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Review

Recent Advances in Chemically Engineered Nanostructures Impact on Ischemic Stroke Treatment

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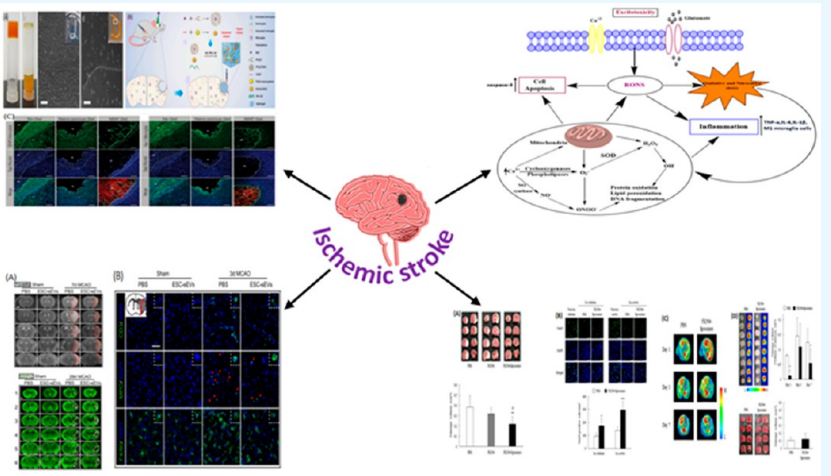

ABSTRACT: Stroke is a serious public health problem that raises expenses for society and causes long-term impairment and death. However, due to restricted blood−brain barrier (BBB) penetration, there are few treatment alternatives for treating stroke. Recanalization techniques, neuroprotective medications, and recovery techniques are all forms of treatment. The ischemic stroke treatment window is too narrow for logical and efficient therapy, and detection is possible only in advanced stages. BBB integrity disruption, neurotoxicity, and the brief half-life of therapeutic thrombolytics are the key molecular pathogenic causes of ischemic stroke. Existing neuroprotective drugs' inability to promote the recovery of ischemic brain tissue after a stroke is another factor that contributes to the disease's progression, chronic nature, and severity. A possible approach to getting around these medication restrictions and boosting the effectiveness of therapies is nanotechnology. In order to get around these drug-related restrictions and boost the effectiveness of therapies for neurological conditions such as stroke, nanotechnology has emerged as a viable option. These problems might be avoided by using nanoparticle-based methods to create a thrombolytic medication that is safe to use after the tissue plasminogen activator (tPA) treatment window has passed. The idea of using biomimetic nanoparticles in the future for the treatment of ischemic stroke through immunotherapy and stem cell therapy is highlighted, along with recent advancements in the study of nanomaterials for ischemic stroke diagnostics and treatment.

INTRODUCTION

The brain is a delicate organ that controls various neuro-regulatory functions, including language and behavior. It has a weak capacity for self-healing and a constrained ability to regrow lost parts.[1] The leading factor in fatalities and disability is stroke.[2] Because it poses such a serious threat to human health and welfare, stroke has drawn a great deal of attention. A stroke is brought on by an embolus or blood clot, which abruptly reduces the brain's blood supply. Cerebral ischemia is the result of a complex cascade of biochemical responses that are caused by a reduction in the delivery of glucose and oxygen, and it is characterized by significantly dropped levels of adenosine triphosphate (ATP) in the mitochondria. The blood−brain barrier (BBB) is essential for defending brain tissue against cerebral ischemic stroke (CIS). The BBB is a system that stops dangerous substances from getting into the bloodstream and going to the brain.[3] Inflammation, oxidative and nitrosative stress, and glutamate excitotoxicity are some of

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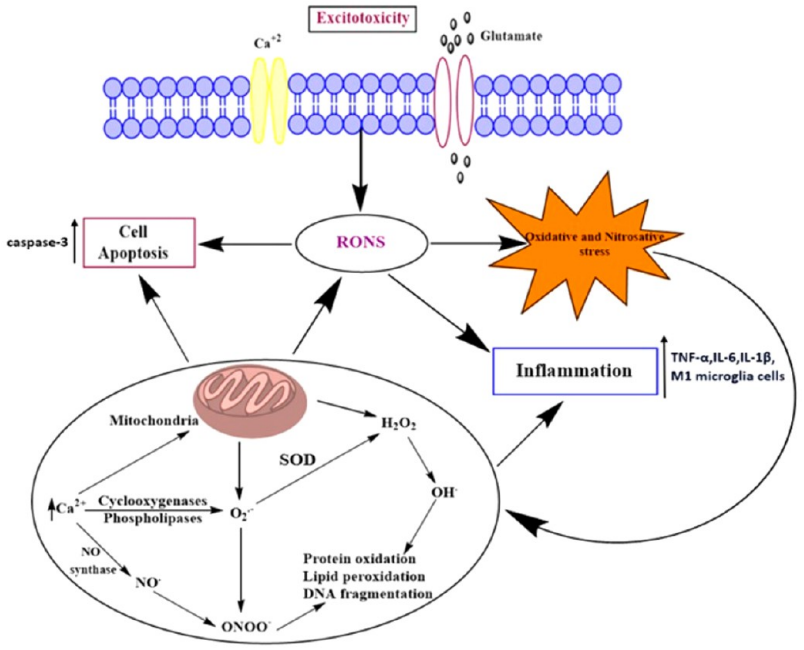

Figure 1. Several variables that have a role in the mechanistic features of oxidative and nitrosative stress in stroke.

the pathophysiologies of the processes involved in brain ischemic stroke, which is a significant factor in ischemic brain damage.[4]

Inflammation, oxidative stress, and immediate glutamate excitotoxicity are all brought on by stroke and contribute to tissue damage. By 2030, it is expected that there will be 7.8 million more related deaths than there will be stroke cases, totaling 23 million.[5] Each year, one in six people worldwide has a stroke, which leads to 5.8 million existing cases and almost 13.7 million new fatalities. In the event of an ischemic stroke, thrombolysis and thrombectomy are used as the most efficient treatments to reestablish blood flow to the brain, preserve brain tissue, and restore brain activity. Intravenous thrombolysis has a treatment window of 4.5 h,[6] while mechanical thrombectomy allows for intervention up to 24 h after the onset of a stroke.[7] In the struggle for nerve repair, microglia are a double-edged sword because they activate in two distinct ways while at rest, known as the M_1 and M_2 phenotypes. A variety of proinflammatory cytokines and mediators are secreted by M_1-like microglia that are triggered by damage-associated molecular patterns (DAMPs), supporting immune-mediated neuronal damage, and inhibiting neurogenesis. By secreting neurotrophic factors and anti-inflammatory cytokines, the M_2 microglia, in contrast, primarily carry out protective actions and provide neuroprotection. Therefore, it is necessary to modulate the microglia's activation state during ischemia.[8]

There have been several significant developments in research on nanoparticles as a potential means of moving molecules between tissues. They can be used as a cutting-edge delivery system to safeguard imaging and therapeutic agents.[9] Delivery methods for nanomedicine have recently attracted some interest as efficient and secure methods for delivering therapeutic agents across the BBB.[5] Because of their advantages of high biocompatibility, high drug-loading efficiency, simple surface modification, and active and passive targets, nanocarriers have been the focus of extensive research.[10]

PATHOPHYSIOLOGY OF STROKE

Excitotoxicity. Excitotoxicity was the first molecular cause of ischemic brain tissue damage to be discovered and thoroughly investigated. When energy is insufficient, the excitatory amino acid glutamate is released quickly and in large amounts, and its reuptake is inhibited.[11] Glutamate plays a crucial role in ischemia via excitotoxic pathogenesis due to its ability to operate as a cytotoxic excitatory neurotransmitter, which is important for neuron disintegration following a stroke. The glutamate receptor subtypes *N*-methyl-D-aspartate (NMDA) and a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) are activated, which may cause excitotoxicity, and this facilitates membrane depolarization and the facilitation of calcium ion influx into neurons.[12] Increased extracellular glutamate levels in the ischemic brain cause localized hyperactivation of ionotropic glutamate receptors, which, in turn, causes excessive Na^+ and Ca^{2+} influx into neurons, which ultimately causes the demise of neuronal cells. In ischemic stroke, glutamate toxicity is a key mechanism of neuronal death.[13]

Gamma Aminobutyric Acid (GABA) Antagonists. Despite attempts to treat the symptoms of stroke patients with chlormethiazole and the GABA agonist diazepam, these drugs were unable to reduce the toxicity caused by glutamate receptors.[14]

Potential Role of Na^+ and Ca^{2+} Ion Blockers. Na^+ channel blockers have been administered as neuroprotective drugs in a variety of stroke models. They slow down neuronal aging and lessen the harm to the white matter. Even though some blockers were used, the desired results were not achieved. For instance, a Phase II clinical trial using sipatrigine, a Na^+ and Ca^{2+} channel blocker, in stroke patients was unsuccessful. In contrast to beta-blockers and diuretics, independent research found that Ca^{2+} channel blockers dramatically reduced the risk of stroke by 13.5%.

Oxidative and Nitrosative Stress. Ischemic tissue faces significant difficulties as a result of oxidative and nitrosative

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stress. Owing to a rise in secondary messenger systems and the creation of free radicals by enzymatic processes (for example, via cyclooxygenase or nitric oxide synthases; Figure 1), these stresses are partially downstream effects of excitotoxicity.[11] Peroxynitrite (ONOO−), a strong oxidizing reactive nitrogen species (RNS) that can harm cells through oxidative processes such as DNA fragmentation and lipid peroxidation, is created when too much oxygen (O_2) reacts quickly with nitric oxide (NO). Strongly reactive oxygen species (ROS) can be produced by peroxynitrite, an oxidizing RNS.[15] Furthermore, NO can affect cells because it controls crucial proteins like metalloproteases, caspases, and glycolytic enzymes.[5] An excess of reactive oxygen and nitrogen species (RONS) results in oxidative damage, which then triggers an inflammatory response, which significantly accelerates the deterioration of ischemic brain tissues.[16] Brain edema, inflammatory conditions, hemorrhage, and death of neuronal cells are all signs of increased BBB permeability and all effects of excessive ROS/RNS production, which also develops a stressed microenvironment and initiates several cellular signaling cascades.[17]

Due to the risk to the cerebral vascular endothelium that high levels of ROS present, they also have a significant impact on blood vessels. Increased cerebrovascular permeability may restrict blood flow to the brain's tissues, causing local edema to worsen and intracranial pressure to rise. Increased cerebrovascular permeability can prevent tissue vascularization in the brain, increasing intracranial pressure and causing localized edema. Energy loss following an ischemic stroke causes lactic acid buildup, which results in acidosis in the neurons. As H^+ concentrations increase, acidosis has a pro-oxidant effect because it speeds up the process by which the superoxide anion (O_2) is transformed into hydrogen peroxide (H_2O_2) and the hydroperoxyl radical (HO_2). Three isoforms of the superoxide dismutases (SODs), which are found in mitochondria and change oxygen (O_2) into hydrogen peroxide (H_2O_2), are produced by cells. Extracellular (EC-SOD, SOD3), manganese (Mn-SOD), and copper−zinc (CuZn-SOD, SOD1) are some of these isoforms. Catalase and the glutathione (GSH) redox system, which is made up of the enzymes glutathione reductase (GR), glutathione peroxidase (GSHPx), and peroxiredoxins (Prdxs), can further decompose H_2O_2 into water and oxygen. It has been demonstrated that ischemic stroke patients benefit from acupuncture, a conventional medical procedure, possibly by reducing oxidative stress. Acupuncture's beneficial effects on the body's antioxidant system have been linked to changes in the molecular signaling systems that control the generation or elimination of ROS. Additionally, it was found that acupuncture improved mitochondrial respiratory activity and mitophagy clearance, both of which indirectly decreased ROS production.[15] Overproducing antioxidant enzymes or saturating the body with antioxidants in terms of lowering the negative effects of ROS on stroke may be just as damaging as extended exposure to free radicals.[18]

