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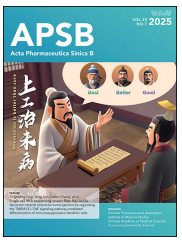

REVIEW

Advances in research on biomaterials and stem cell/exosome-based strategies in the treatment of traumatic brain injury

Wenya Chi[a,†], Yingying He[a,†], Shuisheng Chen[a,†], Lingyi Guo[a], Yan Yuan[a], Rongjie Li[a], Ruiyao Liu[a], Dairan Zhou[b], Jianzhong Du[c,d,*], Tao Xu[b,*], Yuan Yu[a,*]

[a]*Department of Pharmaceutical Science, Faculty of Pharmacy, Naval Medical University, Shanghai 200433, China*
[b]*Department of Neurosurgery, Changzheng Hospital, Naval Medical University, Shanghai 200003, China*
[c]*Department of Gynaecology and Obstetrics, Shanghai Key Laboratory of Anesthesiology and Brain Functional Modulation, Clinical Research Center for Anesthesiology and Perioperative Medicine, Translational Research Institute of Brain and Brain-Like Intelligence, Shanghai Fourth People's Hospital, School of Medicine, Tongji University, Shanghai 200434, China*
[d]*Department of Polymeric Materials, School of Materials Science and Engineering, Tongji University, Shanghai 201804, China*

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Regeneration

Abstract Traumatic brain injury (TBI) is intricately linked to the most severe clinical manifestations of brain damage. It encompasses dynamic pathological mechanisms, including hemodynamic disorders, excitotoxic injury, oxidative stress, mitochondrial dysfunction, inflammation, and neuronal death. This review provides a comprehensive analysis and summary of biomaterial-based tissue engineering scaffolds and nano-drug delivery systems. As an example of functionalized biomaterials, nano-drug delivery systems alter the pharmacokinetic properties of drugs. They provide multiple targeting strategies relying on factors such as morphology and scale, magnetic fields, pH, photosensitivity, and enzymes to facilitate the transport of therapeutics across the blood−brain barrier and to promote selective accumulation at the injury site. Furthermore, therapeutic agents can be incorporated into bioscaffolds to interact with the biochemical and biophysical environment of the brain. Bioscaffolds can mimic the extracellular matrix environment, regulate cellular interactions, and increase the effectiveness of local treatments following surgical interventions. Additionally, stem cell-based and exosome-dominated extracellular vesicle

*Corresponding authors.
E-mail addresses: pharmyuu@163.com (Yuan Yu), xutao@smmu.edu.cn (Tao Xu), jzdu@tongji.edu.cn (Jianzhong Du).
[†]These authors made equal contributions to this work.

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carriers exhibit high bioreactivity and low immunogenicity and can be used to design therapeutic agents with high bioactivity. This review also examines the utilization of endogenous bioactive materials in the treatment of TBI.

1. Introduction

Traumatic brain injury (TBI) is a disorder and dysfunction caused by damage to brain tissue from external forces[1]. The annual incidence of TBI is estimated to be approximately 27−69 million cases worldwide[2] and approximately 4 million cases in China[3]. There are regional variations in TBI care. Over the past two decades, improvements in TBI outcomes in China have been attributed to advancements in legislation, healthcare policy, and clinical management. Chinese craniocerebral trauma experts have authored and published a series of guidelines and consensus reports on topics such as surgical treatment, pharmacologic treatment, intracranial pressure monitoring, decompressive craniectomy, post-traumatic hydrocephalus treatment, coma-awakening surgery, cranioplasty for traumatic cranial defects, and post-traumatic epilepsy prevention and management. These publications have significantly transformed the approach of Chinese neurosurgeons, shifting from experience-based to evidence-based medicine. Additionally, medical facilities in China, particularly neurosurgical intensive care units, have seen substantial improvements since the 1980s. The implementation of modern imaging technologies, including CT, MRI, and Digital Subtraction Angiography, has provided advanced care for TBI patients over the past decade[4].

The clinical treatment outcomes for TBI patients are often unsatisfactory, with most experiencing varying degrees of functional impairment, resulting in a significant societal burden. Research is currently focused on finding ways to protect and regenerate neural function after injury, which represents a critical frontier in the field. The blood−brain barrier (BBB) consists of brain microvascular endothelial cells, peripheral basement membranes, pericytes, outer membrane cells, astrocytes, and tight junction structures[5]. As a physiological barrier, the BBB maintains the safety of the brain's internal environment by preventing most drugs, especially large biomolecules, from effectively reaching the brain. TBI disrupts the tight junctions of the BBB, leading to cell swelling, increased gaps, reduced vascular lumens, and surrounding inflammation. These changes in BBB-related transport protein expression reduce the functional interactions between endothelial cells and neuroglial cells, further compromising the physiological functions of the BBB[6]. With advances in the understanding of pathological mechanisms, the combination of biomaterial technologies[7], nanomedicine systems[8], various stem cell therapies[9,10], and implantable tissue engineering scaffolds has emerged as a highly promising direction for TBI treatment.

2. Pathological mechanisms of TBI

The injury mechanisms of TBI can be divided into primary and secondary injuries, leading to temporary or permanent deficits in neural function[11]. Primary injury refers to the direct effects of external mechanical forces on the head, causing primary damage to neurons, white matter, and the vascular system, such as concussion, contusion, and diffuse axonal injury (DAI)[12]. After the injury, the intracranial contents may undergo a series of molecular, chemical, and inflammatory cascade reactions and release excitatory neurotransmitters, resulting in the progression of secondary injuries, which can include focal injuries such as multiple brain parenchymal hemorrhages, subdural hematomas, or epidural hematomas. In cases of TBI that result in severe intracranial pressure elevation, surgical treatment is often needed[13].

Primary brain injury carries risks of short-term and long-term sequelae, while secondary brain injury is the main driving factor for neural dysfunction and neuronal death[14]. Secondary injury can persist for days, months, or even longer and consists of a series of pathological cascade reactions. The specific mechanisms are related to neurotoxicity, mitochondrial dysfunction, inflammatory response, apoptosis, and vascular dysfunction[15]. The complex interaction of multiple mechanisms leads to neuron death and exacerbates brain injury. A deeper understanding of the mechanisms of secondary brain injury will aid in targeted therapeutic interventions.

Neurotransmitter-mediated excitatory toxicity leads to cell membrane damage caused by glutamate and free radicals. Trauma or ischemic stress causes a large release of glutamate at the injury site, resulting in the overactivation of glutamate-related receptors and voltage-dependent calcium channels. An increase in sodium and calcium ion influx causes calcium overload, which activates protein phosphatases, phospholipases, nucleases, etc. The overactivation of these enzymes leads further to DNA structure breakage, the degradation of proteins that make up the neural cytoskeleton, the disruption of axonal fast transport systems, axonal swelling, delayed axonal rupture, and the disruption of intercellular communication.

Mitochondrial dysfunction. The mitochondria are the main sites for reactive oxygen species (ROS) production, and TBI causes mitochondrial dysfunction, disrupting the mechanisms that maintain the ROS balance[15]. Excessive ROS can cause mitochondrial membrane lipid peroxidation, destroy the membrane structure, and lead to dysfunction[16]. After TBI, ionotropic receptors such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors and *N*-methyl-D-aspartate receptors are activated on the membrane. The opening of Ca^{2+} channels activates intracellular proteins, and excessive channel opening increases mitochondrial membrane permeability and fragmentation, leading to membrane damage and the release of endotoxins and apoptotic factors into the cytoplasm[14]. These molecules in turn activate caspases and cause apoptosis.

