

Advances in nano drug delivery systems for targeted microglia modulation in ischemic stroke treatment

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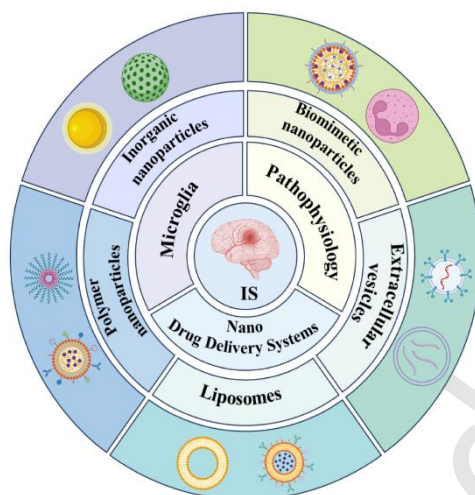
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This review highlights nano-drug delivery systems that enhance BBB penetration and target microglial polarization for ischemic stroke therapy, covering advanced platforms such as liposomes and exosomes.

Review Article

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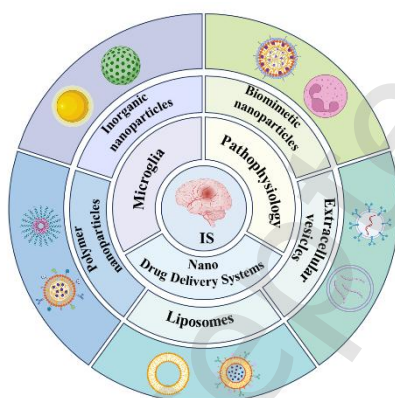
Abstract: Ischemic stroke (IS) progression is closely linked to neuroinflammation from microglial polarization imbalance. Its precise modulation, however, is limited by poor blood–brain barrier (BBB) penetration, low spatiotemporal accuracy, and systemic off-target effects. Nano drug delivery systems (NDDS) offer a promising alternative, utilizing enhanced BBB traversal and targeted lesion accumulation to address these challenges. Therefore, systematically sorting out the design strategies of NDDS targeting the regulation of microglia is of great significance for achieving precise treatment of ischemic stroke. This review outlines the pathophysiological mechanisms of IS and the biological characteristics of microglia, focusing on analyzing their dual regulatory roles in IS progression. Building upon this foundation, it explores NDDS strategies targeting microglial polarization, encompassing advanced

delivery platforms such as liposomes and exosomes. Finally, future directions are proposed to advance NDDS-based therapeutic paradigms.

Keywords: ischemic stroke; neuroinflammation; targeting; polarization of microglia; nano drug delivery system

Graphical abstract

Schematic diagram of a microglia-based nanodrug delivery system for the treatment of ischemic stroke



1. Introduction

According to the latest Global Burden of Disease (GBD) 2021 analysis, stroke remains the second leading cause of death worldwide, surpassed only by ischemic heart disease^[1]. Ischemic stroke (IS), accounting for approximately 85% of all stroke cases with an annual mortality reaching 6.4 million, imposes substantial burdens on individual health and societal development^[2]. Defined as cerebral tissue necrosis caused by the stenosis or occlusion of cerebral blood supply arteries, IS results from cerebral hypoperfusion due to intravascular thrombosis or embolism^[3]. The pathological progression exhibits marked temporal dynamics: as exemplified by the subacute phase (24 hours to 14 days), during which inflammatory cascade and persistent blood-brain barrier (BBB) disruption dominate. This stage-specific evolution of pathogenic mechanisms poses significant therapeutic challenges. Critically, neuroinflammation serves as a core driver of IS pathology, with microglia emerging as central orchestrators of this process. Their rapid activation post-ischemia initiates dual phenotypes: initially amplifying inflammation (M1) through cytokine/reactive oxygen species (ROS) release, then potentially transitioning to repair (M2). Consequently, investigating microglial polarization dynamics and designing targeted strategies offer a promising avenue for developing stage-specific therapeutic paradigms.

Microglia, the principal immune cells of the central nervous system (CNS), play pivotal roles in neural repair and regeneration processes^[4]. These cells exhibit functional complexity in both neurological disorders and IS. Under physiological conditions, quiescent microglia critically maintain cerebral homeostasis, support neurodevelopment, and preserve cognitive functions^[5]. In IS, microglia serve as first responders that rapidly activate and migrate to ischemic regions, where they mitigate neuroinflammation and improve prognosis through efferocytosis of apoptotic neurons. However, excessive microglial activation may induce neurotoxic effects that exacerbate cerebral damage^[6]. Therefore, targeting microglia and modulating their polarization state may represent a novel therapeutic dimension for optimizing IS

treatment.

While microglia represent a critical therapeutic target for modulating post-ischemic inflammation, conventional drug therapies still face delivery barriers such as poor BBB permeability and inadequate cellular specificity. Nano drug delivery systems (NDDS) are driving a paradigm shift in IS treatment through designs engineered to target and modulate microglia.

By leveraging their advantageous characteristics, including nanoscale dimensions (1-100 nm), high drug-loading capacity, controlled release mechanisms, diagnostic potential, and targeted delivery capabilities^[7], NDDS demonstrate breakthrough therapeutic efficacy in neurological disorders such as neurodegenerative diseases^[8], depression^[9], and glioblastoma^[10]. These systems enable precise drug targeting while effectively penetrating biological barriers. Particularly in IS therapy, NDDS demonstrate exceptional capabilities in bypassing the BBB and achieving lesion-specific drug accumulation. Surface engineering strategies further allow precise modulation of microglial polarization states^[11]. Collectively, these advances provide a robust platform for enhancing drug bioavailability within the brain parenchyma, establishing NDDS as a transformative approach for microglia-targeted stroke intervention^[12].

This review comprehensively outlines the pathophysiology of IS and the role of microglia, focusing on the regulatory effects of microglia-targeting NDDS on IS therapy. By deepening the understanding of microglial mechanisms in IS and synthesizing research advances in microglia-targeting NDDS, this work aims to provide theoretical references for designing innovative NDDS. Ultimately, integration of research in this field is expected to promote the development of optimized NDDS, thereby enhancing the therapeutic efficacy of IS treatment.

2. The pathophysiology of ischemic stroke

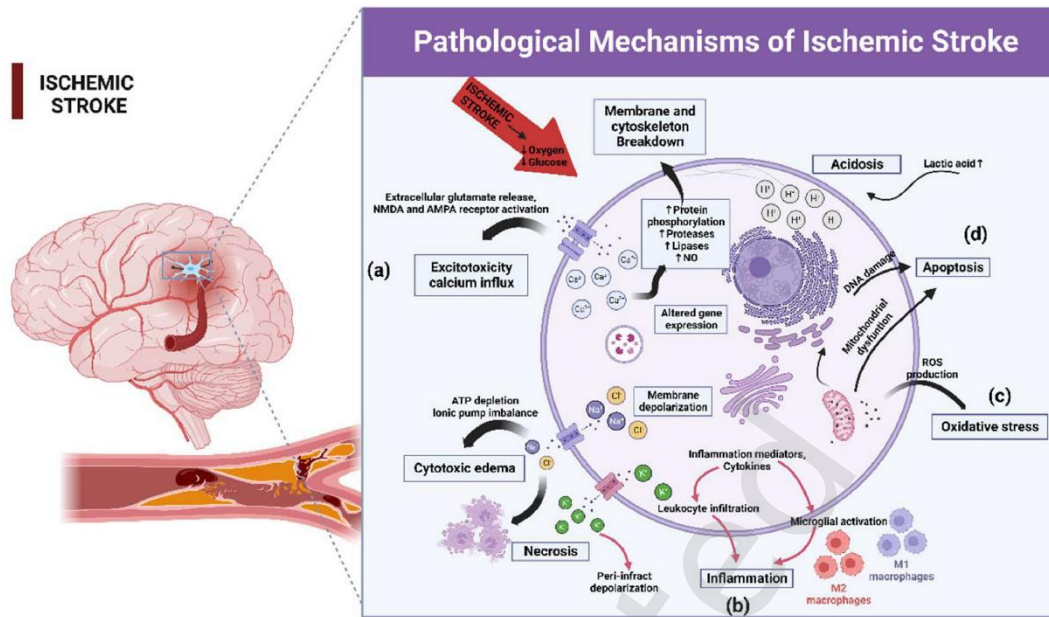


Figure 1. Pathologic mechanisms and cascading injury processes in IS^[13]. (a) Calcium overload leads to neuroexcitotoxicity. (b) Inflammation. (c) Mitochondrial dysfunction leads to oxidative stress. (d) Apoptosis. Copyright 2023, Elsevier.

IS arises from the interruption of regional cerebral blood flow, leading to diminished cerebral perfusion. This hypoperfusion induces ischemia and hypoxia, which disrupt ionic equilibrium, provoke metabolic disturbances, and reduce protein synthesis^[14]. These initial events trigger a cascade of interconnected pathophysiological processes (as illustrated in Figure 1), including excitotoxicity, oxidative stress, BBB disruption, cellular apoptosis, and inflammatory responses. These mutually reinforcing mechanisms ultimately culminate in neuronal apoptosis and subsequent neurological dysfunction. The pathological mechanisms of IS demonstrate temporal dynamics, encompassing four distinct phases: hyperacute, acute, subacute, and chronic. Interconnected pathological mechanisms across these phases form an amplified cascade of injury networks. Within this context, microglia - serving as central immune sentinels in CNS - emerge as a pivotal hub integrating multiple pathological cascades through their dynamic polarization (M1/M2 phenotypic switching).

