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Review

The role of nanomaterials in revolutionizing ischemic stroke treatment: Current trends and future prospects

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SUMMARY

Ischemic stroke has a high disability rate, which leads to irreversible neuronal death. The efficacy of conventional stroke treatments, including thrombolytic and neuroprotective therapies, is constrained by a number of factors, including safety concerns and inefficient drug delivery. The advent of nanomaterials has created new avenues for stroke therapy, facilitating enhanced pharmacokinetic behavior of drugs, effective drug accumulation at the target site, augmented therapeutic efficacy, and concomitant reduction in side effects. Therefore, this paper pioneers a research approach that summarized the development trend and clinical value of nanomaterials in the field of ischemic stroke through bibliometric analysis. This review provides an overview of the pathophysiological mechanisms of stroke and examines the current research trends in the use of nanomaterials in stroke management. It encompasses a multitude of domains, including targeted drug delivery systems, biosensors for the sensitive detection of biomarkers, and neuroprotective nanotechnologies capable of traversing the blood-brain barrier. Moreover, we investigate the challenges that nanomaterials encounter in the clinical translation context, including those pertaining to biocompatibility and long-term safety. These results have provided the clinical value and limitations of nanomaterials in the diagnosis and treatment of ischemic stroke from double perspectives, thereby offering new avenues for the further development of innovative nanotherapeutic tools.

INTRODUCTION

At the current rate of global aging, it is anticipated that ischemic stroke (IS) will become a significant cause of mortality and morbidity.[1–3] Large artery atherosclerosis is the most common pathogenic type of IS, which is prone to produce atherosclerotic plaques.[4] In the event of an atherosclerotic plaque rupturing unexpectedly, a blood clot is immediately formed, which blocks the blood vessel. Finally, the nerve cells would undergo irreversible ischemic necrosis and the patient would become disabled or even die.[5] At present, traditional treatment strategies mainly focus on the hyperacute phase of cerebral infarction, including intravenous thrombolysis and mechanical thrombectomy. Among them, intravenous thrombolysis is the most effective treatment. The therapy can be within 4.5h after thrombosis through intravenous antithrombotic drugs dissolve thrombus, and restore blood flow perfusion in the infarction area.[6] Furthermore, to prevent the recurrence of plaque rupture thrombosis, oral drug treatment will be administered, which will include antiplatelet therapy, lipid-lowering therapy, and neural protection treatment. However, in the narrow time window, drugs frequently encounter blocking due to the blood-brain barrier (BBB) and cannot achieve the best therapeutic effect.[7] This will lead to more patients suffering severe disability and even death. Therefore, there is an urgent need to find more effective treatment measures to improve the diagnosis and treatment of stroke.

The use of nanomaterials in IS therapy and diagnostics has attracted much attention in the field and significant progress has been made. Lv et al. highlighted the potential of nanodelivery systems in this regard, emphasizing their superiority over conventional therapies.[8] Parvez et al. reported promising results with intranasal drug delivery systems, demonstrating their potential for clinical applications.[9] Zhang et al. conducted a comprehensive review of the design aspects of nanomedicinal drugs, highlighting significant innovations in the field of nanomedicine. Their focus included membrane-encapsulated nanoparticles, magnetic nanoparticles, and liposomes.[10] In addition, Tian et al. explored the versatility of liposomes, nanoparticles, and hydrogels, especially their ability to cross the BBB and deliver drugs precisely to their targets.[11] Although each of these studies provided valuable insights from different perspectives, a comprehensive review that systematically assesses the major research trends and recent advances in IS nanomaterial applications is still lacking.

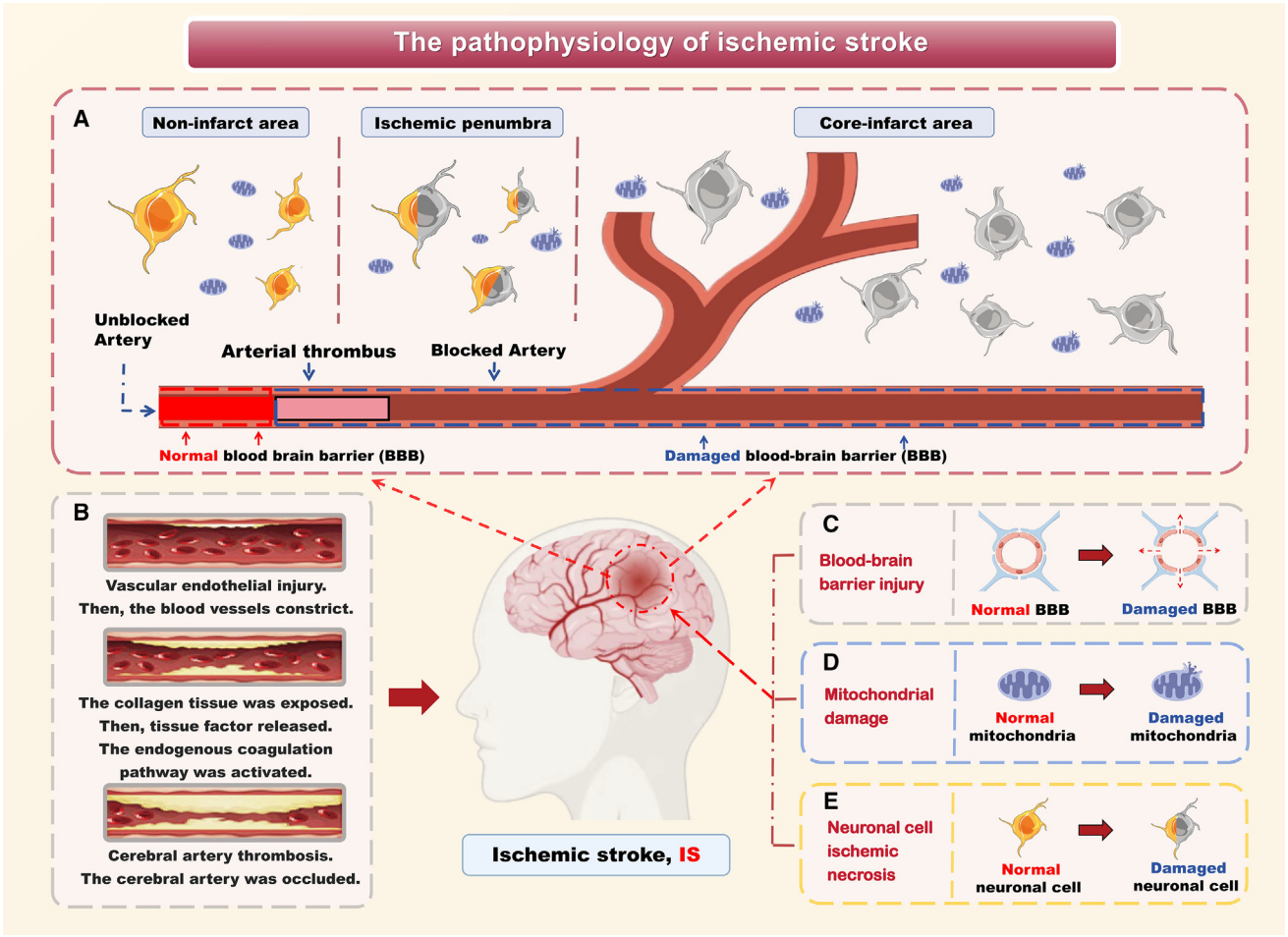


Figure 1. Pathophysiology of ischemic stroke

(A) The sequence of onset for ischemic injury after ischemic stroke onset.
(B) Ischemic stroke results from sudden occlusion of cerebral blood vessels.
(C) Breakdown of the blood-brain barrier.
(D) Initiation of mitochondrial damage.
(E) Necrosis of neurons, glial cells, and other supporting brain cells.

In this study, we first summarized the clinical value and trends of nanomaterials in IS through bibliometric analysis. We provided a trajectory of nanomaterials in IS and identified potential research hotspots and future advances through keyword clustering analysis. In addition, we identify optimal combinations of nanomaterials with diagnostic and therapeutic biomarkers, while also elucidating future directions in nanomedicine development and the integration of these materials with other cutting-edge technologies. These findings provide a dual perspective on the clinical value and limitations of nanomaterials in the diagnosis and treatment of IS, thus providing new avenues for further development of innovative nanotherapeutic tools.

THE PATHOPHYSIOLOGY OF IS

IS is driven by a complex interplay of pathophysiological processes, including excitotoxicity, oxidative stress, mitochondrial dysfunction, inflammatory responses, and disruption of the BBB (Figure 1). When a thrombus blocks a cerebral artery, the resulting deprivation of oxygen and nutrients triggers neuronal injury in the affected region.[12,13] Neurons within the ischemic core rapidly undergo necrosis and apoptosis, forming the infarct core and surrounding ischemic penumbra.[14] In the infarct core, a lack of glucose and oxygen severely reduces adenine nucleoside triphosphate production, while excessive release of excitatory neurotransmitters such as glutamate leads to overstimulation of glutamate receptors, intracellular calcium overload, and excitotoxicity. This cascade drives cell death pathways, including apoptosis, autophagy, and necrosis.[15–17] Mitochondrial calcium overload further exacerbates damage by generating reactive oxygen species, which promote protein nitration, lipid peroxidation, and DNA damage.[18] The ensuing mitochondrial dysfunction activates or inhibits various signaling pathways, intensifies inflammatory responses, and amplifies neuronal apoptosis and necrosis, triggering a cascade of programmed and non-programmed cell death. The BBB, normally a highly

selective barrier composed of endothelial cells, astrocyte endfeet, pericytes, and the basement membrane, is also compromised following IS. Capillary constriction and BBB disruption lead to tight junction dysfunction and allow the infiltration of peripheral immune cells, including microglia, macrophages, and T lymphocytes, into the brain parenchyma.[19] These immune cells adhere to vascular walls and migrate into the central nervous system (CNS), further exacerbating BBB breakdown and promoting cerebral edema. In parallel, neutrophil accumulation in the infarct core amplifies oxidative stress and contributes to additional BBB damage.[20,21] As ischemic injury progresses, patients typically experience acute neurological symptoms, including sudden headache, facial paralysis, limb weakness, speech impairment, and visual disturbances.[22] According to the World Health Organization, one in six individuals worldwide will experience a stroke, with one person dying from a stroke every 6 s and another becoming permanently disabled within the same time frame.[23] Without timely intervention, survivors often face permanent brain damage and loss of function or even death. Therefore, early therapeutic intervention is critical to minimizing brain injury, improving survival, and increasing the likelihood of functional recovery.

