with SDF-1, which is overexpressed in the ischemic regions. The in vivo imaging experiment showed that compared with the naked PLGA nanoparticles or normal NSC membranes coated PLGA nanoparticles (MNPs), the engineered NSCs membranes coated PLGA NPs (CMNPs) significantly enhanced the deposition of PLGA nanoparticles in the ischemic regions. The in vivo treatment experiment showed that, by intravenous injection, the Gly-CMNPs significantly enhanced the survival, reduced the infarct volume and neurological score of tMCAO mice than free glyburide (Fig. 5).

Nimodipine is an L-type voltage gated calcium channel antagonist that can mitigate Ca^{2+} overload in neurons [143]. Oral administration may cause vomiting and gastrointestinal discomfort. To overcome these drawbacks, Orsolya et al. employed chitosan nanoparticles as drug carriers to encapsulate nimodipine, which selectively delivered nimodipine to the ischemic brain and released nimodipine in response to the acidic microenvironment of the ischemic brain tissue [78]. The pH-responsive chitosan nanoparticles did not induce a neuro-immune response at the brain tissue. In addition, the nanoparticles potently inhibited the spreading depolarization and showed improved neuroprotection effect for ischemic stroke.

To sum up, although several excitotoxicity inhibitors have been tested in clinical trials, none of them have demonstrated the neuroprotection effect for stroke patients [127]. On the basis of animal experiments, excitotoxicity inhibitors-based nanoparticles have tremendous potential for ischemic stroke therapy. Studies have shown that the therapeutic effect of excitatory neurotoxicity inhibitors is time-dependent, namely, the protective effect of brain will be weakened beyond a certain time window. Therefore, the specific treatment time window and the administration dosage need to be further investigated.

4.3. Antioxidants-based nanoparticles

Oxidative stress is one of the important mechanisms causing ischemia/reperfusion injury, which derives from the overwhelming generation of reactive oxygen species and/or nitrogen species (RONS) during the process of reperfusion, such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet -}$), hydroxyl radical (•OH), nitric oxide (•NO), and peroxynitrite ($ONOO^-$) [144,145]. The excessive RONS mediate tissue damage and cell apoptosis through destroying the DNA, proteins, and lipids [31,146]. Studies have shown that antioxidants can ameliorate oxidative stress by eliminating RONS. Endogenous antioxidant enzymes, inorganic nanomaterials with reductase activity, and anti-oxidative drugs are the three most studied antioxidants [82,90,147]. Despite their admirable anti-oxidative effects, free antioxidants have a very short half-life and are difficult to cross the BBB. To circumvent their weaknesses, a variety of nanoparticles have been designed to enhance their therapeutic effects against ischemic stroke [148].

4.3.1. Endogenous anti-oxidases-based nanoparticles

Endogenous antioxidant enzymes are antioxidants that produced by the human body, such as melanin, glutathione antioxidant enzymes (GSH-Px), catalase (CAT), and superoxide dismutase (SOD), which have favorable biocompatibility and robust anti-oxidative activity [149–152]. For instance, melanin is a heterogeneous biopolymer synthesized by the melanocytes, and it has been proved to present robust RONS scavenging ability. But the short half-life and water-insolubility hindered its further application for ischemic stroke. To overcome these problems, Liu et al. developed polyethylene glycol (PEG) modified melanin nanoparticles (PEG-MeNPs) (Fig. 6A) [79]. The PEG-MeNPs exhibited broad anti-oxidative activities against multiple RONS. The in vitro cellular experiment showed that the PEG-MeNPs decreased the intracellular $O_2^{\bullet -}$ level and alleviated the inflammatory reaction. The in vivo results further demonstrated that pre-intravenous injection of PEG-MeNPs efficiently decreased the infarct area of the ischemic brain with negligible side effects.

To protect the catalase (CAT) from protease hydrolysis and effectively deliver it to the cerebral ischemic lesions, Zhang et al. constructed cross-linked dendrigraft poly-L-lysine (DGL) nanoparticles (NPs) comprising *cis*-aconitic anhydride-modified CAT that exhibited high

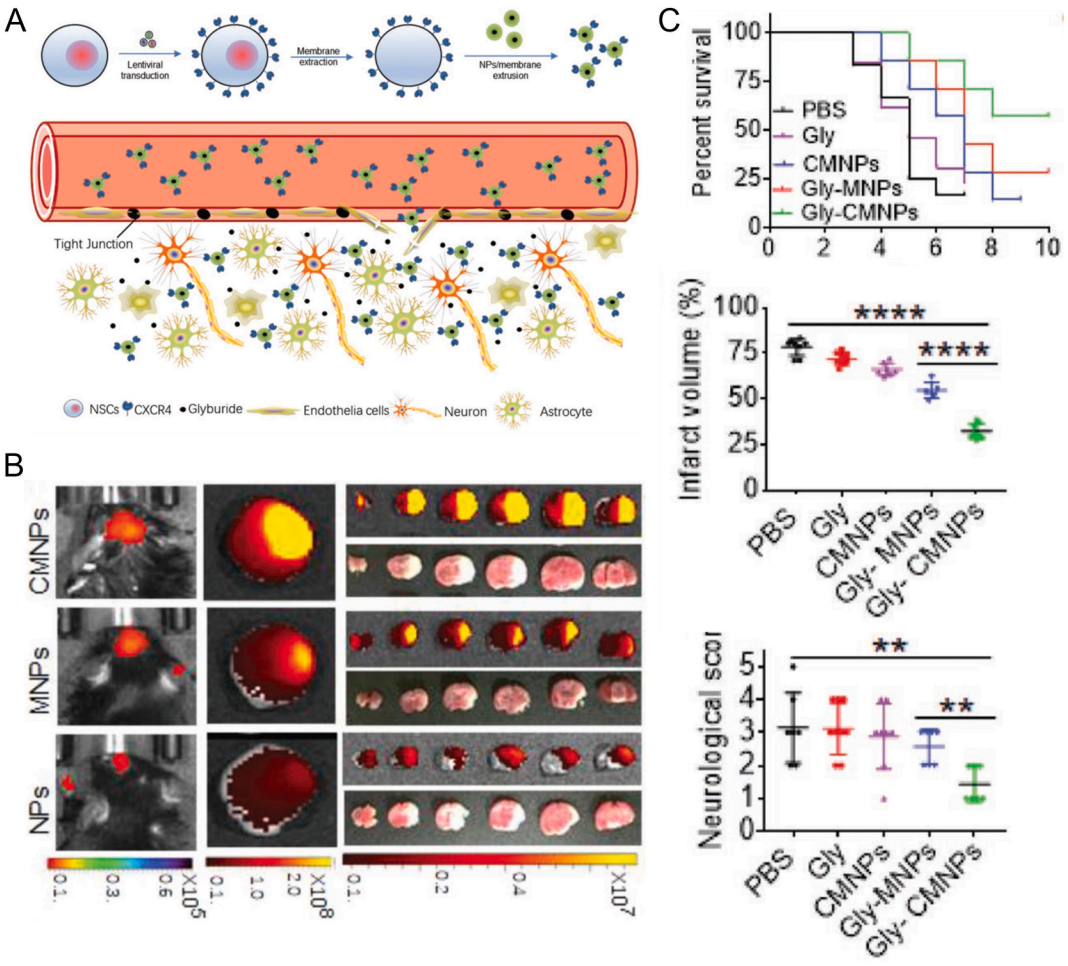


Fig. 5. Engineered neuronal stem cells biomimetic nanoparticles for ischemic stroke treatment. (A) Scheme illustration of engineered neuronal stem cells biomimetic nanoparticles for ischemic stroke treatment. (B) In vivo imaging of PLGA NPs (NPs), MNPs and CMNPs in the ischemic brain. (C) In vivo therapeutic results of Gly, CMNPs, Gly-MNPs and Gly-CMNPs. Reproduced with permission [38]. Copyright 2019, Wiley-VCH.

binding affinity to neutrophils (Fig. 6B) [80]. By phagocytosis, neutrophils acted as vehicles to carry the NPs cross the BBB and targeted to the brain inflammatory regions. Then the neurons ingested the NPs or ingested the exosomes containing NPs by intercellular communications with neutrophils. Through intravenous injection, the delivery efficiency and therapeutic effect of CAT were significantly enhanced in MCAO mice. In another research, to prolong the half-life and improve the BBB permeability of copper/zinc superoxide dismutase (SOD1), Jiang et al. incorporated SOD1 into cross-linked polyion complex (methoxy-poly (ethyleneglycol)-*b*-poly (L-lysine, PEG-PLL) to form a cross-linked nanozyme, which could accumulate at the damaged cerebral vessels and exert satisfying antioxidant effect by intravenous injection [81]. Although endogenous nanozymes have achieved positive effects in animal experiments, most of them have specific substrate selectivity, which prevents their further clinical transformation.

4.3.2. *Inorganic nano-reductases-based nanoparticles*

The robust enzyme-like antioxidant activities of several inorganic nanomaterials have been identified by recently developed nanotechnology, such as ceria nanoparticles, prussian blue nanozymes, carbogenic nanozymes, molybdenum-based polyoxometalate nanoclusters, and Mn_3O_4 nanozymes [82,83,86,153,154]. These inorganic nanomaterials have superior stability than endogenous antioxidants.

It is well known that ceria nanoparticles (CeO_2 NPs) have strong antioxidation and repetitive ROS scavenging activity by transferring electron between Ce^{3+} and Ce^{4+}. CeO_2 NPs have been proven to prevent degenerative diseases of the central nervous system through eliminating ROS. For instance, Kim et al. prepared PEGylated ceria nanoparticles for intravenous injection to protect against ischemic stroke [82]. The ceria nanoparticles were modified with PEG to improve their colloidal stability and prolong their blood circulation time. In another study, He et al. designed CeO_2@ZIF-8 nanoparticles with a nanoscale at about 140 nm for intravenous injection to achieve stronger ROS scavenging activity, in which ceria nanoparticles were capped into bioactive zeolitic imidazolate framework-8 [19]. They found that this rational designed CeO_2@ZIF-8 nanoparticles protected the antioxidant activity of CeO_2 nanoparticles, prolonged the blood circulation time, and promoted the CeO_2 nanoparticles cross the BBB and accumulated at the ischemic brain sites, which together enhanced the therapeutic effect of ischemic stroke.