During the hyperacute phase of IS (minutes to hours), excitatory amino acid toxicity rapidly becomes the central pathological mechanism. In the ischemic core,

energy metabolism collapse triggers a cascade: hypoxia-ischemia induces mitochondrial oxidative phosphorylation impairment, with adenosine triphosphate (ATP) depletion disrupting Na^+/K^+ -ATPase pump activity^[15]. Neuronal membrane depolarization subsequently drives massive glutamate release from presynaptic terminals^[16]. Excessive glutamate overactivates N-methyl-D-aspartate receptors (NMDARs), mediating pathological calcium influx that elevates intracellular Ca^{2+} concentrations to 100-200 times physiological levels^[17]. This calcium overload activates proteases, kinases, lipases, and endonucleases, initiating intrinsic apoptotic pathways that exacerbate neuronal damage^[18]. Simultaneously, ATP and high-mobility group box 1 (HMGB1) released from damaged neurons act as damage-associated molecular patterns (DAMPs) that activate microglial immune sentinel functions through P2X purinoceptor 4 (P2X4) and Toll-like receptor 4 (TLR4) receptors, establishing feedforward inflammatory signaling loops during this critical window^[19].

During the acute phase of IS (6-24 hours post-onset), oxidative stress emerges as the central pathogenic driver. Mitochondrial electron transport chain uncoupling generates excessive superoxide anions ($\text{O}_2^{\cdot -}$)^[20], which are converted into more destructive hydroxyl radicals ($\cdot\text{OH}$) and hydroperoxyl radicals ($\text{HOO}\cdot$) under acidic microenvironmental conditions^[21]. Concurrent activation of nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase 2, NOX2) and intracellular calcium overload establishes a positive feedback loop, synergistically driving ROS production and pathological accumulation^[22]. Excessive ROS inflict damage to cellular lipids, proteins, and nucleic acids, disrupting structural integrity and functional homeostasis, ultimately triggering neuronal apoptosis^[23-25]. Furthermore, ROS stimulate the secretion of inflammatory mediators and activation of inflammasomes. The resultant pro-inflammatory cytokines induce vasodilation, promote leukocyte infiltration, and exacerbate inflammatory cascades, collectively aggravating ischemic brain injury^[26-28]. These oxidative insults further activate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa\beta$) pathway to upregulate matrix metalloproteinase-9 (MMP-9) expression, which specifically degrades tight junction

proteins occludin and claudin-5, inducing the first significant breach in BBB integrity^[29]. Concurrently, microglia complete their polarization toward the pro-inflammatory M1 phenotype. The released interleukin-1 beta (IL-1 β) and tumor necrosis factor- α (TNF- α) synergize with extravasated serum albumin to induce astrocytic end-foot edema, establishing a self-perpetuating cycle of vasogenic cerebral edema through neurovascular unit dysfunction. Ultimately, oxidative damage triggers neuronal apoptosis (mediated by caspase-3 activation) and ferroptosis (via glutathione peroxidase 4 (GPX4) pathway inhibition), thereby accelerating infarct core formation^[30]. In IS, oxidative stress not only arises as a direct consequence of energy metabolism collapse but also acts as a central driver of the vicious cycle of cerebral injury by amplifying inflammatory responses and disrupting the BBB.

The subacute phase (24 hours to 14 days post-onset) is characterized by systemic amplification of inflammatory cascades, during which neuroinflammatory responses peak and dominate the pathological progression. Following cerebral ischemia-induced tissue damage, necrotic cells activate resident immune cells within the CNS, such as astrocytes and microglia, by releasing DAMPs, ROS, and inflammatory cytokines. This activation initiates immune responses and exacerbates neuronal death^[31, 32]. Activated astrocytes drive neuroinflammatory progression through the secretion of pro-inflammatory mediators (e.g., interleukin-6 (IL-6) and TNF- α), while their excessive proliferation forms glial scars that spatially confine the lesion area but concurrently impede axonal regeneration^[33, 34]. As the resident immune cells of the brain, microglia are the first responders to ischemic insults, rapidly activating during the early phase of cerebral ischemia. Activated microglia release pro-inflammatory cytokines, chemokines (e.g., C-C motif chemokine ligand 2 (CCL2), C-X-C motif chemokine ligand 10 (CXCL10)), and neurotoxic agents such as MMPs and ROS, thereby initiating inflammatory cascades^[35, 36]. The secreted chemokines mediate directional migration of peripherally derived immune cells (neutrophils, monocytes, and lymphocytes) adhered to endothelial cells toward the ischemic region. Infiltrating immune cells further amplify neuroinflammation by releasing additional

inflammatory mediators and neurotoxic molecules, propagating a self-amplifying inflammatory cascade. This process disrupts BBB integrity through oxidative stress and protease-mediated degradation of the extracellular matrix, ultimately triggering neuronal apoptosis and exacerbating secondary ischemic injury^[37].

The chronic phase shifts pathological focus to maladaptive remodeling. Persistently activated microglia excessively prune functional synapses via the complement pathway (C1q/C3b), while iron accumulation in the ischemic penumbra triggers collapse of GPX4-mediated ferroptosis resistance mechanisms^[38, 39]. Spatiotemporal dysregulation of vascular endothelial growth factor (VEGF) induces structurally aberrant neovascularization, whose pathological permeability precipitates recurrent cerebral edema. Notably, microglia exhibit metabolic reprogramming during this phase, with increased glycolytic flux and succinate dehydrogenase (SDH) inhibition collectively shaping a pro-reparative M2-like phenotype^[40]. However, persistent ROS leakage from oxidized mitochondrial DNA continues to impede synaptic plasticity recovery. This imbalance underscores the critical need for chronotherapeutic precision: acute-phase interventions must suppress M1-mediated destructive effects, whereas chronic-phase strategies should promote tissue-reparative phenotypic switching via CX3C chemokine receptor1 (CX3CR1) signaling pathways.

The ischemic cascade fundamentally represents spatiotemporal dysregulation of molecular networks. From hyperacute ionic storms to chronic maladaptive remodeling, microglia serve as central regulatory hubs whose phenotypic plasticity orchestrates the entire process: amplifying early injury signals while paradoxically enabling late-stage repair. This dual role provides a strategic entry point for nanotherapeutic design — modulating microglial phenotypes via NDDS could enable acute-phase neuroprotection through targeted intervention while potentially facilitating chronic-phase repair, thereby transcending the spatiotemporal limitations of conventional therapies.

3. Microglia

Microglia, the most extensively studied glial cells in CNS, play a multifaceted

and pivotal role in IS. They interact closely with neuronal and glial populations (including astrocytes and oligodendrocytes) and are critically involved in diverse pathological processes, such as neuroinflammation modulation, neurogenesis facilitation, synaptic remodeling, and BBB repair. These functions position microglia as a promising therapeutic target for IS. During distinct phases of ischemic injury, microglial polarization states dynamically shift between neuroprotective and neurotoxic phenotypes, exerting context-dependent effects on cerebral tissue outcomes. In-depth elucidation of microglial developmental origins, morphological heterogeneity, functional diversity, and their dual-edged roles in the pathological progression of IS will establish the molecular mechanistic foundation for developing stage-adapted precision modulation strategies. This advancement is poised to transcend the limitations of conventional broad-spectrum therapeutic approaches for IS.

3.1 The biological basis of microglia

3.1.1 The origin of microglia

Microglia, the tissue-resident macrophages of CNS, serve as the primary defense mechanism against CNS injury^[41]. Since their initial identification in 1919, extensive research has been conducted to elucidate microglial biology, yet their ontogenetic origin remains academically contentious. Early hypotheses proposed a mesodermal lineage, suggesting migration into the brain during late embryonic developmental stages^[42]. Alternative theories posited a hematopoietic origin from bone marrow-derived monocytes circulating in peripheral blood^[43]. However, the current prevailing view, supported by fate-mapping studies, maintains that microglia originate from primitive yolk sac-derived macrophages that colonize the CNS during early embryonic development^[44].

3.1.2 Morphology of microglia

Microglia exhibit remarkable morphological plasticity that dynamically correlates with their functional states and brain microenvironmental cues^[45]. Under physiological homeostasis, surveillant microglia display a highly ramified

morphology characterized by intricate tertiary and quaternary branching patterns with minimal territorial overlap, featuring a small cell soma and delicate processes—a phenotype conventionally termed the "resting state"^[46]. Upon inflammatory stimulation, microglia undergo stereotypic morphological transformation, transitioning from branched to hypertrophic or rod-shaped configurations. This structural remodeling coincides with enhanced phagocytic capacity and pro-inflammatory responses. Activated microglia are distinguished by somatic enlargement, process retraction, and spherical/rod-shaped profiles, whereas hyperactivated microglia further evolve into amoeboid morphotypes with complete process loss and augmented phagocytic functionality^[47]. These morphological alterations serve as sensitive histopathological indicators of microglial activation states, which exhibit strong spatial-temporal correlations with the severity of neuropathological lesions.