THE OVERVIEW OF NANOMATERIALS

Nanomaterials are those with a diameter of 100 nm or less.[24] The tiny physical structure of nanomaterials generates unique effects, including photoelectromagnetic thermal effects, size effects, and surface interface effects.[25] These material properties play a crucial role in the diagnosis and treatment of stroke. Under normal physiological conditions, the BBB prevents macromolecular material such as peptides, proteins, and nucleic acids enter the intracranial, significantly limiting the diagnosis and treatment of stroke.[26] However, by exploiting the advantages of the tiny physical structure of nanomaterials, more and more macromolecular substances can bypass the BBB and target brain infarction areas, thus entering the function performed by the CNS.[27] In diagnostic imaging, nanomaterials have been widely developed into novel imaging probes for detecting vascular infarction and monitoring the treatment process. In the field of nanodrug design, nanomaterials successfully target drug delivery to the CNS by binding drugs. At present, the developed binding pathways of nanodrugs mainly include binding to nanoparticles, liposomes, nanogels, dendrimers, and other nanomaterials.[28] Not only that, nanomaterials and nanotechnology can develop a new generation of nanotherapeutic tools by combining them with genetic engineering and micro-robotics. These nanotherapeutic tools can selectively accumulate at the site of cerebral infarction and help to improve the pathophysiological process of IS, and can better exert anti-inflammatory injuries, open collateral circulation, reduce cerebral edema, and further restore the oxygen and blood supply to the brain tissue.[29]

THE TRADITIONAL PHARMACEUTICAL TREATMENT FOR IS

Currently, traditional drug therapies are the mainstay of treatment for IS. Some of the traditional drugs have become raw materials for nanomedicine production and play key therapeutic roles in thrombolysis and neural repair, including the thrombolytic drug rt-PA and the neuroprotective drug edaravone.[30] Traditional therapeutic drugs are divided into several categories, including thrombolytics, anti-platelet aggregation medications, fibrinolytics, anticoagulants, and neuroprotective drugs.[31]

Thrombolytic drugs

Thrombolytic therapy represents the most effective treatment strategy during the acute phase of IS.[32] Based on the research progress of thrombolytic drugs, these agents can be categorized into three generations.[33] The first generation includes urokinase and streptokinase, which are fibrinolytic agents capable of dissolving thrombi but lack specificity.[34] The second generation consists of tissue plasminogen activators (t-PA) and recombinant tissue plasminogen activators (rt-PA), marking a significant advancement in the treatment of IS. However, the use of rt-PA is strictly limited by the time window (the effective salvage time window for ischemic tissue is within 4.5 h).[35] The third generation comprises reteplase, recombinant desmoteplase, and tenecteplase, which exhibit characteristics such as high specificity, longer half-lives, and enhanced thrombolytic efficacy.[36] Research on the dosages, effective time windows, and other aspects of these drugs is still in its infancy. Thrombolytic drugs may be considered for patients whose condition meets the criteria for thrombolysis during the window of revascularization. This approach has significantly reduced patient mortality and disability. However, whether third-generation thrombolytic drugs can improve the prognosis of acute IS patients remains to be confirmed by large-scale randomized controlled trials.

Fibrinolytic and anticoagulant drugs

Fibrinolytic and anticoagulant drugs are the main interventions in the management of acute stroke, aimed at dissolving thrombi and preventing their recurrence. Patients who are not eligible for rt-PA thrombolysis may choose to receive fibrinolytic and anticoagulant medications.[37] Current thrombolytic agents include snake venom extracts, defibrin, and ancrod,[38] while anticoagulant therapies include unfractionated heparin, low-molecular-weight heparin, and coumarin derivatives.[39] During the acute phase of stroke, there is an increase in plasma fibrinogen levels and blood viscosity. Thrombolytic agents can effectively reduce plasma fibrinogen levels and exhibit a mild thrombolytic effect, inhibiting thrombus formation. Anticoagulation therapy may offer benefits to specific subgroups of patients with acute IS, but it is currently not recommended for the majority of patients with acute IS without prior selection.[40]

Anti-platelet aggregation drugs

The presence of multiple platelet-activating factors in the pathomechanism of IS leads to a hypercoagulable state of the blood, increasing the risk of thrombosis. Antiplatelet drugs are used to treat and prevent IS by improving the hypercoagulable state of the blood. The most commonly used antiplatelet drugs are aspirin, dipyridamole, and clopidogrel.[41] Aspirin taken within 48 h of a stroke significantly reduces morbidity, mortality, and disability during follow-up and decreases the risk of recurrence. However, it slightly increases the risk of symptomatic intracranial

hemorrhage. The combined use of clopidogrel, aspirin, and dual antiplatelet agents within 12 h of onset for 90 days also reduces the risk of recurrence of IS. However, it increases the risk of bleeding.[42]

Neuroprotective drugs

The primary objective of neuroprotective agents is to interrupt the cascade of events in the ischemic penumbra, which encompasses ion channel blockers, antioxidants, and anti-inflammatory agents. Edaravone, an antioxidant and free radical scavenger, has been shown to improve functional outcomes in patients with acute cerebral infarction.[43] Citicoline, a crucial intermediate in phosphatidylcholine synthesis, its degradation to free radicals and fatty acids during cerebral ischemia may contribute to the recovery of cerebral function following brain injury. The therapeutic effect is achieved by restoring mitochondrial adenosine triphosphatase activity, which activates and enhances brain metabolism and improves the functioning of the superior reticular activating system.[44]

THE DEVELOPMENT OF NANOMATERIALS

The VOSviewer (version 1.6.19), CiteSpace (version 6.2.R4), Excel (version 2021), and GraphPad Prism (version 9.5.1) software were used to summarize the development process of nanomaterials in IS from 2008 to 2023. After careful evaluation of titles and abstracts, 475 studies met the inclusion criteria for this study. From 2008 to 2023, the annual number of publications, H-index, and citation frequency with nanomaterials applied in IS continued to grow (Figures 2A–2D). This study systematically evaluated the citation frequency of each article and selected a series of papers reporting novel nanodrugs (Figure 2E).

Subsequently, we found that China has the maximum number of publication output, the highest H-index, and 3, 354 citations in this field (Figures 3A–3C). In the field of cooperation, China and the United States are the closest partners and the Chinese Academy of Sciences (CAS) is the largest and most central institution in the network (Figures 3D–3F). In addition, the development trend of nanomaterials in stroke was explored through a co-occurrence network diagram and superposition visualization diagram. The keywords are divided into five clusters, and mainly concentrated on ''nanoparticle characteristics'', ''stroke mechanism'', and ''drug design'' (Figures 4A and 4B). Importantly, the evolution of keywords over time shows that ''ischemic stroke'' was identified as the primary research keyword before 2017. But in 2023, the primary research keywords were ''magnetic resonance imaging'' and ''blood-brain barrier'' (Figures 4C and 4D). These results indicated the important role of nanomaterials in the diagnosis and therapy of IS. These nanomaterials are helpful for the design of targeted drugs and diagnostic devices, which will make the diagnosis and treatment of stroke more accurate and efficient.

Following a thorough analysis of the keyword evolution results presented in Figure 4, we identified high-intensity breakout terms such as ''pathological mechanism'', ''mechanism'', ''design'', ''system'', ''*in vitro*'', ''stem cell'', ''immunity'', ''therapy'', and ''therapeutic contrast agent''. By further integrating the principles of word frequency evolution, we recognized key themes for literature review including ''nanodiagnostic tools'', ''nanomedicine'', ''nanotherapeutic direction'', and ''novel nanomedical technology''. Following an exhaustive examination and critical analysis of a multitude of studies, we have identified several key areas for our review. This review encompasses five main areas: ''Nanomedicine technology in stroke diagnostics'', ''Nanodrugs in stroke treatment'', ''Key nanotherapeutic directions for IS'', ''Nanomedicine combined with gene therapy'', and ''Applications of nanorobots in IS''. It provides a comprehensive overview of the diverse applications and recent advancements in the use of nanomaterials in IS. This information is valuable for laboratory researchers seeking design inspiration and for clinical staff interested in the clinical applications of nanomedicine.

THE NANOMEDICINE TECHNOLOGY IN THE DIAGNOSTIC OF STROKE

The application of nanomaterials in marker detection

The detection of biomarkers will be helpful for the rapid diagnosis of stroke. Neuron-specific enolase (NSE), cardiac troponin (cTnI), and N-terminal pro-B-type natriuretic peptide (NT-proBNP) have been used as critical biomarkers for the diagnosis of IS.[45–47] Nanomaterials are involved in the detection of important markers by optimizing or preparing detection reagents to enhance the detection rate of key markers (Figure 5A). Li et al. employed Gold nano bipyramid (Au NBP) as a chromogen to prepare a biosensor for the detection of NSE. In this nanosensor, nine distinct colors of fluorescence changes can be observed in response to varying NSE concentrations. This new NSE biosensor, with a Wide Linearity Range, good selectivity, and stability, facilitates the assessment of conditions in patients.[48] Given that semiconductor nanoparticles, such as quantum dots (QDs), possess superior optical properties. Rezaei et al. have developed a novel biosensor based on quantum dots and aptamers for the sensitive and specific detection of cTnI. This platform offers enhanced accuracy and specificity in cTnI detection, providing valuable assistance in clinical decision-making.[49] Chen et al. have devised an ultrasensitive electrochemical immunosensor for NT-proBNP, which takes advantage of Co-N-C nanosheets with the characteristics of signal amplification and superior biocompatibility. By using appropriate materials, the new sensor exhibits satisfactory sensitivity, higher selectivity, and stability.[50] In short, the application of diagnostic reagents prepared from nanomaterials can lead to a significant increase in the sensitivity of NSE, cTnI, and NT-proBNP detection. Nanomaterials have shown great potential in the rapid diagnosis of IS.