Inflammatory response. After TBI, microglia are highly activated and secrete many inflammatory cytokines. Inflammatory cytokines reduce monoamine neurotransmitter levels in synaptic gaps by reducing vesicle release and increasing transporter

function, or they activate indoleamine 2,3-dioxygenase to decrease the numbers of monoamine monomers. The secondary metabolites generated from the degradation of kynurenine affect the glutamate system and disrupt the brain-derived neurotrophic factor-tyrosine kinase receptor B (BDNF−TrkB) signaling pathway, affecting neural plasticity and regeneration[17]. Additionally, injured glial cells and neurons produce brain-derived extracellular vesicles (EVs) that are rapidly released into the blood, leading to a series of inflammatory responses[18]. With BBB damage, circulating fibrocytes and other inflammation-related macrophages, leukocytes, and cytokines enter brain tissue, exacerbating damage to the BBB and complicating the inflammatory response[19].

Apoptosis. Oligodendrocytes primarily maintain myelin and provide axonal nutritional support, and the activity of ionotropic and metabotropic purinergic receptor family members is key for oligodendrocyte survival. Chronic activation of calcium-permeable P2X7 purinergic receptors (which are highly expressed on the surface of differentiated and mature oligodendrocytes) by the massive release of ATP leads to oligodendrocyte death, demyelination, and axonal injury[20]. As a result of channel opening and Na^+ influx, cells become depolarized and water content increases, leading to swelling. Ca^{2+} entry *via* these channels also contributes to excitotoxicity and apoptosis[21]. Nitric oxide and ROS may induce apoptosis in brain endothelial cells in the presence of glutamate; however, this claim remains controversial.

Secondary ischemia can occur due to vascular spasms, localized microvascular occlusion, and vascular injury. It has been proposed that oxidative stress causes brain edema by disrupting mitochondria, breaking down the BBB, impairing sensory-motor functions, and causing secondary neuronal damage[16]. The secondary injury cascade is often perpetuated and made chronic by oxidative stress. Non-caspase-dependent apoptosis induced by TBI releases mitochondrial proteins into the nucleus, including apoptosis-inducing factor, Smac/DIABLO, endonuclease G, and polymerase-1, which activate upstream signaling molecules and damage neurons and glial cells[22]. When secondary ischemia occurs in combination with axonal injury, in the acute phase, shear stress causes vascular damage and dysfunction in the brain, including the BBB. Pathological mechanisms of TBI are generalized in Fig. 1.

3. Stem cells and exosomes for TBI treatment

In accordance with the pathological features of TBI, regenerative therapies based on stem cells emphasize the repair of damaged nerves by stimulating endogenous or exogenous stem cells (Fig. 2)[23,24]. Neural stem cells (NSCs), mesenchymal stem cells (MSCs), pluripotent adult progenitor cells, and bone marrow mononuclear cells (BMMNCs) can be administered *via* intravenous injection or targeted intracranial injection to treat TBI[25]. Intracranial injection offers a more controllable mode of drug delivery, allowing cells to be rapidly localized to the lesion with relatively better results. However, challenges such as limited retention time and survival of transplanted cells at the administration site can affect the therapeutic outcome. Additionally, this method may cause new trauma or serious complications. As a

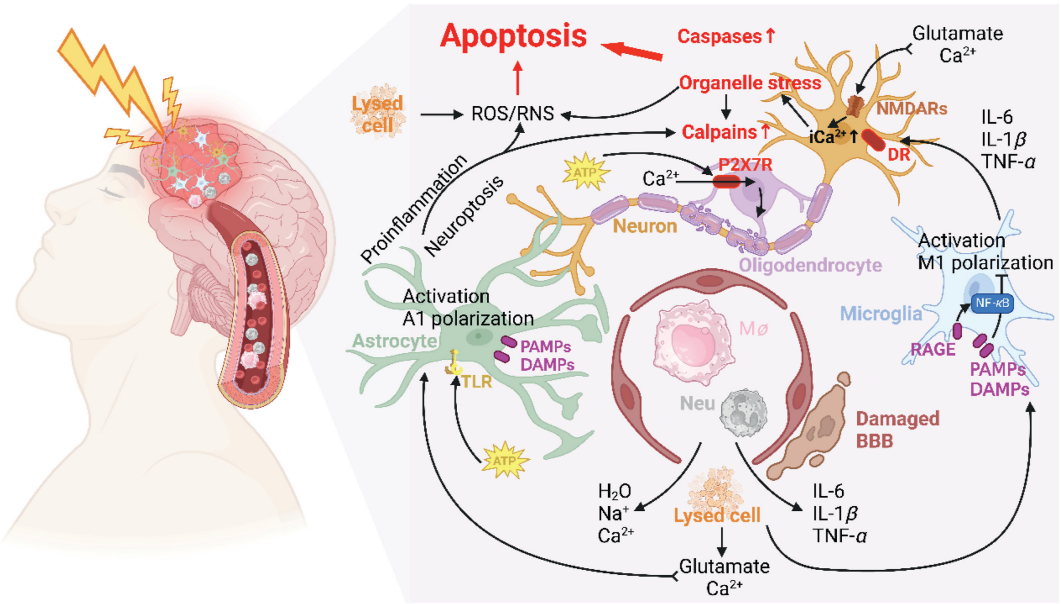

Figure 1 Pathological mechanisms of TBI. Rupture of the BBB leads to edema and changes in ion balance. The release of intracellular components activates microglia and astrocytes, increasing oxidative stress, inflammation, and neural injury. An increase in extracellular Ca^{2+} and glutamate concentrations activates NMDAR receptors, and the mechanical activation and mechanoporation of NMDAR receptors result in increased Ca^{2+} concentrations. Excessive calcium uptake leads to neurotoxicity by decreasing the mitochondrial membrane potential, resulting in neural injury. The production of ROS increases, and after the destruction of the mitochondrial membrane structure, endotoxins and apoptotic factors are released into the cytoplasm, where they activate caspases and cause apoptosis. Simultaneously, the slow, chronic activation of calcium-permeable P2X7 purinergic receptors by a massive release of ATP leads to oligodendrocyte death, demyelination, and axonal injury. Microglia become highly activated and accordingly secrete large quantities of cellular inflammatory factors, which disrupt the BDNF−TrkB signaling pathway and allow circulating fibrocytes and other inflammation-related macrophages, leukocytes, and cytokines to enter brain tissue (Created with BioRender.com).

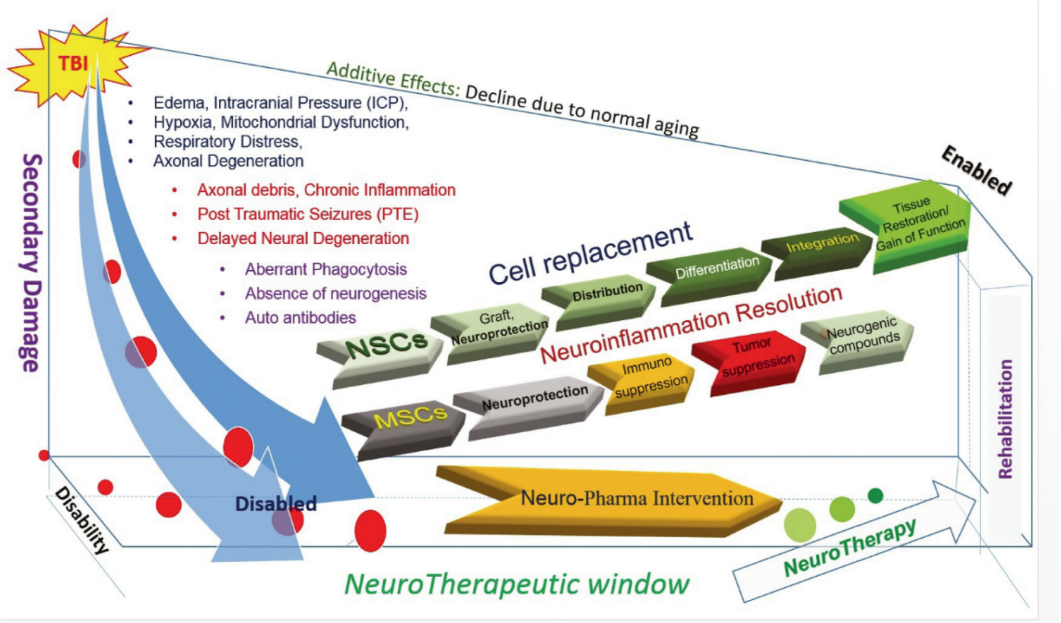

Figure 2 The stem cells rehabilitative schematic summarizes the mechanisms of TBI that lead to debilitating cellular and behavioral injuries and contrasts these with drug-stem cell-based interventions, which promote enhanced cellular survival and behavioral recovery (Reprinted with the permission from Ref. 24. Copyright © 2019 Neuropharmacology).