Inflammation. The pathophysiology of cerebrovascular diseases, especially ischemic stroke, is significantly influenced by inflammation.[12] Inflammation, which is triggered by reduced blood flow, intravascular leukocyte stimulation, brain parenchymal production of proinflammatory cytokines, and ischemic endothelium, can lead to increased tissue injury. Anti-inflammatory therapies have a greater therapeutic window compared to the currently popular therapies, according to reperfusion, which makes them appealing for the treatment of ischemic stroke.[19] In controlling inflammatory reactions in ischemic stroke, glial cells and peripheral immune cells collaborate.[17] Microglia have the capacity to produce inflammatory mediators that cause cell deterioration and death when activated. In the meantime, transforming growth factor-1 beta (TGF-1β), which has neuroprotective properties, can also be produced by microglia.[20] In addition, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), pro-inflammatory cytokines that are responsible for starting the inflammatory response to cerebral ischemia and creating brain damage, can activate gliosis.[16]

Citicoline works as an antioxidant and an anti-inflammatory after ischemic damage by scavenging free radicals.[21] One well-known consequence of cerebral ischemia is the release of a number of damage-related molecular pattern molecules, such as DNA, RNA, ATP, S100 proteins, heparan sulfate, heat shock proteins, and high mobility group protein B1. These are chromatin-associated proteins. The tetracycline derivative minocycline reduces apoptosis and blocks the expression of matrix metalloproteinases and poly(adenosine diphosphate-ribose) polymerase 1 after focal cerebral ischemia.[11] The entire extracellular matrix can be destroyed by the proteolytic enzyme family known as matrix metalloproteinases (MMPs), which also remodel the extracellular matrix. In response to damage, MMPs are expressed more in the brain. Due to its connection to neuroinflammation, MMP-9 inhibition may hasten the recovery process following a stroke.[12] Combination treatment with nanoplatforms recently became popular as a cutting-edge strategy for the potential treatment of inflammatory disorders.[22] Numerous nanomedicines have been created, including those for the treatment of cancer, metabolic syndrome, autoimmune diseases, cardiovascular pathologies, and neurodegenerative diseases, in order to treat diseases with an inflammatory background. A lot of thought went into creating stronger anti-inflammatory drugs, which led to the creation of anti-inflammatory nanomedicines in order to minimize the negative effects of traditional treatment.[23]

Temporospatial regulation is a significant pathogenic element of post stroke inflammation in stroke, according to MRI studies. Monitoring inflammatory responses in the brain parenchyma could help doctors pinpoint patients who would benefit from immunomodulatory treatments. The fact that endothelial cells control leukocyte diapedesis makes them intriguing molecular imaging targets. The existence of an inflammatory penumbra in ischemic stroke has been demonstrated using microsized iron oxide particles (MPIOs), monoclonal antibodies against vascular cell adhesion molecule 1 (VCAM-1), and molecular MRI. Adhesion molecules other than VCAM-1 are responsible for moving leukocytes from the bloodstream to the brain. For instance, ICAM-1 (intercellular adhesion molecule-1) was subjected to molecular MRI in a mouse ischemic stroke model by Deddens and colleagues. With the aid of MPIO directed at ICAM-1, it was possible to determine when activated endothelial cells upregulated ICAM-1 following the onset of ischemia. The peri-infarct region's ICAM-1 expression peaked at 48 h and persisted afterward, demonstrating the presence of an inflammatory penumbra. The ability of imaging with VCAM-1 and ICAM-1 to distinguish between different aspects of stroke pathophysiology is still unknown.[24]

Apoptosis. The term "apoptosis" was first used by Kerr et al. to describe the morphological alterations that occur during the process, including chromatin condensation, nuclear

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membrane disruption, cell shrinkage, and the formation of tiny vesicular bodies called "apoptotic bodies" close to the cell surface.[25] The cellular process known as apoptosis causes controlled cell death. It is essential for fetal development, immune regulation, and tissue growth. The nucleus, mitochondria, and plasma membrane are among the intracellular structures that are destroyed during apoptosis by a group of protease enzymes called caspases.[26] Apoptosis can still happen even though caspase activity is not required for apoptosis to occur because of the important role that caspases play in controlling programmed cell death. Caspase-independent neuronal apoptosis has been observed following ischemia and excitotoxic stimulation with NMDA.[27]

The main form of neurodegeneration brought on by cerebral ischemia in the brain is apoptotic neuronal cell death. The majority of ischemia damage is brought on by apoptosis-mediated neuronal cell death, while necrosis can also lead to cell death in the ischemic brain, which accounts for a higher degree of neuronal death. Additionally, curcumin administration significantly decreased the number of apoptotic neuronal deaths and attenuated transient middle cerebral artery occlusion (MCAO)-induced caspase-3 expression.[28] Apoptosis suppression is one of the neuroprotective techniques used in the treatment of stroke.

Treatment Strategies for Stroke. The stroke treatment approach aims to enhance brain reperfusion through the mechanical or chemical removal of clots. Time plays a critical role in stroke intervention. Invasive recanalization techniques and tissue plasminogen activator (tPA)-induced thrombolysis are commonly employed for treating acute ischemic strokes with certain limitations, such as a narrow therapeutic window (up to 4.5 h) and risk of a cerebral hemorrhage. A recent study proposed that mechanical thrombectomy could be extended to a 24-h window in stroke patients, highlighting the growing necessity for global exploration of this aspect in acute ischemic stroke treatment.[7]

An Inducer of Tissue-Type Plasminogen Activation. The production of tissue plasminogen activators occurs in a laboratory. In addition to treating lung clots, heart attacks, and strokes, it aids in the dissolution of blood clots. It is also being investigated as a potential cancer treatment. It belongs to the class of medications designated as systematic thrombolytics. There is just one medication that the Food and Drug Administration (FDA) has authorized that works: tPA, given intravenously within 4.5 h of the start of symptoms. Only 7% of patients are qualified for the treatment due to the limited therapeutic window.[2] Additionally, tPA can increase the risk of bleeding and has a generally low success rate due to significant infarcts in patients.[5] The advantages of extending the window for tPA treatment are 2-fold: It widens the window for neuroplasticity and decreases the severe negative effects of postponing tPA therapy, increasing the possibility of better functional outcomes following stroke.[29] Furthermore, postponing the initiation of tPA therapy past 4.5 h has been linked to unfavorable impacts, most notably hemorrhagic transformation (HT), which increases the mortality risk for stroke patients. The shortcomings of tPA as a stroke treatment strategy highlight the need for better and more potent substitutes.[30] In addition to neuroprotection, thrombolytic revascularization is essential in ischemic stroke therapy. For ischemic stroke, the only thrombolytic therapy approved by the US-FDA is recombinant tPA (rtPA), which can speed up the plasminogen to plasmin conversion for clot breakup. Due to its low affinity for thrombi and the possibility of neuronal damage from ischemia reperfusion within 4.5 h of the beginning of symptoms (the thrombolysis window), rtPA is not without controversy as an effective treatment option. To accomplish this, rtPA and a neuroprotectant are being used in combination as a therapy to enhance blood flow and lessen ischemia reperfusion injury. A drug delivery system (DDS) that can gradually target the rtPA thrombus while delivering the neuroprotectant to the ischemic brain is necessary for the treatment of ischemic stroke.[31]

Neuroprotective Agents. Neuroprotective substances are used to prevent neuronal cell degeneration or irreversible damage to ischemic neurons in the brain. A cerebral vessel occludes, preventing blood flow to a section of the brain and resulting in an ischemic neuron. It is believed that neuroprotective substances prevent the death of nerve cells. Neuroprotective refers to the preservation of the neuronal structure and/or function to a certain extent. Several neuroprotective medications are also used to treat other diseases. Neuroprotective medications include those for Parkinson's disease, Alzheimer's disease, and ischemic stroke.[32]

The neuroprotective and neurotoxic effects of cerebral ischemia-reperfusion injury are both mediated by NO. In the hippocampus, NO acts as a neuroprotective and anti-inflammatory agent, mediates cellular transduction mechanisms, regulates neuronal plasticity, and inhibits neuronal apoptosis. Therefore, after a stroke, NO may be neuroprotective or restorative.[33] One of the primary factors contributing to the failure of the majority of clinical studies was the lack of methodological rigor in preclinical studies, and neuroprotectant restricted BBB permeability is one of the barriers.

Photothermal Therapy. In order to eliminate tumors, Goldman used laser radiation for the first time in 1966. Due to its numerous advantages, photothermal therapy (PTT) has garnered considerable interest in the medical community because of characteristics like noninvasiveness, low toxicity, simplicity of use, and speedy recovery. The use of conjugated polymer-based nanomaterials for PTT has increased recently as a result of their numerous advantages, including their increased buildup at tumor locations, strong photothermal conversion efficiency, and extended blood circulation times.[34] Attempts to alleviate the symptoms of various illnesses, including cancer, with electromagnetic radiation (typically in the form of infrared wavelengths) are known as PTT. A certain band of light triggers the activation of a photosensitizer in this method, which is a development of photodynamic therapy. In contrast to photodynamic therapy, photothermal therapy interacts with the target cells or tissues without the use of oxygen. In contrast to photodynamic therapy, photothermal therapy interacts with the target cells and tissues without the need for oxygen. According to recent studies, a benefit of photothermal therapy is the use of higher wavelength light, which has lower energy and, as a result, is less damaging to the cells and tissues in its vicinity.[35] Clinical applications for light-controlled drug release in the treatment of strokes are possible.

Crossing the BBB with Drugs. One of the biggest challenges in treating neurological disorders is the difficulty of getting drugs into the brain. Endothelial cells of cerebral microvessels, which encircle the brain, form the BBB, a selective barrier. The BBB controls nutrient and ion transport while guarding the brain against neurotoxic substances to keep it in a state of homeostasis. The BBB's unique anatomy allows

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it to perform its intended function. Tight junctions hold the brain endothelial cells together, as seen in Figure 2; they are unfenestrated. Unfortunately, because of the BBB's extreme selectivity, the majority of drugs are unable to cross it via physiological pathways.[36]

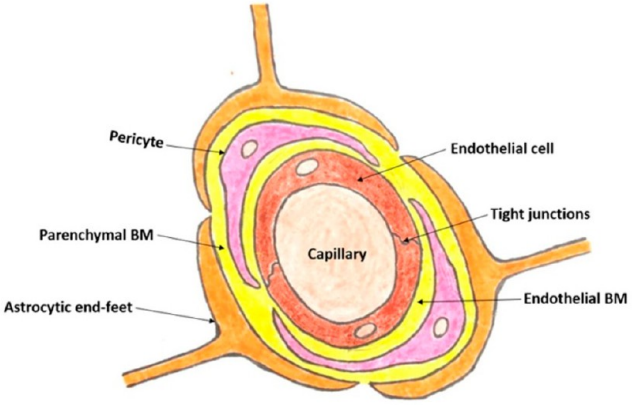

Figure 2. Simplified schematic representation of the blood−brain barrier diagram.

Nanomedicine for the Treatment of Ischemic Stroke. Nanotechnology has recently advanced to the point where it is now a cutting-edge tool for the diagnosis and delivery of drugs used to treat many ailments, such as malignancy and inflammatory conditions. When an ischemic stroke occurs, nanoparticles may be able to prolong medication distribution, boost drug accumulation in diseased sites, and deliver therapeutics across the BBB. Since nanoparticles are larger than small molecules and smaller than cells, they make excellent carriers for therapeutics (drugs) and can be used to control their release for more effective treatments for a variety of diseases. DDSs based on nanoparticles may be able to overcome these difficulties in the treatment of ischemic stroke (Table 1). Several ischemic stroke treatment strategies utilizing nanoparticle-based DDSs are covered in this review.[37] Some of the various kinds of chemically engineered nanostructures used in ischemic stroke treatment are shown in Figure 3.