The applications of nanomaterials in imaging devices

The diagnostic capabilities of imaging devices are expanding with the advantage of the tiny structures of nanomaterials.[51,52] Nanomaterials are being prepared as multifunctional imaging probes or contrast agents and are being applied to diagnose IS (Table 1). These nano-imaging devices not only improve the clarity of imaging but also track and monitor the process of cellular repair. Among them, nano-CT with high MRI sensitivity and fluorescent magnetite nanoclusters (FMNC) play a key role in the imaging of IS (Figure 5B). In conventional radiographic

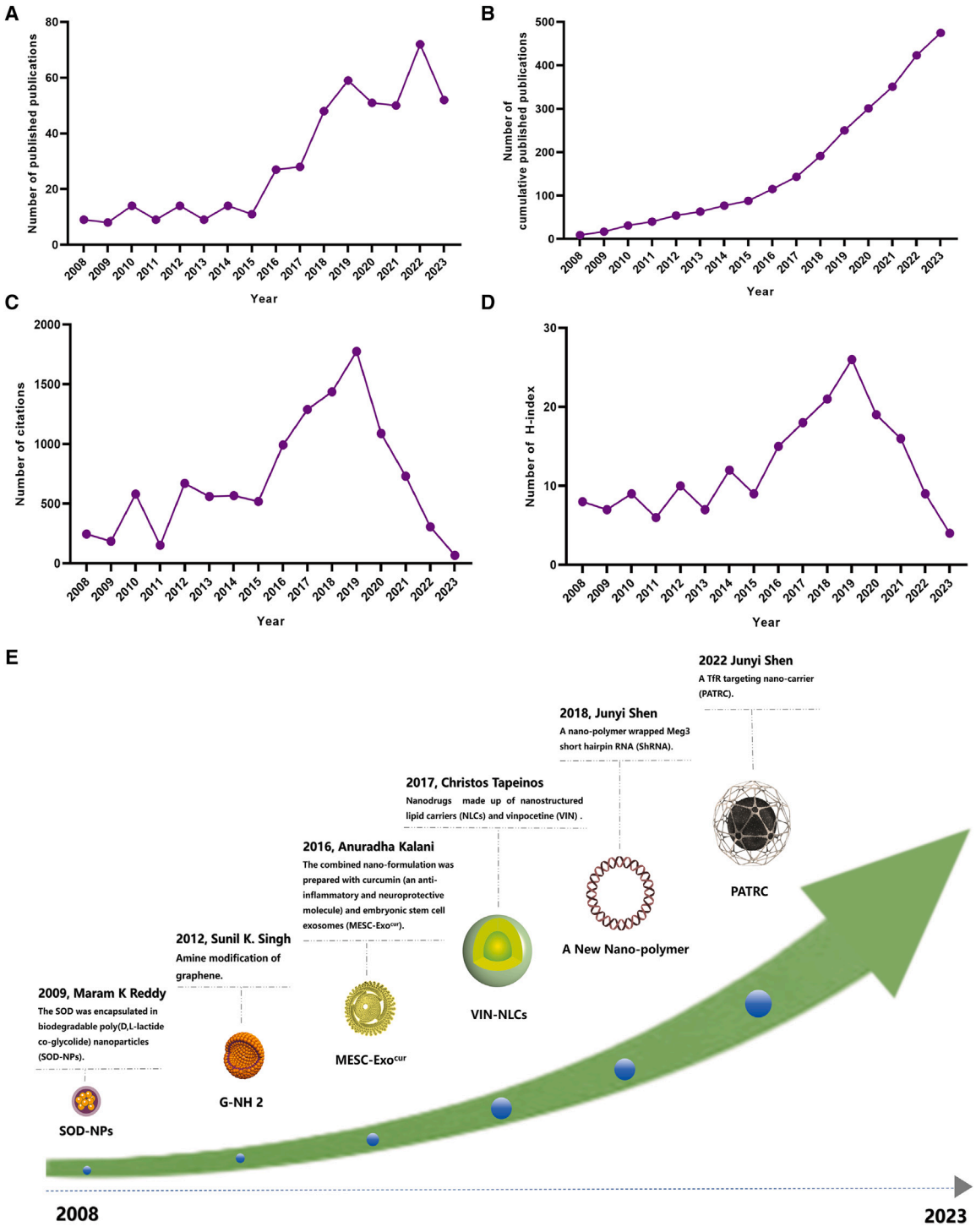


Figure 2. Publication trends in nanomaterials and ischemic stroke research

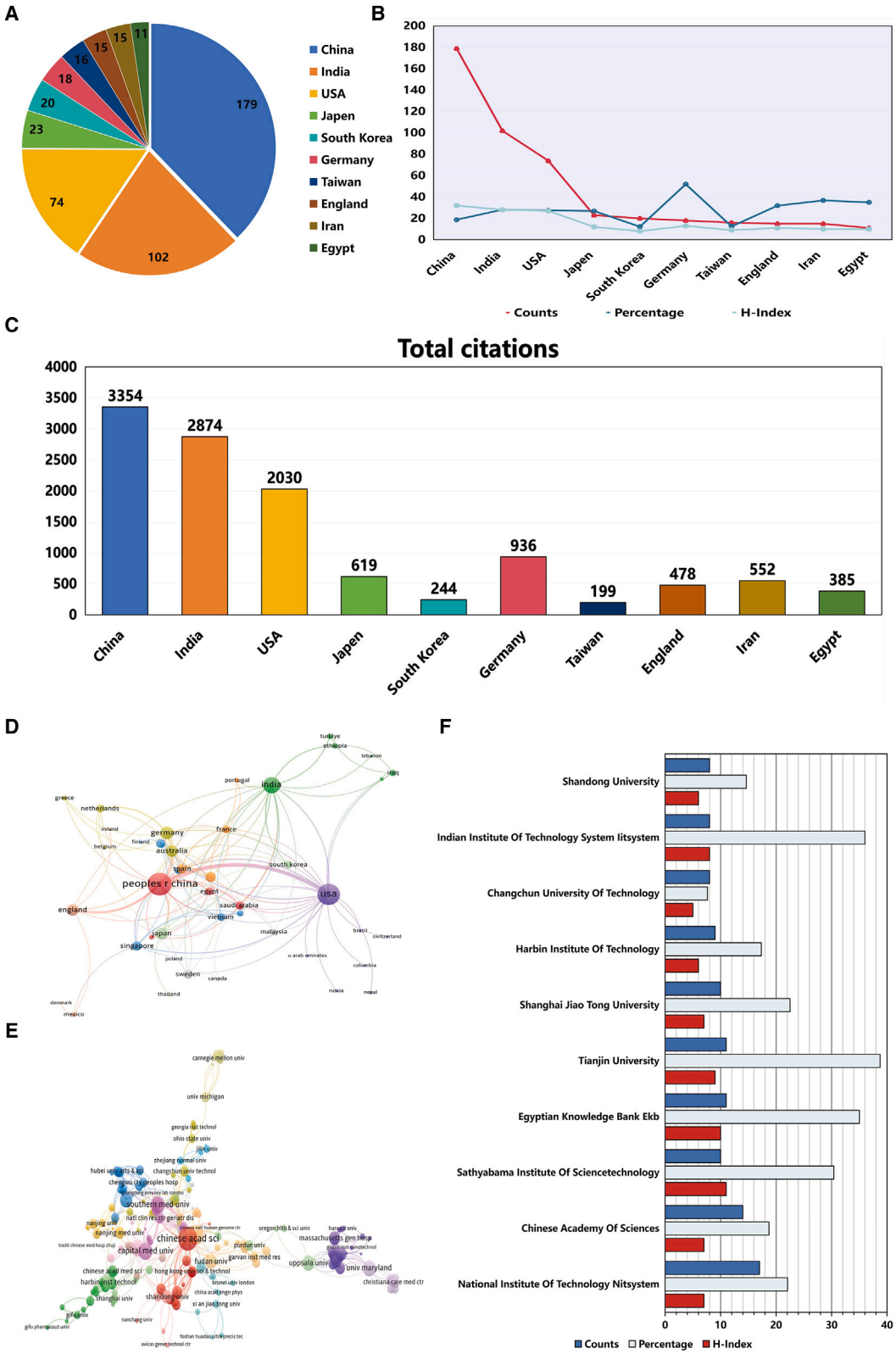
(A) Annual number of publications from 2008 to 2023.

(B) Cumulative number of publications over time (2008–2023).

(C) Total citations of publications each year.

(D) H-index values of the publications across the years.

(E) Chronological timeline highlighting key nanomaterials used in ischemic stroke research.



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techniques, CT images are produced primarily from independently located X-sensors. Due to the differing absorption characteristics of X-rays, cross-sectional images, or ''slices'', of the brain can be produced.[53] Langheinrich et al. model thrombosis in the superior sagittal sinus of the rat. The researchers isolated the mouse brain and performed nano-CT imaging, which allowed them to study the boundary layer of contrast-enhanced vessels and the occluded veins, getting more detailed information about the vascular perfusion region to further study the boundary layer of micro-CT contrast-enhanced vessels and occluded veins. A comparison with the control group demonstrated that nano-CT is capable of visualizing the interface between contrast-perfused and occluded veins, which is more conducive to the grading assessment of the lesion.[54] Furthermore, MRI, a pivotal diagnostic tool for acute cerebral infarction, has emerged as a key target for the modernization and enhancement of medical equipment. In the field of MRI research, the electronic and magnetic properties of nanoparticles are employed to enhance the examination capabilities of imaging equipment.[55] Wang et al. developed a new FMNC with high MRI sensitivity, which enables the tracking of mesenchymal stem cells (MSCs). Experiments conducted in a mouse middle cerebral artery occlusion model demonstrated that FMNC exhibited high cell labeling efficiency and no adverse effects on MSCs.[56] In conclusion, nano-CT and imaging nanoparticles FMNC can accurately assess the extent of ischemic damage and monitor treatment efficacy. This design allows for optimal imaging at the lowest contrast agent concentration, significantly reducing contrast cycle time.

THE NANODRUGS IN THE TREATMENT OF STROKE

Nanomaterials not only improve the diagnostic mode of IS but also play an important role in the treatment of IS. Nanodrugs mainly use two preparation strategies (nanodrug delivery system and nanodrug self-loading system).[65] According to the structural characteristics of traditional drugs themselves, nanodrugs with different preparation strategies can significantly improve pharmacokinetics and pharmacodynamics and benefit stroke patients (Table 2).