result, clinical treatment may opt for intravenous infusion. Intravenous injection is more common in clinical settings because it is easy to administer, less invasive, and reproducible. However, compared to intracranial injection, stem cells delivered *via* intravenous injection often have insufficient homing due to the distance from the target site, thus affecting the therapeutic effect. As research has progressed, the mechanisms of endogenous neurogenesis in adult brains, such as epigenetic mechanisms[26], signaling pathways regulating endogenous stem cell proliferation and differentiation[27], the promoting effects of neurotrophic factors[27], and neural circuit formation[28], have been further elucidated. Several studies have indicated that injury can promote neurogenesis and glial cell formation[29,30]. In TBI patients, the expression of neural stem/progenitor cell markers, such as DCX, TUC4, PSA-NCAM, SOX2, and NeuroD, is increased in the cortex compared to that in normal brain tissue, and the cell proliferation marker Ki67 is significantly increased in the injury area, colocalizing with neural progenitor markers, which indicates trauma-driven neurogenesis[31].

The goal of endogenous stem cell-based regeneration is to complete injury repair by stimulating local neurogenesis and other repair processes, such as through the action of cytokines, hormones, and neurotrophic factors[32,33], thereby promoting endogenous stem cell migration, proliferation, differentiation, and maturation. However, this process presents challenges such as insufficient neuron differentiation and maturation and low cell survival rates[30]. Therefore, exogenous stem cell transplantation has emerged as a highly promising biological therapy. Transplanted stem cells can promote brain injury repair in two ways: by differentiating into new neurons and glial cells to repair the damaged area or through a bystander effect that promotes neuroreparative processes[34]. The bystander effect repair mechanism is related to endogenous stem cells: exogenous stem cells secrete cytokines, extracellular matrix (ECM) molecules, and EVs, which can reduce inflammation, mediate neuroprotection, and stimulate endogenous stem cell proliferation and neurogenesis[35,36]. The bystander effect is specific and is closely related to the functional components secreted by stem cells. In a rat TBI model, genetically modified neural precursor cells from rat embryos that secreted multifunctional neurotrophic factors were shown to promote transplant cell survival and neuron differentiation, significantly improving behaviors indicative of hippocampus-related learning and memory[37]. Furthermore, stem cell transplantation into the brain can establish a biological "bridge" between the injury site and neurogenic niches, enabling endogenous neural cells to migrate to the injury site[38].

3.1. Neural stem cells (NSCs)

NSCs are a type of pluripotent stem cell that can differentiate into neural lineage cells, such as neurons, astrocytes, and oligodendrocytes. These cells are distributed mainly in the hippocampal dentate gyrus, the subventricular zone, and the olfactory bulb[39,40]. Extensive research has shown that transplanted NSCs possess neuroprotective properties and can support functional recovery after acute brain injury by alleviating neuroinflammation, promoting neurogenesis, and inducing angiogenesis[37,41,42]. Wang et al.[43] used tetrahedral framework nucleic acids (tFNAs) to enhance the proliferation of endogenous NSCs inhibit the overactivation of astrocytes and microglia, regulate the neuroinflammatory response in the acute phase, and promote the recovery of cognitive impairment after TBI. Shear et al.[44] performed a year-long evaluation of the survival, migration, and differentiation of NSCs derived from mouse fetal brains and transplanted into injured brain tissue. They found significant improvements in motor and spatial learning functions in the transplant group, with widespread migration of the transplanted cells in the injured brain, primarily expressing the oligodendrocyte precursor cell (OPC) marker NG2. Several studies have shown that when modified NSCs are transplanted into brain-injured animals,

they differentiate into mature neurons that express neurotransmitters or overexpress growth factors such as BDNF, thereby promoting transplant cell survival and neuron differentiation and improving motor and cognitive functions in injured animals[45]. Riess et al.[46] and Boockvar et al.[47] reported that after brain trauma, transplanted immortalized embryonic stem cell-derived NSCs (C17.2 cells) could improve motor functions. The transplanted cells survived for up to 13 weeks and differentiated into mature neurons and neuroglial cells. However, neuroinflammation is one of the primary factors affecting the survival rate of NSCs in brain injury and might be a driving factor of chronic progressive neurodegeneration. After TBI, activated microglia secrete proinflammatory factors such as TNF-α, which stimulate an inflammatory cascade response, promote astrocyte proliferation, and increase ICAM-1 expression, consequently recruiting peripheral immune cells for infiltration and activation, which leads to white matter damage and extensive cell death and inhibits the survival of NSCs[48-50]. In terms of the impact of the inflammatory environment on the NSC survival rate, NSC transplantation alone may not be sufficient to repair damaged tissue, and auxiliary therapies with immunoregulatory characteristics are needed[51]. Microglia collected from brains with stroke or excitotoxic striatal lesions release mitogenic factors, and the culture of NSCs in the presence of these factors can promote proliferation and differentiation into neurons and oligodendrocytes[52].

3.2. *Mesenchymal stem cells (MSCs)*

MSCs are a group of multipotent stromal cells with multidirectional differentiation potential, tissue repair function, and immunomodulatory function. They are widely distributed in tissues such as bone marrow, umbilical blood, fat, and muscles and can differentiate into various cells, such as bone, fat, and nerves. The bystander effect mechanism is considered the main mechanism by which MSCs contribute to tissue regeneration and repair, secreting numerous bioactive molecules, such as anti-inflammatory, antioxidative stress, antiapoptotic, and neural regeneration-related factors[53-55]. The transplantation of IL-10-expressing MSCs into the medial cortex of the frontal lobe after rat brain injury has shown that MSCs provide a favorable microenvironment that promotes the polarization of macrophages from a proinflammatory phenotype (CD86) to an anti-inflammatory phenotype (CD163), resulting in immunomodulatory effects[56]. Studies by Chen et al.[57] have shown that after brain injury, MSCs produce vascular endothelial growth factor (VEGF), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and epidermal growth factor (EGF), inducing functional recovery by promoting neural protection and vascular reconstruction[58].

Bone marrow mesenchymal stem cells (BMSCs) are nonhematopoietic stem cells derived from the mesoderm, and one of the mechanisms of neural repair involves cell migration to the injury site and the secretion of growth factors to repair injured neurons[59]. Watanabe et al.[60] verified the improvement of early neural protection and late functional behavior in brain-injured mice by BMSCs producing TSG-6. Moreover, BMSCs may promote functional recovery by differentiating into neurons and astrocytes[61] and providing nutritional support for endogenous neural regeneration[62,63]. Several clinical trials with BMSCs are underway. In Cramer et al.'s trial[64], 61 chronic TBI patients were intracranially injected with different doses of allogeneic BMSCs (SB623). No dose-dependent toxicity was observed. However, a statistically significant improvement in the Fugl-Meyer motor score was observed after 24 weeks of treatment, and the highest dose group (5.0×10^6 cells) showed the greatest improvement in motor status.

Cell modification or its combination with drugs can effectively increase the efficacy of stem cell therapy. Although brain trauma can be treated without changing the original cell phenotype, improvements in the homing of BMSCs to the injury site are still needed. Fibroblast growth factor 21 (FGF21) reportedly promotes the migration of various cell types, and BMSCs overexpressing FGF21 significantly increase the homing ability of brain injury cells[65]. In a TBI mouse model, genetically modified BMSCs overexpressing the anti-inflammatory cytokine IL-10 not only increased mitochondrial autophagy and reduced cell apoptosis[66], but also enhanced immune regulation and promoted functional recovery[56]. In addition, synergistic effects can enhance the therapeutic response; for example, treatment with both BMSCs and erythropoietin[67] can reduce apoptosis in the ischemic penumbra, which is conducive to the proliferation of endogenous cells in the periventricular region[68].