3.1.3 The functions of microglia

Microglia, constituting approximately 10% of brain cells in the adult human brain, play an indispensable role in maintaining cerebral homeostasis^[48]. As brain-resident innate immune cells, microglia exhibit phagocytic capabilities essential for combating invading pathogens, clearing cellular debris, toxins, and foreign substances within CNS, thereby preserving neural microenvironmental equilibrium^[49]. Furthermore, microglia critically regulate neurodevelopment and functionality through synaptic pruning and neural circuit remodeling. Following brain injury, activated microglia rapidly migrate to lesioned areas, where they release pro-inflammatory cytokines (e.g., IL-1 β , TNF- α , IL-6) and cytotoxic mediators including nitric oxide (NO), ROS, and prostaglandins (PGs) ^[50]. Notably, microglia serve as specialized antigen-presenting cells (APCs) within the CNS—a unique capability given the absence of alternative APCs capable of traversing the intact BBB. Upon antigen phagocytosis, microglia present processed antigens via major histocompatibility complex (MHC) molecules while concurrently secreting interleukins and chemokines to orchestrate neuroimmune responses.

Simultaneously, microglia exert neuroprotective and reparative functions post-injury by secreting growth factors (e.g., brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1)) and neurotrophins that promote neuronal survival and axonal regeneration^[51]. They actively participate in synaptic reconstruction and neural network plasticity, thereby facilitating structural and functional recovery of neural tissue.

3.2 The role of microglia in ischemic stroke

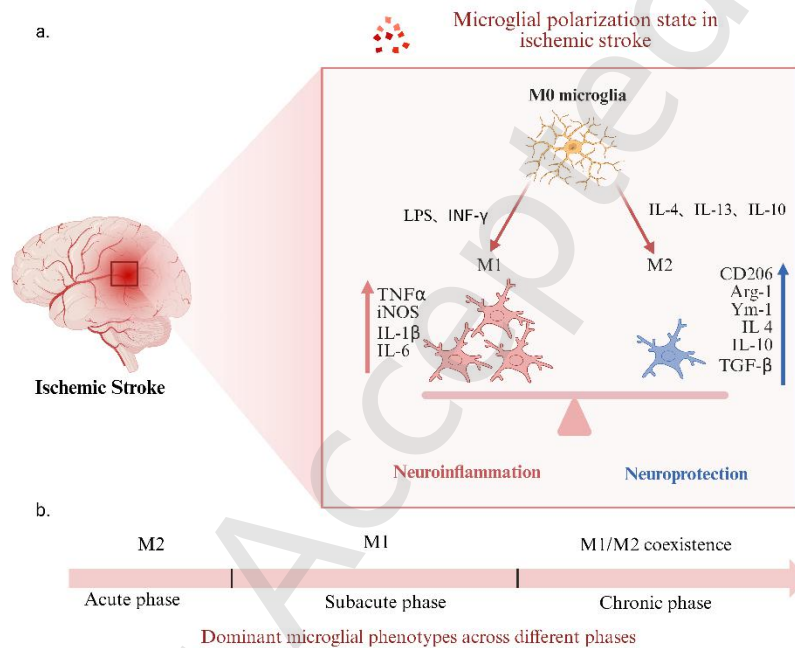


Figure 2. Microglial changes in ischemic stroke. a) Microglial polarization state in ischemic stroke. b) Dominant microglial phenotypes across different phase.

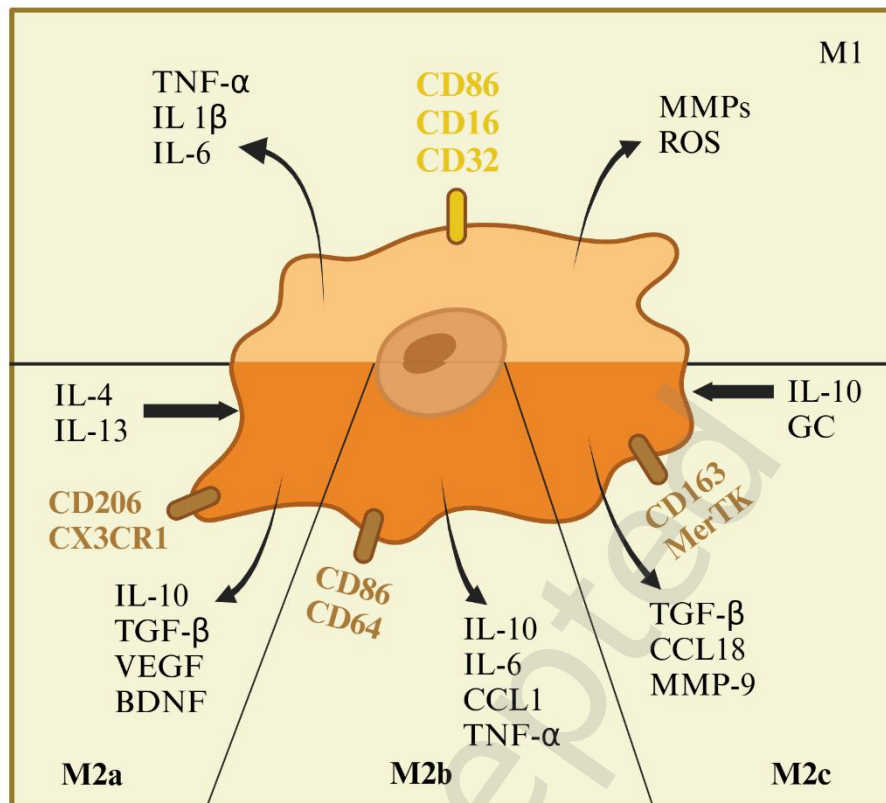


Figure 3. Schematic diagram of the typing of microglia

Following the onset of IS, the brain initiates a cascade of complex inflammatory responses, with microglial activation representing the primary event^[52]. Microglia are recognized as the earliest cellular responders to ischemic injury, rapidly detecting and reacting to pathological stimuli. Their phenotypic plasticity, termed microglial polarization, is dynamically regulated by extracellular signals, microenvironmental cues, and temporal progression post-injury^[53]. Functioning as a double-edged sword in CNS, polarized microglia exhibit a functional dichotomy: neurotoxic (pro-inflammatory, M1) or neuroprotective (anti-inflammatory, M2) phenotypes^[54].

Activated microglia polarize into two principal phenotypes: classically activated M1 (pro-inflammatory) and alternatively activated M2 (anti-inflammatory). Figure 2 depicts microglial polarization in IS. M1-polarized microglia release pro-inflammatory mediators (e.g., $\text{TNF-}\alpha$, $\text{IL-1}\beta$, IL-6 , Interferon- γ ($\text{IFN-}\gamma$)) and generate ROS, amplifying neuroinflammation and exacerbating ischemic damage. In contrast, resting microglia transition to the M2 phenotype via alternative activation pathways mediated by T helper 2 cell (Th2) cytokines (Interleukin-4 (IL-4),

Interleukin-13 (IL-13) , Interleukin-10 (IL-10)). M2-polarized microglia secrete anti-inflammatory cytokines (IL-10, transforming growth factor- β (TGF- β)), growth factors, and neurotrophins (e.g., BDNF, Glial-cell-line-derived neurotrophic factor (GDNF)), enhancing post-ischemic neuroplasticity, promoting angiogenesis, facilitating debris clearance, and maintaining tissue homeostasis^[55].

Post-ischemia, microglia undergo morphological and functional plasticity in response to local stimuli, adopting divergent polarization states that either promote neuroprotection through debris clearance and trophic support or exacerbate pathological damage via excessive cytokine release and oxidative stress. During the acute phase of stroke, the ischemic-hypoxic microenvironment rapidly activates the M1 pro-inflammatory phenotype of microglia. Through signaling pathways such as TLR4/NF- κ B, activated microglia release excessive pro-inflammatory cytokines (e.g., TNF- α , IL-1 β , IL-6) and ROS, exacerbating BBB disruption, neuronal apoptosis, and excitotoxicity^[56]. The polarization imbalance between M1 and M2 microglia is governed by several key signaling cascades. Specifically, the NF- κ B pathway serves as a central driver of M1 polarization: upon ischemic injury, IKK-mediated I κ B α degradation allows NF- κ B nuclear translocation and subsequent transcription of pro-inflammatory genes (e.g., iNOS, IL-6, TNF- α), with p65 Ser536 phosphorylation representing a critical intervention node for nanocarrier-based inhibition^[57]. The STAT family exerts dichotomous effects: STAT1 phosphorylation promotes M1 polarization, while STAT3 activation facilitates M2 polarization via IL-4/IL-13 signaling, suggesting that modulating the p-STAT1/p-STAT3 balance offers a dual-targeting strategy for nano-delivery systems. In contrast, PPAR γ functions as a key negative regulator of neuroinflammation: upon agonist binding, it transrepresses NF- κ B activity and skews microglia toward an M2-like phenotype, with its nuclear translocation serving as a tractable target for agonist-loaded nanocarriers. The polarization regulatory mechanisms elucidated above, along with their intervention nodes, can be rationally exploited by nano-delivery systems. Simultaneously, they shift their metabolic profile from oxidative phosphorylation to aerobic glycolysis to

rapidly generate ATP and sustain inflammatory bursts. This stage-specific metabolic reprogramming manifests as suppressed mitochondrial respiratory function and lactate accumulation, creating a vicious cycle of energy crisis and oxidative stress that amplifies secondary damage in the ischemic core region.