Application of nanodrug delivery system technology in IS

Nanodrug delivery systems (nano-DDS) encapsulate drugs in nanoparticles to enhance their penetration across the BBB and control targeted drug release.[26] Utilizing the nano-DDS technique, edaravone and rapamycin (RAPA) demonstrate enhanced permeability across the BBB. Concurrently, rt-PA effectively executes thrombolytic action at the site of infarction. The incorporation of the nano-DDS technique approach significantly augments the therapeutic efficacy of these conventional pharmaceuticals at the infarction locale (Figure 6A). The administration of rt-PA intravenous thrombolytic therapy represents a significant advancement in the treatment of stroke, offering a potential avenue for a cure for many patients. Rt-PA is a major enzyme in thrombolysis, catalyzing the conversion of fibrinogen to fibrinolytic enzymes and subsequently degrading the fibrin network. Nevertheless, rt-PA is also associated with complications, including a short half-life, low targeting ability, and unexpected bleeding.[74] Xu et al. developed platelet membrane-camouflaged polymeric nanoparticles (nanoplatelets) for the delivery of thrombolytic drugs, rt-PA, to local thrombus sites. Nanomaterial-encapsulated rt-PA was found to have significantly enhanced thrombolytic activity and reduced risk of bleeding compared to free rt-PA when administered to mice with pulmonary embolism and mesenteric arterial thrombosis.[70] Bao et al. have developed an efficacious treatment agent for stroke based on monodisperse ceria nanoparticles, which were loaded with edaravone and modified on their surface with Angiopep-2 and poly(ethylene glycol) (E-A/P-CeO_2). In *in vitro* animal experiments, the E-A/P-CeO_2 features highly effective BBB crossing via receptor-mediated transcytosis, enabling access to brain tissues. Additionally, the loaded edaravone and ceria nanoparticles demonstrate a combined effect on the elimination of reactive oxygen species. The nanoparticles display excellent tissue compatibility properties and afford cerebral ischemic tissue protection.[71] In addition, the capacity of RAPA to act on multiple pathways in the ischemic cascade response confers a significant advantage over other neuroprotective agents, potentially prolonging the time window for IS therapy. Cheng et al. used nanotechnology to design a pH-sensitive theranostic RAPA-loaded nanoparticle system. The system was able to take advantage of the fact that stroke tissues have a low pH microenvironment to release RAPA stably. Moreover, the nanodrug delivery system also exhibited acid-enhanced MRI and near-infrared fluorescence imaging signal properties, which can improve RAPA tracing and diagnostic accuracy. In a rat model of transient middle cerebral artery occlusion (tMCAO), the RAPA-loaded nanoparticle system demonstrated neuroprotective effects.[72] These researches suggested that nano-DDS technology enhances drug targeting ability and solubility in the body, reduces drug clearance rate, and prolongs drug action on the CNS.

Application of nanodrug self-loading system technology in stroke

Nanodrug self-loading system (nano-DSS) is a drug packaging system that is composed of small molecule prodrugs and nanodrugs.[75] Following the use of nano-DSS technology, curcumin and VIN can cross the BBB and deliver highly concentrated active ingredients into the CNS (Figure 6B). Tapeinos et al.

Figure 3. Global distribution and collaboration networks in nanomaterials and ischemic stroke research

(A) Top 10 most productive countries/regions in terms of publication volume.

(B) Number of publications, percentage of total citations, and H-index for the top 10 contributing countries.

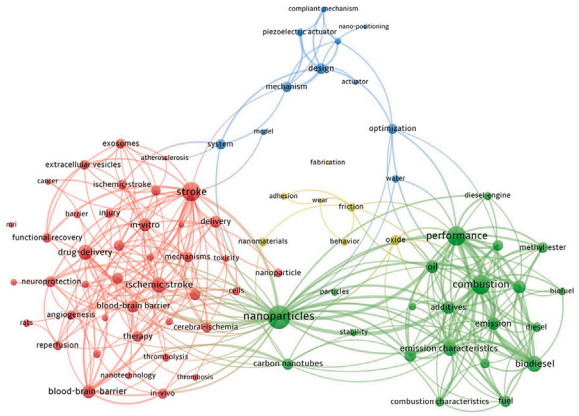
(C) Total citation counts from the top 10 contributing countries.

(D) Co-authorship network among the top contributing countries.

(E) Co-authorship network among the leading research institutions.

(F) Number of publications, percentage of total citations, and H-index for the top 10 research institutions by publication volume.

A



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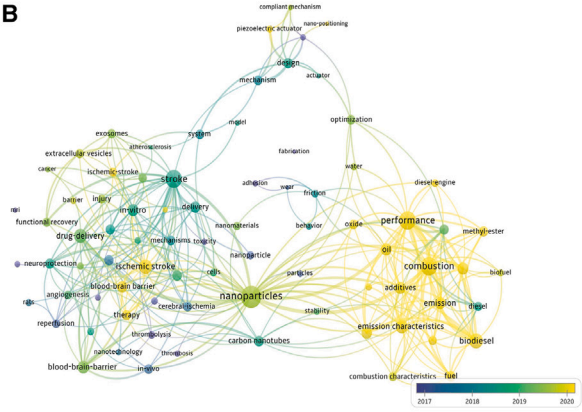

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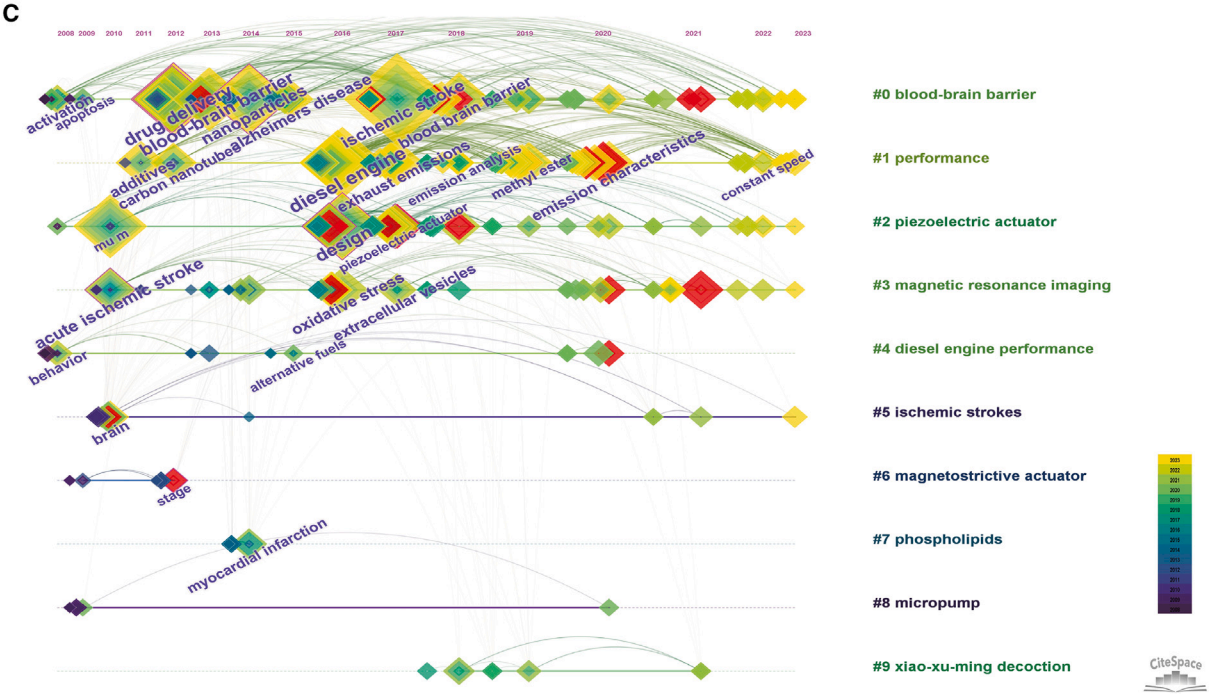

D

Top 25 Keywords with the Strongest Citation Bursts

Keywords	Year	Strength	Begin	End	2008 - 2023
focal cerebral ischemia	2010	2.72	2010	2016	
in vivo	2012	2.63	2012	2015	
drug delivery	2012	2.62	2012	2015	
stage	2012	2.51	2012	2017	
mechanism	2016	3.98	2016	2019	
design	2016	3.42	2016	2017	
single cylinder	2016	2.62	2016	2017	
driven	2016	2.42	2016	2018	
large stroke	2017	3.67	2017	2018	
neurodegenerative diseases	2017	2.52	2017	2019	
system	2017	2.27	2017	2019	
optimization	2018	3.68	2018	2019	
therapeutic agent	2018	3.36	2018	2019	
in vitro	2013	2.65	2018	2019	
blood brain barrier	2018	2.48	2018	2019	
methyl ester	2019	2.55	2019	2020	
diesel fuel	2020	3.52	2020	2023	
stem cells	2020	3.46	2020	2021	
inflammation	2020	2.96	2020	2021	
biodiesel	2020	2.94	2020	2023	
functional recovery	2016	2.65	2020	2021	
therapy	2021	3.67	2021	2023	
piezoelectric actuator	2017	3.16	2021	2023	
parkinsons disease	2021	2.58	2021	2023	
neurological disorders	2021	2.15	2021	2023	

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prepared Vin-loaded NLC (VIN-NLC) by using a high-pressure homogenization method. Get VIN-NLC small, uniform particle size, coating with high efficiency. *In vitro* release studies showed that VIN-NLC exhibited the sustained release characteristics of VIN without an obvious burst release effect. *In vivo* pharmacokinetic studies showed that the Vin-NLC formulation had a relative bioavailability of 322% after oral administration in Wistar rats compared to VIN suspension. The NLC formulation significantly improves the oral bioavailability of VIN and offers promising prospects for oral administration of poorly soluble drugs.[76] Kalani et al. used curcumin (an anti-inflammatory and neuroprotective molecule) and embryonic stem cell exosomes (MESC-exo^{cur}) to investigate the potential role of nanomedicine in neurovascular recovery. The study was divided into the MESC-exo^{cur} intervention group and the control group. MESC-exo^{cur} treatment reduced neurological scores, infarct volume, and edema after IR injury. Compared with the control group, the IR group after MESC-exo^{cur} treatment showed reduced inflammation. Studies have shown the potential of combining embryonic stem cell exosomes and curcumin to aid neurovascular recovery after ischemia-reperfusion injury in mice.[73] Overall, nano-DSS technology has significant potential to increase drug concentration at the lesion site, particularly in improving oral drug solubility and bioavailability.

Nanomaterials with therapeutic properties

The utilization of nanomaterials is currently being extended to additional therapeutic avenues for stroke. Some nanomaterials have been observed to exert therapeutic effects independently. Nanodiamonds have shown great potential due to their unique physicochemical properties, including a nanostable inert core, tunable surface, intrinsic fluorescence without photobleaching, negligible toxicity, and the ability to form complexes with drugs.[77] The ability of nanodiamonds to penetrate the BBB and target specific affected regions of the brain may shed light on the underlying etiology of stroke and serve as a possible therapeutic approach in the future. Furthermore, nanodrugs offer novel avenues for the management of inflammatory disorders.[78] Huang et al. developed a hemostatic agent with anti-inflammatory properties by incorporating carboxyl-functionalized biodegradable polyurethane nanoparticles (PU NPs) and evaluated its function using a rat neurosurgical model.[79] The results indicated that gelatin containing PU NPs reduced brain edema, suppressed the gene expression levels of pro-inflammatory M1 biomarkers, increased the gene expression levels of anti-inflammatory M2 biomarkers, and reduced the activation of inflammatory cells at the implantation site compared to conventional gelatin. Research indicates that PU NPs-containing hemostatic agents exhibit anti-inflammatory and neuroprotective potential *in vivo*, which may be beneficial in the treatment of diseases such as stroke, traumatic brain injury, and neurodegenerative diseases. Kukaliia et al. suggested that C60-Arg adducts may be a promising material for the treatment of ischemic and hemorrhagic stroke.[80] The water-soluble fullerene adduct, C60-Arg, is blood-compatible, non-cytotoxic, and non-genotoxic, and possesses significant antioxidant activity. The protective effect of the C60-Arg adduct is likely due to its antioxidant properties, ability to penetrate the BBB, and the breakdown of L-arginine residues to release nitric oxide, which promotes vasodilation.