Prussian blue was approved by the FDA in 2003 as a detoxifier for thallium and cesium because of their excellent bio-safety [155,156]. Recently, Prussian blue nanoparticles have been reported to present robust RNOS scavenging activity owning to their endogenous antioxidant enzyme-like activity. For example, Zhang and coworkers constructed hollow Prussian blue nanozymes (HPBZs) with large specific surface area and an average diameter of 65 nm [83]. The HPBZs exhibited outstanding RNOS scavenging property and neuroprotective effect both in vitro and in vivo (Fig. 7A). The HPBZs enhanced the viability of H_2O_2-damaged SH-SY5Y cells from 17.15% to 50.13% and significantly reduced the cerebral infarct volume of MCAO rats by intravenous injection.

Molybdenum-based polyoxometalate (POM) nanoclusters present

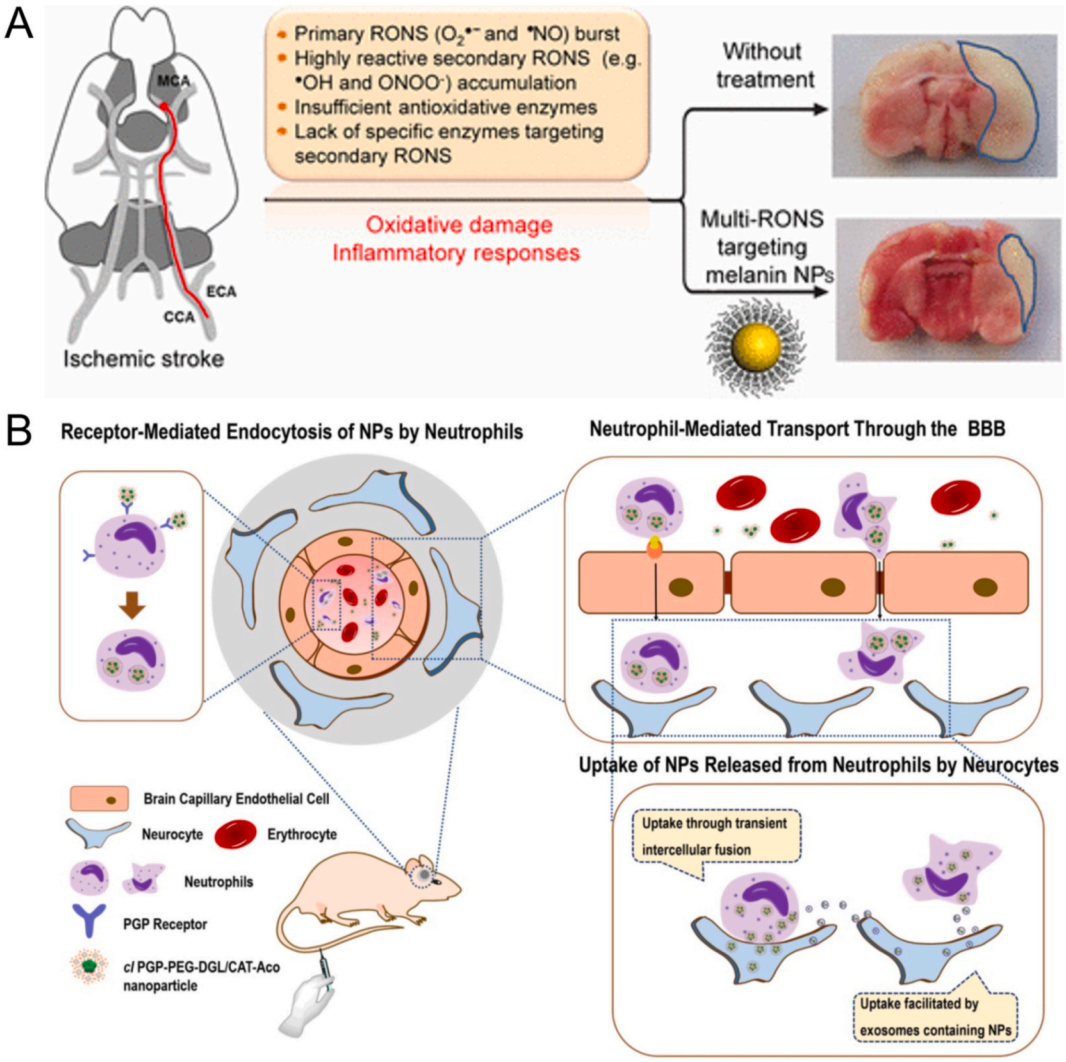


Fig. 6. Designing nanoparticles for circumventing drawbacks of endogenous antioxidants. (A) Antioxidant therapy with PEG-MeNPs for ischemic stroke. Reproduced with permission [79]. Copyright 2017, American Chemical Society. (B) Polymeric nanoparticles containing *cis*-aconitic anhydride-modified catalase specifically target neutrophils for ischemic stroke therapy. Reproduced with permission [80]. Copyright 2017, Ivyspring international publisher.

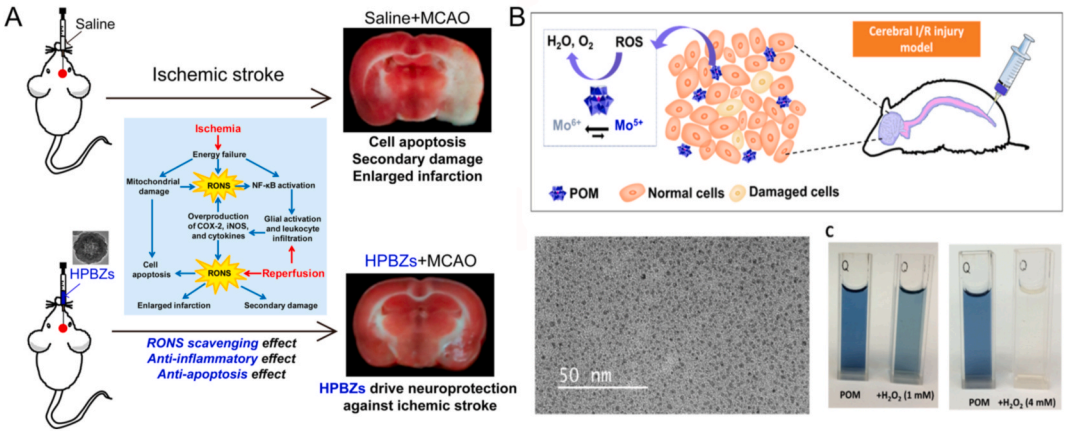


Fig. 7. Designing inorganic nanoreductases based nanoparticles for enhanced ischemic stroke therapy. (A) Schematic illustration of HPBZs for preventing ischemia/reperfusion injury. Reproduced with permission [83]. Copyright 2019. American Chemical Society. (B) Schematic illustration of POM clusters in preventing ischemia/reperfusion injury and characteristics of POM clusters. Reproduced with permission [84]. Copyright 2019. American Chemical Society.

remarkable antioxidative effect through changing their reduction and oxidation states [153]. They have been proved effective for Alzheimer's disease and acute kidney injury. Li and coworkers reported Molybdenum-based polyoxometalate nanoclusters (POM) for ischemic stroke therapy through intrathecal injection [84]. The intrathecal injection enables POM nanoclusters to bypass the BBB and quickly reach the cerebral ischemic regions. Both the in vitro and in vivo results demonstrated that POM nanoclusters ameliorated ischemia/reperfusion injury through effectively inhibiting oxidative stress and inflammation response (Fig. 7B).

Carbon nanomaterials have received extensive attention due to their important anti-oxidative and redox regulation functions [157,158]. Samuel et al. developed poly (ethylene glycol) hydrophilic carbon clusters (PEG-HCCs) that could act as antioxidants similar to superoxide dismutase [85]. Similarly, Mu et al. developed an ultrasmall carbogenic nanozyme that showed a 12-fold better antioxidative activity than ascorbic acid and behaved effective for traumatic brain injury treatment [86]. Lee et al. prepared amine-modified single-walled carbon nanotubes (a-SWNTs). They found that pre-treatment rats with a-SWNTs through lateral cerebral ventricle injection could efficiently protect neurons damage caused by ischemic stroke [87].

Apart from these inorganic nanozymes mentioned above, Mn_3O_4 has also been used as antioxidant for ischemic stroke therapy. For example, Shi et al. attached T7 peptides with a natural erythrocyte, the engineered erythrocyte was then extruded with Mn_3O_4 to get Mn_3O_4@nanoerythrocyte-T7 (MNET) with a mean size of about 200 nm [88]. By intravenous injection, the well-designed MNET presented the following significant advantages for ischemic stroke therapy: (i) enhanced the BBB permeability with the help of T7 peptides; (ii) before thrombolysis, protected the neurons through scavenging free radicals and releasing oxygen; (iii) after thrombolysis, reduced the oxidative stress through scavenging free radicals and storing oxygen.

Even though these inorganic nanoreductases are highly reductive and easy to construct and modify, there are still many challenges for their clinical transformation. The biggest one is the long-term toxicity in vivo. Despite the fact that a large number of studies have confirmed the low acute toxicity of the inorganic nanomaterials, it remains to be verified whether they can be eliminated from the body and whether they will cause long-term toxicity.

4.3.3. *Antioxidant drugs-based nanoparticles*

Antioxidant drugs are substances with antioxidant activities that are extracted from natural plants or artificially synthesized, such as Tempol, edaravone, succinobucol, and curcumin [89,90,92,95]. Although they are simple to synthesize or extract, their poor water solubility, short half-life, and incompetent to cross the BBB remarkably restricted their clinical application. Nanoparticles become a feasible strategy to improve the therapeutic outcomes of antioxidant drugs in the treatment of ischemic stroke.