However, prolonged ischemia drives phenotypic switching toward the M1 state^[58]. During the subacute and reparative phases of IS, microglial phenotypes progressively transition toward the M2 anti-inflammatory/reparative phenotype. IL-4 and IL-13 upregulate markers such as Arginase-1 (Arg1) and Mannose Receptor (CD206) through activation of the STAT6/PPAR γ signaling pathway, thereby promoting phagocytic clearance of necrotic debris and secretion of anti-inflammatory factors (e.g., IL-10, TGF- β) to suppress excessive inflammatory responses^[59]. During this phase, microglia revert to an oxidative phosphorylation-dominant metabolic mode, relying on fatty acid oxidation (FAO) and the tricarboxylic acid (TCA) cycle to sustain energy production for supporting angiogenesis, synaptic remodeling, and neural stem cell migration^[60]. However, the chronic post-stroke inflammatory microenvironment may impede M2 polarization, leading to compromised tissue repair and pathological scar formation.

Microglia exhibit dynamic phenotypic polarization during the pathological progression of IS, with their M1/M2 balance exerting bidirectional regulatory effects on disease outcomes. Consequently, microglia are recognized as a critical therapeutic target for IS intervention^[61, 62]. During the acute phase, M1-polarized microglia exacerbate BBB disruption and neuronal excitotoxicity through the release of pro-inflammatory cytokines (TNF- α , IL-1 β) and ROS. Conversely, the transition toward M2 phenotype in the subacute phase promotes phagocytic clearance of necrotic debris and neurovascular unit repair via secretion of anti-inflammatory mediators such as IL-10 and TGF- β . Given that their temporal activation states dictate functional consequences, precisely modulating the phenotypic switching window of microglia holds critical therapeutic significance for interrupting inflammatory cascades and enhancing neural remodeling. Nevertheless, conventional

pharmacological agents face limitations in achieving precise microglial phenotype regulation due to insufficient BBB penetrability. Emerging strategies based on NDDS, which utilize stimuli-responsive carriers to achieve lesion-targeted accumulation and controlled release of immunomodulatory molecules, provide innovative solutions to overcome these therapeutic bottlenecks.

4. Microglia-Targeting Nano Drug Delivery Systems for Ischemic Stroke Therapy

The pathological progression of IS is closely associated with microglia-mediated neuroinflammatory responses, while its complex pathological microenvironment presents multiple therapeutic barriers. Targeted modulation strategies based on NDDS provide innovative therapeutic avenues to overcome these challenges by enabling spatiotemporally precise drug delivery and immune microenvironment. Leveraging their nanoscale size effects, surface modifiability, and stimuli-responsive release properties, NDDS achieve efficient BBB penetration and enable phenotype-specific modulation of microglia through precision targeting, thereby pioneering novel therapeutic avenues for IS treatment^[63]. This section details the classifications of NDDS (liposomes, polymeric/inorganic/biomimetic nanoparticles, extracellular vesicles), their drug-loading strategies, and mechanistic roles in modulating microglial polarization. Their central therapeutic mechanism in IS treatment lies in direct or indirect modulation of the microglial M1/M2 polarization balance, thereby alleviating microglia-induced neuroinflammation, mitigating IS symptoms, and providing a framework for innovative therapeutic strategies.

4.1 Liposomes

Table 1. Liposomal drug delivery system based on microglia for the treatment of IS

Drug delivery systems	Drugs	Targeting method	Targeting results	Ref.
9-AA/L-PEG-c	9-AA	NR4A1/IL-10/SOC	Inhibits	[64]
RGD		S3	neuroinflammation	

				Regulates microglial polarization	
TF/PS/DSS-LPs	DSS	PSR		Inhibits inflammatory responses	[65]
		TLR4/NF- κ B		Regulates microglial polarization	
LITA	Acetate	ERP effects		Neuroprotection	[66]
				Regulates microglial polarization	
IGF1R-targeted Lipo/SAA	SAA	Conjugation with IGF1R antibody		Neuroprotection	[67]
				Regulates microglial polarization	

Liposomes are artificial vesicular structures composed of a phospholipid bilayer, forming spherical vesicles with diameters ranging from 25 nm to 1000 nm in aqueous solutions^[68]. As the first-generation nanoparticles initially developed in 1965, liposomes serve as synthetic carriers for gene and drug delivery^[69]. These spherical vesicles are formulated using naturally derived, non-toxic phospholipids and cholesterol. Owing to their biocompatibility, ease of surface modification, and controllable drug release kinetics, liposomes are well-suited for delivering biotechnological pharmaceuticals^[70]. By shielding drug molecules from degradation, enhancing cellular and tissue penetration, and optimizing biodistribution profiles, liposomes reduce systemic toxicity while improving therapeutic efficacy. Their biodegradability, low toxicity, and biocompatibility have led to the clinical approval of numerous liposome-based formulations^[71]. Liposomal encapsulation significantly alters the pharmacokinetic profiles of payloads, particularly for proteins and peptides. Furthermore, surface modifications such as polyethylene glycol (PEG) conjugation prolong circulation half-life and extend therapeutic duration^[72].

Emerging studies have explored liposomal delivery systems for IS therapy. In this context, liposomes can transport diverse therapeutics—including

anti-inflammatory agents, antioxidants, neurotrophic factors, and small nucleic acids—to ischemic brain regions via ligand-mediated targeting. This precision delivery enhances drug bioavailability at lesion sites while minimizing off-target effects, offering a promising strategy to mitigate secondary brain injury and improve neurological outcomes.

Wang et al. developed a bifunctionalized liposomal system (9-AA/L-PEG-cRGD) to achieve precise delivery of 9-aminoacridine (9-AA) - an anti-inflammatory repurposed drug - to activated microglia in ischemic brain lesions during the subacute phase of IS (3-14 days post-ischemia), while systematically elucidating its molecular mechanisms regulating phenotypic polarization. Through high-throughput screening of FDA-approved drug libraries, the team first identified 9-AA as a novel activator of the orphan nuclear receptor NR4A1, demonstrating its dose-dependent upregulation of NR4A1 expression in microglia. Mechanistically, 9-AA activates the "NR4A1/IL-10/SOCS3 signaling axis", significantly suppressing pro-inflammatory cytokine expression (TNF- α , IL-1 β , and IL-6 reduced by 60-80%) while elevating anti-inflammatory markers (IL-10, ARG-1, and CD206 mRNA levels increased 2-3 fold), thereby driving microglial transition from pro-inflammatory M1 to reparative M2 phenotypes. To enhance targeting precision, surface modification with cRGD peptides enabled specific binding to integrin $\alpha\beta3$ receptors overexpressed on ischemic microglia, combined with PEGylation to prolong systemic circulation, resulting in 4.7-fold higher drug accumulation in ischemic brains. In transient middle cerebral artery occlusion (tMCAO) rat models, single intravenous administration of 9-AA/L-PEG-cRGD (5 mg/kg) reduced cerebral infarct volume by 51.3% within 3 days with MRI-confirmed edema resolution, while long-term assessments showed motor coordination recovery to 82.5% of normal levels in Rotarod tests. This study not only establishes microglial phenotypic switching as a therapeutic cornerstone in stroke management but also pioneers a paradigm for nanomedicine-enabled drug repurposing - employing an integrated "drug screening-mechanistic

deconvolution-targeted delivery" strategy to synergize neuroinflammatory modulation with neural repair^[64].

Min Bai et al. developed dual-functional liposomes (TF/PS/DSS-LPs) that precisely regulate neuroinflammation through a dual-targeting strategy: transferrin (TF)-mediated BBB penetration and phosphatidylserine (PS)-directed microglial targeting. The liposomes utilize DSPE-PEG2000-TF as surface ligands to exploit transferrin receptor (TfR) overexpression on brain endothelial cells for BBB traversal. Upon reaching brain tissue, PS mimics the "eat-me" signals of apoptotic cell membranes by specifically binding PS receptors (e.g., Triggering Receptor Expressed on Myeloid cells 2 (TREM2), Brain-specific Angiogenesis Inhibitor 1 (BAI1)) on microglia, triggering liposomal internalization. Mechanistically, the encapsulated danshensu (DSS) suppresses pro-inflammatory cytokines via TLR4/NF- κ B pathway inhibition and orchestrates epigenetic reprogramming to downregulate M1-associated genes (CD86) while upregulating M2 markers (Arg1, CD206). Furthermore, the liposomes exhibit inflammation-responsive drug release, achieving 70% DSS payload discharge within 12 hours under acidic inflammatory conditions (pH 5.0), thereby enhancing targeted anti-inflammatory efficacy. Experimental validation demonstrated a 3.2-fold increase in cerebral drug concentration, significant reduction in infarct volume, and improved neurofunctional outcomes through M1/M2 balance restoration during reperfusion^[65].

These studies underscore the potential of engineered liposomal systems in IS therapy. By leveraging liposomes' advantages—including tunable targeting and biocompatibility—novel delivery systems can be designed to optimize clinical outcomes through precise modulation of microglial dynamics.