Ruppert et al.[69] explored the effects of adipose-derived mesenchymal stem cells (ADMSCs) on neuroinflammation after TBI by administering ADMSCs early and late post-injury. Rats were subjected to controlled cortical impact (CCI) to induce moderate to severe brain injury, followed by intravenous injection of ADMSCs at 3 and 14 days post-injury. The early administration of ADMSCs effectively reduced M1-type microglial cell numbers and might have promoted neurogenesis in the hippocampal region; the delayed administration of ADMSCs significantly increased M2-type microglial cell numbers and significantly elevated the M2/M1 cell ratio. These results suggested that ADMSCs can effectively regulate the inflammatory response, creating a more favorable anti-inflammatory environment for recovery in the early stages of TBI. However, some issues still surround the further clinical application of MSCs, such as the source, processing, and culture of cells as well as the cell heterogeneity, administration route, quantity, and concentration[63]. Additionally, long-term safety studies are important for developing MSC-based treatment for TBI.

Studies have shown that the therapeutic capacity of MSCs *in vivo* can be increased by genetic modification, which is usually achieved using viral vectors or nonviral vectors[70]. The genetic modification of stem cells can improve their functional and other properties, thus increasing their potential therapeutic efficacy. However, due to the genotoxicity and tumor risk of genetic modification, genetically modified MSCs have not yet been used for the treatment of TBI, and there is still a long way to go before their future clinical application.

3.3. *Embryonic stem cells (ESCs)*

ESCs are a type of cell isolated from early embryos or primordial gonads and are characterized by unlimited *in vitro* proliferation, self-renewal, and multidirectional differentiation abilities. Studies involving predifferentiated ESCs or neural precursor cells have been conducted in animal models of conditions such as TBI and neurodegenerative diseases[71]. Human ESCs separated from fetal brains can differentiate into NSCs, and when transplanted into the brain after cortical contusion, they can survive for 6 weeks and migrate to the contralateral cortex, where they differentiate into neurons and astrocytes[72]. Skardelly and colleagues transplanted predifferentiated human ESCs into the brains of rats with severe cortical injuries. They observed the formation of new blood

vessels, an increase in astrocyte numbers, an increased survival rate of neurons in the injury border region, and a reduction in brain injury volume[73], indicating that the ESCs had a positive therapeutic effect on cortical injuries. However, despite the observed therapeutic efficacy following experimental TBI, ESC transplantation carries certain risks and challenges, such as tumorigenicity, ethical concerns, limited tissue availability, and immune rejection[71,74]. These factors consequently limit the utilization of ESCs.

3.4. Adipose-derived stem cells (ADSCs)

ADSCs can be easily extracted from fat, are abundantly available, have low immunogenicity and immunomodulatory properties, and can differentiate into different cell lineages[75]. Results of a large number of clinical trial studies have shown that they have great potential for treating a wide range of diseases[76]; however, fewer clinical applications have been reported in TBI. Tajiri et al.[77] found that intravenous injection of human adipose-derived stem cells (hADSCs) protected rats against TBI-induced neurodegeneration as well as motor and cognitive deficits. Interestingly, their efficacy varied depending on the age of the rats, with hADSCs showing reduced efficacy in aged rats, which may be due in part to reduced cell homing to the spleen[77]. Studies have shown that ADSCs can inhibit inflammation and improve memory deficits after TBI by modulating the NLRP3 signaling pathway and extracellular signal-regulated kinase 1/2[78,79]. In addition to inflammatory homing properties, ADSCs can be modified in response to exogenous signals. For example, ADSCs labeled with superparamagnetic iron oxide (SPIO) have been shown to preferentially migrate to magnetic arrays *in vitro*[80]. In conclusion, ADSCs are highly promising therapeutic cells for TBI, the establishment and standardization of good manufacturing practices regarding the isolation and culture of ADSCs is essential to establish ADSCs therapies as legitimate medical therapies.

3.5. Other stem cells for TBI treatment

Multipotent adult progenitor cells (MAPCs) can be easily isolated from a patient, and the use of these cells possess the advantages of enabling autologous transplantation and avoiding ethical concerns. Mechanistically, these cells upregulate the anti-inflammatory response at the injured site by interacting with spleen cells[81], ultimately causing microglia to transition from an activated to a reparative state[82].

Induced pluripotent stem cells (iPSCs) can be differentiated from somatic cells such as fibroblasts or peripheral blood mononuclear cells using various reprogramming factors (Oct3/4, Sox2, c-Myc, and Klf4)[83]. These cells display morphological, surface antigen, and epigenetic traits specific to pluripotent cells, similar to those of human ESCs, and their use can thus avoid ethical concerns about the use of ESCs for biomedical research and clinical treatment[84]. Hasselmann and others on the differentiation of iPSCs into microglia confirmed the expression of microglial markers such as IBA1 and CD45 by iPSCs[85]. Currently, iPSCs are generated by transfection with retroviruses or lentiviruses, and the integration of viral vectors into the genome might lead to insertional mutations, limiting their practical application. However, the ongoing development of new strategies to prevent cellular genomic modification may increase the safety of iPSCs in the future.

In conclusion, stem cell therapy holds potential as an alternative treatment for central nervous system (CNS) injuries and subsequent functional deficits. However, stem cell therapy faces many hurdles, such as low cell survival and integration rates, teratogenicity, tumorigenicity, and ethical issues. Studies have shown that minimally invasive therapies can improve stem cell retention, reduce trauma, improve efficacy, and reduce the risk of complications[86,87]. The minimally invasive interventional magnetic resonance imaging approach allows for real-time intraoperative magnetic resonance imaging to guide the injection cannula to the target and track cell delivery to the brain[88]. Stem cells administered intranasally can cross the olfactory epithelium and travel through areas such as the periosteum of the turbinate bone, the cribriform plate, and so on, to reach the subarachnoid space[89]. In another study, stem cells administered intranasally could migrate to the primary and distal injury sites of TBI, which in turn improved cognitive function and enhanced spatial learning in TBI rats[90]. In clinical trials, umbilical cord mesenchymal stem cell transplantation has been shown to significantly improve neurological function in patients with TBI sequelae[91]. After six months of treatment, patients receiving umbilical cord MSCs transplantation demonstrated statistically significant improvements in motor function, sensitivity, and balance compared to the control group. Additionally, these patients showed enhanced mobility, locomotion, communication, and independent living skills. Intravenous MSCs reduced leukocyte, neutrophil, and inflammatory factor levels, and the majority of patients experienced varying degrees of improvement in overall health function during follow-up[92]. Tian et al.[93] found that BMSCs therapy is safe and effective for patients with TBI complications, such as persistent vegetative state and motor disorders. Wang et al.[94] investigated the safety, feasibility and biological effects of NSCs transplantation. The results showed that most patients experienced varying degrees of neurological improvement during the follow-up period. No patient deaths or serious adverse events were observed after transplantation. Additionally, higher levels of serum NGF and BDNF were detected post-transplantation compared to pretreatment levels. In endogenous stem cell therapy, the focus lies in stimulating differentiation and guiding new neurons derived from endogenous stem cells to the injury site, thereby promoting vascular formation and cell survival. Key goals in exogenous stem cell transplantation include minimizing adverse side effects such as epilepsy or tumor formation[95], improving the body's low-growth-factor environment, and overcoming the immune rejection of allogenic stem cell transplantation[96]. These studies will help optimize the dose, timing, and route of stem cell-mediated clinical cell therapies. The stem cells for TBI treatment are listed in Table 1[37,41,42,44-47,53-56,61-68,72,73,81,82,97-100].