Tempol is a nitroxide radical compound, which scavenges ROS through simulating SOD and inhibiting lipid peroxidation [159]. Hisayuki et al. developed a novel core-shell (polyethylene glycol [PEG]-b-poly [4-(2,2,6,6-tetramethylpiperidine-1-oxyl) aminomethylstyrene]) nanoparticle containing Tempol (RNPs) [89]. The RNPs prolonged the half-life and reduced the side effect of Tempol. tMCAO mice were injected with RNPs through the common carotid artery, and were detected around endothelial and neuronal cells in the ischemic lesions. The endothelial tight junctions were preserved and the neuronal apoptosis was suppressed in the ischemic lesions. Furthermore, the infarction size and neurological deficit score were significantly reduced. Thus, an intra-arterial injection of RNPs provided an infarct brain protection effect.

Edaravone (EDV), a small-molecule ROS scavenger, is the only clinically approved neuroprotective agent for ischemic stroke treatment. To prolong the circulation half-life and enhance the cerebral uptake of EDV, Jin et al. developed an edaravone micelle (EDV-AM), in which EDV was encapsulated into the mixture of methoxypoly (ethylene glycol)-b-poly (D,L-lactic acid) (MeO-PEG-PLA), purine nucleotide derivative CGS21680-methoxypoly (ethylene glycol)-b-poly (D,L-lactic acid) (CGS-PEG-PLA), and near-infrared fluorophore IR783B-DiR-methoxypoly (ethylene glycol)-b-poly (D,L-lactic acid) (IRB-PEG-PLA). The average diameter of EDV-AM was about 20 nm. By intravenous injection, EDV-AM actively opened the tight junctions of the BBB and therefore facilitating the specific delivery of EDV to the cerebral ischemia [90]. The in vitro experiment demonstrated that the EDV-AM up-regulated the permeability of the endothelial monolayer. Magnetic resonance imaging (MRI) proved the superior treatment effect of EDV-AM that free EDV. In addition, diffusion tensor imaging (DTI) showed that EDV-AM effectively accelerated the axonal remodeling of the ipsilesional white matter and improved nerve functions of photo-chemically induced permanent MCAO mice. Consequently, the EDV-AM holds potential for improving therapeutic efficiency of edaravone. In another study, Hou and coworkers reported cRGD peptide modified liposomes loaded with edaravone (ER-cRGDLs) [91]. The engineered liposomes actively targeted the surface receptors of leukocytes (mainly monocytes and neutrophils), which would carry the ER-cRGDLs to migrate into the BBB and effectively deliver therapeutic agents to the injured brain cells. Through intravenous injection, ER-cRGDLs showed better therapeutic effects in tMCAO rats than free edaravone.

Succinobucol (SCB) is a derivative of probucol, which has stronger antioxidant and anti-inflammatory effects than probucol. To improve the solubility and brain targeting of SCB, our group develop a 4T1 cancer cell-inspired nanovehicle (MPP/SCB), in which a pH sensitive nanoparticle (PP/SCB) was formed by loading SCB in polymer of methoxy poly (ethylene glycol)-block-poly (2-diisopropyl methacrylate) (PEG-PDPA), and PP/SCB was then coated with 4T1 cancer cell membrane to form MPP/SCB with a mean hydrodynamic diameter of 68 nm [92]. By virtue of the platelet endothelial cell adhesion molecule-1 (CD138) and vascular cell adhesion molecule 1 (VCAM-1) overexpressed on 4T1 cancer cell membrane, MPP/SCB could preferentially target the ischemic stroke lesions and effectively penetrate the BBB to exert therapeutic effect. Through intravenous injection, MPP/SCB showed remarkable neuroprotective effects on tMCAO rats.

Moreover, studies have shown that curcumin, a traditional Chinese medicine component, can eliminate ROS and mitigate inflammatory response. However, the clinical application of curcumin is hindered by its poor water solubility and instability properties. Benefiting from the BBB penetration of mesenchymal stem cells (MSCs) and their affinity for damaged cells, Wu et al. functionalized curcumin liposomes with mesenchymal stem cell membrane to form engineered MSC-Lipo with an average size of 70 nm [93]. The engineered MSC-Lipo were more likely to assemble at the surface of the endothelial cells, thereby promoting the interaction between MSC-Lipo and the endothelium. The in vivo distribution experiment on MCAO mice showed that MSC-Lipo preferred to distribute at the cerebral ischemic regions. More importantly, compared with the un-modified liposome, intravenous injection of MSC-Lipo significantly reduced the brain infarct volumes and enhanced the survival rate of MCAO mice (Fig. 8). Similarly, Tian et al. conjugated cyclo (Arg-Gly-Asp-D-Tyr-Lys) peptide [c (RGDyK)] on the surface of mesenchymal stromal cell (MSC)-derived exosomes (cRGD-Exo). Furthermore, the engineered exosomes were applied to load curcumin (cRGD-Exo-cur) [94]. The cRGD-Exo-cur could recognize the integrin $\alpha_v\beta_3$ on reactive cerebral vascular endothelial cells and preferentially accumulate at the cerebral ischemic regions. In addition, intravenous injection of cRGD-Exo-cur suppressed the inflammatory response and cellular apoptosis. In another study, to improve the solubility and stability of curcumin, Wang et al. encapsulated curcumin into copolymer of poly (ethylene glycol)-b-poly (D,L-lactide) (mPEG-*b*-PLA) to form nanoparticles (NPcurcumin) for ischemia/reperfusion injury therapy [95]. Compared with free curcumin, NPcurcumin significantly reduced the infarct volumes and improved the neuronal functions by intravenous injection.

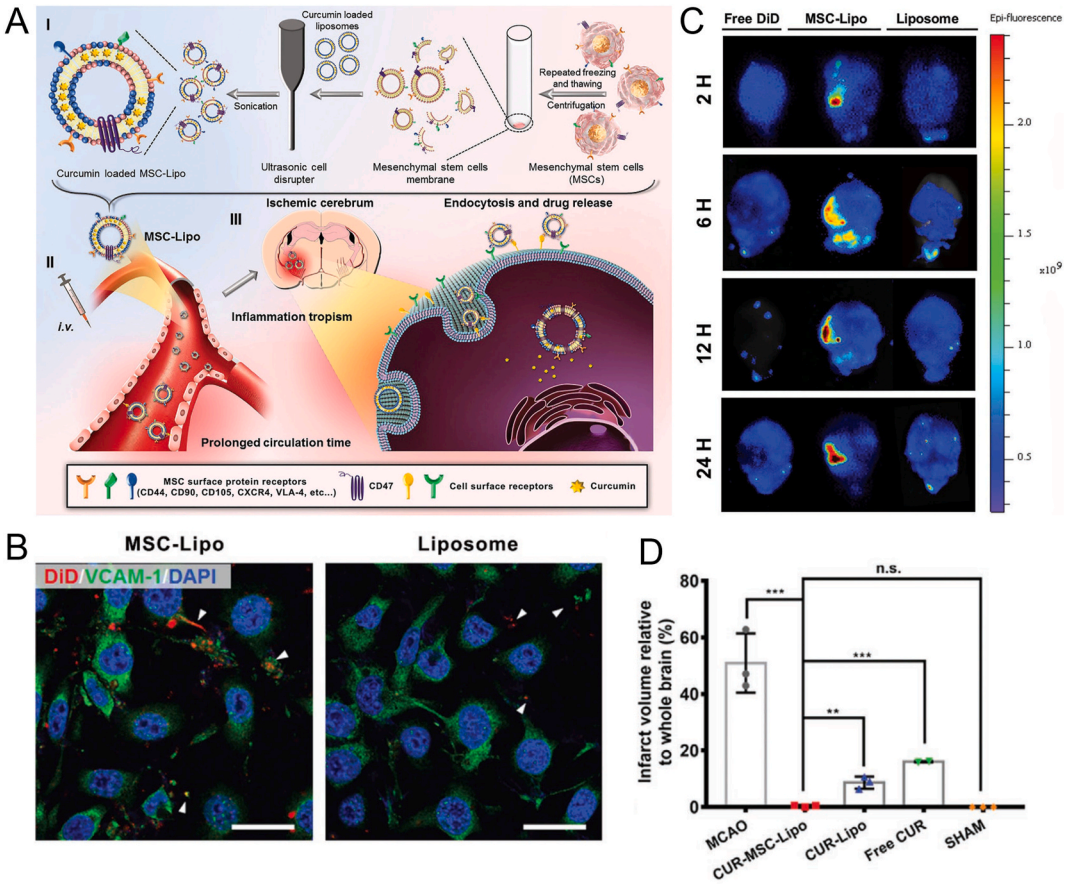


Fig. 8. Mesenchymal stem cells bioengineered curcumin liposomes for targeted therapy. (A) A schematic diagram of preparation of MSC-Lipo and targeted therapy for ischemic stroke. (B) VCAM-1 binding assay of MSC-Lipo and liposomes. (C) Cerebral ischemic regions targeting ability of free DiD, liposomes, and MSC-Lipo on MCAO mice. (D) Quantitative analysis of infarct volume in each group. Reproduced with permission [93]. Copyright 2020, Wiley-VCH.