4.2 Polymer nanoparticles

Table 2. Microglia-based polymer nanoparticle delivery system for the treatment of IS

Drug delivery	Cargo	Targeting method	Targeting results	Ref.
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systems				
PLGA PNP	PINK1 siRNA	Passive targeting	Neuroprotection	[73]
			Inhibits inflammatory responses	
PLGA PNP	PER	Passive targeting	Inhibits inflammatory responses	[74]
mPEG-b-PLA	Curcumin	Passive targeting	Inhibition of M1 microglial activation	[75]
CPLB/RAPA	CREKA	CREKA peptide	Reduces oxidative stress	[76]
	combines with microthrombus	to bind fibrin in microthrombi	Promotes microglial M2-type polarization	

Polymeric nanoparticles (PNPs) are nanostructures composed of polymers with dimensions ranging from 10 to 100 nm^[77]. As one of the most prevalent nanomaterials in nanomedicine, PNPs have emerged as highly promising drug delivery systems for CNS therapeutics over the past decade, owing to their nanoscale dimensions, high specific surface area, and tunable porosity^[78].

PNPs are broadly categorized into natural and synthetic types. Natural polymeric nanoparticles, derived from naturally occurring biomolecules (e.g., chitosan, albumin), serve as ideal carriers due to their exceptional biocompatibility and biodegradability^[79].

In contrast, synthetic polymeric nanoparticles enable precise regulation of BBB penetration through their customizable structures and physicochemical properties. Typically integrated into drug delivery systems, these nanoparticles are formed via emulsification of polymeric materials such as polyethylene glycol (PEG)^[80, 81], polylactic acid (PLA), and poly(lactic-co-glycolic acid) (PLGA)^[82, 83]. These nanoparticles achieve controlled release and targeted delivery of poorly soluble drugs through encapsulation or surface modification, thereby reducing systemic toxicity and off-target side effects during in vivo administration, and laying the technical foundation for microglia-targeted stroke therapy.

PLGA, a synthetic copolymer, is widely utilized as a drug carrier due to its excellent biocompatibility, stability, and biodegradability, with safety approvals from the U.S. Food and Drug Administration (FDA) and European Medicines Agency^[84]. Recent studies have extensively explored PLGA nanoparticles for targeted delivery of therapeutic agents (e.g., drugs, RNA) to ischemic brain regions in stroke treatment^[85, 86].

Seung Gyu Choi et al. established a photothrombotic IS mouse model to investigate the role of PINK1, a key mitophagy regulator in microglia, and developed a nanoparticle-based therapeutic strategy targeting microglial modulation during the acute phase. They engineered PLGA nanoparticles loaded with PINK1 siRNA (PINK1 NPs), which demonstrated preferential microglial uptake, achieving 85% suppression of PINK1 expression. This intervention promoted microglial polarization toward an anti-inflammatory phenotype and enhanced phagocytic capacity by 40%. Mechanistic studies revealed that PINK1 silencing reduced microglial migration by 40% and decreased neuronal toxicity induced by microglial secretory factors by 35%. Pharmacological depletion of microglia using PLX3397 completely abolished the neuroprotective effects of PINK1 NPs, confirming microglia-dependent therapeutic efficacy. The treatment attenuated mitophagy-associated neuronal damage, reducing infarct volume by 32% and improving neurological deficit scores by 45% on modified Bederson scales. This study elucidates the dual role of the microglial PINK1 pathway in ischemic injury and establishes nanoparticle-mediated siRNA delivery for PINK1 inhibition as a novel therapeutic paradigm. The findings provide a framework for microglia-targeted nanotherapeutics in stroke management, emphasizing spatiotemporal control of neuroimmune interactions through precision delivery systems^[73].

Shin et al. systematically investigated the neuroprotective mechanisms of perampanel (PER)-loaded PLGA nanoparticles in modulating microglial polarization using a neonatal rat photothrombotic stroke model. The study revealed that intracisternally administered PLGA nanoparticles exhibited preferential uptake by

microglia (59%), significantly surpassing neuronal (26.6%) and astrocytic (14%) internalization rates. PER-PLGA treatment significantly suppressed AMPA receptor-mediated microglial activation, reducing mRNA expression of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) by 45-68% while upregulating M2 phenotype markers Ym1 and Arg1 protein levels by 2.3-3.1 fold. In vitro experiments further confirmed PER's direct induction of M2 polarization in BV2 microglia, maintaining anti-inflammatory properties under LPS- or CoCl₂-induced inflammatory/hypoxic conditions. This work elucidates microglial phenotype switching through PLGA-mediated PER delivery, offering a novel strategy for perinatal stroke treatment through immunomodulatory targeting of microglia^[74].

Polyethylene glycol (PEG) coating (PEGylation) is a widely adopted strategy to enhance nanoparticle targeting efficiency. PEG layers prevent aggregation, opsonization, and phagocytic clearance, prolonging systemic circulation^[72]. Building on PEGylated proteins' success, PEG's impact on nanocarrier pharmacokinetics remains a research focus. Wang et al. developed curcumin-encapsulated nanoparticles (NPcurcumin) using mPEG-b-PLA copolymer, demonstrating its potent inhibitory effects on M1 microglial polarization. Double immunofluorescence labeling of Iba-1+/CD68+ cells revealed significantly reduced activated M1 microglia in the ischemic penumbra of NPcurcumin-treated groups compared to PBS and free curcumin controls. ELISA and qPCR analyses showed approximately 60% reduction in pro-inflammatory cytokine expression (TNF- α , IL-1 β) versus stroke models, with NPcurcumin significantly outperforming unencapsulated curcumin. The study innovatively elucidated NPcurcumin's dual mechanism: maintaining BBB integrity while blocking signaling pathways driving pro-inflammatory microglial transformation, thereby mitigating neuroinflammatory cascades. These findings provide critical experimental evidence for microglial polarization-targeted therapies in IS^[75].

Polymeric nanoparticles exhibit considerable promise for IS therapy due to their tunable drug release profiles and BBB penetration. However, limitations persist,

including stringent purification requirements and organic solvent dependency during synthesis, necessitating further optimization for clinical translation.

4.3 Inorganic nanoparticles

Table 3. Inorganic nanoparticle delivery system based on microglia for the treatment of IS

Drug delivery systems	Targeting method	Targeting results	Ref.
Au7Ag1 NCs	Passive targeting	Regulates microglial polarization	[87]
CeNP nanoparticles	Passive targeting	Reduces oxidative stress	[88]
CeO ₂ @PAA-LX W7	LXW7 binds $\alpha v\beta 3$ integrin	Regulates microglial polarization Reduces oxidative stress	[89]
Ang2-MMC@V X765	Angiopep-2 (Ang2) binds to LRP receptors on the BBB	Reduces oxidative stress Regulates microglial polarization Inhibits pyroptosis	[90]

Inorganic nanomaterials are nanoparticles synthesized via physical or chemical methods, exhibiting diverse morphologies with diameters ranging from 1 to 100 nm^[128]. These materials facilitate surface functionalization and enable stimuli-responsive drug release through electrostatic interactions, hydrophobic forces, or enzyme-sensitive covalent bonding. Their advantages in drug delivery—including facile synthesis, high payload capacity, biocompatibility, and tunable surface properties—coupled with exceptional thermal, optical, magnetic, and catalytic properties, have garnered significant attention for IS therapy^[91–93].

Gold nanoparticles (AuNPs) are widely employed in nanomedicine due to their superior biocompatibility and unique optical characteristics^[94]. Zhu et al. systematically elucidated the regulatory mechanisms of Au7Ag1 nanoclusters (NCs)

on neuroinflammation in IS reperfusion injury through in vitro and in vivo studies. Using an oxygen-glucose deprivation/reperfusion (OGD/R) model in BV2 murine microglial cells, the team demonstrated that Au7Ag1 NCs significantly reduced the secretion of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) and suppressed M1 polarization markers (Inducible Nitric Oxide Synthase (iNOS), CD86), as confirmed by qRT-PCR and flow cytometry. Concurrently, the NCs upregulated mRNA and protein levels of M2 anti-inflammatory markers (CD206, Arg-1). In vivo experiments in middle cerebral artery occlusion (MCAO) mice revealed that Au7Ag1 NCs treatment increased M2 microglia (CD206-positive/Iba1-positive) to 67.8% in ischemic brains, while reducing M1 microglia (iNOS-positive/Iba1-positive) to 34.8% via immunohistochemical analysis. These findings indicate that Au7Ag1 NCs alleviate neuroinflammation and enhance tissue repair by promoting microglial polarization toward the M2 phenotype. This dual regulatory effect provides a novel nanozyme-based therapeutic strategy for IS, targeting both inflammatory resolution and neural recovery^[87].

In addition to gold nanomaterials, other inorganic materials have been explored for nanodrug delivery in IS. Ceria nanoparticles (CeNPs) demonstrate therapeutic potential across multiple CNS disorders, including IS^[95], intracerebral hemorrhage^[96], and neurodegenerative diseases^[97]. Notably, CeNPs exhibit catalase- and superoxide dismutase (SOD)-mimetic properties, enabling them to function as potent ROS scavengers, thereby conferring robust neuroprotection^[98, 99]. While CeNPs are recognized for their antioxidative efficacy in mitigating neural damage in cerebral pathologies, their underlying mechanisms remain incompletely elucidated. Zeng et al. engineered tailored CeNPs capable of efficiently neutralizing diverse ROS species and reprogramming microglia from a pro-inflammatory (M1) to an anti-inflammatory (M2) phenotype under pathological conditions. Pre-treatment with these nanoparticles inhibited pro-inflammatory signaling cascades, transforming microglia-mediated interactions with neurons from detrimental to protective. This study not only elucidates the neuroprotective mechanisms of CeNPs but also proposes a novel

strategy to rebalance the immune microenvironment via microglial phenotypic modulation^[88].