THE KEY NANOTHERAPEUTIC DIRECTIONS FOR IS

The application of nanomaterials in the inflammatory microenvironment of stroke

Inflammation plays a crucial role in the pathogenesis of various diseases, including neuroinflammation, myocarditis, metabolic disorders, and uveitis, resulting in significant damage to organs and tissues.[81–83] Particularly in stroke, inflammation is a potentially fatal factor. In the pathomechanism of IS, the inflammatory microenvironment accelerates the progression of the disease by recruiting inflammatory cells and directly injuring endothelial cells (EC), vascular smooth muscle cells, and extracellular matrix through inflammatory factors such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and IL-6.[84,85] Furthermore, IL-1β exacerbates vascular and neural damage by increasing the expression of endothelial adhesion molecules, triggering the migration of white blood cells to the ischemic area, and initiating an inflammatory response.[86] Given its significance in stroke pathology, inhibiting vascular inflammation is essential for slowing down disease development. In recent years, utilizing functional nanomaterials to modulate the inflammatory microenvironment has emerged as a promising strategy for treating stroke.

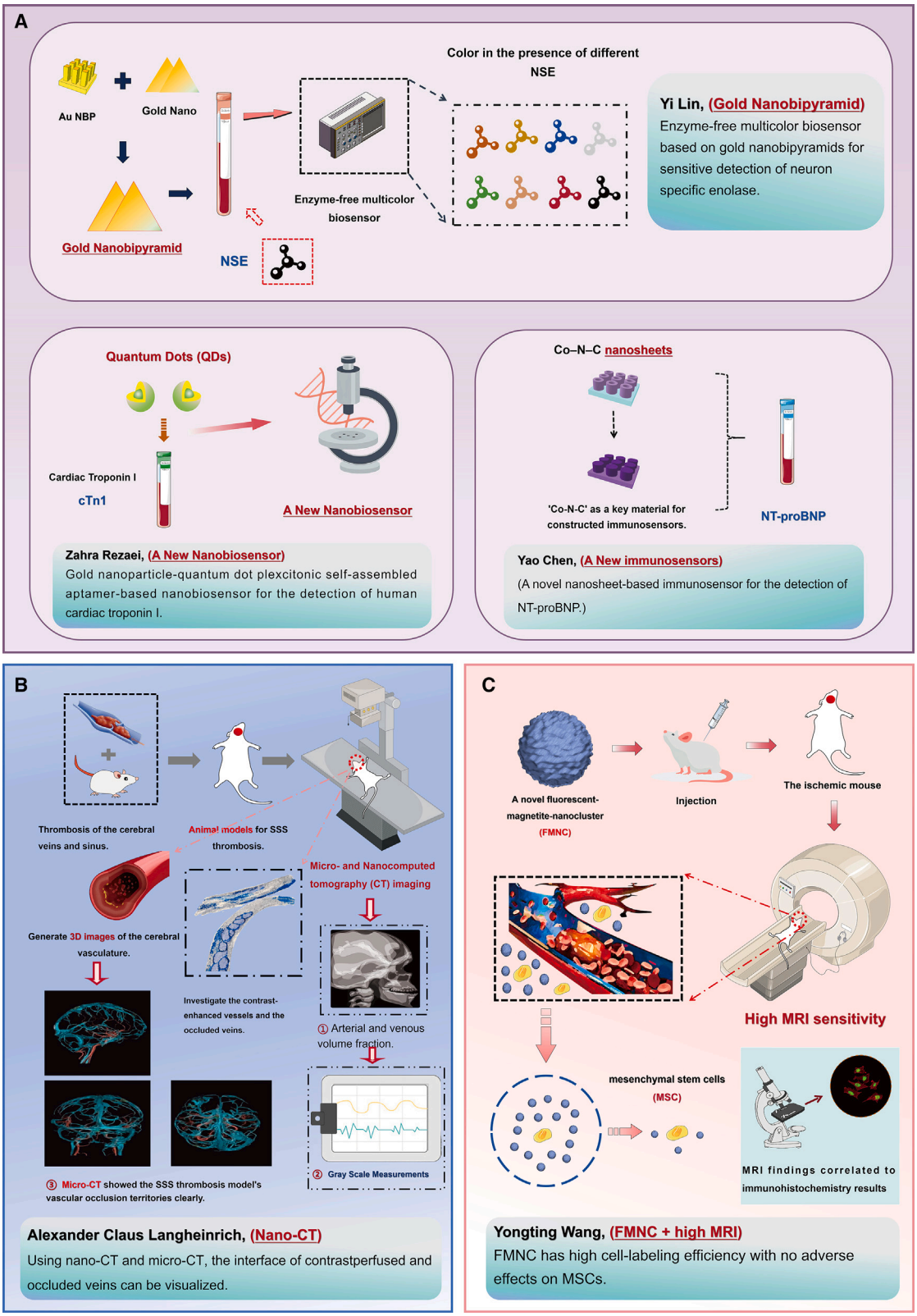
Li et al. designed curcumin-loaded triblock copolymer nanocells (NC) and utilized rats to investigate the brain's anti-inflammatory effects of NC after transient cerebral ischemia/reperfusion (I/R) injury.[87] The levels of NC were found to be higher than those of free curcumin in plasma and organs, including the brain, heart, and kidney. In comparison with the control group, the expression of NF-κB, IL-1β, IL-6, and TNF-α was significantly upregulated in rats after induction of I/R, leading to increased inflammation. These findings suggest that NC has a therapeutic effect on an animal model of stroke with cerebral ischemia/reperfusion (I/R) injury by down-regulating NF-κB-p65 proteins and inflammatory cytokines. Li et al. engineered macrophage-camouflaged honeycomb manganese dioxide (MnO_2) nanorods loaded with fingolimod (FTY) to salvage the ischemic hemidiaphragm.[88] The MnO_2 nanorods could consume an excessive amount of hydrogen peroxide (H_2O_2) and convert it into the required oxygen (O_2), as well as break down and release cargoes in acidic lysosomes. This process reduces oxidative stress, promotes the conversion of M1 microglia to M2 type, ultimately reversing the pro-inflammatory microenvironment and enhancing the survival of damaged neurons.

Figure 4. Keyword analysis of research hotspots

(A and B) The network of keywords co-occurring.

(C) The timeline map of keyword co-occurring.

(D) The Top 25 keywords with the strongest citation bursts.



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Yuan et al. designed a new type of nanodrug called TPCD NPs composed of a pharmacologically active oligosaccharide material (TPCD).[89] This material was prepared by covalently conjugating a free radical scavenging compound (Tempol) and a hydrogen peroxide-eliminating phenylboronic acid pinacol ester molecule on β-cyclodextrin. The therapeutic efficacy achieved through their antioxidant properties includes anti-inflammatory effects as well as anti-apoptotic effects. The TPCD NPs also act as reactive oxygen species-labeled nanocarriers effectively loading and triggering the release of anti-inflammatory peptide Ac2-26 resulting in an anti-inflammatory nanotherapeutic (ATPCD NP). The *in vivo* efficacy of ATPCD NPs was significantly enhanced compared to TPCD NPs, mainly due to their additional anti-inflammatory activity. Interestingly, Khuwaja et al. designed a novel curcumin nanoparticle, which, in a rat model of IS, significantly improved motor function and muscle strength, increased the activity of antioxidant enzymes such as glutathione peroxidase, glutathione, glutathione S-transferase, superoxide dismutase, and catalase, and reduced oxidative stress and inflammatory responses.[90]

The application of nanomaterials in nerve and vascular regeneration

In the aftermath of a stroke, ischemic or focal lesions of the cerebral cortex give rise to microenvironmental alterations that stimulate endogenous neural repair by influencing the morphology of neurons and glial cells. Nevertheless, the adaptive modifications initiated by this pathological process have a restricted capacity to facilitate the restoration of impaired neural function.[91] The application of nanomaterials and nanotechnology allows therapeutic substances to significantly improve changes in the microenvironment of the injury, by protecting mitochondrial function and inhibiting neuroinflammation and neuronal apoptosis. Moreover, the use of nanomaterials can assist in the formation of blood vessels in the brain and improve the plasticity of neural cells, helping patients recover neural functions. Oxidative stress and mitochondrial damage are the main mechanisms of IS with ischemic reperfusion injury. Cerium oxide nanoparticles are used as carriers to carry dl-3-*n*-butyl-phthalimide (NBP-CeO_2NPs) for the combined treatment of IS.[92] In mouse models, NBP-CeO_2NPs also showed excellent ability to clear reactive oxygen species (ROS), protect mitochondria, and inhibit neuroinflammation and neuronal apoptosis. Neurobehavioral tests showed that NBP-CeO_2NPs improved vascular regeneration after IS, significantly improving sensory-motor function and spatial learning ability. Zhang et al. reported a multifunctional *in situ* hydrogel delivery system for the combined delivery of an ROS-responsive nanoparticle, carrying statin calcium (DSPE-se-se-PEG@AC NPs) and β-nerve growth factor, to improve brain ischemic injury.[93] In a rat model of IS, the hydrogel significantly reduced the volume of cerebral infarction by regulating inflammatory response, rescuing apoptotic neurons, and promoting angiogenesis and neurogenesis.