4.4. Anti-inflammatory agents-based nanoparticles

Inflammatory response occurs throughout the pathological process of ischemic stroke. Before thrombolysis, the oxidation and anti-oxidation homeostasis of the brain is disrupted, resulting in mitochondrial dysfunction, cell apoptosis and necrosis. The intracerebral microglia are thus activated and release inflammatory factors and cytokines. Subsequently, peripheral leukocytes are recruited, and pro-inflammatory mediators such as IL-1, TNF-α, and ICAM-1 are up-regulated, causing inflammation in the cerebral ischemic area [28]. After ischemia/reperfusion, the oxidative stress induces the activation and aggregation of peripheral immune cells, and stimulate inflammatory factors secretion, thereby aggravating the inflammatory response. In addition, abundant leukocytes accumulate at the vascular endothelial cells in the penumbra, resulting in “no-reflow” phenomenon of the microvascular [64]. Anti-inflammatory agents such as Resolvin D2, piceatannol, 9-Aminoacridine, and Tanshinone IIA have been proved beneficial for ischemic stroke therapy in preclinical trials [20,160–162]. They can inhibit microglia polarization, reduce the expression of inflammatory factors, or suppress inflammatory cells infiltration. To improve the druggability and bioavailability of these anti-inflammation agents, different kinds of nano-formulations have been designed.

Piceatannol, a hydroxylated analog of resveratrol, has been testified to alleviate inflammation through preventing neutrophils adhesion [56]. However, it is difficult for free piceatannol to selectively target inflammatory neutrophils. Inspired by the specific cell-cell recognition between platelets and inflammatory neutrophils, Tang and coworkers encapsulated piceatannol and superparamagnetic iron oxide (SPIO) into PLGA nanoparticles and then coated the nanoparticles with platelet membrane to form platelet-mimicking nanoparticles (PTNPs) for the recognition, intervention, and monitoring of inflammatory neutrophils [96]. The PTNPs specifically recognized the adherent neutrophils and then released piceatannol to prevent neutrophils adhesion to the endothelial cells, therefore alleviating inflammation. In addition, the co-delivery of SPIO (SPIO-PTNPs) helped to monitor the infiltration of neutrophils. The in vivo MRI showed that mice treated with SPIO-PTNPs through intravenous injection exhibited significant MRI contrast effect in the ischemic hemisphere. The in vivo therapeutic experiment showed that, compared with bare PLGA nanoparticles, PTNPs significantly inhibited neutrophils infiltration and reduced the infarct size of ischemic brain tissue (Fig. 9).

Resolvin D2 (RvD2), a derivative ofω-3 fatty acids, can reduce the interaction between neutrophils and endothelial cells by inducing endothelial cells to produce NO. In addition, RvD2 can also bind to the G-protein-coupled receptor (GPR18) on neutrophils, thereby inhibiting neutrophil infiltration and stimulating neutrophil apoptosis [160,163,164]. However, after intravenous injection, the bioavailability of RvD2 is reduced, since RvD2 tends to bind to plasma proteins. To enhance the bioavailability of RvD2, Dong and coworkers loaded RvD2 into neutrophil membrane-derived nanovesicles (RvD2-HVs) for alleviating ischemic stroke-induced inflammation [20]. The RvD2-HVs could specifically adhere to the inflamed brain endothelium during reperfusion. The in vivo experiments showed that, through intravenous injection, RvD2-HVs was more effective than RvD2 in preventing neurological damage inflicted by reperfusion in tMCAO mice.

Orphan nuclear receptor NR4A1 participates in the microglia and macrophage-mediated inflammation [165,166]. Wang et al. found that 9-aminoacridine (9-AA) is a novel NR4A1 activator, which shows

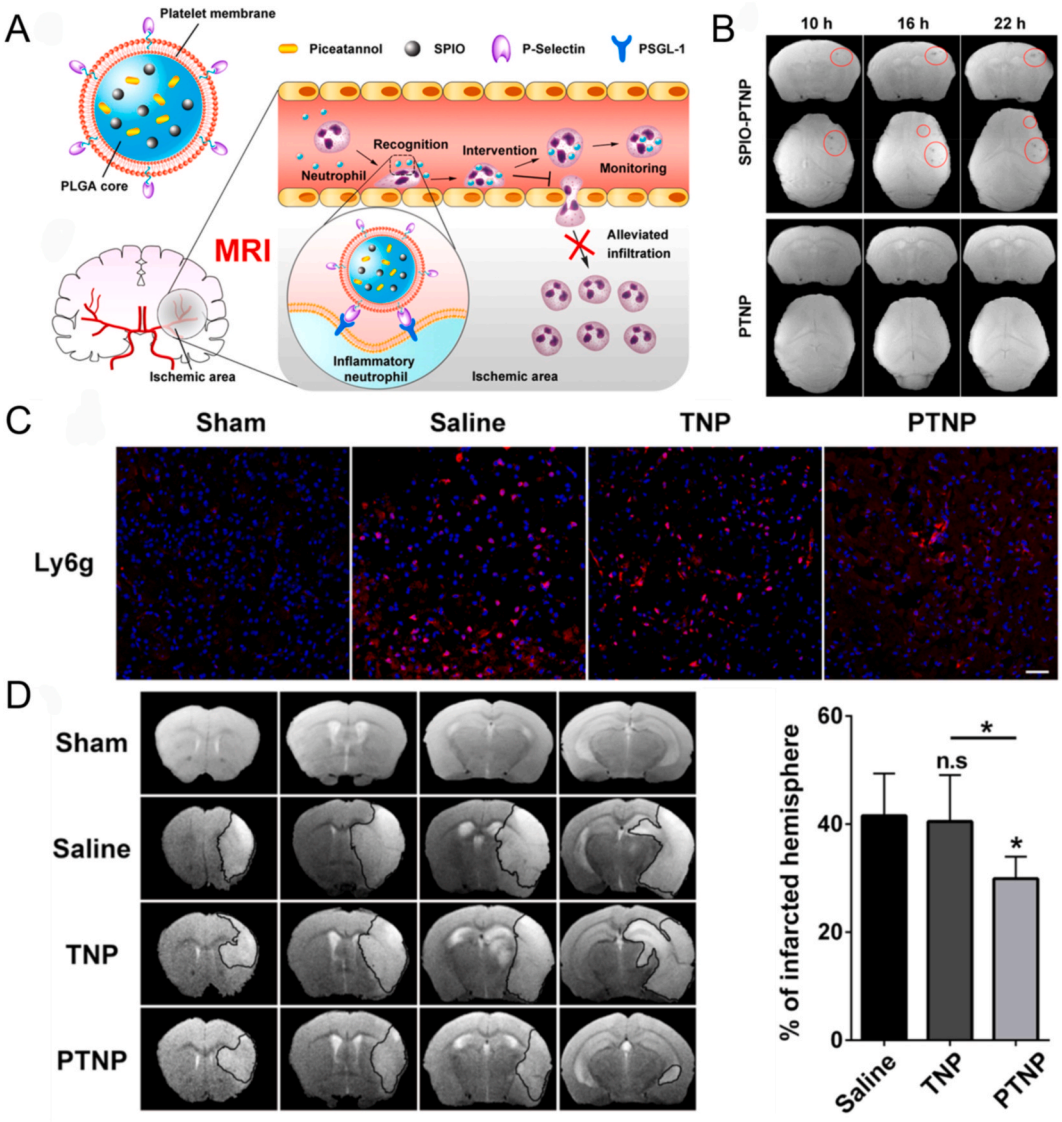


Fig. 9. Platelet-mimetic nanoparticles for ischemic stroke treatment by interacting with inflammatory neutrophils. (A) Scheme diagram of PTNPs-mediated recognition, intervention, and monitoring of neutrophils for ischemic stroke treatment. (B) MRI images of tMCAO mice injected with SPIO-PTNPs and PTNPs. (C) Confocal laser scanning images of neutrophil infiltration stained with anti-Ly6g antibody in ischemic brain after treatment with saline, TNPs (bare PLGA nanoparticles), and PTNPs. (D) In vivo therapeutic experiment of PTNPs for ischemic stroke. Reproduced with permission [96]. Copyright 2019. American Chemical Society.

anti-inflammatory activity and regulates the activation of microglia through the NR4A1/IL-10/SOCS3 signaling pathway [97]. They loaded 9-AA into PEG/cyclic Arg-Gly-Asp (cRGD) peptide dual-modified liposomes (9-AA/L-PEG-cRGD) to prolong the circulation time and increase the brain deposition of 9-AA, thereby dramatically reducing the cerebral infarct volume and ameliorating the neurological function of tMCAO rats by intravenous injection. Short chain fatty acids such as acetate also have been proved useful for inflammation attenuation [167]. To prolong the half-life and reduce the gastrointestinal irritation of acetate. Po-Wah et al. loaded acetate into liposomes and treated tMCAO rats by intraperitoneally injection [98]. The liposomes prolonged the blood half-life and reduced the gastrointestinal tract irritation of acetate, and achieved effective anti-inflammatory therapy for ischemic stroke.

In nature, some plant extracts such as tanshinone IIA, scutellarin, and 3-n-Butylphthalide (dl-NBP) have been identified to present anti-inflammatory effects for ischemic stroke therapy [56,99,100]. For example, Tanshinone IIA (TIIA) is the main active ingredient of the traditional Chinese medicine Salvia miltiorrhiza, which presents anti-inflammatory effect through modulating PPARγ and p38MAPK inflammatory signaling pathways. However, its therapeutic effect is severely limited by short half-life and poor BBB permeability. To prolong the circulation time and enhance the targeted delivery of tanshinone IIA, Liu et al. developed cationic bovine serum albumin-conjugated tanshinone IIA PEGylated nanoparticles (CBSA-PEG-TIIA-NPs) with a mean diameter of 118 nm. Compared with free tanshinone IIA, intravenous injection of CBSA-PEG-TIIA-NPs significantly reduced the inflammatory cytokines and decreased the infarct volumes of tMCAO rats [99]. Scutellarin is a traditional Chinese medicine which can curb cerebral ischemia/reperfusion injury through down-regulating the inflammatory cytokines, inhibiting neutrophils infiltration, and modulating the HMGB1/TLRs/MyD88/TRIF/NF-kB inflammatory signaling pathway. To improve the solubility and BBB penetration ability of scutellarin, Dang et al. designed a low density lipoprotein receptor (Angiopep-2) and neutrophils receptor (N-acetylated proline-glycine-proline) co-modified nanocarrier (hydroxyl-terminated polyamidoamine dendrimer) for effectively delivering scutellarin to the cerebral ischemia sites [100]. Through intravenous injection, the dual-targeting nano-formulation significantly improved the neuroprotective effect of scutellarin in

tMCAO rats than free scutellarin.