Liposomes as Delivery Vehicles. Liposomes are the first generation of drug delivery nanocarriers employed in research and the pharmaceutical sector. Drugs can be shielded from enzymatic degradation and prevented from clearing the blood by the use of liposomes, which are made of biocompatible and biodegradable molecules.[37] Neuroprotective liposomal DDSs are used for the treatment of ischemic stroke. Alec Bangham first described liposomes, which are bilayered vesicles made of pure lipids or lipid and cholesterol mixtures, in the middle of the 1960s. The realm of medicine has long utilized liposomes from vaccination as delivery systems or adjuvants because of their adaptability and flexibility. According to Li et al., the desired properties of liposomes can be achieved by changing their surface structures, particle sizes and charges, manufacturing methods, lipid compositions, *etc.*[38] As vaccine carriers, cationic liposomes are employed more frequently than anionic liposomes because they better penetrate macrophages and dendritic cells (DCs). They have the ability to avoid cellular endosomal−lysosomal degradation. In either a passive or active manner, liposomes are essential for drug loading.[39] Drug loading takes place during liposome formation, and interactions between the drug and lipids control uptake and retention. The ability of liposomes to trap aqueous buffers is necessary for passive encapsulation of drugs that are water-soluble, with the effectiveness of the trapping typically being constrained by the volume and drug solubility. It is possible to achieve 100% trapping effectiveness with active entrapment using pH gradients. Using a liposomal DDS, neuroprotective drugs are being explored for treating ischemic strokes.[40] Mireia Campos-Martorell et al.'s study indicates that liposome charge is essential for their accumulation in the area of the infarcted brain following transient MCAO. Additionally, simvastatin delivery in brain tissue was unaffected by encapsulation in neutral liposomes. Thus, simvastatin encapsulation may be an effective method for ensuring that the medication reaches the brain, increasing bioavailability and minimizing potential side effects.[41] For delivering neuroprotective drugs to the brain, preventing *in vivo* degradation, facilitating BBB passage, and encouraging drug accumulation in the ischemic area, liposomes are flexible, effective delivery systems.[42] The use of liposomal DDS in the therapy of ischemic stroke has a focus on recent developments in nanoparticle DDS. By disrupting the BBB, liposomes can increase the therapeutic advantages of neuroprotectants, accelerating the creation of new medications and removing those that are already failing from the market.[43] Since it sterically stabilizes the liposome and reduces particle aggregation and opsonin recognition, the hydrophilic polymer polyethylene glycol (PEG), when surface-attached with liposomes functionalized with it, is known as "stealth liposomes" (Figure 4).[44]

The study discovered that the PEG-liposomes accumulated in the ischemic zone after being injected into MCAO rats during ischemia. It has been demonstrated that the immunosuppressant FK506 (tacrolimus), which is recommended in many countries, has neuroprotective benefits due to its ability to block calcineurin activity (Figure 5).[45] FK506-liposomes significantly reduced oxidative damage during the ischemia state, aggravating damage to the cortex after reperfusion. They also reduced the deficits in motor function brought on by reperfusion and improved brain damage. This demonstrates the potential efficacy of liposomal DDS as a prereperfusion therapy for ischemic stroke.[46] In rats following transient MCAO, by considerably reducing the brain infarct size and ischemic brain damage and promoting the restoration of long-term neurological function, 9-aminoacridine (9-AA) was found to have these beneficial effects by Wang et al. This "from drug discovery to drug delivery" process offers a practical therapeutic strategy for treating ischemic stroke by using liposomal 9-AA, a nuclear receptor 4A1 (NR4A1) activator, to suppress neuroinflammation.[4] According to Fukutaet al., PEG-Lip builds up in the ischemic zone of MCAO animals in a time-dependent manner and does so before brain damage progresses. Inferring that tPA/Fasudil-Lip combination treatment may lower the risk of cerebral hemorrhage following thrombolytic therapy, Fasudil-Lip treatment prior to tPA administration reduced BBB permeability and blocked MMP-2 and -9 activation. In comparison to tPA alone or tPA/free Fasudil, the therapeutic time window (TTW) of tPA was significantly extended by the tPA/Fasudil-Lip therapy. This shows that thrombolytic therapy, Fasudil-Lip+tPA therapy, and liposomal DDS therapy may all improve therapeutic outcomes and have potential as ischemic stroke treatments.[47] By adding Fasudil-Lip, t-PA should have a higher TTW and be more effective in treating ischemic strokes.[48] By using engineered liposomal techniques that prevent the drugs from degrading *in*

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Table 1. Various Nanostructures for the Treatment of Ischemic Stroke

S. No.	Nanoparticles	Type of Study	Outcome
	Liposome		
1	Engineered liposomal strategies[42]	*In Vivo*	passage through the BBB and increasing the therapeutic effectiveness of neuroprotective drugs
	FK506 liposomes[46]	*In Vivo*	suppress the expansion of oxidative stress and brain cell damage
	liposomal 9-aminoacridine[4]	*In Vivo*	suppress neuroinflammation
	Polymeric nanoparticles		
2	Dextran polymer nanoparticles[52]	*In vitro and In vivo*	protective effects on glutamate-induced cytotoxicity in PC-12 cells and reduce ischemic brain damage
	Dendrimers		
3	PAMAM dendrimers[56]	*In vitro and In vivo*	reduce cytotoxicity, prolong blood circulation half-life, and reduce blood clotting
	G5G2.5 tecto-dendrimer[58]	*In vitro and In vivo*	reducing neuroinflammation to mitigate secondary neuronal death
	Hydrogel		
4	Genipin-cross-linked sericin hydrogel (GSH)[61]	*In vitro and In vivo*	growth of neurons and high cell survival rate and allows the cells to continuously proliferate
	Metal nanoparticles		
5	Ca-MOF (metal−organic framework)@miR-124 nanoparticles[62]	*In vivo*	decreased the ischemic area to almost normal levels by day 7
	Ag and Cu nanoparticles[72]	*In vivo*	NP intoxication reduces oxidative stress and ischemia, attenuates brain edema, and protects against heat stroke
	Carbon nanoparticles		
6	Hydroxyl fullerenes nanoparticles[85]	*In vitro and In vivo*	suppress ROS and RNS
	PEG-HCCs[83]	*In vitro and In vivo*	antioxidants that squelch ROS such as superoxide and hydroxyl radicals
	Fullerenol nanoparticles[84]	*In vivo*	decrease brain edema and mRNA expression levels of MMP-9 and IL-6
	Carbon nanotubes		
7	Amine-modified single-walled carbon nanotubes[79]	*In vivo*	low levels of apoptotic, angiogenic, and inflammation markers indicated that the brain was protected from ischemia
	Exosomes		
8	M2 microglia-derived exosomes[96]	*In vitro and In vivo*	reduced infarct volume and achieved comparable neuroprotective effect
	Serum-derived exosome[97]	*In vitro and In vivo*	inhibition of endothelial cell apoptosis and autophagy-mediated BBB breakdown
	Nanoprobes in stem cell therapy		
9	cobalt protoporphyrin IX (CoPP)-loaded mesoporous silica nanoparticle (CPMSN) into a 125I-conjugated/spermine-modified dextran polymer (125I-SD) [CPMSN@125I-SD] nanoprobe[103]	*In vivo*	increased the survival of mesenchymal stem cells and promoted neurobehavioral recovery

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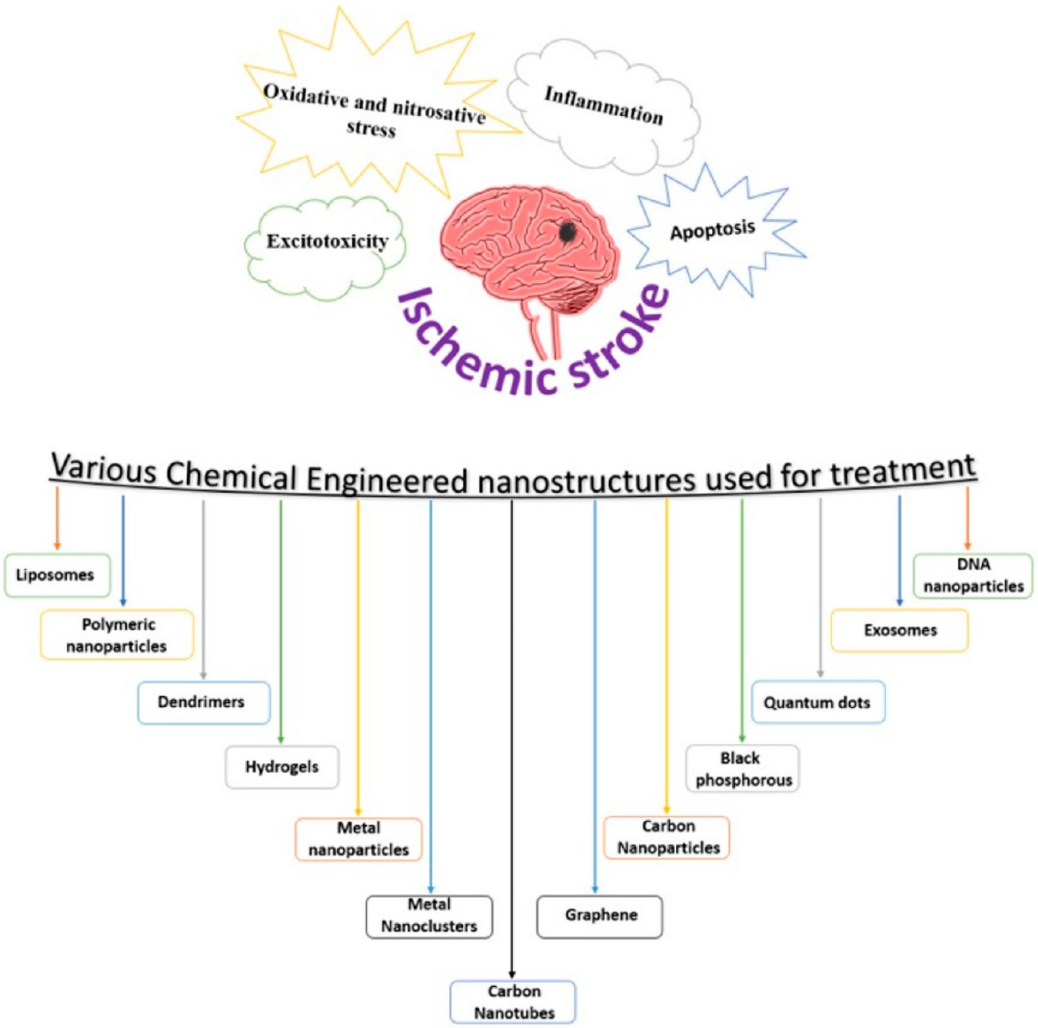

Figure 3. Schematic illustration for various chemically engineered nanostructures impacting ischemic stroke treatment.