Endothelial colony-forming cells (ECFCs) are commonly regarded as the most suitable cell tool for achieving neovascularization.[94] The vascular repair potential of ECFCs can be enhanced through genetic or pharmacological means, specifically by strengthening specific angiogenic signaling pathways. Conductive polymers (3-hexylthiophene-2,5-diyl), also known as P3HT, H_2O_2 in the gap between nanomaterials and cell membranes, stimulating the entry of extracellular calcium ions through transient receptor potential vanilloid 1 (TRPV1) channels. This TRPV1-dependent calcium ion influx, induced by H_2O_2, promotes the proliferation and vascular formation of ECFCs. Neovascularization is a critical repair process following ischemia and a viable treatment for improving neurological dysfunction. Targeted drug delivery is achieved by covalently binding the Pro-His-Ser-Arg-Asn (PHSRN) peptide to the surface, which binds to the abundant integrin α5β1 in cerebral vessels of ischemic tissue.[95] Subsequently, smooth muscle agonist (SAG), a hedgehog signaling pathway activator, is coupled to PHSRN-HES through pH-dependent electrostatic adsorption. SAG@PHSRN-HES nanoparticles can release more SAG in the acidic environment of ischemic brain tissue. Most importantly, SAG@PHSRN-HES promotes neovascularization and the integrity of the BBB through the synergistic mechanism of PHSRN and SAG, thereby improving neural plasticity and neurofunctional recovery. Additionally, a near-infrared light-driven nanophotosynthetic system, fabricated by Wang et al., can produce oxygen and absorb carbon dioxide, rescuing neurons from ischemia for the treatment of stroke.[96] By delivering auto luminescent microalgae and upconversion nanoparticles capable of internal photosynthesis to the brain, near-infrared light with excellent tissue penetration ability is converted into visible light by upconversion nanoparticles, activating microalgae to produce oxygen *in vivo*, enhancing neovascularization, reducing infarction, promoting brain tissue repair, and improving neuronal function recovery.

NANOMEDICINE COMBINED GENE THERAPY

In the etiology of stroke, metabolic risk and behavioral factors accounted for a large proportion, and genetic factors accounted for a small proportion. Several GWAS identified approximately 32 risk loci and 18 associated pathways associated with stroke or stroke subtypes.[97] These loci have attracted the attention of researchers. Gene therapy for stroke mainly focuses on genes encoding stroke-related proteins or reducing the expression of stroke-related genes. Among them, miRNA and plasmid DNA are two hot spots in stroke gene therapy. Many miRNAs and plasmid DNAs play a key role in gene therapy for stroke by binding to nanomaterials (Table 3). These studies show that miRNA and plasmid DNA usually need to be combined with nanoparticles or inorganic nanomaterials to enter the human body and play a role in gene regulation.[98,99]

Figure 5. Applications of nanomaterials in stroke diagnosis and detection

(A) Nanomaterial-based biosensors for detecting critical biomarkers in stroke.

(B) nano-CT is capable of visualizing the interface between contrast-perfused and occluded veins, which is more conducive to the grading assessment of the lesion.

(C) Novel nanomaterials enable cell labeling and cell tracking with the help of MRI technology.

Table 1. Recent works exploiting smart diagnostic nano-agents for MR imaging in ischemic stroke

Material	Design	Effectiveness	*In vivo* model	Technique	Reference
magnetic nanobubbles (MNBs)	magnetic nanobubbles (MNBs)	magnetic resonance imaging and ultrasound imaging surveillance of NSCs based on MNB labeling can be leveraged to provide NSC therapeutic outcomes.	Mouse in a stroke model	MRI	Li et al.[57]
a low-immunogenic nanoprobe	The nanoprobes were constructed by attaching self-peptides to the surface of Fe_3O_4 nanoparticles as invisible coatings.	The nanoprobes were better able to show stroke progression, through SWI and combined DWI tracing.	Mouse in a stroke model	MRI	Zhang et al.[58]
PAMNs	A platelet membrane biomimetic magnetic nanocarrier, loaded with L-arginine and γ-Fe_2O_3 magnetic nanoparticles (PAMNs).	The PAMNs can specifically target different types of damaged blood vasculature.	Mouse in a stroke model	MRI	Li et al.[59]
neutrophil-camouflaged magnetic nanoprobes (NMNPs)	NMNPs are composed of an inner core of superparamagnetic iron oxide (SPIO)-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles and a biomimetic outer shell of a neutrophil membrane.	The neutrophil-camouflaged magnetic nanoprobe could serve as a highly safe and selective nanoprobe for neuroinflammation imaging.	Mouse in middle cerebral artery occlusion model	MRI	Tang et al.[60]
superparamagnetic iron oxide (SPIO) nanoparticles	an MRI-visible nanomedicine is developed to co-deliver functional peptides and superparamagnetic iron oxide (SPIO) nanoparticles into NSCs.	This nanomedicine not only promotes the survival and migration ability of neural stem cells (NSCs) but also allows an *in vivo* tracking of transplanted NSCs with MRI.	Mouse in a stroke model	MRI	Wang et al.[61]
specific Fe_3O_4-Arg-Gly-Asp (RGD)	Molecular probes were prepared from an integrin $\alpha v\beta 3$-specific Fe_3O_4-Arg-Gly-Asp (RGD)	Fe_3O_4 -RGD nanoprobes bind to collaterals.	Mouse in middle cerebral artery occlusion model	MRI	Wang et al.[62]
SPIONs	Contrast agents were prepared from PEGylated supermagnetic iron oxide nanoparticles (SPIONs).	The ability of the PEGylated SPIONs to highlight BBB damage by MRI.	Mouse in a stroke model	MRI	Deng et al.[63]
MIRB	MRI or Xenogen imaging combined with the labeling of SPIO-Molday ION Rhodamine-B (MIRB)	MIRB-labeled $CD4^{(+)}$ T cells can be longitudinally visualized in the mouse brain after cerebral ischemia.	Mouse in a stroke model	MRI	Jin et al.[64]
FMNC	Embedding individual magnetite nanoparticles (NPs) into a polystyrene scaffold coated with two layers of silica and a layer of rhodamine.	FMNC-labeled MSCs migrated to and accumulated in the ischemic region after FMNC-labeled MSC transplantation.	Mouse in middle cerebral artery occlusion model	MRI	Wang et al.[56]

Application of nanotechnology in miRNA

Inhibition of pathogenic miRNAs, including miR-124, miR-141-3p, and miR-195, may contribute to the treatment of stroke. The researchers used nanoparticles to wrap the gene locus inhibitors and send the gene locus inhibitors into the CNS, thereby reducing the expression of pathogenic genes and achieving the purpose of curing stroke. Yang et al. delivered miR-124 using the Ca-MOF@miR-124 nano delivery system. In this study, the effect of NSCs on accelerating the directional differentiation of neurons was evaluated by cell experiments. The potential of the nano delivery system to treat IS was evaluated in a stroke mouse model. The results showed that Ca-MOF@miR-124 nanoparticles could promote the differentiation of NSCs into mature neurons with electrophysiological functions within 5 days. Ca-MOF@miR-124 nanoparticles reduced the ischemic area to almost normal levels by day 7.[107] Dhur et al. used a liquid solvent evaporation technique to encapsulate PNA and PS anti-mirS-141-3P probes in poly(lactic-co-glycolic acid) (PLGA) nanoparticles. The *in vivo* study was carried out in a mouse model of IS. After treatment, miR-141-3p levels in brain tissue and infarct injury were

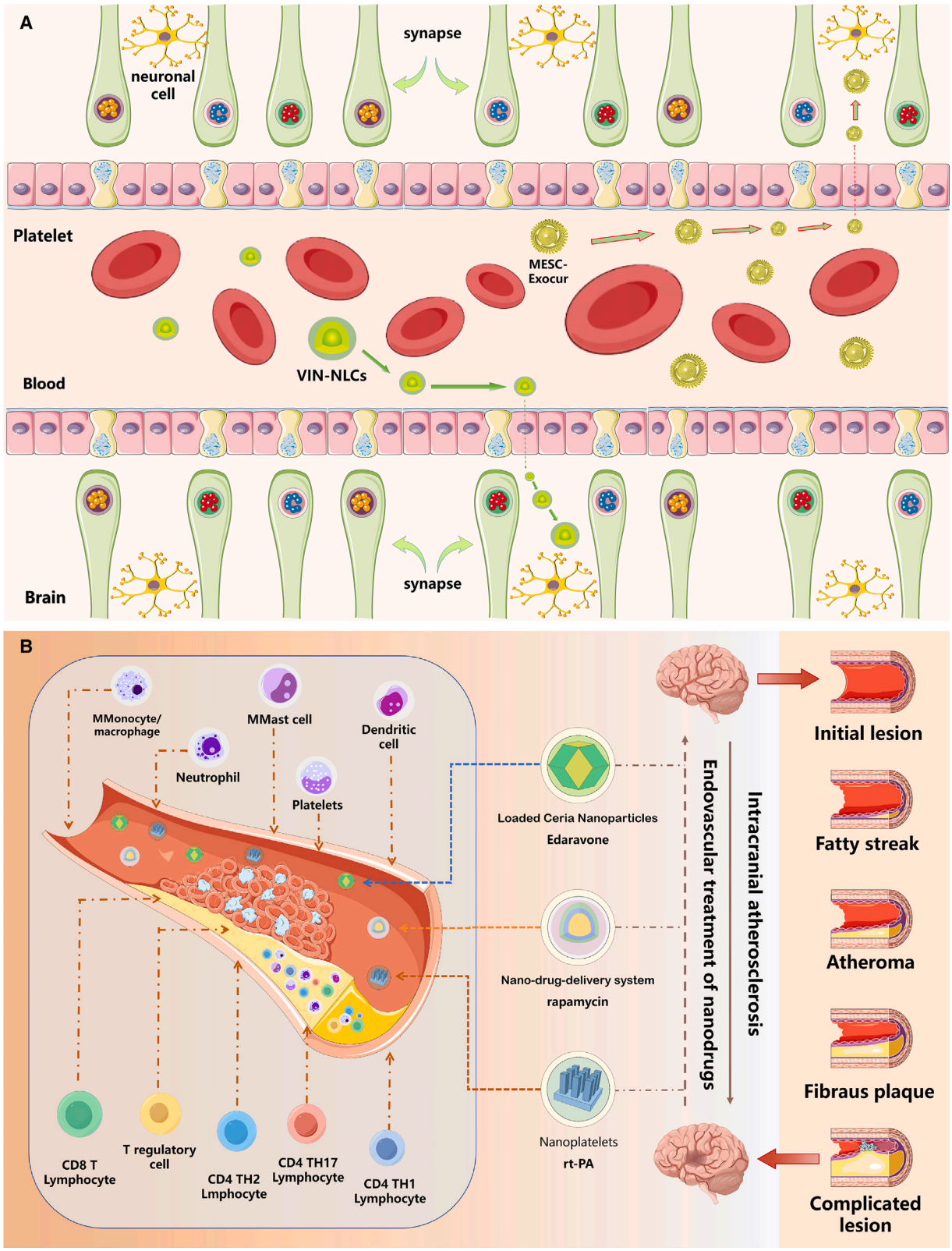
Table 2. The targeted application of nanomedicine in ischemic stroke