4.5. Stem cells and genes-based nanoparticles

Exogenous stem cells transplantation and genes therapy have been proved beneficial in the treatment of many brain-related diseases [168–170]. Cerebral ischemia not only leads to neuronal cells necrosis or apoptosis, but also induces the expression of various genes, inducing or suppressing brain damage. First, stem cells differentiate into neurons, astrocytes, or oligodendrocytes in the brain lesions through their own differentiation potential, thereby replacing damaged or apoptotic brain cells. Second, by secreting neurotrophic factors and expressing therapeutic genes in the stroke lesions, stem cells promote the repair and regeneration of damaged brain tissue. Third, some cytokines secreted by stem cells through the paracrine pathway contribute to the reconstruction of the vascular structures in the transplanted area. Fourth, target genes are directly transferred to the injured cells to express functional proteins, thus facilitating functional reconstruction after cerebral ischemia [171–173]. Taken together, these functions synergistically endow stem cells and genes with great potential for ischemic stroke therapy.

4.5.1. Stem cells based-nanoparticles

Stem cells are a type of highly undifferentiated cells with self-renewal ability and multi-directional differentiation potential. Different sources of stem cells have been proposed for ischemic stroke treatment, including neuronal stem cells (NSCs), mesenchymal stem cells (MSCs), induced pluripotent stem cells (PSCs), and endothelial progenitor cell (EPCs), among which NSCs and MSCs are the most widely reported [174]. However, the harsh microenvironment of ischemic stroke reduces the survival rate and proliferation activity of stem cells, which severely limits their therapeutic effects [175]. Transfection of functional nanoparticles into stem cells can enhance the viability and cytokines secretion ability of stem cells, thereby improving their therapeutic efficacy for ischemic stroke.

Brain-derived neurotrophic factor (BDNF) is a neurotrophic protein secreted by NSCs. When cerebral ischemia occurs, BDNF can prevent Ca^{2+} influx, combat excitotoxicity, reduce the free radicals generation, and promote the survival of neuronal cells [176–178]. On this basis, in combination with the therapeutic function of NSCs, transfection of BDNF plasmid into NSCs could improve the expression BDNF and further promote the recovery of the damaged brain tissues. For example, Jiang et al. used ROS-responsive charge-reversal poly [(2-acryloyl)ethyl (pboronic acid benzyl)diethylammonium bromide] (B-PDEA) to condense BDNF plasmids into polyplex nanoparticles (cationic B-PDEA/DNA polyplexes), the polyplexes were then transfected into NSCs for efficient BDNF gene transfection [101]. In response to the high concentration of intracellular ROS, B-PDEA had a charge reversal to disintegrate the polyplexes and rapidly release the condensed DNA. The cellular experiment proved that the ability of NSCs to secrete BDNF was significantly improved after transfection of the multi-complex. In addition, after transplantation of the genetically modified NSCs, the BDNF in the mice brain was also significantly enhanced. More importantly, compared with the non-transfected NSCs (NSCs), intravenous injection of the polyplexes transfected NSCs (BDNF-NSCs) significantly improved the cerebral ischemia in tMCAO mice (Fig. 10). Myelin-associated proteins, such as oligodendrocyte myelin glycoprotein and Nogo-A, can inhibit the axonal regeneration and neuronal differentiation, thereby impeding the recovery of ischemic stroke. Lu et al. assembled diblock copolymers of poly (etherimide) and poly (D, L-lactide) with hydrophilic superparamagnetic iron oxide nanoparticles (SPIONs) to form an MRI-visible nanocarrier (SPION-CP). The SPION-CP then complexed siRNA capable of silencing NgR gene (siNgR) via electrostatic interaction to form MRI-visible siRNA nanomedicine (siRNA/SPION-CP) for transfection of NSCs [102]. After injecting to the striatum ipsilateral of the ischemic hemisphere, The siRNA/SPION-CP enabled the in vivo imaging of transfected NSCs and silencing of NgR genes that mediate the synthesis of Nogo-A. Most importantly, compared with the scrambled siRNA nanomedicine, the siRNA/SPION-CP remarkably enhanced the differentiation of NSCs and promoted the functional recovery of the ischemic brain.

MSCs are widely concerned due to their alternative sources, weak immunogenicity, and better ethical feasibility [179,180]. However, the poor homing efficiency of MSCs impedes their treatment for ischemic stroke [181,182]. In a study, Zhang et al. fabricated MFION-based gene complexes by combining magnetosome-like 1D ferrimagnetic iron oxide nanochains (MFIONs) with pDNA through electrostatic interactions with PEI. The MFION-based gene complexes were then applied to transfect MSCs [103]. By tail vein injection, the 1D MFIONs not only facilitated the internalization of MSCs, resulting in high gene transfer efficiency in MSCs, but also upregulated the expression of homing-related chemokine receptor CXCR4 to further improve the homing of MSCs in the cerebral ischemic regions. Moreover, the multi-functional engineered MSCs significantly reduced the mortality of ischemic mice and recovered the ischemic brain function (Fig. 11). In another research, Yao et al. proposed a core-shell nanoparticle (CPMSN@^{125}I-SD) in which a cobalt protoporphyrin IX (CoPP)-loaded mesoporous silica nanoparticle (CPMSN) was encapsulated into ^{125}I-conjugated/spermine-labeled dextran polymer (^{125}I-SD). The core-shell nanoparticles were then loaded into bone marrow stromal cells (BMSCs) to form an all-in-one theranostic nano-platform [104]. By intracerebral injection, the loading of CPMSN realized the real-time photoacoustic (PA) imaging of the transplanted MSCs in the mouse brain. Besides, the controlled release of CoPP protected BMSCs from oxidative stress. Meanwhile, the ^{125}I-SD shell realized the single-photon-emission computed tomography (SPECT) nuclear imaging of the ischemic mouse brain. What's more, this versatile nano-platform maintained superior therapeutic effect for ischemic stroke than normal BMSCs.

Similar to other stem cells, EPCs-based therapies have also brought great potentials for ischemic stroke therapy. However, the weak homing ability and survival rate after transplantation limited their clinical application [183]. In a study, Wang et al. found out that silencing hif-prolyl hydroxylase 2 (PHD2) could enhance the expression of C-X-C chemokine receptor type 4 (CXCR4) and hypoxia-inducible factor-1α (HIF-1α), which then enhanced the targeting and survival ability of EPCs. They transfected EPCs with superparamagnetic iron oxide (SPIO)/PHD2 silencing siRNA (siPHD2) nanocomplexes (Alkyl-SPIO/-siPHD2). On the one hand, the Alkyl-SPIO/siPHD2 could track the migration of EPCs. On the other hand, the Alkyl-SPIO/siPHD2 could effectively silence PHD2, therefore elevating the homing and survival ability of EPCs [105]. After intracardiac injection of Alkyl-SPIO/siPHD2-transfected EPCs, the infarct volumes of the stroke mice were significantly reduced than the normal EPCs group.

4.5.2. Genes-based nanoparticles

Cerebral ischemia can induce or inhibit the expression of multiple genes. The purpose of gene therapy is to transfect exogenous genes into the penumbra or change the endogenous genes expression of neuronal cells, so as to prevent the neuronal damage by improving the hemodynamics or changing the cellular biological mechanisms [184]. Angiogenesis genes, vascular endothelial growth factor (VEGF) genes, antioxidant genes, anti-apoptotic genes, and hypoxia-specific genes have been proved effective for post-stroke therapy [185–188]. However, naked DNA or RNA has poor targeting ability and low transfection efficiency, which has been the major challenge to gene therapy for ischemic stroke. Genes-based nanoparticles are important vectors for genes transfection and the key for successful gene therapy.

Studies have found that angiogenesis after ischemic stroke can promote neuron regeneration, improve cerebral blood flow, and eliminate necrotic tissues, which is of great importance for ischemic stroke therapy. For example, hypoxia-inducible factor 1-α (HIF-1α) can stimulate