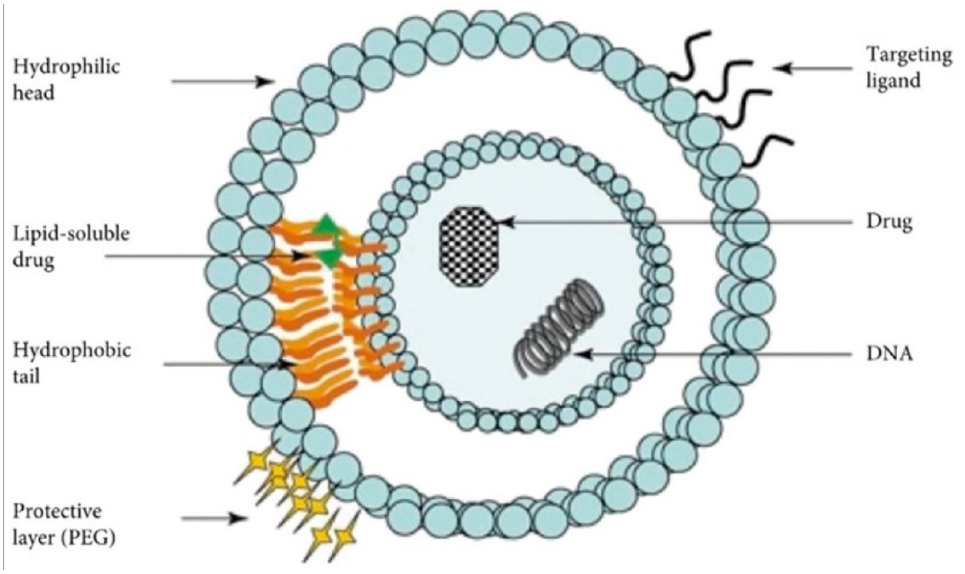

Figure 4. Simplified representation of a liposome with an aqueous core and lipid bilayer that each house a medication (drug and DNA) that is either hydrophilic or hydrophobic. The liposome surface allows for the introduction of targeting ligands and a PEG coating for active and passive targeting, respectively, reproduced with permission from Allahou et al. Copyright 2021, Hindawi.[44]

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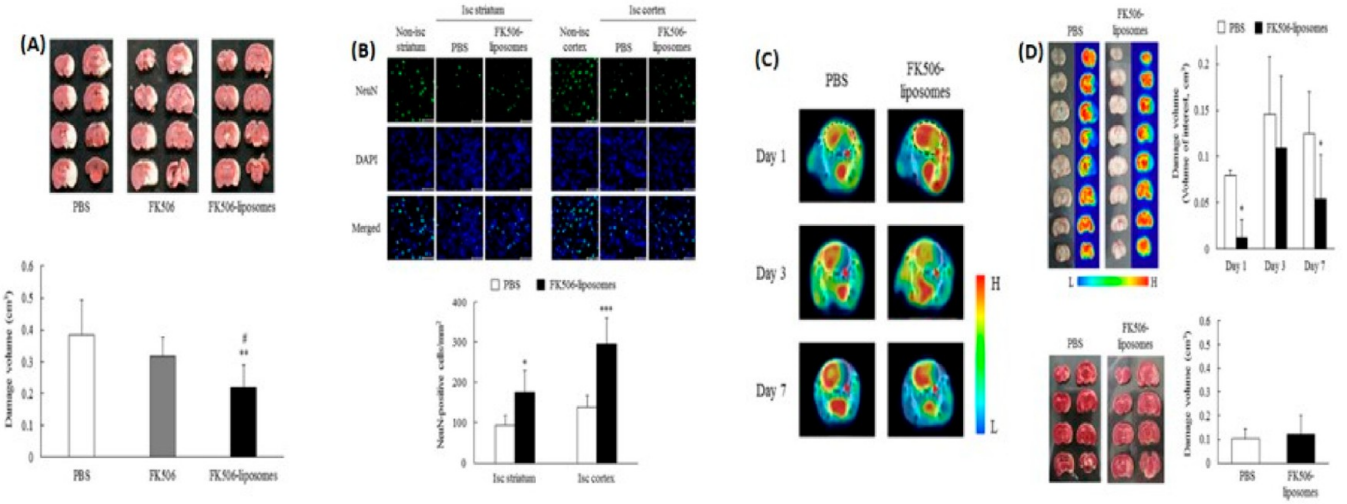

Figure 5. (A) FK506-liposomes reduced brain damage in t-MCAO animals 3 days after reperfusion. Rats were administered PBS, 100 μg/kg of FK506, or 100 μg/kg of FK506 liposomes intravenously. 2 mm coronal brain slices were cut 3 days after reperfusion and stained for 30 min at 37 °C with 2% TTC. ImageJ evaluated brain volume. (B) Frozen brain slices were made 7 days after reperfusion and immunostained for NeuN and counterstained nuclei with DAPI. Confocal laser scanning microscopy captured fluorescence pictures of the striatum and cortex, with areas labeled as ischemic and nonischemic. NeuN-positive cells were counted to obtain quantitative data. The neuroprotective impact of FK506-liposomes was assessed by using the MC-I-specific PET probe [18F] BCPP-EF. PET scans were performed at 1, 3, and 7 days after reperfusion, using [18F] BCPP-EF (10 MBq/rat) in 60 min scans. Standardized uptake value (SUV) pictures were created by reconstructing PET images from 10 to 30 min. The right cerebral hemisphere is shown by red arrows, and the bar displays the relative degree of signal strength (C−D). The volume of brain injury was calculated by acquiring VOIs in the reconstructed PET images at days 1, 3, and 7, and ImageJ was used to compute the injured brain volume as determined by TTC staining at day 7. The data are displayed as the mean SD (n = 5), reproduced with permission from Tatsuya Fukuta et al. Copyright 2016, Springer Nature.[45]

vivo, make it simpler for them to cross the BBB, and promote their accumulation in the ischemic region, recent studies have increased the therapeutic efficacy of neuroprotective drugs.[42]

Polymeric Nanoparticles As Delivery Vehicles. Polymeric nanoparticles (PNPs), which can have active molecules surface-adsorbed onto their polymeric cores or trapped within, are microscopic particles with sizes ranging from 1 to 1000 nm. For the delivery of targeted medication for the cure of a range of diseases, polymeric NPs have shown great promise. PNPs have recently piqued the interest of researchers due to their unique properties brought on by their small size. PNPs have the ability to hide drugs and other physiologically active molecules from the environment, boost bioavailability, and enhance the therapeutic index, which is one benefit of using them as drug carriers.[49] For surface modification and pharmacokinetic properties, PNPs use polymers like polylactide (PLA), poly(lactic-*co*-glycolic acid) (PLGA), poly(methyl methacrylate) (PMMA), or poly(amidoamine) (PAMAM). Without a change in their structural integrity, micelles can transport both hydrophilic and hydrophobic medications. The most common kind of polymeric nanoparticle is called PLGA, and it frequently has a sphere-like shape. It can also control steady drug release and is easy to produce and modify.[50] Surfactants such as (T80) and poloxamer can be applied to the PNP surfaces to give them long-circulating properties and BBB-crossing capacity. Targeting is improved by surface modification with polyethylene glycol (PEG), and NPs are driven to the target site by a magnetic field.[51] The bioengineered boronic ester-modified dextran polymer nanoparticles used by Wei Lvet al. in their work as ROS-responsive nanocarriers for the treatment of ischemic stroke dramatically increase NR2B9C circulation, target ischemic regions, and lessen brain damage in MCAO rats.[52] The poly(propylene sulfide) (PPS)-NPs are cytotoxic but also exhibit notable antioxidant and anti-inflammatory activities. Intravenous administration of NPs caused rapid accumulation in the areas of the brain that were affected by ischemia. According to immunohistochemistry, there is a reduced infarct volume, BBB deterioration, neuronal degeneration, and neuroinflammation as well as improved neurological function recovery, as shown by behavioral analyses. According to research by Olivera Rajkovic et al., an *in vitro* evaluation of (PPS)-NPs for the treatment of ischemic stroke revealed remarkable antioxidant properties, a decreased infarct volume, and improved neurological function. Strong evidence for PPS-NPs' efficacy as an antioxidant therapy for stroke is provided by the therapeutic window for these molecules, which can last up to 3 h.[53] In order to deliver medications to the brain while avoiding physiological, biological, and BBB barriers, PNPs have proven to be an effective method. By targeting therapeutically active molecules to brain cells, ligand-conjugated polymeric nanoparticles can be used to treat neurological diseases and brain cancer in a safe and efficient manner. The potential of ligand-mediated PNPs to address CNS conditions has received a great deal of research attention. The therapeutic efficacy of ligand-based PNPs is superior to that of standard formulations and PNPs without ligands, with less toxicity and undesirable side effects, according to *in vitro* and pharmacokinetics models. However, more clinical research and studies are required to better understand the effectiveness and security of ligand-mediated PNPs.[51] Haoan Wu et al. developed a unique antioxidant poly(2,2′ thiodiethylene, 3,3′-thiodipropionate) (PTT) NP as a vehicle to effectively provide medicine to an ischemic brain. In addition to providing antiedema and antioxidant benefits in a straightforward formulation, ASPTT NPs provide targeted therapeutic treatment for stroke, with the

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ACS Omega 2023, 8, 45188−45207

potential for successful stroke treatment through glyburide-loaded delivery.[54] The primary goal of the study is to investigate the therapeutic potential of rod-shaped PLGA nanoparticles encapsulated in piceatannol for the treatment of ischemic stroke. By preventing neutrophil adhesion to the endothelium, these nanoparticles stop neutrophil infiltration through the BBB. Lower levels of inflammatory cytokines are present in the ischemic brain as a result of increased endocytosis of the aspect ratio of 5 (AR5) nanoparticles. This strategy offers fresh perspectives on the treatment of ischemic stroke.[31]

Dendrimers as Delivery Vehicles. Dendrimers are regarded as potential treatment options for stroke because they have demonstrated effectiveness in preventing deep vein thrombosis and therapeutic effects against prion diseases. By delivering neuroprotective drugs like *N*-acetyl cysteine (NAC), an anti-inflammatory compound with promising clinical outcomes, and coating MR agents, dendrimers have the potential to treat stroke.[55] Dendrimers could pass through the broken BBB and get inside the brain. A secure *in vivo* DDS for a stroke scenario was made by functionalizing the cationic PAMAM dendrimers of various generations with PEG. This was done in order to increase the half-life and reduce the cytotoxicity of blood circulation. Rhodamine B isothiocyanate (RITC) was used as a tiny surrogate drug and for tracking because it was fused to the dendrimer's backbone covalently. As a function of dendrimer production and functionalization level, PEGylation markedly increased the biocompatibility of PAMAM. The PEGylated RITC-modified dendrimers had no negative effects on the integrity of an *in vitro* BBB model's integrity. Following hypoxia brought on by oxygen-glucose deprivation, functionalized dendrimers were still safe to interact with bEnd.3cells and rat primary astrocytes, which together make up the *in vitro* BBB model. The reduction in PAMAM interaction and absorption by endothelial cells caused by PEG modification suggest that transport across a BBB leak caused by localized brain ischemia may be facilitated. The functionalized dendrimers did not hemolyze when they came into contact with red blood cells, in contrast to the unaltered dendrimers. It is intriguing to note that the dendrimers treated with PEG prevented blood coagulation, which could be another helpful effect in the context of stroke.[56] A natural anti-inflammatory and antiapoptotic enzyme is heme oxygenase-1 (HO-1). In order to treat ischemic stroke, the HO-1 gene was delivered into the brain using dexamethasone-conjugated PAMAM generation 2 (PAMAM G2-Dexa). Together with plasmid DNA (pDNA) and PAMAM G2-Dexa, these complexes were stable. When delivering pDNA to Neuro2A cells, PAMAM G2-Dexa outperformed polyethylenimine (PEI, 25 kDa), dexamethasone-conjugated PEI (PEI-Dexa), and PAMAM G2. In an animal stroke model, the therapeutic efficacy of PAMAM G2-Dexa/pHO-1 complexes was assessed. PAMAM G2-Dexa was used to deliver pHO-1 into the ischemic brain more effectively and with a therapeutic effect greater than that of PEI25k and PEI-Dexa. In light of this, gene therapy for ischemic stroke may benefit from PAMAM G2-Dexa/pHO-1 complexes.[57] After brain ischemia, the G5G2.5 tecto-dendrimer is an excellnt drug delivery vehicle for specifically targeting reactive glial cells.[58]