Nanomedicine	Preparation method	Models	Affection	Reference
PATRC	Polymer-based	The mouse model of t-MCAO.	The PATRC group showed stronger lumen formation ability, reduced penetration of the BBB, reduced cerebral infarction volume, and promoted microvascular regeneration in the cerebral infarction area.	Shen et al.[66]
A-BAM NPs	Polymer-based	The mouse model of t-MCAO.	A-BAM NPs can be employed as an antioxidant agent for stroke treatment as well as a carrier to improve the therapeutic benefits of NA1.	Zhang et al.[67]
pPMNP-rtPA	Polymer-based	The mouse model of t-MCAO.	Dual-targeted pPMNP-rtPA could reduce the clot lysis time for reperfusion by 40% when compared to free rtPA at the same drug dosage.	Chen et al.[68]
CsA@HFn	Polymer-based	The mouse model of t-MCAO.	CsA@HFn showed a significant therapeutic effect on cerebral ischemia/reperfusion injury by alleviating the apoptosis of neurons, nerve inflammation, and the damage of BBB in the ischemic area.	Liu et al.[69]
PNP-PA	Polymer-based	The mouse model of t-MCAO.	The tailor-designed nanoplatelets efficiently accumulate at the thrombi, eliciting a significantly enhanced thrombolysis activity compared to free rt-PA.	Xu et al.[70]
E-A/P-CeO_2	Polymer-based	The mouse model of middle cerebral artery occlusion (t-MCAO).	The E-A/P-CeO_2 with low toxicity and excellent hemo/histocompatibility can be used to effectively treat strokes due to great intracephalic uptake enhancement, and effectively protect the BBB.	Bao et al.[71]
RAPA/Gd^{3+}-loaded nanoparticles	Polymer-based	The mouse model of t-MCAO.	The nanoparticles demonstrated good stability and biocompatibility and could efficiently load rapamycin, followed by its rapid release in acidic environments, thereby improving therapeutic accuracy.	Cheng et al.[72]
MESC-exo^{cur}	Exosomes	The mouse model of t-MCAO.	Restored NeuN-positive neurons, reduced vascular inflammation, and alleviated tight and adherent junctions following IR injury.	Kalani et al.[73]

differentially reduced. Compared with PNa-based anti-MIR, PNa-based anti-MIR showed better efficacy.[108] Cheng et al. injected miR-195 carrying nanoparticles intravenously into rats with acute ischemic or hemorrhagic stroke. The results showed that miR-195 reduced brain lesion size and improved functional recovery in both types of stroke rats. IS can reduce the injured brain volume by 45%, and hemorrhagic stroke can be reduced by about 30%. The therapeutic window period between stroke onset and miR-195 treatment may be as long as 6 h.[109] In conclusion, miRNA is a potential pathway for gene therapy of IS. Nanoparticle encapsulation of miRNA can be used as an effective strategy for miRNA delivery. Nanoparticles can effectively prevent miRNA from being degraded by nuclease and help miRNA to enter the CNS to play a therapeutic role.

Application of nanotechnology to plasmid DNA

Plasmid DNA is a stable gene editing tool and can replicate independently of host cells. Plasmid DNA can contain more than 10 kb of foreign DNA fragments, and its infection efficiency is close to 100%.[108] Since the BBB does not allow the passage of large molecules, plasmid DNA cannot enter the CNS directly. However, when plasmid DNA is coated with nanomaterials, it can cross the BBB and enter the CNS to exert therapeutic effects. The use of inorganic nanomaterials (polyethylene glycolic acid, PGA) as carriers was able to smoothly deliver plasmid DNA to the CNS and exert gene therapy effects (Figure 7A).[109] Shen et al. from Nanjing University have developed a highly efficient non-viral gene therapy vector using γ-poly glutamic acid as the carrier. They created the Meg3 shRNA plasmid as a means of promoting neovascularization and improving neurological function. It will be translated into mice by knocking out the Meg3 gene and conjugating it to the MPO antibody. For this experiment, rats with cerebral infarction were divided into groups receiving MPO or untreated controls. According to the results, MPO enhanced the migration and the formation of tubes within brain microvascular endothelial cells *in vitro*, and it increased the capillary density. In comparison with Meg3 or the control plasmid-treated group, MPO treatment upregulated angiogenesis-associated genes VEGFA and VEGFR2. As a result of this study, MPO was found to target brain targets and significantly promote angiogenesis,



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which made it a viable treatment method for cerebral infarction. In a word, Plasmid DNA wrapped by nanomaterials has no tissue toxicity and can be more stable in gene therapy.[110]

APPLICATIONS OF NANOROBOTS IN IS

Thrombosis represents a significant global health concern, posing several challenges to clinical treatment. These include the low bioavailability of thrombolytic drugs, recurrent thrombosis, ischemic-hypoxic injury, and neurodegeneration.[111] Furthermore, the complexity of the vascular network makes interventions in sub-millimeter vessels extremely challenging.[112] Nanorobotics presents a precise solution for endovascular therapy. Presently, researchers can control and navigate nanorobots through various fluid environments using chemicals, external magnetic fields, and even moving cells. Remarkably, the nanorobots developed as intelligent devices with high spatial accuracy not only ensure targeted drug delivery for different types of thrombi but also minimize side effects and enhance the efficacy of thrombolytic therapy. Following treatment, these nanorobots can be self-recycled or degraded, offering new prospects for the treatment of IS (Figure 7B).[113]

Wang et al. developed a recyclable magnetic colloidal microcluster consisting of tPA-anchored Fe_3O_4@$mSiO_2$ nanorobots (tPA-nbots) for tPA-mediated thrombolysis with the help of balloon catheter-assisted magnetic actuation and X-ray fluorescence imaging system (CMAFIS).[114] By deploying the tPA-nbots transcatheter device in the vicinity of the thrombus, the tPA-nbots microclusters could be magnetically driven precisely to thrombi in sub-millimeter vessels by the CMAFIS system. After thrombolytic therapy, the tPA-nbots can be recovered via the CMAFIS system.[115] Yin et al. utilized smart DNA nanodevices for precise delivery and metering of t-PA. They utilized DNA origami to integrate DNA nanosheets with pre-designed t-PA binding sites and thrombin-responsive DNA fasteners. The fastener is an interlocking DNA triple structure that acts as a thrombin recognizer, threshold controller, and open switch. After loading t-PA and injecting it intravenously, these DNA nanodevices rapidly target the site of thrombosis, track circulating microemboli, and expose active t-PA only when thrombin concentrations exceed a threshold. Yang et al. present a biocompatible swarming magnetic nanorobot designed for safe and efficient targeted thrombolytic therapy *in vivo*.[116] This innovation was achieved through the meticulous grafting of magnetic beads with heparan phospholipid polymer brushes under an alternating magnetic field **B**(t). The dense surface charge of heparan phospholipid polymer brushes enabled the swarming nanorobots to exhibit reversible cluster-free remodeling, low hemolysis, antibioadhesion properties, and self-anticoagulant capabilities within a high ionic strength blood environment. Both *in vitro* and *in vivo* experiments validated their efficacy in synergizing thrombolysis via ''motion-targeted'' drug delivery and mechanical disruption. Furthermore, upon completion of thrombolysis and cessation of **B**(t), the nanorobots disintegrate into dispersed particles within the bloodstream, facilitating their safe circulation and subsequent phagocytosis by immune cells without inducing significant organ damage or inflammatory lesions.

BIOSAFETY OF NANOMATERIALS

Nanomaterials have considerable potential for application in the field of IS due to their inherent excellent physicochemical properties, which provide ideal functionality for disease cure (e.g., diagnostics, imaging, drug delivery, and therapy). However, prolonged, nonspecific accumulation of these nanomaterials in tissues may lead to toxicity, thus hindering their large-scale clinical application.[117] Therefore, further understanding of the design of nanomaterials and their metabolic pathways *in vivo* will be crucial for IS applications and advancing clinical trials in the future.

Excitingly, the last 10 years of research have demonstrated improved safety of nanomaterials, particularly in terms of biocompatibility, controlled drug release, local therapeutic effect, and BBB permeability.[118,119] Nanomaterials have shown good biocompatibility and minimal systemic toxicity in the treatment of IS. Nanomaterials can reduce harm to healthy tissues and improve therapeutic efficacy through targeted drug delivery and precise drug release rate control. Nanomaterials also can repair the vascular endothelial barrier, inhibit inflammatory responses, enhance microcirculation function, and provide neuroprotection.[120] In addition, nanomaterials exhibit biocompatibility and stability, enabling them to significantly mitigate damage to blood vessels and nerve cells. As a result, pharmaceuticals and medical devices will likely find wider potential applications for nanomaterials in future research. We contend that as the potential of nanomaterials with rapid *in vivo* clearance and degradable nanomaterials is further investigated, a greater number of nanomaterials will complete the clinical translation process and continue to enhance patient outcomes.

LIMITATIONS OF THE STUDY

In the diagnosis of stroke, nanomaterials can enhance subtle changes in the lesion area to aid clinicians in diagnosis. However, the combination of nanomaterials and markers is not stable, leading to the removal of nanomaterials as foreign bodies. It is essential to further improve the stability of the combination of nanomaterials and markers to ensure that nanomaterials can exist stably in the blood and continue to perform diagnostic functions. Additionally, understanding the metabolic pathways and potential side effects of nanomaterials is necessary to ensure their safety and efficacy in treatment. The therapeutic effects and side effects of nanomaterials are significantly influenced by the preparation method. Researchers can ensure consistency

Figure 6. Two nanoparticle design strategies for targeted stroke therapy

(A) Targeted nanoparticle drug delivery systems transport therapeutic agents (e.g., edaravone, rt-PA, rapamycin) directly to the ischemic brain regions, promoting thrombolysis, inhibiting platelet aggregation, and protecting vascular endothelial function.

(B) Self-assembling drug nanosystems (e.g., VIN-NLCs, MESC-EXO^{cur}) enhance drug loading capacity and enable efficient penetration of the BBB for improved therapeutic delivery.