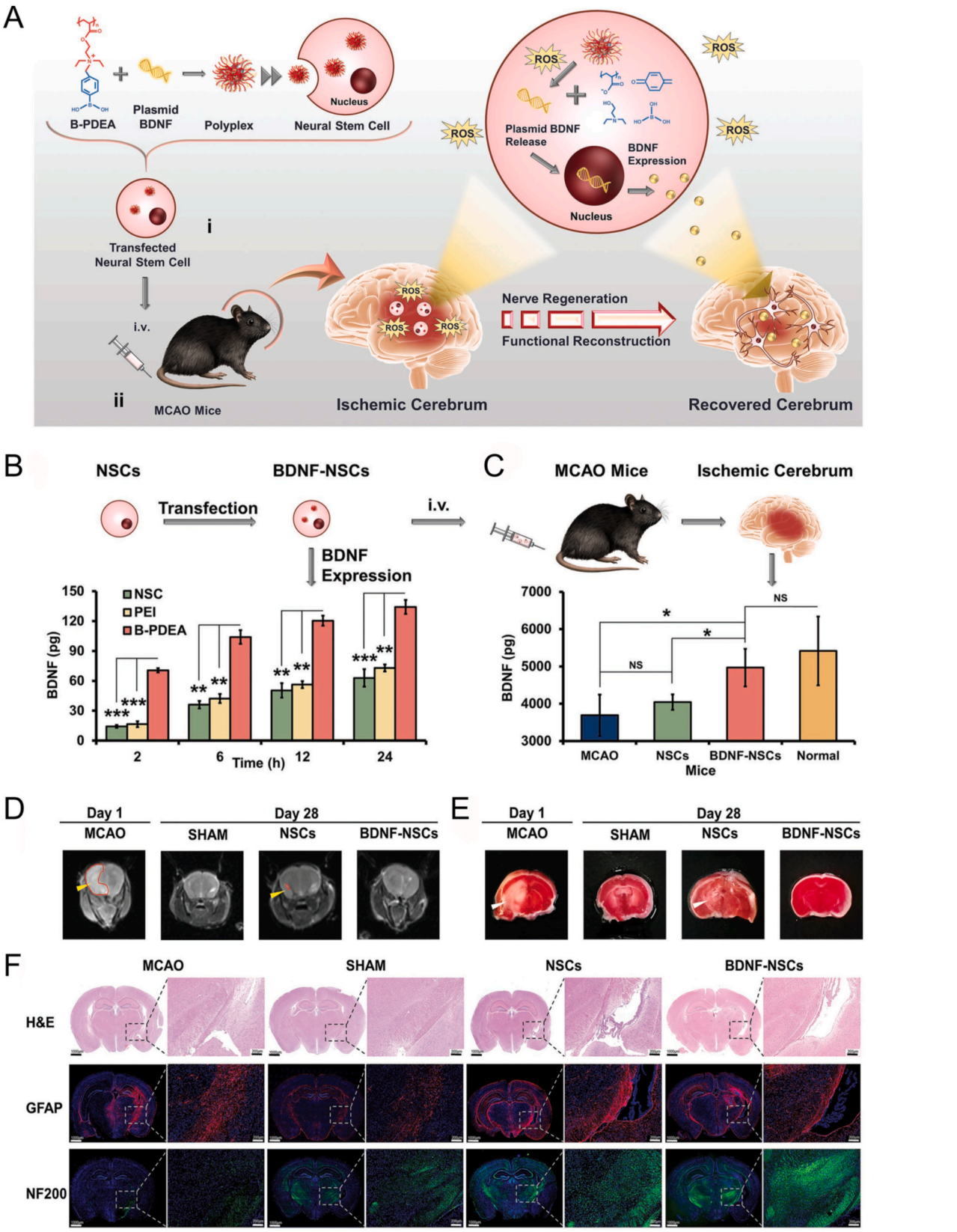


Fig. 10. Bioengineered NSCs for efficient ischemic stroke treatment. (A) Scheme diagram of ROS responsive B-PDEA/BDNF plasmids polyplexes transfected NSCs for ischemic stroke therapy. (B) BDNF expression of NSCs transfected with BDNF polyplexes of PEI or B-PDEA at different time points. (C) BDNF expression in normal mice or tMCAO mice transplanted with NSCs or B-PDEA/BDNF polylexes transfected NSCs. (D) Representative MRI and (E) TTC staining images of the tMCAO mice with different treatments. (F) Representative H&E staining, glial fibrillary acidic protein (GFAP) staining, and NF200 staining images of the tMCAO mice with different treatments. Reproduced with permission [101]. Copyright 2019, Wiley-VCH.

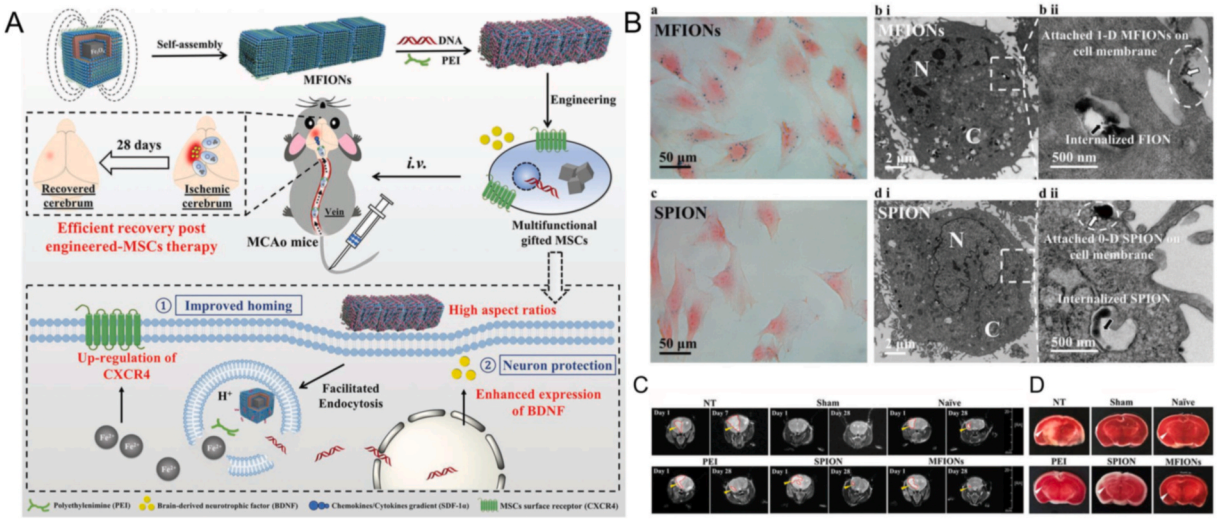


Fig. 11. MFIONs-based gene complexes engineered MSCs for efficient post-stroke recovery. (A) Scheme diagram of MFIONs-based gene complexes engineered MSCs for the recovery of ischemic stroke. (B) Representative prussian blue staining and TEM images of cellular internalization of 1D MFION-complexes (MFION) and 0D SPION-complexes (SPION). (C) Representative MRI images and TTC staining images of the MCAo mice after receiving different treatments. Reproduced with permission [103]. Copyright 2019, Wiley-VCH. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

angiogenesis. To enhance the targeting ability of HIF-1α, Deng et al. conjugated RGD peptides (arginine-glycine-aspartic acid) with hyperbranched cationic polymer (DMAPA-Amyp) to obtain RGD-DMAPA-Amyp. The obtained RGD-DMAPA-Amyp were then interact with HIF-1α plasmid DNA (HIF-1α-AA) to form RGD-DMAPA-Amyp/HIF-1α-AA nanoparticles, which could effectively target the vascular endothelial cells in cerebral ischemia [106]. In zebrafish model, RGD-DMAPA-Amyp/HIF-1α-AA markedly promoted angiogenesis than the non-targeted nanocarrier group (DMAPA-Amyp/HIF-1α-AA). Moreover, through carotid injection, RGD-DMAPA-Amyp/HIF-1α-AA significantly promoted the recovery of tMCAO rats.

Heme oxygenase-1 (HO1) is an antioxidant enzyme that can degrade heme into carbon monoxide, biliverdin and iron ions, which exert anti-inflammatory effects through reducing the expression of adhesion molecules and inhibiting leukocyte recruitment after ischemic stroke [189,190]. Therefore, increasing the expression of HO1 gene in the penumbra can mitigate reperfusion injury. In a study, Oh et al. fabricated self-assembled nanoparticles (HSAP-NP/pHO1) composed of hypoxia-specific anti-RAGE peptide (HSAP), deoxycholate-conjugated polyethylenimine-2k (DP2k), and HO1 plasmid (pHO1) for ischemic stroke therapy [109]. Compared with HSAP alone or the DP2k/pHO1 complex, HSAP-NP/pHO1 increased the genes delivery and expression in the ischemic tissues and showed effective therapeutic effect for MCAO rats by stereotaxic injection. Similarly, Oh et al. used a non-viral carrier deoxycholic acid-conjugated PEI2k (DA-PEI2k) to effectively deliver HO1-mRNA to the cerebral ischemic sites [88]. The HO1-mRNA/DA-PEI2k complex exhibited higher gene expression than HO1-pDNA (HO1-plasmid DNA)/DA-PEI2k and significantly reduced the infarct volume of MCAO rats by stereotaxic injection. High mobility group box 1 (HMGB1) is an endogenous danger signaling molecule secreted by necrotic cells, macrophages and monocytes, which can aggravate inflammation. Knockdown of HMGB1 has been proved effective for ischemic stroke therapy [191–193]. To enhance the delivery efficacy of HMGB1 siRNA, Kim and coworkers used the biodegradable PAMAM dendrimer of e-PAM-R as the carrier for intranasal delivery of HMGB1 siRNA (e-PAM-R/siRNA) [110]. The in vivo experiment indicated that intranasal delivery of e-PAM-R/siRNA complex efficiently realized the knockdown of HMGB1 and markedly suppressed the infarct volume of MCAO rats.

It is reported that M2 microglia exhibit neuroprotective effect and improve the post-stroke outcomes [194,195]. In a study, Song et al. extracted exosomes from M2 microglia [111]. After tail vein injection of the M2 microglia-derived exosomes into tMCAO mice, the infarct volume and the neuronal apoptosis were significantly attenuated. They demonstrated that miR-124 derived from microglial exosomes promote neuronal function recovery by inhibiting neuronal inflammation. Complement component C3 (C3) activation is closely related to the microglial neurotoxicity after cerebral ischemia/reperfusion. Inhibition of C3 with C3 siRNA is promising for ischemic stroke treatment. The BBB is a major obstacle for C3 siRNA delivery to the microglia. In a study, Wang et al. prepared C3-siRNA-encapsulated cationic lipid assisted poly (ethylene glycol)-block-poly (D, L-lactide) (PEG-PLA) nanoparticles (NP_{siC3}) to cross the BBB and effectively deliver C3-siRNA into the microglia [107]. By intravenous injection, NP_{siC3} significantly decreased the C3 expression in microglia, reduced the microglial neurotoxicity, and then improved the functional recovery of tMCAO mice than naked siRNA.

Given the current results of preclinical trials, stem cells and genes are highly promising for ischemic stroke therapy. In addition, small-scale preliminary clinical investigations have also demonstrated the safety and feasibility of stem cells and genes therapy. These findings boosted the researchers' confidence. Yet, large-scale randomized controlled trials are required to further verify their efficacy and safety. Beforehand, the following issues need to be taken into consideration: (i) exogenous stem cells, mRNA, or DNA may trigger immunogenicity with the body, how to eliminate these rejections remains a proposition to be solved [196,197]; (ii) although non-viral gene vectors have low immunogenicity, polycationic non-viral vectors such as PEI, chitosan, and dendrimers are prone to accumulate in liver, spleen, and lung, resulting in toxic side effects to the human body [198]; (iii) stem cells and gene therapies must strictly follow the ethical morality requirements.