Due to the robust enzymatic antioxidant activity of cerium oxide, cerium oxide nanoparticles (CeO₂ NPs) have emerged as promising free radical scavengers. Notably, these cerium oxide nanoparticles exhibit superior antioxidant properties in modulating microglial polarization. For instance, the dual oxidation states of ceria nanoparticles (CeNPs), Ce³⁺ and Ce⁴⁺, serve as advanced ROS scavengers, with the former eliminating •OH and O₂ •⁻, while the latter specifically targets H₂ O₂. Jia et al. established an inflammatory model using lipopolysaccharide (LPS)-induced activation of BV2 microglial cells. They constructed a CeO₂@PAA structure by combining CeO₂ with polyacrylic acid (PAA), followed by an EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide)-catalyzed reaction to conjugate biotinylated LXW7, successfully synthesizing a novel biocomposite carrier (CeO₂@PAA-LXW7). The study demonstrated that both LXW7 and cerium oxide nanoparticles (CeO₂@PAA) effectively suppressed LPS-induced BV2 microglial activation, reduced inflammatory cytokine production and nitric oxide (NO) release, decreased intracellular ROS levels, and inhibited phosphorylation of FAK and STAT3. The combined application of CeO₂@PAA-LXW7 exhibited enhanced anti-inflammatory and neuroprotective effects, potentially through synergistic mechanisms involving LXW7's ability to modulate integrin pathways and CeO₂'s radical scavenging capacity, thereby amplifying therapeutic efficacy^[89].

Despite advantages like high drug-loading efficiency and facile surface engineering, clinical translation of inorganic nanoparticles faces challenges in biodegradability and long-term safety. Certain metal-based nanoparticles exhibit uncontrolled degradation kinetics, risking persistent bioaccumulation and toxicity^[100]. These limitations necessitate further research to optimize biocompatibility while retaining therapeutic functionality.

In addition, some NDDS targeting microglia employ a passive targeting strategy, primarily relying on carriers or active components (e.g., antioxidants) to elicit

responses to the diseased microenvironment (e.g., scavenging ROS, inhibiting the NF- κ B pathway), thereby achieving short-term efficacy in neuroimmunomodulation. However, this mechanism remains inherently non-specific, and the induced phenotypic switch (e.g., shifting microglia toward the M2 phenotype) constitutes a secondary effect at the systemic level rather than precise, cell-type-specific programming. Although this approach offers certain advantages during acute inflammatory phases, it still faces limitations such as off-target risks, ambiguous mechanisms of action, and a narrow therapeutic window. Therefore, the field urgently needs to shift from passive microenvironment modulation to active cell-specific targeting. Future breakthroughs will depend on developing intelligent NDDS capable of specifically recognizing cell subpopulations and responding to pathological signals, ultimately enabling precise immunomodulation.

4.4 Biomimetic nanoparticles

Table 4. Biomimetic nanoparticle delivery system for microglia therapy for IS

Drug systems	delivery	Modified strategies	Targeting method	Targeting results	Ref.
SNM-NPs		Neutrophil membrane	Membrane receptors recognize inflammatory signals SHp peptide binds to neurons	Regulates microglial polarization ; Improves nerve function	[101]
Ma@(MnO ₂ +FTY)		Macrophage membranes	macrophage membrane proteins (CD44 and CD11b) to bind ICAM-1/P-selectin	Regulates microglial polarization; Reduces oxidative stress	[102]
miRNA/TPHL@PM		Platelet membranes	Platelet-neutrophil hitchhiking, T7	Regulates microglial polarization;	[103]

peptide targeting. Reduces brain damage

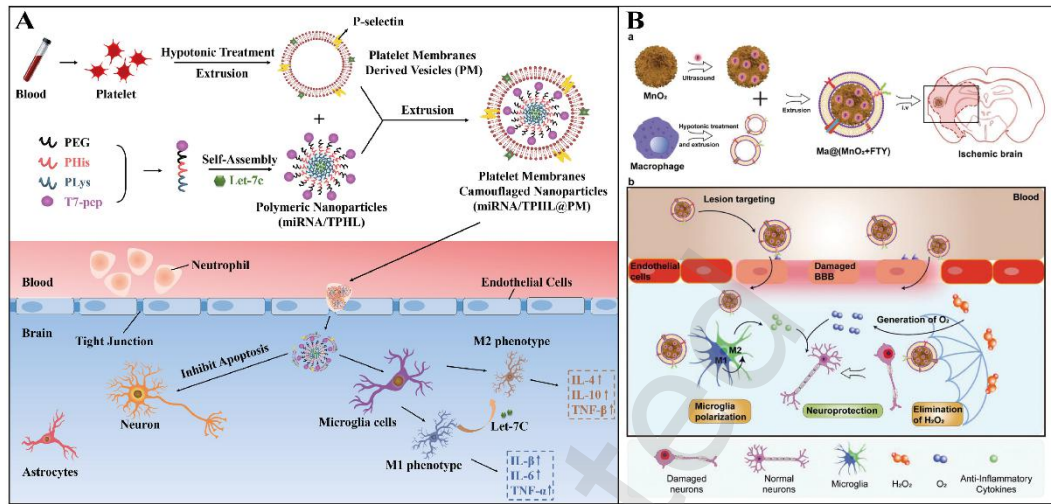


Figure 4. A) Platelet-camouflaged miRNA nanoparticles mitigate neuronal apoptosis and microglial dysregulation in cerebral I/R injury^[104]. B) Illustration of Ma@(MnO₂+FTY) nanoparticles formation and salvation of damaged neurons in ischemic brain^[102].

Biomimetic nanoparticles (BioM NPs) refer to nanoscale materials engineered by mimicking the structural and functional characteristics of biological entities in nature. These particles integrate the biosafety of natural materials with the advantages of synthetic systems, achieving chemical, physical, or morphological mimicry of biological structures to enable targeted drug delivery and efficient intracellular transport. Current evidence confirms BioM NPs as high-performance drug delivery platforms, offering enhanced biocompatibility and intrinsic affinity toward natural biological targets^[105]. To date, diverse cell membrane-functionalized nanoparticles—including erythrocyte-, neutrophil-, platelet-, exosome-, and macrophage-derived variants—have been developed. These systems exhibit superior biointeraction capabilities for precise drug delivery, targeted therapy, and tissue regeneration. Notably, they demonstrate significant potential in ameliorating cerebral ischemia and mitigating neurotrauma and neurological dysfunction through mechanisms such as inflammation modulation and neural circuit repair.

4.4.1 Neutrophil Membrane-Functionalized Biomimetic Nanoparticles

Neutrophils play a pivotal role in IS and thrombosis formation, establishing them as a critical therapeutic target for both prevention and treatment of IS^[106]. Following the onset of IS, neutrophils are recruited and infiltrate into the brain within 12 hours, reaching peak levels within the first day. Subsequently, their numbers gradually decrease by day 3 post-stroke^[107].

Dong et al. developed a ROS-responsive biomimetic nanoparticle system (SNM-NPs) coated with neutrophil membrane (NM) and stroke-homing peptide (SHp) for precision modulation of the inflammatory microenvironment following IS. The study focused on microglial regulatory mechanisms: SNM-NPs exploit neutrophil membrane-embedded chemokine receptors (CXCR2 and CXCR4) to target cerebral ischemia-reperfusion injury (CIRI) sites, while SHp modification enhances nanoparticle accumulation in inflammatory foci surrounding damaged neurons where microglia exhibit heightened activity. In vitro oxygen-glucose deprivation/reperfusion (OGD/R) models demonstrated that SNM-NPs reduced microglial activation markers (Iba1 expression decreased by approximately 60%) and suppressed pro-inflammatory cytokine release (IL-1 β , IL-6, TNF- α reduced by 45-65%) through ROS scavenging, concurrently inhibiting the NLRP3 inflammasome activation pathway. In rat middle cerebral artery occlusion/reperfusion (MCAO/R) models, SNM-NPs treatment decreased activated microglia counts by 50% and reduced neuronal apoptosis by 90% in ischemic brains. Mechanistically, SNM-NPs achieved ROS-triggered co-release of edaravone (Edv) and beta-cyclodextrin derivatives (RCD), synergistically eliminating ROS while suppressing caspase 3-mediated apoptotic pathways, thereby indirectly promoting microglial transition from pro-inflammatory to anti-inflammatory phenotypes. This work establishes a novel drug delivery strategy for modulating microglial activity through microenvironment-targeted intervention, offering dual neuroprotection and immunomodulation in stroke therapy^[101].

4.4.2 Macrophage Membrane-Functionalized Biomimetic Nanoparticles

Macrophages are essential components of the innate immune system, distributed across various tissues and playing pivotal roles in the monocyte-macrophage

system^[108]. In the brain, microglia represent the predominant macrophage population. The monocyte-macrophage system possesses inherent capabilities for morphological adaptation and diapedesis across the BBB. Leveraging these properties, macrophage membrane-engineered drug carriers demonstrate enhanced BBB penetration potential, offering promising therapeutic efficacy for cerebral disorders^[102].