Hydrogels as Delivery Vehicles. With the use of hydrogels, cells, large-molecule proteins, and small-molecule drugs and the regeneration of damaged brain tissue after an ischemic stroke can all be delivered. Especially noteworthy are hydrogels' advantages in evading the BBB and selectively deploying therapeutic payloads.[59] The use of hydrogels as suitable scaffolding biomaterials for the delivery of cells and drugs as well as the regeneration of damaged tissues and organs has grown in importance. The polymer in a hydrogel, a semisolid two-component system made of water and a polymer, builds up a massive network that traps the water. The rheological characteristics of the hydrogels show that they are viscoelastic by nature. Biomaterials known as injectable hydrogels are injected frequently while still liquid and solidify immediately. Depending on whether they exhibit chemical or physical cross-linking, these hydrogels can be divided into different categories. Injection-ready hydrogels ought to be breathable, nontoxic, and biocompatible and have the ideal balance of mechanical stability and biodegradability. Injectable hydrogels that are more mechanically stable have improved cell-based tissue regeneration and delivery systems because they can control how quickly entrapped growth factors, nutrients, and metabolites diffuse. For a variety of clinical therapeutic uses, such as orthopedic, cardiovascular, endovascular, and dentistry, injectable hydrogels have received a lot of attention recently.[60] Providing therapeutic drugs or acting as an extracellular matrix (ECM) from outside the body that is ideal for the growth of brain tissue are two common goals of the use of biomaterials in ischemic strokes. Hydrogels are a significant class of biomaterial that have so far found widespread applications in drug delivery and cell transportation. Hydrogels may therefore prove useful for investigating poststroke biological changes and evaluating novel therapeutic strategies. The potential efficacy of in situ injectable hydrogels as DDS for the treatment of various diseases, including stroke, has drawn a lot of interest. Hydrogels' ability to extend and even regulate the release of medicine after one administration is one of their key benefits.[59] One of the very vital uses of injectable hydrogels in this context is the neovascularization of ischemic tissues/organs, including the ischemic heart, musculoskeletal system, *etc*. In comparison to natural hydrogels, synthetic hydrogels have advantages because they allow for precise control over material characteristics like polymerization, degradation, and mechanical stiffness. By hybridizing native ECM with organic biopolymers, they can engineer ECMs with specific biophysical and biochemical characteristics. To create synthetic peptide-based hydrogels that mimic growth factors and show their effectiveness in promoting therapeutic angiogenesis in humans and primates, more research is required.[60] Anti-inflammatory drugs can be added to hydrogels as drug carriers to improve bioavailability. A mouse that had just experienced a stroke was given an injection of gelatin microspheres (GMS) containing osteopondin. The time that osteopondin was released after GMS encapsulation was significantly increased. After a stroke, there was an improvement in neuroprotection and a decrease in inflammation due to the microspheres' delivery.[5] Hyaluronan (HA) microporous annealed particles were prepared by Darling and colleagues for use in stroke-related brain repair. In the peri-infarct region, HA microporous annealed particles, as opposed to nonporous gels, may successfully encourage the recruitment of endogenous neural progenitor cells and vascular regeneration. They employed porous HA-Tet microporous annealed particle (MAP) scaffolds in a different study that had previously been shown to be biocompatible with lessened inflammatory response and astrogliosis in the ischemic stroke mouse brain. By incubating MAX8 hydrogel systems with

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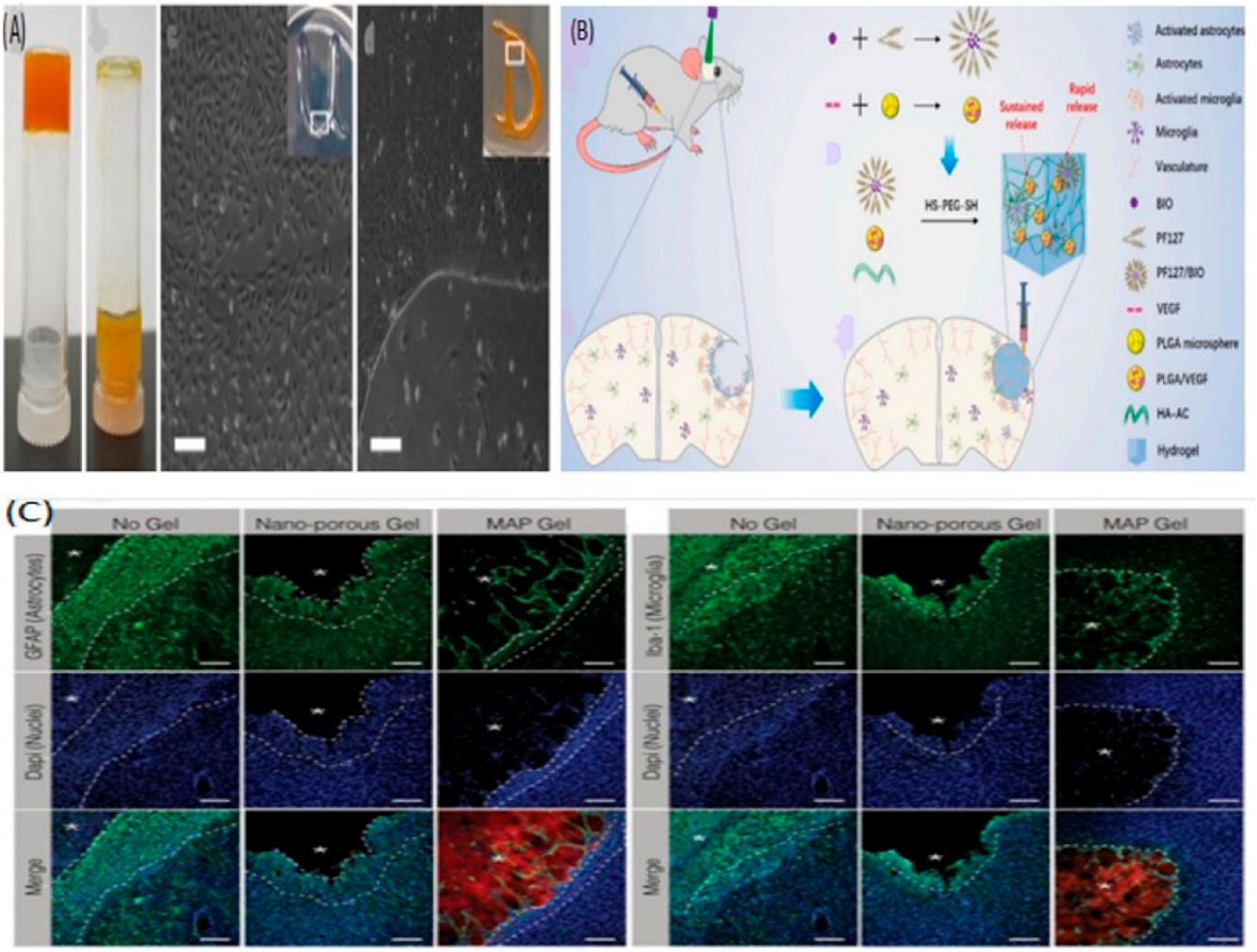

Figure 6. (A) Curcumin-loaded MAX8 hydrogels are described. MAX8 hydrogels with or without curcumin implanted into DAOY cells. (B) Schematic illustration of the applications of injectables in the treatment of ischemic strokes in mice. In the PT model in rats, blood flow disruption, microglia activation, and astrocyte activation all occurred. Injection of a dual-functional hydrogel into the infarct area. (C) Fluorescent pictures of GFA and IBA-1 staining demonstrating the astrocytic and microglial response following a stroke (scale bar: 100 μm), reproduced with permission from Ying Bai et al. Copyright 2022, MDPI.[59]

PC12 cells, Bai et al. demonstrated that brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) produced from hydrogel preserved their activity effectively and increased their half-life. The physiochemical properties of the hydrogel remained constant, as well. In addition to delivering growth factors, small-molecule drugs like curcumin have allegedly also been loaded into MAX8 hydrogels; Figure 6 shows the hydrogel system. Based on these findings, the MAX8 device may serve as a platform for ischemic stroke treatment since it can deliver a range of therapeutic drugs into the stroke cavity for time-release.[59] Tissue engineering techniques that use hydrogels to deliver cells and neurotropic cytokines to injured areas are promising options for neuronal restoration. This approach frequently encounters problems like low *in vivo* cell survival rates, ineffective encapsulation, and cytokine loss. To get around these limitations, a biomaterial was created that can create a matrix to enhance transplanted cells' *in vivo* survival and reduce *in vivo* cytokine loss. Genipin-cross-linked sericin hydrogel (GSH) provides excellent support for *in vitro* neuronal adhesion and development. Unexpectedly, the sericin protein possesses both neurotrophic and neuroprotective properties by nature. It promotes axon extension and branching while guarding against hypoxia-related cell death in primary neurons. The GSH promotes unrestricted cell division and has a high rate of cell survival after transplantation in living tissue. In the treatment of ischemic stroke, sericin's dual neurotrophic and neuroprotective properties as well as GSH's potential function as a neuronal cell delivery mechanism may be useful.[61]

Metal Nanoparticles As Delivery Vehicles. Metallic nanoparticles are safe and biocompatible, and their surfaces are negatively charged, which can be altered to transport a variety of chemicals. Due to free electrons on the surface, some metallic and metal oxide nanomaterials, such as platinum nanoparticles (PtNPs) and nanoceria, exhibit high ROS-scavenging activity with stable chemical properties.[50] According to Yang et al., guided neuronal maturation of transplanted neural stem cells (NSCs) is enhanced by the use of a Ca-based metal−organic framework (Ca-MOF@miR-124 nanoparticles). The nanoparticles integrate into NSCs and successfully inhibit nuclease degradation, enhancing their therapeutic effects *in vitro* and reducing the infarct area *in vivo*. This novel approach may lead to an increase in the use of miR-124 and NSC therapy for neurodegenerative diseases and traumatic

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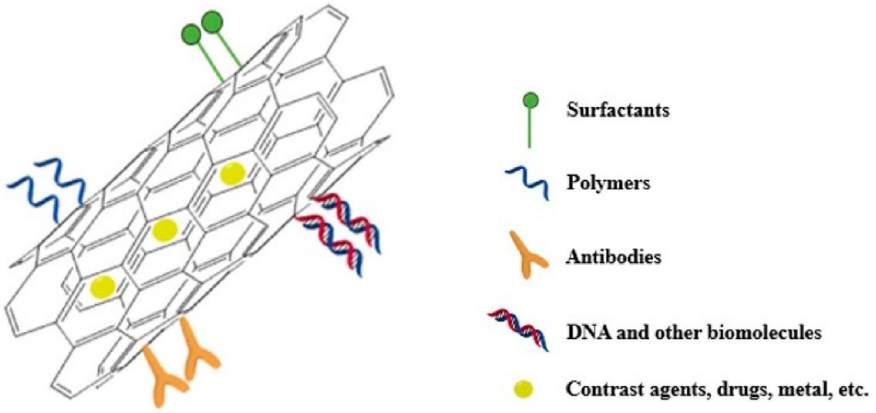

Figure 7. Schematic illustration for use in biological applications. Carbon nanotube surfaces have been functionalized and loaded with surfactants, polymer, antibodies, DNA, contrast agents, drugs, metals, and other biomolecules.