3.6. Exosome therapy for TBI treatment

EVs are endogenous vesicles released by cells, and they are divided into exosomes (Exos), microvesicles, etc., based on the biogenesis, size, and biophysical properties[101]. As endogenous particles, EVs can evade capture by the mononuclear phagocyte system and can carry biologically active components to target specific cells for signal transduction, immune regulation, or other purposes[102], such as crossing the BBB to communicate with neurons and glial cells in the brain[103]. EVs from various cell types have been studied for downregulating the inflammatory response[104], regulating synaptic plasticity, and inducing neuronal regeneration[105]. They derived from NSCs, MSCs, astrocytes,

Table 1 The stem cells for TBI treatment.

Stem cell type	Source	Recipient	Administration	Function & mechanism	Ref.
NSCs	Rat embryos	Rat model of TBI	Intraventricular injection	The secretion of multifunctional neurotrophic factors after gene modification promotes graft survival and neuronal differentiation	37
	Rat embryos	Mouse model of TBI	Intraventricular injection	Reduced neuroinflammation, promoted neurogenesis, and induced angiogenesis	37,41,42
	Immortalized ESCs	Mouse model of TBI	Cortical-hippocampal interface injection in ipsilateral or contralateral hemisphere; ipsilateral or contralateral corpus callosum injection	Improved the motor function of mice	46,47
	Ten-week-old (postconception) human forebrain tissue	Rat model of TBI	Cortical injection at the damaged site	Migrated to the contralateral cortex and differentiated into neurons and astrocytes	72
	Mouse	Mouse model of TBI	Striatum injection	The motor ability and spatial learning ability of mice were improved, and the recovery continued until 1 year after transplantation	44
	Rat	Rat model of TBI	Cortical injection	Predifferentiated into mature neurons expressing neurotransmitters, or overexpressed growth factors that promote cell survival and neuronal differentiation	45
MSCs	Human	Patients with TBI	Intravenous injection	Secreted anti-inflammatory,	53,54
	Rat	Rat model of TBI	Intraventricular injection	antioxidative stress, and anti-apoptosis factors, promoted nerve regeneration and vascular reconstruction, and induced functional recovery	55
	Rat	Rat model of TBI	Intraventricular injection	Engineered MSCs overexpressing IL-10 promoted the polarization of M1 macrophages to M2 macrophages and played an immunoregulatory role	56
	Rat	Rat model of TBI	Tail vein injection	BMSCs differentiated into neurons and astrocytes and provided nutritional support for endogenous nerve regeneration to promote functional recovery	61−63
	Human	Patients with chronic brain trauma	Intracranial implantation	Improved motor function of patients	64
	Mouse	Mouse model of TBI	Intraventricular injection	The homing ability of BMSCs overexpressing FGF21 to brain injury was significantly improved	65
	Rat	Rat model of TBI	Intraventricular injection	BMSCs overexpressing IL-10 increased mitophagy, reduced inflammatory damage, and improved immune regulation	66
	Rat	Rat model of TBI	Tail vein injection	BMSCs combined with erythropoietin reduced apoptosis and promoted endogenous cell proliferation in the subventricular zone	67,68
ESCs	Human	Rat model of TBI	Intraventricular injection, tail vein injection	Promoted angiogenesis, reduced astrocytes, reduced the area of injury, increased the survival rate of neurons in the edge of injury, and improved long-term motor function	73

(*continued on next page*)

Table 1 (*continued*)

Stem cell type	Source	Recipient	Administration	Function & mechanism	Ref.
	Human	Mouse model of TBI	Intracranial implantation	Reduced neuronal cell death, recovered microvessel density, and promoted neurogenesis in the ipsilateral subventricular zone and dentate gyrus of the hippocampus	97
	Human	Mouse model of TBI	Intracranial implantation	Induced vascularization and reduced glial scar, improved spatial learning and memory improved	98
MAPCs	Rat	Rat model of TBI	Tail vein injection	The interaction with spleen cells upregulated the anti-inflammatory response, and microglia changed from an activated state to a repair state	81,82
	−	Rat model of TBI	Tail vein injection	Reduced activated microglia/ macrophages in the dentate gyrus of the hippocampus, improved cognitive behavior	99
	−	Rat model of TBI	Tail vein injection	Improved cognitive behavior, and attenuated activated microglia/ macrophages in the dentate gyrus	100

−, not applicable. NSCs, neural stem cells; MSCs, mesenchymal stem cells; ESCs, embryonic stem cells; MAPCs, multipotent adult progenitor cells; TBI, traumatic brain injury; BMSCs, bone marrow mesenchymal stem cells.

microglia, and endothelial cells, which have exhibited the therapeutic activities for TBI (Fig. 3)[105-107]. Exos are nanometer-sized vesicles, formed by invagination inside lysosomes, have diameters of approximately 30−150 nm and contents including various nucleic acids, proteins, and other biologically active substances, and they play essential roles in cell-to-cell communication[108]. Exos can regulate the inflammatory response, neuronal function and plasticity, and modulate BBB permeability and cellular responses to brain injury, providing nourishment and promoting synapse genesis for brain injury repair[109]. Compared to cell therapy, Exos therapy has the advantages of low tumorigenicity and immunogenicity[110]. Furthermore, the small size of Exos allows free passage through vessel walls and the ECM, and they demonstrate strong homing ability[111], making them suitable as biological markers for the dynamic pathophysiology of brain injury or as carriers for therapeutic molecules.

3.6.1. Astrocyte-derived Exos (AD-Exos)

Astrocytes are a type of glial cell that play vital roles in brain development, physiological functions, and pathological processes. AD-Exos inhibit post-TBI neuroinflammation by participating in interactions between astrocytes and microglia. Microglia have two distinct phenotypes that play key roles in neuroinflammation: the M1 phenotype produces high levels of proinflammatory cytokines (IL-1β, IL-12, and TNF-α), promoting inflammation, whereas the M2 phenotype secretes anti-inflammatory cytokines such as IL-10, thereby inhibiting inflammation[112]. After brain injury, microglia are rapidly activated and polarized, and activated AD-Exos containing miR-873a-5p inhibit ERK phosphorylation and NF-κB signaling, promote microglial polarization to the M2 phenotype, suppress proinflammatory factor release, and reduce neuroinflammation, thus minimizing post-TBI brain damage, brain edema, and neurological deficits[113]. Additionally, apoptosis is an essential pathological mechanism of TBI[114], and the Bcl-2 protein family plays a crucial role in apoptosis: Bcl-2 has antiapoptotic effects, whereas the Bcl-2-associated protein Bax promotes apoptosis. The regulation of Bcl-2 and Bax expression may be a potential target for TBI treatment[115]. Wang et al.[116] loaded plasmids expressing Bcl-2 and Bax shRNA into AD-Exos and examined their therapeutic effect on a TBI mouse model after intraventricular injection. The results showed that AD-Exos significantly upregulated Bcl-2 protein expression in neurons, silenced the Bax gene, and inhibited neuronal apoptosis, indicating that AD-Exos can not only regulate microglial polarization to inhibit neuroinflammation but also carry exogenous nucleic acid drugs to enhance therapeutic efficacy.

3.6.2. Microglia-derived Exos (MD-Exos)

An increasing number of studies have shown that MD-Exos mediate interactions between microglia and neurons after TBI through many microRNAs (miRNAs). After TBI, the level of miR-124-3p in MD-Exos increases, and miR-124-3p can be transferred to damaged neurons to inhibit excessive neuronal autophagy, resulting in a protective effect against neuronal injury[117]. MiR-124-3p can also promote microglial M2 polarization or directly target PDE4B to inhibit mTOR signaling activity, thus suppressing neuronal inflammation and promoting neurite growth[118]. MD-Exos-derived miR-124-3p can also promote Aβ protein hydrolysis by targeting the hippocampal neuron Rela/ApoE signaling pathway, alleviating post-TBI neurodegenerative degeneration and improving cognition (Fig. 4)[119]. In a study by Zhao et al.[120], MD-Exos overexpressing miR-5121 targeted RGMa to promote neurite growth and synapse recovery, significantly improving motor coordination in TBI mice.