5. Nanoparticles-mediated combination therapy approach for effective treatment of ischemic stroke

Since the occurrence and progression of ischemic stroke involve complex pathophysiologic mechanisms, treatment regimens targeting merely a singular pathological mechanism are often insufficient to achieve satisfactory results. For the sake of more effective benefits, comprehensive approaches that combining therapeutic agents against

different pathological targets are required. Compared with monotherapy, combination treatments such as thrombolytic agent combined with antioxidant, antioxidant combined with anti-inflammation agent, and anti-edema agent combined with antioxidant achieved more effective effects and less side effects in preclinical trials. The combination therapy based nanoparticles have been designed to obtain better outcomes over ischemic stroke. Table 3 summarizes the combination therapy-based nanoparticles for effective treatment of ischemic stroke.

5.1. Nanoparticles that combined thrombolytic agents and neuroprotective agents

In view of the fact that thrombolysis and neuroprotection are the two most indispensable strategies for ischemic stroke treatment. Nanoparticles that combined thrombolytic agents and neuroprotective agents have been designed for the combination therapy of ischemic stroke [199,200]. For example, Xu et al. developed a bioengineered thrombin-responsive "nanoplatelet" with a mean hydrodynamic size of 167.2 ± 1.6 nm with core-shell structure (tP-NP-rtPA/ZL006e) for synergistic ischemic stroke therapy [199]. The excitotoxicity inhibitor (ZL006e)-loaded dextran derivative polymeric nanoparticle core was coated with platelet membrane shell coupled with thrombin-cleavable Tat-peptide-coupled rtPA. Firstly, the nanoplatform could target the thrombus sites and responsively release rt-PA by the upregulated thrombin. Subsequently, the exposure of Tat-peptide enhanced the BBB penetration ability of the nanoparticles for specific delivery of ZL006e to the ischemic regions. Compared with free ZL006e + rtPA, the bioengineered "nanoplatelet" exerted a significant thrombolytic and neuroprotective effect for tMCAO rats by intravenous injection (Fig. 12). In another research, Mei et al. conjugated the antioxidant 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl (4-amino-TEMPO) with Methoxy-poly (ethylene glycol)-b-poly (chloromethylstyrene) (MeO-PEG-*b*-PCMS) to obtain cationic PEG-*b*-PMNT copolymers, which then self-assembled with anionic poly (acrylic acid) (PAAc) and t-PA to form pH-sensitive polyion complex nanoparticles (t-PA@iRNP) for the combination of thrombolysis and anti-oxidation therapy of ischemic stroke [200]. After intravenous injection, the nano-sized t-PA@iRNP significantly prolonged the half-life of t-PA and enhanced the antioxidative effect of 4-amino-TEMPO in MCAO mice.

5.2. Nanoparticles that combined different kinds of neuroprotective agents

Combining neuroprotective agents with different mechanisms into a simple formulation that modulates various cell types can significantly enhance the therapeutic effects of ischemic stroke. For example, Lu et al. conjugated poly (ethylene glycol) (PEG) with phenylboronic ester-modified polylysine and fibrin-binding pentapeptide CREKA to obtain amphiphilic copolymers (CPLB). CPLB were then assembled with Rapamycin (RAPA) to form microthrombus-targeting and ROS responsive micelles (CPLB/RAPA) with an average hydrodynamic size of 65 nm for combination therapy of ischemic stroke [50]. Firstly, through binding to the fibrin in the microthrombus, CPLB/RAPA actively targeted the cerebral ischemic regions. Secondly, by intravenous injection, the ROS responsive release property of the micelles eliminated the proliferating ROS to ameliorate the oxidative stress and protect the disrupted BBB. More importantly, RAPA released from the micelles induced the protective autophagy of neurons and promoted the polarization of microglia from the pro-inflammatory M1-type to the anti-inflammatory M2-type, thereby enhancing the survival of neurons and reducing the inflammatory reaction of ischemia/reperfusion injury (Fig. 13). In another study, Deng et al. employed betulinic acid nanoparticles (BA NPs) as drug carriers to deliver glyburide to the brain lesion for combination therapy [201]. Glyburide is an antagonist of the sulfonylurea receptor 1 (SUR1) transient receptor potential melastatin 4 (SUR1-TRPM4) cation channel, which can alleviate cerebral edema. BA is a natural compound that acts as an antioxidant agent. By intravenous injection, the multifunctional nanoparticles enhanced the delivery of glyburide and achieved the combination effects of anti-edema and anti-oxidation for tMCAO rats.

Combination therapy based on different antioxidants significantly reduce the oxidative stress of ischemic stroke. For instance, Bao et al. loaded edaravone into Angiopep-2 and PEG modified ceria nanoparticles (E-A/P–CeO_2) for combinational therapy of ischemic stroke [202]. The well-designed E-A/P–CeO_2 consists of three important advantages: (i) by tail vein injection, the modification of PEG enhanced the blood circulation time and stability of ceria nanoparticles; (ii) the modification of Angiopep-2 helped ceria nanoparticles cross the BBB via specifically binding to the low density lipoprotein receptor-related protein over-expressed on the brain endothelial cells; (iii) E-A/P–CeO_2 exerted combined ROS elimination effect by both edaravone and ceria nanoparticles.

Additionally, combination therapy based on genes and chemical drugs have also been applied for ischemic stroke therapy. For example, Hyun et al. established a conjugation of dexamethasone to polyethylenimine (PEI2k) for the co-delivery of dexamethasone and heme oxygenase-1 (HO-1) plasmid DNA for synergistic treatment of ischemic stroke [203]. Dexamethasone played an anti-inflammatory role while

Table 3
Summary of combination therapy-based nanoparticles for effective treatment of ischemic stroke.

Design	Mechanism of action	Effectiveness	Administration route	Reference
Thrombin-responsive "nanoplatelet" containing rtPA and ZL006e	Thrombolysis; Anti-excitotoxicity	Target the thrombus sites; Responsively release rt-PA; Enhance the BBB penetration ability	Intravenous injection	[199]
Self-assembled polyion complex nanoparticles containing tissue plasminogen activator (t-PA) and antioxidant (4-amino-TEMPO)	Thrombolysis; Anti-oxidation	Prolong the half-life of t-PA; Enhance the antioxidative effect of 4-amino-TEMPO	Intravenous injection	[200]
Microthrombus-targeting and ROS responsive micelles encapsulating rapamycin	Scavenging of RONS; Anti-inflammation; Inducing protective autophagy of neurons	Target the thrombus sites; ROS responsively release rapamycin; Combination therapy	Intravenous injection	[50]
Betulinic acid nanoparticles carrying glyburide	Anti-edema; Anti-oxidation	Enhance the delivery of glyburide; Achieve the combination of anti-edema and anti-oxidation effect	Intravenous injection	[201]
Angiopep-2 and PEG modified ceria nanoparticles loading edaravone	Anti-oxidation	Enhance the blood circulation time; Enhance the BBB penetration effect; Achieve combined ROS elimination effect	Intravenous injection	[202]
Nanocarrier for co-delivery of dexamethasone and heme oxygenase-1 plasmid DNA	Anti-inflammation; Anti-oxidation	Enhance the targeted delivery of dexamethasone and HO-1 plasmid DNA; Achieve efficient combinational therapy effect	Intracerebral injection	[203]
Dexamethasone-loaded R3V6 peptide micelles delivering HO-1 plasmid DNA	Anti-inflammation; Anti-oxidation	Show the combined anti-inflammatory and antioxidant therapeutic effect	Intracerebral injection	[204]

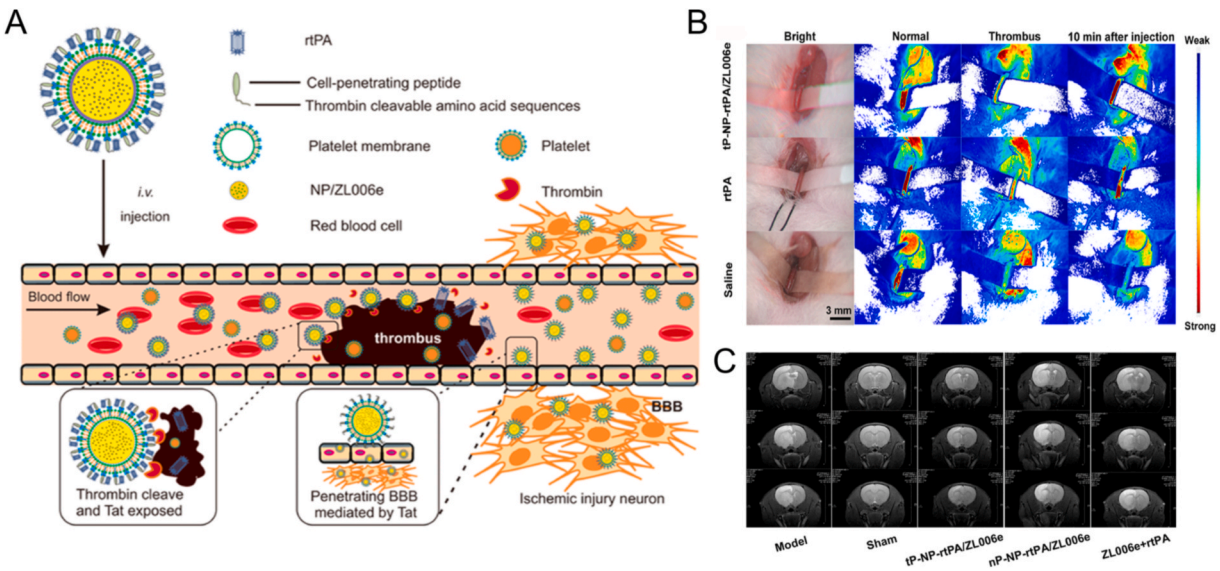


Fig. 12. Biomimetic nanoparticles co-delivering thrombolytic agent and antioxidant for synergistic ischemic stroke therapy. (A) Schematic design of a bioengineered thrombin-responsive "nanoplatelet" with core-shell structure (tP-NP-rtPA/ZL006e) for synergistic ischemic stroke therapy. Reproduced with permission. (B) Representative bloodstream recovery images of the rat common carotid artery thrombolysis induced by $FeCl_3$ in each group. (C) Representative MRI images of the brain in each group. Reproduced with permission [199]. Copyright 2019, American Chemical Society.