nerve damage.[62] In contrast to ferrumoxtran-10 nanoparticles, which were successful in the MRI imaging of the BBB, iron oxide nanoparticles performed well in the imaging of inflammation in acute brain ischemia. This application is closely related to the monitoring or selective targeting of human mesenchymal stem cells for the treatment of stroke.[55] According to Huang et al. (2016), manganese-based nanomaterials have manganese as the active core and naturally occurring enzyme activity.[63] Divalent manganese is necessary for phosphotransferase and hydrolysis in addition to photosynthesis. For ribonucleotide reductase, catalase, peroxidase, and SOD, the more expensive manganese functions as the redox center in the mitochondria.[64,65] The first manganese nanoparticle with superoxide dismutase activity to be described is manganese phosphate ($Mn_3(PO_4)_2$).[66] Additionally, the scientists found that Mn_3O_4 nanoparticles' dendritic structure had larger pores and the ability to produce catalase and superoxide dismutase.[67] Jia Yao et al. used a hydrothermal process to make Mn_3O_4 NPs, and it was shown that they are more stable and active than natural enzymes, showing a strong ROS scavenging function *in vitro*.[68] These results lay the foundation for upcoming clinical studies on the use of anti-inflammatory drugs in ROS-mediated inflammation.[69] CeO_2 nanoparticles coated with PEG/PLGA polymer matrixes were shown to be an effective barrier against ischemic brain stroke in research by Gao et al. For the purpose of increasing the activity of the nanoparticles in the brain tissues, the copolymeric groups work as a BBB delivery method. The findings imply that a PEG/PLGA matrix can increase the effectiveness of brain stroke treatment.[70] Tri-Mn (Salen) cryptands were created based on the natural catalases' antioxidant properties, and Yingying Ning et al. studied them *in vivo* as potential antioxidant metallodrugs. The "active site" of these catalase-based biomimetics has Mn centers close to one another. It was hypothesized that this structural arrangement would encourage more effective H_2O_2 dismutation because of cooperative effects. Operationally, it was significantly more effective than the monomeric Mn (Salen) control at producing oxygen. Then, further comprehensive studies were conducted to investigate its potential as an antioxidant metallodrug.[71] Prussian blue (PB) is the sole application for which the FDA has granted approval. It has been discovered that PB nanoparticles (PBNs) with Proof of Delivery (POD) activity can catalyze H_2O_2, stop the production of ROS, and inhibit the activity of enzymes such as SOD and catalase. PBNs have the ability to inhibit SOD and catalase, and they harm mice's livers by blocking the portal vein. Additionally, in ischemic livers, small PBNs prevented the increase in lipid peroxides.[69] After 4 h of heat stress, the BBB was considerably more likely to break down to endothelial barrier antigen (EBA) and radioiodine than in the saline-treated group compared to the control value. After 4 h of heat stress, compared to the EBA leakage under the same circumstances, the radioiodine leakage was often somewhat greater. The brain's radioiodine and EBA leakage after heat stress were markedly accelerated by treatment with Ag or Cu NPs. Radioiodine extravasation and EBA leakage in the control group were significantly sped up by NP treatment alone.[72]

Metal Nanoclusters As Delivery Vehicles. An ultrafine nanoscale particle known as a metal nanocluster has entirely different properties from macroscopic metals. Instead of the usual surface plasmon resonance absorption in the visible region, they frequently display visible-to-near-infrared fluorescence. Particularly, the noble metallic (Au, Ag, Cu, *etc.*) nanoclusters (NMNCs) provide numerous application possibilities as fluorescence sensing probes in the field of biomedicine. The advantages of these more recent fluorescent materials over older fluorescent probes such as organic fluorescent dyes, fluorescent proteins, and fluorescent quantum dots (QDs) are their strong photoluminescence, compatibility with living cells, and ease of availability. The initial gold nanoclusters created by bovine serum albumin (BSA) were used as models by Wu et al. for *in vivo* fluorescence imaging of microscopic organisms. The BSA-produced gold nanoclusters had a maximal fluorescence emission peak with a size of about 710 nm, which made it simple to distinguish them from the spontaneous fluorescence of living things.[73] In this investigation, Lan Xiao et al. looked at the neuroprotective effects of gold nanoclusters (AuNCs) functionalized with dihydrolipoic acid (DHLA-AuNCs) on the polarization of microglial cells. NF-kB signaling is decreased, and cell survival is increased as a result of DHLA-AuNCs' potent suppression of pro-inflammatory processes. The administration of DHLA-AuNCs to BV2 cells, a murine microglial cell line, improved tissue injury brought on by stroke and decreased astrocyte activation, indicating DHLA-AuNCs as a potential therapeutic agent for central nervous system (CNS) disorders.[74]

In order to avoid cerebral I/R damage, large brain accumulations of antioxidants are highly sought. Researchers Shiyong Li et al. used polyoxometalate (POM) nanoclusters as

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nanoantioxidants with preferential brain absorption to protect neurons from cerebral I/R damage. Advanced positron emission tomography (PET) imaging was used to continuously and noninvasively monitor the full absorption of nanoantioxidants in the brain. The total amount of nanoantioxidants absorbed in the brain was continuously and noninvasively assessed using sophisticated PET imaging. The outcomes demonstrated that POM nanoclusters successfully scavenged ROS to lessen oxidative stress and soon reached the ischemic penumbra after intrathecal injection. The infarct size was decreased, and neurological function was restored in cerebral I/R-damaged rat models. Intrathecal injections of nanoantioxidants are a great therapeutic approach to reducing cerebral I/R damage.[75] To address the limitations of current anti-ROS natural enzymes and small molecules, a technique for synthesizing CeO_2 @ bovine serum albumin (BSA) nanoclusters was recently developed. These nanoclusters demonstrated potential for reducing depressive-like symptoms and pathological alterations, such as impaired neuroinflammation and neuroprotection.[76]

Carbon Nanotubes As Delivery Vehicles. Carbon nanotubes (CNTs) are promising nanomaterials with a unique one-dimensional structure that offers numerous applications in biology and medicine. These nanoparticles can pass across cell membranes and are nonimmunogenic, nontoxic, biocompatible, and photostable. Additionally, they have a large specific surface area and an extremely low density. Their special properties make it possible to conjugate and encapsulate therapeutics, which makes them a promising advancement in the field. Due to their hollow tube-like structure shown in Figure 7, CNTs are advantageous for use in DDSs. Molecular targets that are dispersible and biocompatible can be held in the hollow inner cavity by capillary and adsorption action. In rat models of stroke, the ischemic sites were targeted with the atrial natriuretic peptide (ANP) antibody. The purification and functionalization processes were conducted with vertical alignment, which increased loading capacity.[77] Depending on how they affect the nervous system on their own or if they can transport other drugs, carbon nanotubes are likely to be used. Along with the nanotube's own neuroprotective properties in an ischemia model, hydrophobic carbon nanotubes (HPCNTs) impregnated with subventricular zone neural progenitor cells (SVZ NPCs) may be used to repair damaged brain tissue after a stroke, as suggested by *in vivo* research.[78] In order to heal damaged parts of the brain, it has been suggested that scaffolds contain stem cells. CNTs have shown promise in this respect, producing favorable results when utilized as scaffolds in neural cells and brain tissues. CNT-treated rats with amine-modified single-walled nanotubes showed reduced tissue damage and improved motor function after induced stroke, indicating better brain recovery and reduced apoptotic, angiogenic, and inflammation markers.[79] CNT-mediated RNA interference offers therapeutic opportunities against stroke by silencing caspase-3. Peri-lesional stereotactic administration of a caspase-3 siRNA reduced neurodegeneration and promoted functional preservation in the rodent motor cortex. This approach can be applied to various neuropathological conditions, offering therapeutic and functional benefits.[80] Zhang et al. demonstrated the strong mechanical properties and electrical conductivity of CNTs, revealing morphological characteristics similar to those of neurons. These similarities may enhance neuronal interaction and improve neuronal management and information processing. CNTs are intriguing materials for neural tissue engineering because of their mechanical and electrical characteristics, compatibility with neurons, and ability to repair damaged neural tissue. Roman et al. demonstrated the therapeutic potential of PEG-modified single-walled nanotubes (SWNTs) for the treatment of neurodegenerative disorders by stimulating neuroregeneration in a rat model. This study highlights the potential of CNT-based substrates for neuroregeneration. In addition to regenerative matrices and CNS therapy delivery systems,[81] CNT nanomaterials have the potential to be used in neuronal implants, electrodes, and neuroprosthetics.[82] Before being used to target inflammatory biomarkers in patients with ischemic stroke, this carbon nanotube system needs to be strengthened and further tested for clinical use.[77]

Graphene Oxide As Delivery Vehicles. Carbon atoms arranged in a single layer, or graphene, have special mechanical, optical, thermal, electronic, and magnetic properties. Potential uses for these characteristics in neurotherapeutics include functional neurosurgery, spinal surgery, neuro-oncology, neuroimaging, and peripheral nerve surgery. Liu et al.'s study indicates that the noncovalent stacking binding of hydrophobic drugs is improved by adding a PEG chain to graphene oxide (GO) nanoparticles. The chemical functionalization of graphene is therefore necessary for the development of novel drugs that specifically target the CNS. These remedies aim to break through the sharp edges, which may disrupt the cytoskeleton, impair cell movement, and have other negative effects, making these properties of graphene somewhat hazardous. Neuroregeneration may specifically benefit from graphene's remarkable ability to penetrate. A high specific surface area, hydrophobic interactions, and electrostatic interactions are provided by graphene and GO for effective drug delivery. These systems can be used to treat genetic disorders in systemic, targeted, and local systems, guaranteeing DNA degradation and high transfection.[81] There are not many studies that support the use of GO in the treatment of strokes. Because the BBB's paracellular tightness is momentarily reduced after ischemia, reduced graphene oxide (rGO) is briefly able to enter the hippocampal region through blood circulation. The brief opening of the BBB brought about by the rGO had no obvious negative effects. Because of the rGO's brief increase in BBB permeability, drug distribution to the lesion site can be evaluated. The significant potential of graphene group materials to treat neurological disorders was demonstrated by the increased BBB permeability following the use of rGO.[5]

Carbon Nanoparticles As Delivery Vehicles. Antioxidants were made of carbon nanoparticles with the best possible reactivity profiles toward ROS. Hydrophilic carbon clusters (PEG-HCCs) are nanoparticles that have PEG functionalization. They are pieces of pi-conjugated monatomic layer graphene-like domains that are 3−40 nm in size and heavily edge-oxidized. The PEG-HCCs have a 2 to 3 h *in vivo* half-life and are nontoxic. Antioxidants called PEG-HCCs inhibit ROS such as superoxide and hydroxyl radicals in cell-free systems, *in vitro* and *in vivo*. PEG-HCCs, however, respond significantly faster than any fullerene-based system. In comparison to most enzymes with a single active site, the PEG-HCCs were found to convert superoxide to oxygen at a rate of >20,000 s^{-1}. This implies that the PEG-HCCs have a large number of active sites. For this reason, PEGHCCs are unique in terms of therapy. It has been demonstrated that PEG-HCCs perform therapeutic functions in preclinical

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ACS Omega 2023, 8, 45188−45207

models that cannot be achieved by using enzymes or small molecules, and this may portend the utilization of treatments based on active carbon nanoparticles. As a result, PEGHCCs could eventually play a significant role in the management of both acute injuries and chronic diseases. The HCCs' extensive π-conjugated domains, similar to those found in some fullerene derivatives, allow the PEG-HCCs to function as potent active antioxidants in addition to their passive drug delivery applications.[83] Mahsa Sarami Foroshani et al.'s research indicates that fullerenol nanoparticles can lessen brain edema and BBB disruption by maintaining the BBB's integrity and the brain's microvasculature after ischemia-reperfusion damage, which improves neurological dysfunctions. Fullerenol nanoparticles also decreased MMP-9 and interleukin-6 (IL-6) mRNA expression levels during brain ischemia-reperfusion.[84]

Water-soluble fullerene derivatives have been shown by Hu et al. to be very effective at halting cell death and apoptosis. Malondialdehyde (MDA) and nitrate levels in the brain were significantly lowered after fullerenol was administered during cerebral ischemia, per Shamsi Darabi and Mohammad Taghi Mohammadi's studies. Different free radicals (ROS and RNS) are known to be particularly susceptible to being neutralized by water-soluble fullerene derivatives in biological contexts. Both *in vitro* and *in vivo*, hydroxyl fullerenes inhibit ROS and RNS in a variety of cell types. When fullerenol nanoparticles were administered while brain ischemia was occurring, the neurological dysfunction, edema, and infarction of the ischemic brain were all significantly decreased.[85]

Black Phosphorus As Delivery Vehicles. Black phosphorus (BP), specifically phosphorene, is evolving into a significant molecule due to its distinct structure and properties. The broadband-adjustable direct band gap of BP ensures a wide range of powerful light absorption. Since BP exhibits a strong near-infrared extinction coefficient, excellent fluorescence imaging, photothermal therapy, photoacoustic imaging, and other sectors may all benefit from its high photothermal conversion efficiency and enticing fluorescence quantum yield.[5] Due to its high potential for reduction, BP shows promise in the creation of treatments for diseases linked to ROS. By scavenging ROS, BP nanosheets have been demonstrated to lessen oxidative-stress-triggered cell apoptosis in preliminary *in vitro* cell studies. This benefit makes BP nanosheets a powerful candidate for treating diseases caused by ROS. Transition-metal ions, such as Cu^{2+}, may contribute to neurodegenerative diseases (NDs) by causing biological tissues to release toxic ROS, which, in turn, causes neuronal cells to undergo apoptosis. It may be possible to establish the removal of Cu^{2+} ions from BP in order to treat ND and encourage neuronal regrowth. According to Chen et al., photothermal impact following NIR-laser irradiation may cause 2D BP nanosheets to more effectively absorb Cu^{2+} ions for ND therapy and may also increase BBB penetration.[86]