3.6.3. Mesenchymal stem cell-derived Exos (MSC-Exos)

MSC-Exos are widely used in research on nervous system diseases, and MSC-Exos therapies have been proven to improve TBI and restore brain function[121,122]. In models of TBI and hemorrhagic shock, MSC-Exos were found to upregulate genes related

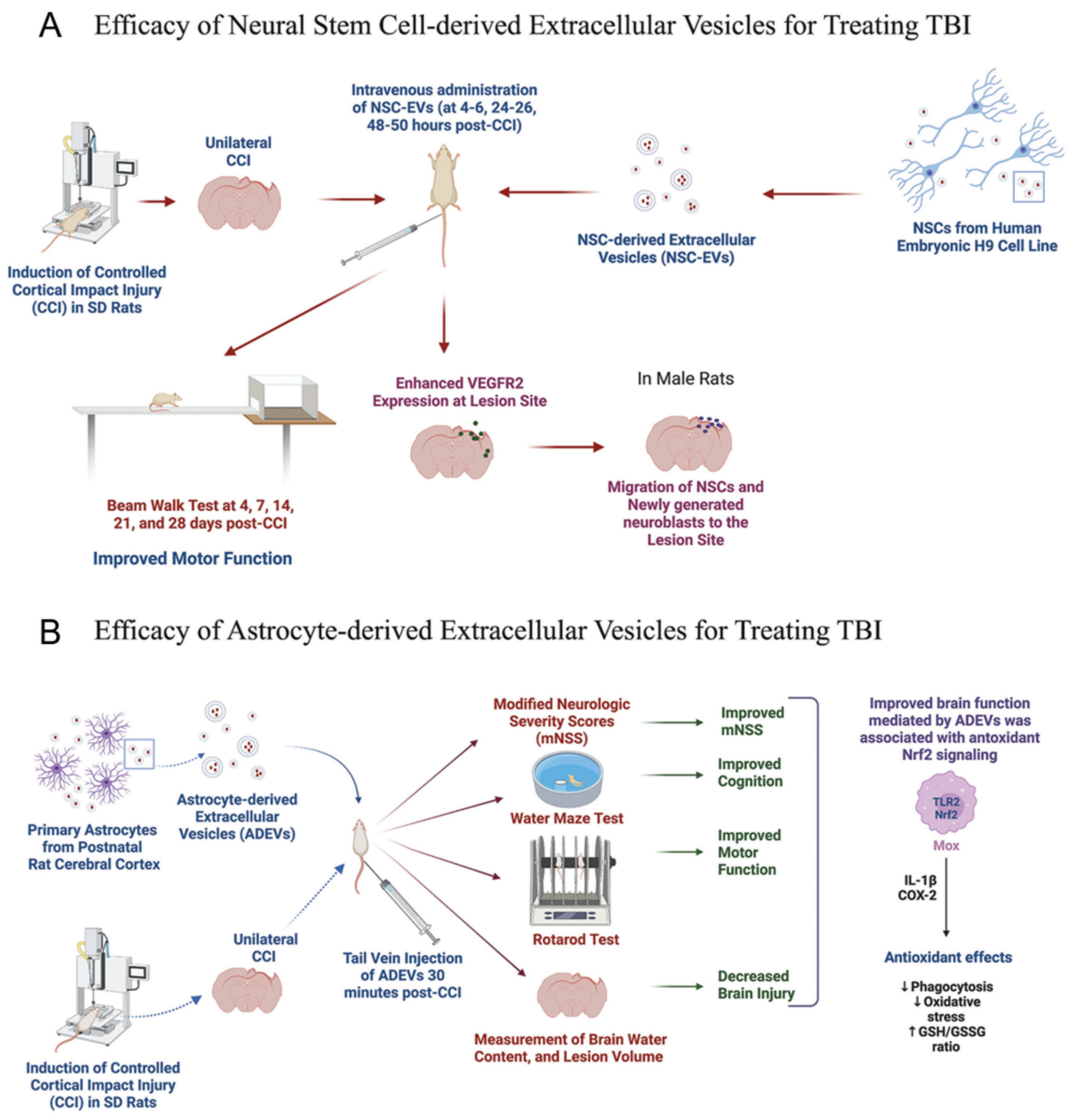

Figure 3 Mechanism of action of EVs in TBI treatment. (A) Schematic diagram of how EVs mediate neural repair and regeneration. After CCI injury, intravenous administration of NSC-EVs increases the expression of VEGF receptor-2 in the brain cortex and promotes the migration of endogenous NSCs and possibly newly generated neuroblast cells to the injury site. (Reprinted with the permission from Ref. 107. Copyright © 2023, Stem Cells Transl Med). (B) Schematic diagram of the mechanism by which EVs inhibit oxidative stress. Early administration of EVs derived from astrocytes after TBI can reduce tissue damage and motor and cognitive deficits by activating the Nrf2 signaling pathway, which has antioxidant effects (Reprinted with the permission from Ref. 107. Copyright © 2023, Stem Cells Transl Med).

to neurogenesis, neural plasticity, synapse formation, and BBB stability while downregulating genes related to neuroinflammation, reactive gliosis, and cell death; moreover, they reduced the brain injury area and swelling, alleviated neural damage, and increased the neural cognitive recovery rate[123]. Other experiments have shown that intravenous injection of MSC-Exos can promote vascular remodeling and neurogenesis and reduce neuroinflammation, somewhat promoting functional recovery in TBI rats[124]. Some of these effects are related to the miRNAs in Exos, such as miR-9, miR-124, and miR-125b, which can regulate key cytokines, such as IL-1β, thereby promoting angiogenesis and neurogenesis[125,126]. After induction by BDNF, MSC-Exos with high expression of miR-216a-5p significantly increased the migration ability of PC12 cells, inhibited cell apoptosis, and promoted sensory-motor function and spatial learning function recovery in TBI rats[127]. Moreover, MSC-Exos can regulate microglial polarization, thereby promoting recovery after TBI. Ni et al.[128] and Zhao et al.[129] showed that MSC-Exos *in vivo* can promote the transition of M1 microglia/macrophages to the M2 phenotype, resulting in reduced secretion of proinflammatory factors and increased secretion of anti-inflammatory and neurotrophic factors. These changes inhibited early neuroinflammation, exerted neuroprotective effects, and significantly improved functional recovery after TBI in mice. MSC-Exos can also noninvasively deliver therapeutic drugs, such as specific miRNAs, to the brain, producing alternative stem cell treatment effects. miR-124 can regulate microglial function under physiological conditions, and Yang et al.[130] constructed pSUPER-miR-124 plasmids to transfect BMSCs and obtained miR-124-loaded Exos, which promoted the M2-type polarization of microglia after tail vein