HO-1 had an antioxidant effect. In addition, by stereotaxic injection, the nanocarrier enhanced the delivery of dexamethasone to the cerebral ischemic sites while dexamethasone facilitated the nuclear delivery of HO-1 plasmid DNA for efficient combinational therapy of ischemic stroke. Similarly, Lee et al. developed dexamethasone-loaded R3V6 peptide micelles (R3V6-Dexa) as a gene carrier for the delivery of HO-1 plasmid DNA (pSV–HO–1) to form into pSV–HO–1/R3V6-Dexa complex [204]. Through stereotaxic injection, the complex showed the combined anti-inflammatory and antioxidant therapeutic effects in tMCAO rats.

6. Conclusions and perspectives

The occurrence and progression of ischemic stroke involve multiple pathological mechanisms, including cerebrovascular occlusion, excitatory neurotoxicity, oxidative stress, and inflammatory response, etc. Due to the unique characteristics of the blood-brain barrier, most of the effective therapeutic agents cannot reach the cerebral ischemic regions. The failure of specifically delivering therapeutic drugs to the ischemic sites results in poor clinical outcomes. Inspired by the distinguished brain targeting and BBB penetration ability of nanoscale particles, researchers have designed various functional nanoparticles-mediated emerging treatment approaches to the ischemic regions. The nanoparticles predominantly comprise recanalization-based nanoparticles, neuroprotection-based nanoparticles, and combination therapy-based nanoparticles, which have made some progress for efficient ischemic stroke therapy in preclinical trials.

The progress of nanoparticles-mediated approaches for effective ischemic stroke treatment are mainly reflected in the following points: (i) improve the blood stability and prolong the blood circulation time of therapeutic substances; (ii) enhance the brain targeting and BBB permeability of therapeutic agents; (iii) reduce the toxicity of therapeutic agents; (iv) improve the viability of stem cells or increase the transfection efficiency of therapeutic genes; (v) co-deliver therapeutic agents with different targets into one nano-formulation for synergistic therapy. Therefore, nanoparticles might possess a broad prospection in clinical application for ischemic stroke. Despite the rapid development of nanoparticles, some issues still need to be taken into consideration, and their clinical transformation is greatly challenged. First, considering the complex pathological mechanisms of ischemic stroke, nanoparticles usually merely aim at one or two of the pathological mechanisms of ischemic stroke and failed to provide comprehensive protection for the central nervous system. Paradoxically, co-delivering different therapeutic agents into a single nano-delivery system adds the complexity of the nanoparticles and hampers their clinical translation. Second, animal models of cerebral ischemia were mostly constructed through the suture-occluded method or photochemical method, which were different from the pathogenesis of human patients. Third, although the brain targeting effect of nanoparticles can be enhanced by brain-targeted modification or biomimetic approaches, the BBB remains a huge challenge for nanoparticles to penetrate, and a large proportion of nanoparticles still failed to reach the cerebral ischemic regions. Finally, research on the intracerebral fate of the nanoparticles need to be further clarified to guarantee the long-term safety of nanoparticles for clinical application.

In general, intravenous injection is the favorable route of nanoparticles for ischemic stroke therapy because of its operation convenience, high administration dosage, and minimal invasion. After intravenous injection, the in vivo destiny of the nanoparticles depends on their particle sizes, surface targeting groups, and surface charges, etc. Typically, nanoparticles would interact with plasma proteins and form the "protein corona" on the surface of nanoparticles, which impedes their brain targeting and BBB permeability [205]. The non-invasive approach of intranasal route that bypass the BBB and rapidly reach the effective drug concentrations has been reported. Yet, the delivery dosage through intranasal administration is small, while the movement of the cilia and mucus further reduces the delivery of drugs to the brain [206]. Additionally, the retro-orbital and intrathecal routes can also bypass the BBB and directly deliver therapeutic substances to the brain. However, these two routes are limited by the high injection techniques and the extremely suffering of patients, respectively. Moreover, to protect the viability of stem cells and improve the transfection efficiency of RNA or DNA, some nanoparticles based on stem cells or gene therapy have been used for highly-invasive intracranial administration. In view of these facts, according to the different action mechanisms of nanoparticles and their targeting mechanisms, the optimal therapeutic effects can be achieved by choosing the appropriate administration routes.

In summary, there is still a huge gap between the preclinical trials and clinical applications of nanoparticles for ischemic stroke therapy. Considering the distinct structural features of the brain and the complex injury mechanisms of ischemic stroke, nanoparticles need to be

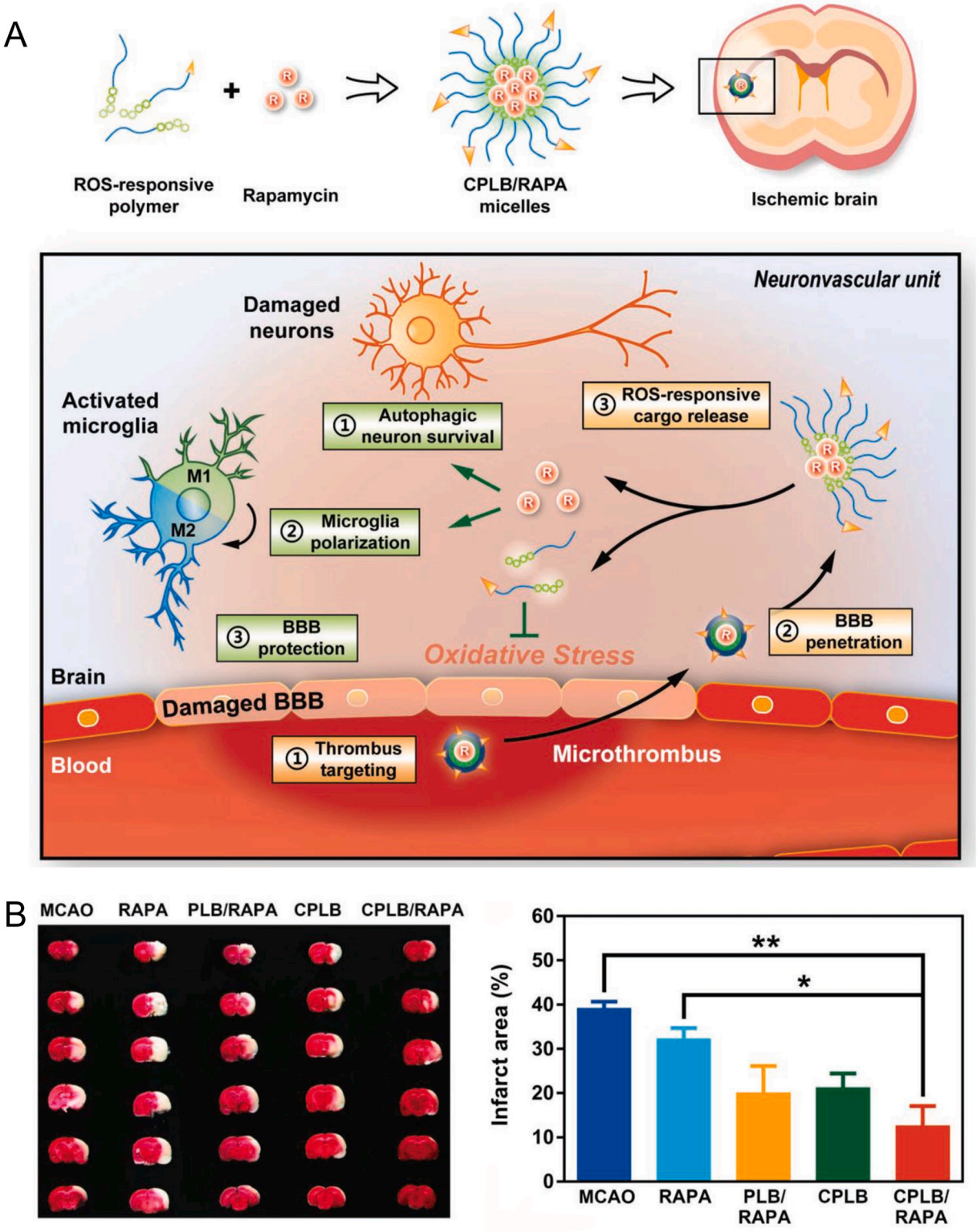


Fig. 13. Microthrombus-targeting and ROS responsive micelles for the combined treatment of ischemic stroke. (A) Schematic illustration of microthrombus-targeting and ROS responsive micelles (CPLB/RAPA) for synergistic ischemic stroke therapy. Reproduced with permission. (B) Representative TTC staining images of the brain slices and cerebral infarct area in different group. Reproduced with permission [50]. Copyright 2019, Wiley-VCH.

rationally designed by combining their BBB penetration ability and the microenvironment of the ischemic regions, thereby enhancing their targeting delivery to the lesion. Moreover, the pathological mechanisms of ischemic stroke and the effective therapeutic drugs should be further intensively explored, so as to recover the impaired neurological functions, and reduce the disability and mortality of human patients. With the development of nanomedicine and the deep understanding of the pathological mechanisms of ischemic stroke, the biological functions of nanoparticles should be further improved to promote the clinical transformation of these nanoparticles for efficient ischemic stroke

treatment.

Declaration of competing interest

The authors declare no conflict of interest.

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