Quantum Dots As Delivery Vehicles. Quantum dots (QDs), which contain metals, were found to exhibit a moderate level of toxicity in kidney organoids by He et al. The nephrotoxicity of BPQDs has been shown by renal tubular epithelial cells from both mice and humans.[87] Utilizing a liquid phase exfoliation technique, Sun et al. created ultrasmall BPQDs while avoiding long-term toxicity. These nanoparticles have strong photothermal killing power and are biocompatible. Under physiological circumstances, BPQDs with PEG modification have improved stability and dispersion. *In vivo* experiments show that they have excellent photoacoustic imaging properties and the potential for bioluminescent imaging. Through liquid-phase exfoliation in chloroform, Lee et al. discovered that BPQDs were biocompatible and water dispersible. Their maximum fluorescence, which is caused by band gap transitions or the electrons and holes recombining in a radiative manner, is observed at 437 nm under 377 nm excitation. The use of BP-nanosheets (NSs) for the treatment of cancer has recently attracted more attention due to their distinct properties. Drugs, targeting molecules, photosensitizers, and magnetic nanoparticles can all be carried by BPNSs given their extensive surface areas and negative charge. The ability of BPNSs to produce single oxygen molecules makes them particularly suitable for use as photosensitizers in photodynamic therapy (PDT). Additionally, BPNSs can be used for administering medication due to their relatively high surface area. Along with these benefits, BPNSs are appropriate for use in biomedicine because of their outstanding biocompatibility and biodegradability.[88] When GO binds to nucleic acids, it causes the formation of hydrophobic and nontoxic graphene quantum dots (GQDs), which are hydrophobic and nontoxic. Due to their ability to separate fibrils into monomers and intercalate fibrillary proteins, GQDs have been studied as potential drug candidates. According to *in vitro* research, GQDs can prevent the propagation of synuclein fibrils in Parkinson's disease. Biotin-labeled GQDs were used to examine the BBBs *in vivo* permeability.[89] An implant site neuron network may be supported by a more resilient graphene layer, which would also provide the close contact necessary between the electrodes and the desired neurons for effective long-term recording. Consequently, the continued development of neuroelectronic devices that use graphene for intracortical interactions may be facilitated. A variety of substrates, including optical fibers and 3D electrical probes, can be coated with this substance and might reduce the skepticism that deep brain interfaces, which are used in many scientific fields, currently encounter in basic medicine and neurology.[90]

Fluorescent carbon nanoparticles and carbon quantum dots (CQDs) are an emerging class of carbon nanomaterials that have just come into existence as potential competitors to conventional semiconductor quantum dots. Additionally, CQDs have desirable benefits of low toxicity, ecological sustainability, low cost, and straightforward production procedures because of their identical optical characteristics. Additionally, physicochemical processes are made possible by CQD surface passivation and functionalization. CQDs have been extensively used since their development in a wide range of industries, including nanomedicine, photocatalysis, electrocatalysis, chemical sensing, and biosensing.[91]

Selenium quantum dots (SeQDs) have an ultrasmall size and can quickly penetrate the BBB. Xian Guo et al. claim that the SeQDs' fluorescence properties may be used to identify and monitor Alzheimer's disease (AD). The SeQDs exhibit potent free-radical scavenging ability, broad-spectrum antioxidant activity, and protection of cells from oxidative stress brought on by various stressors. *In vivo* research demonstrates that SeQDs may effectively treat AD, significantly improve memory impairment in AD mice, and improve their capacity for learning and memory. SeQDs can also persistently accumulate in the brain following fast BBB transit. Because of this, employing SeQDs to treat AD has several advantages over using traditional single-target medications and opens up

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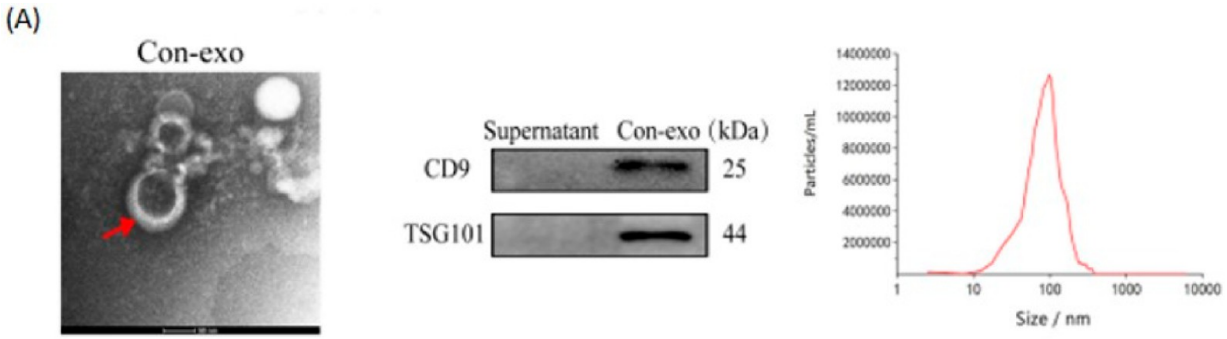

Figure 8. Transmission electron microscopy (TEM), immunoblotting, and nanoparticle tracking analyses were utilized for the isolation and characterization of serum exosomes. The size of the particles was examined, and exosomal markers including CD9 and TSG101 were found, reproduced with permission from Huang et al. Copyright 2022, Frontiers.[97]

new opportunities for combining the prevention and treatment of neurodegenerative illnesses.[92]

Exosomes As Delivery Vehicles. Endosomes create exosomes, which are separate extracellular vesicles with diameters ranging from 30 to 150 nm. They were first discovered in 1983, while examining the transferrin receptor fate as sheep reticulocytes developed into red blood cells, and were given the name "exosomes" in 1987.[93] Brain cells secrete exosomes under physiological conditions, and exosome production starts in the numerous multivesicular bodies seen in neurons. The heterocellular communication that controls brain function is significantly influenced by these brain exosomes. These exosomes are derived from neurons and contain glutamate receptor subunits GluR2 and GluR3, as well as the neuronal-specific protein L1 cell adhesion molecule (L1CAM), which suggests that neuronal exosomes are involved in controlling neuronal function. Exosomal release from cultured mature neurons is induced by glutamatergic synaptic activity. The BBB is regulated by an interaction between brain endothelial cells and neuronal exosomes.[94] The benefits of exosome-based therapies for poststroke neurovascular remodeling include the connections between exosomes and stroke. For patients who have suffered an ischemic stroke, exosomes offer a promising and practical treatment option. Exosomes are minuscule, and extracellular vesicles are harmless and easily traverse the BBB as a brand-new shuttle without triggering immune reactions.[95] Due to these characteristics, exosomes are a perfect adjunct therapeutic strategy for postinjury regeneration. Exosomes, for instance, can be created to be the most effective delivery vehicles for medications, bioactive compounds, and particular immune modulators. Different types of exosomes are produced and released after a stroke. Exosomes produced by brain cells may cross the BBB to enter the cerebrospinal fluid or peripheral circulation, where they may function as potential biomarkers for the diagnosis and prognosis of stroke. In response to a stroke, blood and endothelial cells release exosomes into the blood. Studies have looked at how proteins and nucleic acids in circulating exosomes change in relation to stroke. Since temporary ischemia had no discernible effects on serum miR-126, it is plausible that exosomal miR-126 is more sensitive and focused on ischemia circumstances. Exosomal miR-126 was discovered to decline after 3 h and to revert to normal at 24 h. Mesenchymal stem cell (MSC) transplantation has been demonstrated in both animal models and clinical trials to support poststroke recovery. Exosome release is one of the paracrine mechanisms that stem cells are thought to primarily use to exert their therapeutic effects according to mounting evidence. It has been convincingly demonstrated that MSC-derived exosomes aid in stroke recovery in ischemia. Exosome administration methods for stroke can be broadly categorized into two groups: systemic administration and local administration. Systemic administration includes intravenous administration by the tail vein, femoral vein, or internal jugular vein and intraarterial administration through the common carotid artery, intraperitoneal administration, and intranasal administration. The most popular method for administering exosomes in rodent models of ischemic stroke is through the tail vein. When exosomes were labeled with gold nanoparticles after cerebral ischemia, it was discovered that the number of exosomes delivered to the brain was two times greater than that with intravenous delivery. For noninvasive exosome administration in ischemic stroke, intranasal delivery was more effective and promising. Exosomes produced by stem cells or other cells are becoming more popular as a treatment for cerebral ischemia. However, the dosage, frequency, and delivery methods of engineered exosomes remain unknown. For a treatment to be effective, it is essential to translate and comprehend exogenous exosome distribution, pharmacokinetics, and clinical effects.[93] Exosomes created by MSCs are responsible for the advantages of cell therapy for traumatic brain injury (TBI) and stroke. Animal models of stroke, traumatic brain damage, and other neurological illnesses may exhibit improved neurological outcomes when MSC-derived exosomes are used alone. Exosomes from engineered MSCs that contain a particular miRNA have more pronounced therapeutic effects in stroke and TBI than exosomes from naive MSCs.[94] Using exosomal miR-124 and its downstream target, ubiquitin-specific protease 14 (USP14), Song et al. demonstrated that M_2 microglia-derived exosomes decreased ischemic brain damage and maintained neuronal survival. Exosomes made by M_2 microglia present an ischemic stroke treatment option that may be effective.[96] The serum exosomes (labeled Con-exo) were examined by TEM to ascertain their shape; see Figure 8. The distribution of size and concentration using a multiple-laser analyzer to examine serum exosome ZetaView R Tracking Analyzers for Nanoparticles (f-NTA) (Particle German Metrix) anti-CD9 and anti-TSG101 antibodies are used in Western blotting to validate the exosome surface markers.[97] Therefore, more research on the use of exosomes to treat cerebral ischemia will be necessary. It will concentrate on elucidating the precise mechanism by which exosomes function to treat cerebral ischemia as well as the therapeutic use of exosomes to encapsulate and modify medications. Future

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research should also encourage the development of fresh exosome therapies and improve patient outcomes by further reducing stroke-related mortality, disability, and overall quality of life.[98]