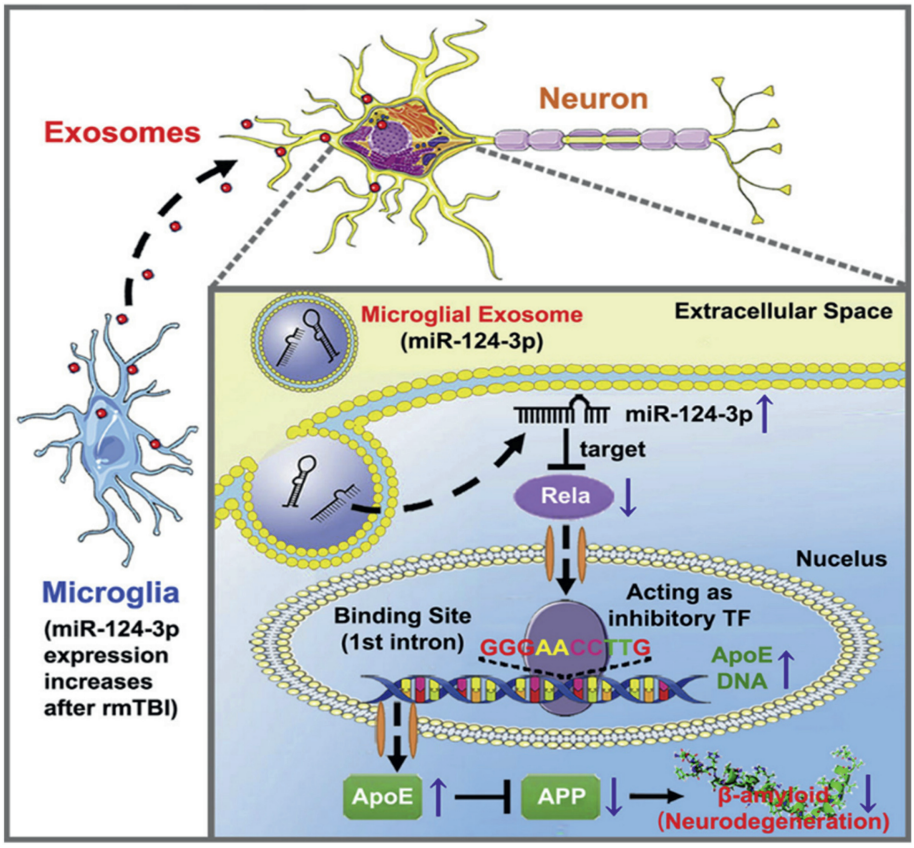

Figure 4 Schematic diagram of the alleviation of neurodegeneration by Exos. The expression of miR-124-3p in microglia increases after TBI and miR-124-3p in Exos is subsequently transferred into hippocampal neurons to alleviate neurodegeneration by targeting the Rela/ApoE signaling pathway (Reprinted with the permission from Ref. 119. Copyright © 2020 Mol Ther).

administration, further improving hippocampal neurogenesis and functional recovery after brain injury.

3.6.4. Neural stem cell-derived Exos (NSC-Exos) and endothelial cell-derived Exos (EC-Exos)

NSC-Exos and EC-Exos can also be used for the treatment of TBI. NSCs have the potential to improve function after TBI[131], and transplanted NSCs mainly promote the regeneration and functional recovery of neural tissue through paracrine factors, such as Exos[132]. Moreover, NSC-Exos have been shown to have neuroprotective effects, significantly reducing neuronal apoptosis, microglial cell activation, and neuroinflammation[133]. NSC-Exos can inhibit the CASP2 signaling pathway and promote neuronal proliferation by transferring miR-150-3p, thus inhibiting neuronal apoptosis after brain injury[134]. Abedi et al.[135] indicated that human NSC-Exos have superior effects than parental cells in terms of sensorimotor functional recovery and neurogenesis after TBI. EC-Exos can significantly promote the growth of neuronal axons, facilitate the formation of endothelial microvessels, and increase the repair and regeneration of injured nerves[136]. These compounds can also induce M2 macrophage polarization. Moreover, Exos secreted by human umbilical cord blood endothelial colony-forming cells can inhibit the expression of *in situ* phosphatase tension protein homolog (PTEN), thereby inhibiting the activation of downstream apoptosis signaling pathways and preventing neuronal apoptosis[137]. In addition, Exos secreted by endothelial colony-forming cells can reduce the degradation of tight junction proteins in TBI mice, which promotes the integrity of the BBB in TBI mice. The Exos for TBI treatment are listed in Table 2[113,116-120,123,124,127,128,130,135,137].

As mentioned previously, stem cells and EVs/Exos have been extensively studied for the treatment of TBI. Stem cells promote regeneration through direct differentiation and paracrine signaling, while EVs primarily exert their effects through the transfer of bioactive molecules. (*e.g.*, proteins, RNAs)[138]. Stem cells possess multipotency and the ability to facilitate comprehensive repair. They can differentiate into various cell types, directly contributing to tissue regeneration, and modulate the injury environment by secreting a broad range of bioactive molecules. In comparison, EVs have a lower risk of immune rejection and tumorigenicity and can be engineered to deliver specific therapeutic molecules to target cells[139]. EVs are also more stable and easier to store and transport, making them more practical for clinical applications[140]. Some studies suggest that Exos may have better therapeutic effects than parental stem cells. Abedi et al.[135] found that NSC-derived Exos have superior effects compared to NSCs in terms of sensorimotor functional recovery and neurogenesis after TBI. Wen et al.[141] demonstrated that BV2 cells transformed into an anti-inflammatory phenotype after co-culture with BMSCs or BMSC-Exos for 48 h. Exos were found to have stronger anti-inflammatory effects compared to BMSCs. The efficacy of EVs varies depending on their cell origin. Yang et al.[142] compared the efficacy of EVs derived from different cell types in the treatment of TBI through a network meta-analysis. The results indicated that

Table 2 The Exos for TBI treatment.

Derived cell	Active ingredient	Recipient	Administration	Function & mechanism	Ref.
Astrocytes	miR873a-5p	Mouse model of TBI	Lateral ventricle injection	MiR-873a-5p attenuated microglia-mediated neuroinflammation and improved neurological deficits by inhibiting the NF-κB signaling pathway	113
	Bcl-2, Bax shRNA	Mouse model of TBI	Intraventricular injection	The modified Exos reduced apoptosis and ameliorated neural and functional deficits	116
Microglia	miR124-3p	Mouse model of TBI	Tail vein injection	Increased miR124-3p inhibited neuronal autophagy and inflammation, contributed to neurite outgrowth, alleviated neurodegeneration, and improved cognitive outcomes	117−119
	miR-5121	Mouse model of TBI	Tail vein injection	MiR-5121 promoted neurite outgrowth and synapse recovery by directly targeting RGMa	120
MSCs		Swine model of TBI and hemorrhagic shock	Left external jugular vein injection	Exos treatment increased gene-associated neurogenesis, neuroplasticity, synaptogenesis, and BBB stability and decreased gene-associated neuroinflammation and reactive gliosis	123
		Rat model of TBI	Tail vein injection	Exos treatment effectively promoted endogenous angiogenesis and neurogenesis and reduced inflammation	124
	miR-216a-5p	Rat model of TBI	Tail vein injection	Increased miR-216a-5p significantly inhibited apoptosis and increased numbers of immature neurons	127
		Mouse model of TBI	Retro-orbital injection	BMSC-Exos inhibited early neuroinflammation by modulating the polarization of microglia/macrophages	128
	miR-124	Rat model of TBI	Tail vein injection	MiR-124 promoted M2 polarization of microglia and improved hippocampal neurogenesis and functional recovery	130
NSCs		Rat model of TBI	Intralesional injection	NSC-Exos promoted sensorimotor functional recovery and neurogenesis after TBI	135
ECs		Mouse model of TBI	Tail vein injection	EC-Exos attenuated the decrease of tight junction proteins, reduced brain edema, and protected the integrity of the BBB	137

TBI, Traumatic brain injury; Exos, exosomes; MSCs, mesenchymal stem cells; BMSC-Exos, bone marrow mesenchymal stem cells-derived Exos; NSCs, neural stem cells; NSC-Exos, neural stem cell-derived Exos; ECs, endothelial cells; EC-Exos, endothelial cell-derived Exos; BBB, blood−brain barrier.

EVs derived from adipose tissue might be the most effective option for early TBI treatment based on the modified neurological severity score. In contrast, EVs derived from MSCs showed superior efficacy in improving neural function and spatial memory during the later stages of TBI. Due to the small number and sample size of the studies included in this analysis, and the uneven quality of the included studies, the results are useful but not entirely reliable.

However, stem cells can consistently release beneficial paracrine factors, whereas EVs have a relatively short half-life and may not maintain sufficient levels in affected areas. This implies that effective treatment with EVs may necessitate longer treatment cycles, more frequent applications, and higher doses of medication[143,144]. In addition, it is important to consider that each experimental modeling method, animal model, administration method, and dosage varies, making direct comparisons unsuitable. To accurately discuss and compare the therapeutic effects of different types of stem cells and EVs/Exos, it is essential to ensure consistency in experimental conditions.

4. Nano-drug delivery systems (NDDSs) for the diagnosis and treatment of TBI

Nanomedicines are normally defined as nanotechnological medicines intended for therapeutic or diagnostic applications with dimensions controlled within the nanoscale range (1−1000 nm)[145]. Many NDDSs prepared from various materials, including lipids, polymers, proteins, metals, and inorganic materials, have been reported over the past few decades[146]. NDDSs effectuate transport across biological barriers by a variety of mechanisms. Actively and passively targeted nanomedicines have been explored as imaging tools, as well as therapeutics, in recent years and have shown impressive preclinical and clinical potential[147].