Li et al. developed macrophage membrane-coated manganese dioxide (MnO_2) nanospheres ($\text{Ma} @ (\text{MnO}_2 + \text{FTY})$) loaded with fingolimod (FTY) for IS therapy. These biomimetic nanoparticles actively accumulate in ischemic brain regions by targeting overexpressed cell adhesion molecules on damaged vascular endothelium. Both FTY and $\text{Ma} @ \text{MnO}_2$ treatments reduced M1 marker (CD16/32) expression while increasing M2 marker (CD206) levels in microglia, indicating their capacity to drive anti-inflammatory M2 polarization. $\text{Ma} @ (\text{MnO}_2 + \text{FTY})$ treatment further enhanced M2 polarization, significantly elevating the M2/M1 ratio. $\text{Ma} @ (\text{MnO}_2 + \text{FTY})$ modulates microglial polarization through multiple signaling pathways, including STAT3 and NF- κ B. It suppresses NF- κ B pathway activation, decreasing pro-inflammatory cytokine (TNF- α , IL-1 β) secretion and p65 nuclear translocation, while concurrently activating the STAT3 pathway to enhance anti-inflammatory cytokine (IL-10) production and phosphorylated STAT3 (p-STAT3) levels. This dual regulatory mechanism drives microglial transition from M1 to M2 phenotypes, reverses inflammatory microenvironments, and enhances neuronal survival^[109].

4.4.3 Platelet Membrane-Functionalized Biomimetic Nanoparticles

Platelet membranes (PM) retain the antigenic diversity of platelet surface proteins, enabling immune evasion and leveraging inherent biological advantages such as prolonged circulation and inflammation tropism^[110]. PM-functionalized nanoparticles (NPs) bind to neutrophil surface molecules and migrate toward inflammatory sites via neutrophil-derived chemotaxis^[111]. Capitalizing on these mechanisms coupled with superior biocompatibility, PM-engineered nanoparticles efficiently traverse the BBB,

achieving precise drug delivery to ischemic brain lesions^[111, 112].

Zhao et al. developed platelet membrane-camouflaged nanoparticles (miRNA/TPHL@PM) loaded with microRNA-Let-7c. These nanoparticles retained the inherent functional properties of platelet membranes (e.g., immune evasion) while evading immune clearance. Leveraging neutrophil-mediated chemotaxis toward inflammatory sites, the PM-camouflaged nanoparticles efficiently traversed the BBB, achieving targeted delivery to ischemic lesions. Both miRNA/TPHL and miRNA/TPHL@PM treatments reduced CD16 (M1 marker) expression and elevated CD206 (M2 marker) levels in microglia, promoting anti-inflammatory M2 polarization. miRNA/TPHL@PM further enhanced this effect, significantly increasing the M2/M1 ratio. Mechanistically, this polarization shift was mediated via Notch1 signaling pathway modulation, suppressing pro-inflammatory cytokine secretion (TNF- α , IL-1 β) and augmenting anti-inflammatory IL-10 production. In a murine MCAO model, miRNA/TPHL@PM treatment markedly decreased M1 microglia (CD16+) density and increased M2 microglia (CD206+) populations in ischemic brain tissue. This study demonstrates that PM-camouflaged nanoparticles represent an effective gene delivery system capable of modulating neuronal and microglial functions, highlighting their therapeutic potential for ischemic brain injury^[103].

4.5 Extracellular vesicles

Table 5. Microglia-based exosome drug delivery system for IS

Drug delivery systems	Origin	Targeting method	Targeting results	Ref.
LPS-Ex	RAW	Passive targeting	Regulates microglial polarization Reduces neuronal damage Reduces inflammation	[113]
miR-30d-5p-A DSC-Exos	ADSC	miR-30d-5p binds to 3'UTR of	Regulates microglial polarization	[114]

		Beclin-1 and Atg5	Reduces inflammation; Reduce infarct size	
miR-126+ exosomes	ADSC	Passive targeting	Reduce infarct size Reduces inflammation	[115]
hUMSC-Exos	hUMSC	Passive targeting	Regulates microglial polarization Reduces neuronal damage	[116]

Extracellular vesicles (EVs) are natural nanoparticles secreted by cells, primarily categorized into three subtypes based on size and origin: exosomes, microvesicles (MVs), and apoptotic bodies^[117]. Exosomes, a subset of EVs with diameters ranging from 40 to 160 nm, are released by various organs, tissues, and cells, playing pivotal roles in biological processes such as cellular metabolism, immune regulation, and neural regeneration^[118, 119]. Functioning as intercellular communication vectors, exosomes transport bioactive molecules—including proteins, lipids, and nucleic acids—to modulate recipient cell functions^[117]. Compared to synthetic nanocarriers (e.g., liposomes), exosome-based drug delivery systems exhibit distinct advantages for CNS disease treatment, including enhanced BBB permeability, superior stability, low immunogenicity, and high delivery efficiency. These attributes position exosomes as a promising therapeutic modality for CNS disorders^[120].

Yan Zheng et al. investigated the regulatory effects of lipopolysaccharide (LPS)-stimulated macrophage-derived exosomes (LPS-Ex) on microglial polarization during the acute phase of IS, proposing a novel therapeutic strategy against post-stroke neuroinflammation. They demonstrated that LPS-Ex efficiently penetrated the BBB and induced phenotypic switching of LPS-activated BV2 microglia from pro-inflammatory M1 (characterized by elevated CD80 and iNOS expression) to anti-inflammatory M2 phenotypes (marked by increased CD206 and Arg1 levels) *in vitro*, achieving 88% neuronal survival rates. In a rat MCAO/R model, intravenous LPS-Ex administration significantly elevated M2 microglial proportions (2.7-fold increase) in ischemic brains while reducing pro-inflammatory mediators, ultimately

decreasing infarct volume by 40% and improving neurological function scores by 35% on Garcia scales. This study reveals LPS-Ex-mediated microglial polarization as a mechanism to reprogram post-ischemic inflammatory microenvironments, establishing exosome-based precision modulation as a promising therapeutic paradigm for IS^[113].

Small molecule-loaded exosomes play critical roles in modulating therapeutic responses. MicroRNAs (miRNAs), constituting ~41.72% of exosomal RNA, represent the most abundant and extensively studied nucleic acids^[121]. miRNAs post-transcriptionally regulate M1/M2 polarization-related genes to reprogram microglial phenotypes^[121]. Exosomes from diverse cellular sources—particularly adipose-derived stem cells (ADSCs) and mesenchymal stem cells—demonstrate therapeutic potential for IS via microglial polarization modulation. Jiang et al. investigated miR-30d-5p-enriched exosomes derived from ADSCs in acute ischemic stroke (AIS). Using a rat MCAO model and in vitro oxygen-glucose deprivation (OGD)-treated microglia, they demonstrated enhanced M1 polarization (elevated pro-inflammatory factors including iNOS and IL-6) with concurrent suppression of M2 polarization (reduced anti-inflammatory markers such as CD206, IL-4, and IL-10) following ischemic injury. Mechanistic studies revealed this polarization imbalance was closely associated with dysregulated autophagy, characterized by upregulation of autophagic markers (Beclin-1, Atg5, and LC3-II). Intervention with miR-30d-5p-overexpressing ADSC-derived exosomes suppressed autophagy through miR-30d-5p-mediated targeting of the 3'UTRs of Beclin-1 and Atg5, achieving a 65% reduction in autophagic flux. This inhibition facilitated microglial transition from M1 to M2 phenotypes, increasing CD206-positive cells by 2.8-fold in vitro, while in vivo results showed a 41.3% decrease in cerebral infarct volume and a 54% reduction in neuronal apoptosis (assessed by TUNEL staining). The study establishes exosome-mediated miRNA regulation of autophagy-microglial polarization crosstalk as a novel therapeutic target for IS^[114].

Geng et al. investigated the regulatory role of miR-126-modified exosomes

derived from ADSCs in modulating microglial responses after IS. Intravenous administration of miR-126-enriched exosomes significantly suppressed the activation of Iba1-positive microglia and reduced cerebral expression of pro-inflammatory cytokines TNF- α and IL-1 β in a rat MCAO model. In vitro experiments demonstrated that miR-126+ exosomes inhibited oxygen-glucose deprivation (OGD)-induced hyperactivation of BV2 microglia, as evidenced by a 38% reduction in cell viability via CCK-8 assays, alongside decreased secretion of TNF- α (62% reduction) and IL-1 β (55% reduction). Mechanistic studies revealed that these exosomes delivered miR-126 to microglia, directly targeting the TLR4/NF- κ B signaling pathway to suppress pro-inflammatory phenotypic transformation. Notably, exosomes with miR-126 knockdown (miR-126- exosomes) exacerbated microglial activation and neuroinflammation, increasing neuronal apoptosis by 2.1-fold. This study highlights ADSC-derived exosomes as vehicles for miR-126-mediated regulation of microglial inflammatory responses, promoting post-stroke neural recovery and offering a novel therapeutic strategy for targeted neuroinflammatory intervention^[115].

Human mesenchymal stromal cell-derived exosomes have been explored for IS therapy, including those from human umbilical cord mesenchymal stem cells (hUC-MSCs). Zhang et al. investigated the effects of human umbilical cord mesenchymal stem cell-derived exosomes (hUMSC-Exos) on microglial-mediated neuroinflammation following IS. In vitro, treatment with hUMSC-Exos significantly reduced pro-inflammatory cytokine (IL-6, TNF- α , IL-1 β) expression in oxygen-glucose deprivation (OGD)-activated BV2 microglia. Mechanistic studies revealed that the anti-inflammatory effects of hUMSC-Exos depended on their miRNA cargo, particularly miR-146a-5p, which suppressed NF- κ B activation by targeting the IRAK1/TRAF6 signaling pathway. In vivo, intravenous administration of hUMSC-Exos in a mouse MCAO model reduced cerebral infarct volume, improved neurological scores, and shifted microglial polarization by decreasing pro-inflammatory M1 (IBA-1⁺ CD16⁺) while increasing anti-inflammatory M2 (IBA-1⁺ CD206⁺) populations. These findings highlight hUMSC-Exos as a novel

therapeutic strategy to modulate microglial responses and mitigate neuroinflammation during the acute phase of IS^[116].