Table 3. Nanoparticle-mediated delivery of miRNAs for treatment of ischemic stroke

Gene	Design	Models	Target	Reference
miR-195	miR-195 carried by nanoparticles.	Mouse in a stroke model	miR-195 reduced the size of brain damage and improved functional recovery in both types of stroke rats.	Cheng et al.[100]
miR-141-3p	The PNA and PS anti-miRs were encapsulated in PLGA nanoparticles.	Mouse in a stroke model	Post-treatment differentially reduced both miR-141-3p levels in brain tissue and infarct injury.	Dhuri et al.[101]
miR-124	PEG-PLGA nanoparticles loaded with miR-124.	The mouse model of t-MCAO.	PLGA/miRNA-124 conjugated with PEG and RVG29 alleviated the symptoms of cerebral ischemia-reperfusion injury.	Hao et al.[102]
miR-21	miR-21 was encapsulated in nanocapsules.	Mouse in a stroke model	Nanocapsules encapsulating miR-21 are effective in improving infarct volume, neurological deficit, and histopathological severity.	Liu et al.[103]
miR-181a	AMO181a-chol-loaded RBP-Exo (RBP-Exo/AMO181a-chol)	The mouse model of t-MCAO.	The combined effects of RBP and AMO181a-chol exerted neuroprotective effects in the ischemic brain.	Kim et al.[104]
miR-214-3p	Hypo-Exo loaded with anti-miR-214-3p	Mouse in a stroke model	The regulation pathway centered on miR-214-3p has a down-regulation effect on the PTEN/Akt signaling pathway, which helps to improve neurological function after ischemia/reperfusion events.	Wu et al.[105]
miR-760-3p	ADSC-Exo contains miR-760-3p	Mouse in a stroke model	ADSC-Exo containing miR-760-3p inhibited neuronal ferroptosis.	Wang et al.[106]

and stability in nanomedicine by improving reproducibility and control during the preparation process. Overcoming these limitations requires enhancing nontoxicity and targeting of nanomaterials while implementing strict monitoring during fabrication processes. In future research, collaboration among researchers and institutions will optimize the performance of nanomaterials, ultimately overcoming limitations for their application in stroke treatment with hopes for a cure.

CONCLUSION AND PERSPECTIVES

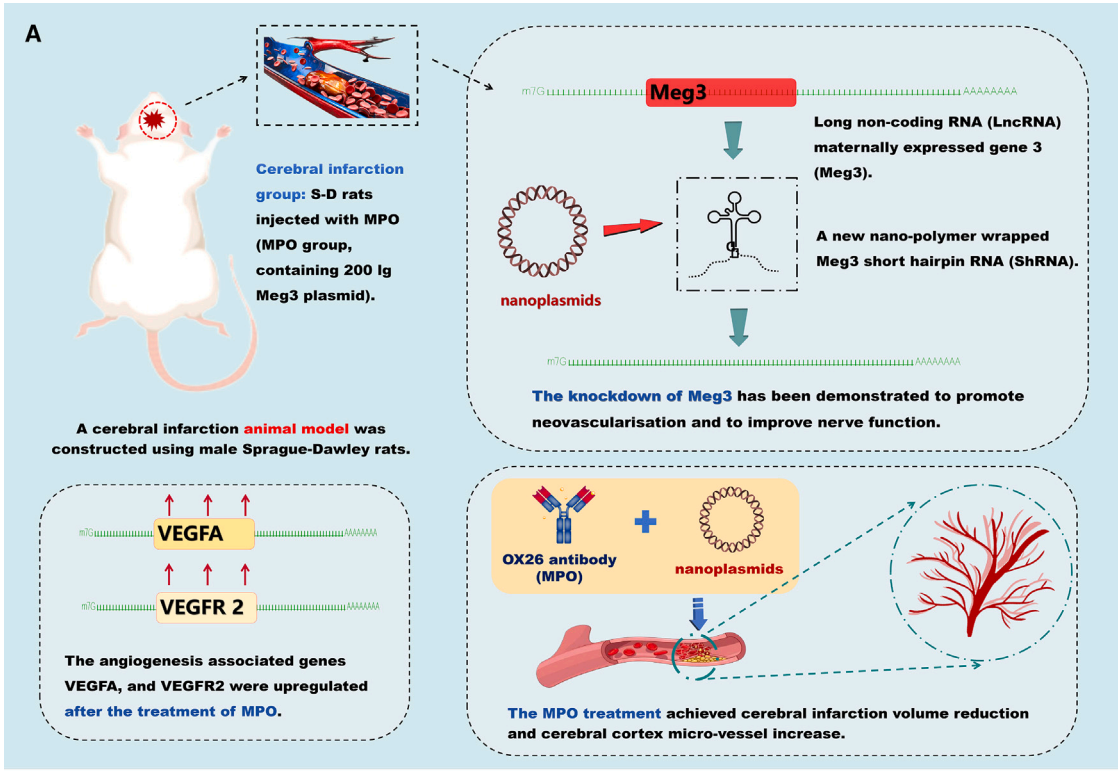
The application of nanotechnology in the diagnosis and treatment of IS offers transformative potential, marking a significant shift from traditional therapeutic approaches. Nanomaterials, with their unique physicochemical properties, show promise in overcoming barriers such as the BBB, enabling precise drug delivery and enhancing diagnostic capabilities. Through a comprehensive bibliometric analysis, this review provides a quantitative assessment of global research trends, identifying key contributors, institutions, and collaborative networks in nanomaterial research for IS. This approach offers valuable insights into emerging research hotspots and evolving directions, guiding future innovations and research efforts in the field.

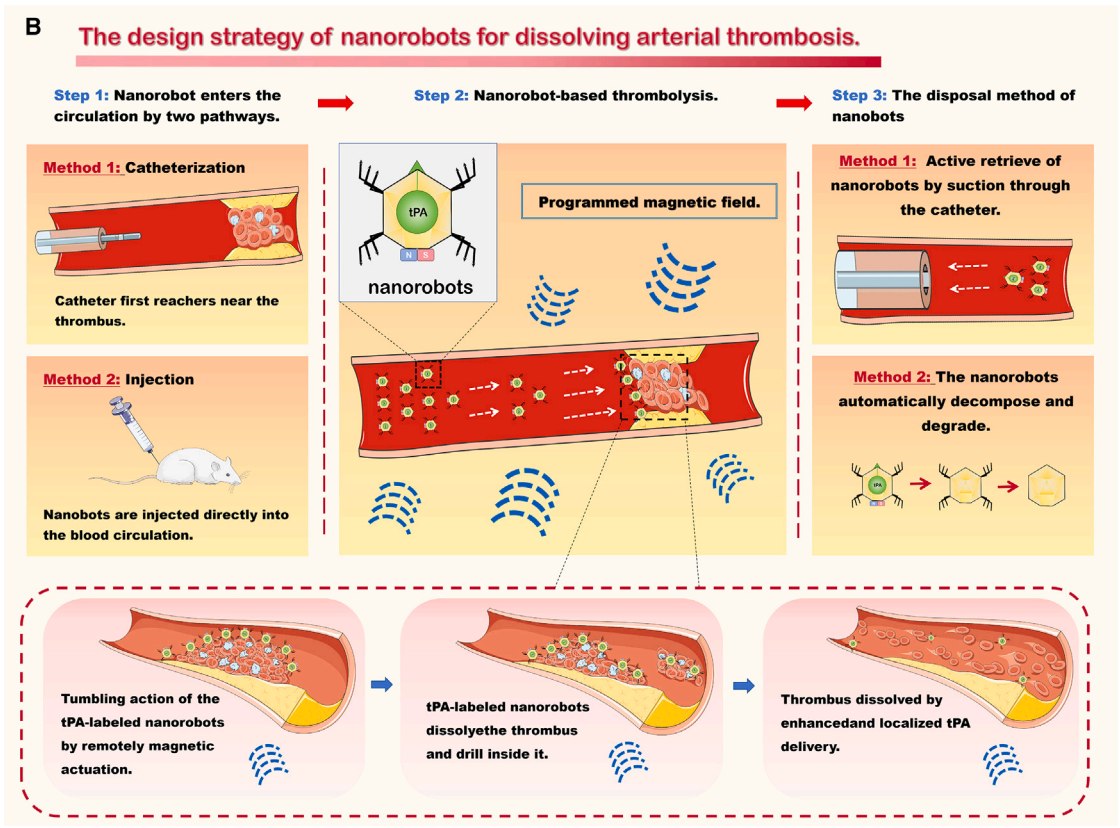
Despite these advancements, important considerations regarding the long-term biological safety of nanomaterials remain. While many studies report promising biocompatibility and therapeutic efficacy, there are still concerns about potential toxicity, tissue accumulation, and the long-term effects of nanomaterials *in vivo*. These issues call for continued research to better understand the metabolism, clearance, and potential adverse effects of nanomaterials as they advance toward clinical application. Refinement of nanomaterials is necessary to enhance their safety profile, improve targeting precision, and ensure consistency in therapeutic outcomes across diverse patient populations.

Looking forward, integrating nanotechnology with emerging fields such as gene therapy, stem cell-based interventions, and precision medicine presents a promising frontier for stroke treatment. The combination of nanomaterials with gene editing tools or regenerative medicine could offer new opportunities for repairing ischemic damage beyond the scope of current treatments. Furthermore, the convergence of nanotechnology with AI has the potential to drive personalized therapeutic interventions, allowing for real-time monitoring and adaptive treatment strategies that respond to the dynamic nature of stroke.

The development of biodegradable or transient nanomaterials could address concerns regarding long-term toxicity, offering materials that perform therapeutic functions and are subsequently metabolized or excreted safely. To advance this field, interdisciplinary collaboration will be essential, particularly in understanding how these materials interact with biological systems over time. Additionally, addressing regulatory and ethical considerations will be critical to ensuring the safe and effective translation of nanomedicine into clinical practice.

In conclusion, nanotechnology holds great promise in revolutionizing the diagnosis and treatment of IS. While challenges related to biological safety and long-term effects remain, the potential benefits of nanomaterials, particularly when integrated with other advanced therapeutic technologies, are substantial. With sustained research efforts and collaborative innovation,





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nanomedicine is poised to play a central role in the future of stroke therapy, offering new hope for improved patient outcomes and quality of life worldwide.

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AUTHOR CONTRIBUTIONS

Y.W. and H.C. conducted the literature search, data collection, and statistical analysis, and they jointly authored the manuscript; L.Q., X.L., M.D., and B.S. were responsible for reviewing and organizing the literature; H.G. designed the study, conceived the research approach, and revised the manuscript. All authors contributed to the final article and approved the manuscript for submission.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Figure 7. Advanced nanotechnology strategies for vascular regeneration and thrombolytic therapy in ischemic stroke

(A) Nanoplasmids facilitate vascular regeneration at stroke sites by silencing Meg3 and enhancing the expression of VEGFA and VEGFR2.

(B) Conceptual illustration of nano-thrombolytic robots, detailing their mechanisms of action, including intravascular release, targeted thrombolysis, and strategies for post-treatment drug clearance.

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