DNA Nanoparticles As Delivery Vehicles. DNA has recently been used to create novel nanomaterials with an unheard-of functionality thanks to programmable assembly technology. For a variety of biological applications, including targeted therapies, molecular diagnostics, biosensing, antimicrobial therapy, anticancer techniques, and tissue regeneration, tetrahedral DNA (TDN) is used as a nanocarrier. TDNs are readily editable and have a strong ability to regulate cellular behavior because of the physicochemical makeup of DNA. Increased cell migration, osteogenic differentiation capacity, anti-inflammatory and ROS-scavenging capacity, and chondrocyte phenotypic maintenance capacity are some of these characteristics. These characteristics make TDN appropriate for application in bone tissue regeneration and repair as a tailored nanocarrier for medical treatments or as a bioactive nanomaterial.[99] Jungju Oh et al. studied the treatment of ischemic stroke and created a self-assembling nanoparticle made of deoxycholate-conjugated polyethylenimine-2k (DP2k), HO-1 plasmid, and hypoxia-specific antireceptor for advanced glycation end-product (RAGE) peptides. RAGE overexpression and inflammation are both present in the ischemic brain. The human serum amyloid P component (HSAP) was created by using recombinant DNA technology and the RAGE-binding domain of high mobility group box-1. There is an inhibition of RAGE-mediated signal transduction. The HSAP-containing nanoparticle (HSAP-NP) may act as both a specific ligand for receptor-mediated transfection and a cytoprotective agent due to its unique affinity for RAGE. By preventing the positive feedback of RAGE-mediated signal transduction, the cytoprotective medication HSAP-NPs decreased RAGE expression on the surface of brain cells. As a result, inflammatory reactions, apoptosis, and ROS were reduced in hypoxic cells. Indicating that HSAP-NPs more effectively delivered the genes to ischemic tissues, hypoxia-inducible factor-1α (HIF-1α) positive cells expressed more genes than HIF-1α negative cells did. The cytoprotective peptide HSAP showed dual actions as a RAGE-positive cell-specific ligand and cytoprotective peptide. HSAP increased the efficiency of HSAP-NP's gene delivery to ischemic tissues. Furthermore, HSAP's cytoprotective and anti-inflammatory characteristics lessened tissue damage in the ischemic brain.[100] According to Sabine Sellner et al., the development of nanomedical remedies with fewer side effects may be aided by the use of DNA nanotubes as a platform for the targeted administration of glucocorticoids.[101] Nucleic acids with a tetrahedral structure (tFNAs) are extremely biocompatible and have a variety of biological activities. *In vitro*, the tFNA inhibits ischemic cascades (excitotoxicity and oxidative stress) to reduce the death of neurons (SHSY-5Y cells) brought about by oxygen-glucose deprivation/reoxygenation. It significantly decreased the infarction volume from 33.9% to 2.7% and significantly enhanced the ischemic hemisphere's microenvironment by increasing erythropoietin expression and lowering inflammation. This reduced cell apoptosis, reversed neuronal loss, and attenuated neurological deficits *in vivo* in transient-MCAO rat models. The tFNA is an effective ischemic stroke treatment option and a reliable pleiotropic nano-neuroprotectant.[102]

Immunotherapy. Innate immune cells enter the meninges and brain during the acute phase, where they can damage tissue from ischemia while also acting as a preventative measure. Injured brain cells send signals of danger into the bloodstream, which activates systemic immunity. This process also causes significant immunodepression, which promotes life-threatening infections. A brain-specific adaptive immune response that is sparked by antigen presentation in the chronic phase and contributes significantly to poststroke morbidity may be the cause of neuropsychiatric sequelae. Following brain injury, DAMPs and cytokines leak into the bloodstream and boost the systemic immunity. Neurohumoral pathways are also activated by brain damage, and these pathways support immune activation, inflammation, and, ultimately, immunodepression.[104] Numerous proinflammatory mediators, including TNF-α, IL-1β, and IL-6, are released by activated microglial cells M_1, disrupting the BBB and making many immune cells more permeable. If specific cytokines are used to encourage microglial polarization in culture, the M_1 and M_2 phenotypes may be observed. When compared with the M_1 phenotype, which favors an inflammatory response, the M_2 phenotype encourages healing and recovery. $Ly6C_{low}$ and $Ly6C_{hi}$ monocytes that enter the tissue after 1 week of ischemia develop M_2-type microglia-like traits. Human ischemic-infarct-derived macrophages initially showed proinflammatory traits, but as the lesion progressed, they acquired anti-inflammatory traits. Monocytes and macrophages contribute to the reduction of inflammation, the protection of the vascular system, and the restoration of function through their phagocytic and vasculoprotective activities.[105]

Through damaged BBBs or lymphatic drainage of cerebrospinal fluid (CSF), brain-produced DAMPs and cytokines produced during ischemic injury enter the systemic circulation. These cytokines stimulate the immune system in lymphoid organs, which results in inflammation.[104] When an ischemic stroke occurs, the rapid activation of immune cells disrupts the BBB. Blood-borne immune cells that invade the BBB become more permeable, disrupt microcirculation, and release molecules linked to inflammation. Microglia, astrocytes, and pericytes are among the cerebral immune cells that have been underappreciated in ischemic stroke.[106] The advent of stem cell nanotechnology has made it now feasible to track biological processes and enhance treatment accuracy in stem cell therapy for an ischemic stroke. Nanoparticle-based labeling agents are often used. It has proven effective to employ photoacoustic contrast agents, Prussian blue nanoparticles, and semiconducting polymer nanoparticles for real-time imaging-guided cellular injection in the mouse brain. Superparamagnetic iron oxide nanoparticles have been used in MRI to track transplanted stem cells. For stem cell treatment, it would be useful to create an efficient imaging nanoprobe with antioxidant defense capabilities. Stem cell therapy would benefit from the creation of an efficient imaging nanoprobe with antioxidant protective properties.[103]

In order to restore the damaged brain circuit after an ischemic stroke and replace lost neurons, neural stem cell (NSC) transplantation is a promising therapeutic approach.[107] NSCs' basic biological characteristics may be used to get around some of the challenges that stroke therapy now faces. NSCs may be used in addition to other therapies, such as tPA. In fact, there is a possibility that these therapies will work best together. Particularly in stroke patients, the anti-inflammatory, antiapoptotic, pro-angiogenic, and pro-regenerative qualities of

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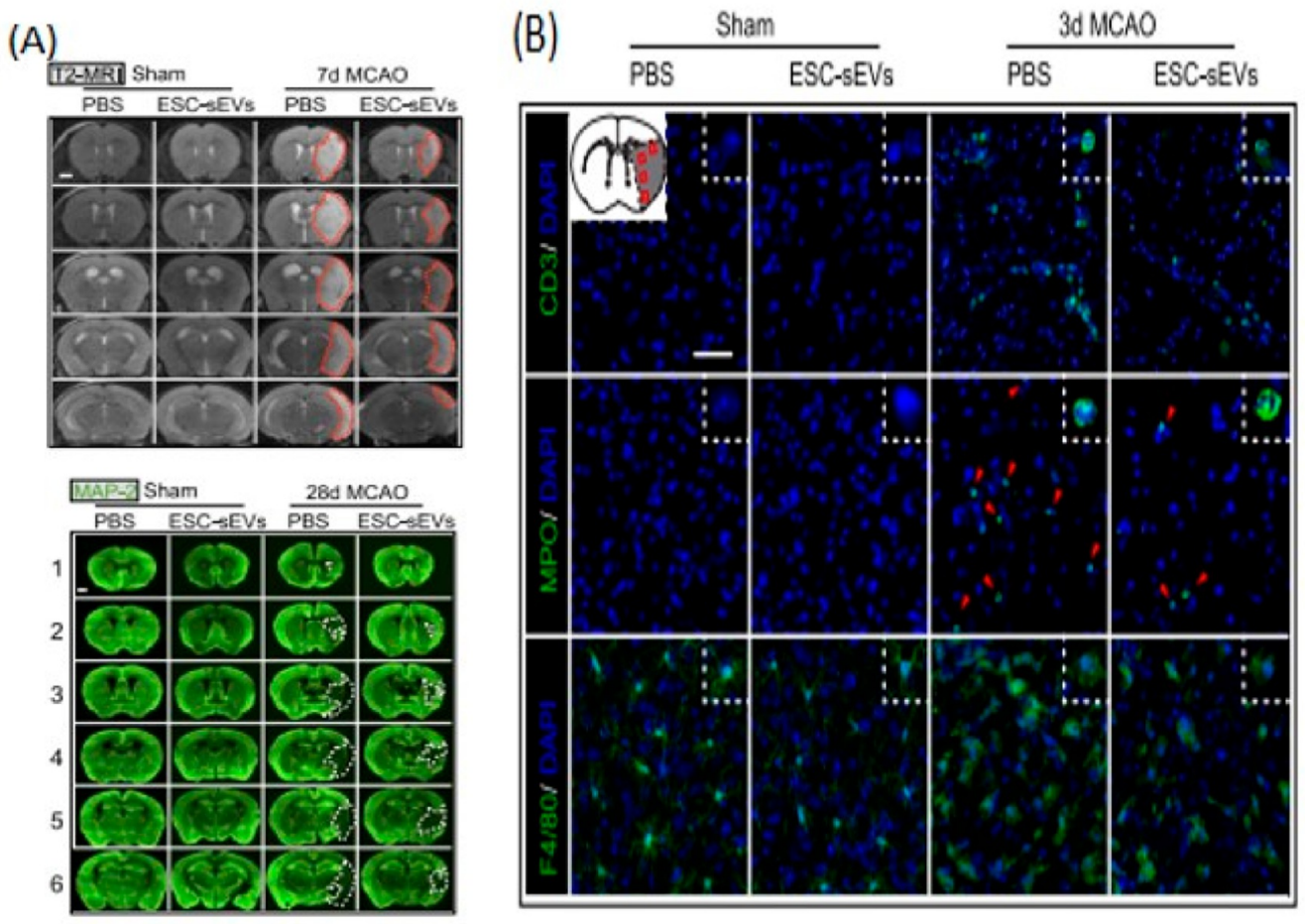

Figure 9. (A) Subacute and chronic stages of ischemic stroke tissue loss and long-term neurological impairments were reduced by ESC-sEV therapy. At days 7 and 28, respectively, MRI scanning and MAP-2 staining were used to measure brain infarction, and behavior tests were carried out to evaluate the sensorimotor functions up to 28 days following stroke. Seven days after a stroke, T2-weighted MRI scans were used to quantify cerebral infarction (n = 6−11 per group). mm is the scale bar. MAP-2 staining images and tissue loss estimation 28 days poststroke, n = 6−14/group. 1 mm is the scale bar. Leukocyte migration and neuroinflammation were prevented by ESC-sEV therapy during the acute stage of stroke as depicted in fluorescent images, reproduced with permission from Yuguo Xia et al. Copyright 2021, American Chemical Society.[109]

NSCs help lessen the adverse effects of tPA therapy.[108] According to a study by Bingling Lin et al., theranostic nanomedicine is used to distribute Pnky long noncoding RNA (lncRNA) targeting small interfering RNA/antisense oligonucleotides (siRNA/ASO) and superparamagnetic iron oxide nanoparticles (SPIO) into NSCs. The Pnky lncRNA is silenced by this nanomedicine, which also makes it possible to track NSCs using *in vivo* magnetic resonance imaging (MRI). It was essential for the morphological and functional healing of the stroke-damaged brain that NSCs enhance neuronal differentiation.[107] Yuguo Xia et al. identified the therapeutic function of small extracellular vesicles (ESC-sEVs) derived from embryonic stem cells (Figure 9), concentrating in particular on their function in immunomodulation following ischemic stroke. Transient MCAO is followed by the intravenous administration of ESC-sEVs. By drastically lowering leukocyte infiltration, inflammatory cytokine expression, neuronal death, and infarct volume after an ischemic stroke, ESC-sEVs help to reduce long-term neurological impairments and tissue loss. It is interesting that after a stroke ESC-sEVs significantly increase the number of regulatory T cells (T_{regs}).[109] To help clinical trials succeed and broaden the patient inclusion criteria for stroke treatment, more research is still required.[108]

CONCLUSION

Ischemic stroke is a significant health risk due to its pathophysiology, which includes excitotoxicity, oxidative stress, inflammation, and apoptosis. Nanomedicine and therapeutic techniques can help reduce strokes by addressing these factors. However, the high selectivity of traditional medications prevents them from crossing the BBB. Nanoparticles, such as hydrogels, nanoparticles, exosomes, quantum dots, and liposomes, can successfully cross the BBB and deliver medications to lesion areas. These nanoparticles are crucial for disease detection and treatment, as they possess high conductivity, chemical stability, and catalytic activity, making them valuable in biomedicine. Nanoparticles offer numerous advantages, including multifunctionality, multivalency, and higher permeability, making them useful in various diseases. The combination of thrombolytic medications and neuroprotective agents in biocompatible nanostructures is the main advance in treating ischemic stroke. Future research will examine how stem cells and innate cells might help stroke patients' tissues renew and repair damaged ones.

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■ ASSOCIATED CONTENT

Data Availability Statement

The data used in this investigation may be accessed via the linked supporting materials, the publication itself, or by contacting the corresponding author.

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Notes

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