Cell-derived exosomes exert beneficial effects on cerebral ischemia-reperfusion (I/R) injury. Liu et al. demonstrated that bone marrow mesenchymal stem cell-derived exosomes (BMSC-Exos) alleviate NLRP3 inflammasome-mediated neuronal pyroptosis by shifting microglial polarization from M1 to M2 phenotypes, thereby conferring neuroprotection against I/R injury^[122]. Cheng et al. revealed a novel mechanism by which mesenchymal stromal cell (MSC)-derived exosomes (MSCs-Exo) mitigate cerebral I/R injury via miR-26a-5p delivery. Their study showed that miR-26a-5p, upregulated in MSCs-Exo, reduces oxygen-glucose deprivation (OGD)-induced BV-2 cell apoptosis and infarct volume in MCAO mice. MSCs-Exo suppressed microglial apoptosis through CDK6 downregulation, offering a novel therapeutic strategy for I/R injury^[123].

Despite their potential, natural exosomes face limitations in targeting specificity and delivery efficiency. Engineered exosomes—modified by loading drugs, proteins, nucleic acids, or lipids—have advanced their application in IS therapy. Xintong Liu et al. developed mannose-modified luteolin-loaded platelet-derived exosomes (lut/man-pEXO), which precisely target ischemic cerebral vasculature and microglia, enhancing drug accumulation and inducing neuroprotection of the neurovascular unit^[124]. These exosomes inhibit M1 activation while promoting M2 polarization, attenuating neuroinflammation and ischemic damage.

In summary, both natural and engineered exosomes regulate microglial activation by suppressing M1 polarization, promoting M2 conversion, reducing inflammation, and inhibiting apoptosis, thereby modulating IS pathology. Exosomes bypass endolysosomal degradation, enabling direct cytosolic drug delivery. However, clinical translation faces challenges including limited understanding of long-term safety, scalability of isolation protocols, heterogeneity, and immunogenicity. Addressing these issues is critical for advancing exosome-based therapies.

These biomimetic nanoparticles enhance drug delivery efficiency and therapeutic

outcomes through improved targeting precision, ensuring accumulation at pathological sites. Simultaneously, they reduce immunogenicity and systemic toxicity while prolonging circulation time for sustained drug release.

4.6 Comparative analysis and selection strategy of NDDS platforms

The aforementioned NDDS platform exhibits certain differences in terms of the mechanism and efficiency of penetrating the blood-brain barrier, targeting specificity, drug loading capacity, biological safety, and preparation complexity. For BBB penetration, liposomes and extracellular vesicles primarily leverage membrane fusion or receptor-mediated transcytosis, especially when functionalized with BBB-targeting ligands. Polymeric and inorganic nanoparticles, by contrast, depend more heavily on passive transport enhanced by optimized physicochemical properties and, when needed, surface-conjugated targeting ligands. Biomimetic systems offer a hybrid strategy: natural cell membranes confer immune evasion and active targeting, yet their BBB penetration efficiency is often constrained by the stability and functional integrity of membrane-associated proteins after engineering. With respect to targeting efficiency, ligand-functionalized polymeric nanoparticles and biomimetic systems achieve the highest lesion specificity when properly designed, whereas inorganic nanoparticles exhibit moderate targeting that relies heavily on surface engineering and microenvironment-responsive behavior. In terms of drug loading and scalability, liposomes and polymeric nanoparticles are the most versatile and clinically established platforms. Biomimetic systems and extracellular vesicles, despite superior biosafety and prolonged circulation, face substantial challenges in preparation complexity and batch-to-batch consistency. Inorganic nanoparticles offer robust physicochemical stability and stimuli-responsive release profiles, but their long-term biosafety and biodegradation remain open questions that require careful case-by-case evaluation.

On the basis of this comparison, we suggest that NDDS selection should be tailored to the therapeutic context. For instance, liposomes or polymeric nanoparticles may be preferred for acute-phase intervention where rapid clinical translation is a

priority, whereas biomimetic systems or extracellular vesicles may be more suitable for sustained immunomodulation in the subacute phase. This context-dependent strategy, rather than a one-size-fits-all assumption, offers a more rational framework for NDDS design in ischemic stroke therapy.

5. Challenges and Prospects

Despite the immense potential of microglia-based NDDS in IS treatment, their clinical translation faces multiple challenges. First, the phenotypic heterogeneity of microglia: During different ischemic phases, microglia polarize into pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes, and their dynamic shifts impose stringent requirements on the targeting efficiency and temporal regulation of NDDS. Current nanocarriers struggle to precisely distinguish or selectively modulate specific subpopulations, potentially exacerbating neuroinflammation due to phenotypic imbalance. Furthermore, the traditional M1/M2 dichotomy-based phenotypic classification system may fail to fully reflect their functional complexity, given the intrinsic differences in cellular environments and injury response mechanisms between macrophages and microglia. Experimental evidence reveals significant heterogeneity between CNS-resident microglia and peripheral macrophages in transcriptomic profiles^[125], metabolic reprogramming patterns^[126], and immunomodulatory functions^[127], underscoring the need to establish more refined subpopulation identification criteria for precise therapeutic strategies. Second, the conflict between BBB penetration and targeting specificity: Although modifying targeting peptides (e.g., TREM2 antibodies or CX3CR1 ligands) enhances microglia-specific delivery, the size, surface charge, and modification density of nanocarriers require meticulous optimization to avoid nonspecific immune clearance or off-target effects. Third, long-term biosafety concerns: Degradation byproducts of nanomaterials (e.g., liposomes or metal-organic frameworks) may induce chronic microglial activation or neurotoxicity, necessitating systematic evaluation of their metabolic pathways and immunocompatibility. Fourth, spatiotemporal regulation challenges: Post-ischemic microglial activation exhibits spatiotemporal specificity, yet

most existing NDDS rely on passive targeting and lack intelligent responsiveness to lesion microenvironmental cues (e.g., pH, ROS, or enzyme activity), resulting in mismatched drug release and pathological progression.

Beyond these biological and material-related barriers, additional hurdles arise when considering clinical translation. For instance, scale-up production must ensure batch-to-batch consistency, as manufacturing variations can substantially alter nanocarrier biodistribution and efficacy. Furthermore, current animal models inadequately reflect human ischemic stroke complexity, particularly regarding patient heterogeneity and comorbidities such as hypertension and diabetes, which may affect microglial polarization and therapeutic outcomes.

To address these challenges, future research should focus on: (1) leveraging single-cell sequencing technology to decode molecular signatures of microglial subpopulations, identifying biomarkers and surface characteristics to guide the design of phenotype-specific targeting ligands; (2) developing dual-responsive nanocarriers that integrate microenvironment-sensitive materials (e.g., ROS-responsive polymers) with external stimuli (e.g., focused ultrasound) for on-demand drug release^[128]; (3) utilizing gene-editing tools (e.g., CRISPR-Cas9) to co-deliver therapeutics targeting polarization-related signaling pathways (e.g., NF- κ B or STAT6). Through interdisciplinary innovation, microglia-based NDDS could overcome existing limitations, offering precise and personalized solutions for IS therapy.

6. Conclusions

IS remains a life-threatening disease characterized by intricate pathological mechanisms and limited therapeutic efficacy, leading to poor clinical outcomes. Current treatments, such as mechanical thrombectomy and thrombolytic therapy, aim to restore cerebral blood flow but are constrained by narrow therapeutic time windows, hemorrhagic risks, and ischemia/reperfusion injuries. These limitations underscore the urgent need for innovative strategies to address unmet clinical demands.

Central to IS progression is neuroinflammation driven by microglial activation. As pivotal immune regulators in the brain, microglia dynamically exert dual roles in

neuroinflammation and tissue repair through dynamic polarization (M1/M2 phenotypes). Targeting microglial activation or polarization has shown therapeutic potential in preclinical studies, with recent evidence suggesting that modulating microglial polarization may provide novel therapeutic strategies for IS. By regulating microglial polarization to mitigate inflammatory responses and reduce infarct volume, this approach offers a promising framework for future research on NDDS-based IS therapies, potentially opening new horizons for IS treatment.

Building on this, NDDS targeting microglia offer a revolutionary therapeutic framework for alleviating neuroinflammatory responses and reducing cerebral infarct volume through precise modulation of M1/M2 polarization states. The core advantages of this strategy lie in overcoming BBB limitations, achieving microglia-specific targeted delivery with spatiotemporally controlled drug release, thereby directly reprogramming the microenvironment to synergistically suppress inflammation and promote repair. However, clinical translation urgently necessitates addressing three critical bottlenecks: optimization of material biocompatibility, precise control of off-target effects, and systematic validation of long-term safety. Future research must focus on functionalized design of NDDS carriers to accelerate the transformation of such precision modulation strategies into clinical therapies for IS by enhancing targeting specificity and therapeutic efficacy.

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