4.1. Factors influencing the ability of nanoparticles across the BBB

Nanoparticles can carry diagnostic molecules and drugs for targeted delivery to target organs, tissues, cells, or even specific

organelles. For TBI treatment, nanoparticles can transport drugs to injured brain tissue through receptor-mediated or adsorption-mediated endocytosis[148]. However, brain injury involves a series of dynamic pathophysiological processes, such as vascular leakage and neuroinflammation, that cause BBB dysfunction and temporary opening, which can allow nanoparticles to be passively delivered to TBI sites in this time window and to accumulate in the brain parenchyma[149,150]. The delivery of nanoparticles to the brain presents challenges related to circulation and targeting, such as rapid clearance in the blood and nonspecific recognition and uptake by macrophages, leading to inflammation[151]. Moreover, improving brain delivery efficiency is crucial. Nanoparticles can be modified with specific peptides, proteins, or antibodies. After binding to receptors on target cells, these nanoparticles are internalized and can transport drugs inside cells or across cells into damaged brain tissue[8]. Factors such as the physicochemical properties, morphology, size, and ligands modifications of nanoparticles are the primary factors affecting BBB transport and target site accumulation.

4.1.1. Hydrophilic modification of nanoparticles

The hydrophilic modification of nanoparticles can reduce their clearance by the MPS, increasing their stability in the blood circulation. Polyethylene glycol reduces nonspecific affinity between nanoparticles and proteins due to its hydrophilicity and steric hindrance effects, thereby reducing the adsorption of plasma complement[152]. Concurrently, the length and surface density of PEG chains affect the persistence of nanoparticle adhesion to the endothelium and nanoparticle circulation time, which in turn can affect tissue distribution[153].

4.1.2. Targeted ligand modification of nanoparticles

Receptor-mediated transcytosis begins with the binding of ligands to receptors, followed by receptor-mediated endocytosis, which allows ligand-coupled nanocarriers to transport into the brain. Ligand-modified nanoparticles often target receptors or transporters on the surface of BBB endothelial cells, such as lipoprotein receptors[154], transferrin[155], lactoferrin[156], and melanotransferrin receptors[157]. Additionally, the concept of multistage targeted delivery involves decorating the nanoparticle surface with BBB-penetrating ligands and targeting ligands for brain cells such as neurons, not only facilitating the central transport of nanoparticles but also enhancing accumulation in specific brain tissues[158].

4.1.3. Size of nanoparticles

The size of nanoparticles plays an important role in their biological distribution, cellular uptake, elimination, and transport in the CNS[149]. Generally, nanoparticles with a diameter smaller than 200 nm are internalized mainly through clathrin-mediated pathways, while larger particles (>500 nm) are internalized through caveolin-mediated pathways[159]. Furthermore, there is a noticeable negative correlation between the size of nanoparticles and the BBB penetration rate, and nanoparticles approximately 100 nm in size accumulate at higher levels in damaged brain tissue[160].

4.1.4. Surface charge of nanoparticles

The surface charge is another important factor that affects the permeability of nanoparticles through the BBB and is directly related to clearance and stability in the blood circulation. Due to the presence of glycoproteins, BBB endothelial cells are negatively charged, the positively charged nanoparticles can form electrostatic interactions with negatively charged cell membranes, leading to internalization and thus increasing their penetration through the BBB[161]. Additionally, the charge of nanoparticles can affect the formation of protein coronas on their surface; most positively charged nanoparticles and some negatively charged and neutral nanoparticles can bind with blood proteins, affecting the receptor-mediated transcytosis of nanoparticles through the BBB[162].

4.1.5. Shape of nanoparticles

The shape of nanoparticles is important for their cellular uptake and internalization. The most common shape is circular or spherical, and these particles can be rapidly internalized by cells. Nanoparticles of other shapes (rod-like, cubic, and discoidal) are receiving increased amounts of attention due to their cellular internalization and drug loading efficiency[163]. Rod-like nanoparticles exhibited stronger adhesion than spherical nanoparticles under both static and flow conditions, resulting in higher internalization by cerebral vascular endothelial cells. In addition, discoidal, cubic-cage, and star-shaped nanoparticles demonstrated shape-dependent biodistribution and cellular binding[164], which could potentially increase BBB penetration.

4.2. Inorganic nanomaterials for the diagnosis and treatment of TBI

Oxidative stress plays a key role in the pathogenesis of TBI[165]. Due to their unique physicochemical properties and outstanding ability to regulate oxidative stress, inorganic nanomaterials have been used in the diagnosis and treatment of TBI; examples include carbon-based nanomaterials[166-170] and metallic nanoparticles[171-173]. In addition to exploiting the inherent properties of these inorganic nanomaterials, researchers have used functional group modification to introduce multiple new functions, such as active transport across the BBB, improved antioxidative stress efficiency, and the ability to reduce neuronal apoptosis[174].

4.2.1. Carbon-based nanomaterials

Carbon nanotubes (CNTs) are a type of three-dimensional (3D) carbon-based nanomaterial with good cell membrane penetration ability, high drug loading capacity, high specific surface area, and easy modification[175]. Kafa et al.[176] reported that amino-functionalized multiwalled carbon nanotubes (MWNTs) could pass through brain endothelial cells by energy-dependent endocytosis. Five minutes after intravenous injection in mice, MWNTs were distributed in the cerebral capillaries and brain parenchyma. Ischemia is a crucial mechanism of secondary injury in TBI, and the activation of Caspase-3 is associated with post-TBI brain tissue loss and cell apoptosis. Functionalized carbon nanotubes (f-CNTs) prepared by introducing ammonium groups into MWNTs through 1,3-dipolar cycloaddition can increase the transfection efficiency of Caspase-3 siRNA, reducing Caspase-3 levels by 56.0%, and repeated neuroprotective effects can be achieved with a single dose[177]. Additionally, polyethylene glycol-functionalized hydrophilic carbon clusters (PEG-HCCs) are a type of non-cytotoxic, biocompatible carbon-based nanoparticle that can quench superoxide anions and simultaneously scavenge ROS by efficiently loading superoxide dismutase, thereby alleviating the reduction in cerebral blood flow accompanying TBI[178]. Marcano et al.[170] combined PEG-HCCs with P-selectin antibodies to rapidly target damaged brain endothelial cells for the treatment of TBI-related vascular dysfunction. PEG-HCCs were shown to

quickly restore brain perfusion and oxidative balance in a TBI model, almost entirely preventing motor dysfunction and reducing lesion size by 61%[179].

Carbon dots (CDs) are a type of carbon-based nanoparticle with high water solubility, desirable chemical inertness, and advantageous functionalization ability. Seven et al.[166] prepared GluCDs from D-glucose, and their uptake mechanism depended on glucose transport proteins. After passing through the rat BBB, GluCDs were distributed in the gray matter, which was consistent with neuronal aggregation, revealing the neuronal targeting ability of GluCDs. GluCDs exhibit significant potential as drug delivery carriers for the treatment of neurodegenerative diseases and brain trauma. Free radical-induced oxidative damage and nitrosative stress are key factors in the post-TBI neuroinflammatory response and are closely related to ROS and reactive nitrogen species. Ouyang et al.[168] reported that antioxidant CDs efficiently scavenged highly active NO· and $ONOO^-$, thereby reducing ROS levels and lipid peroxidation, enhancing superoxide dismutase activity and glutathione levels, and increasing survival in a mouse model of TBI. Researchers have also prepared L-lysine polymerized peroxidase-like CDs, which effectively promoted cell vitality in the presence of hydrogen peroxide (H_2O_2)/lipopolysaccharide through H_2O_2 decomposition, thus supplying oxygen, simultaneously scavenging free radicals, and easing oxidative stress and the inflammatory response in mice[180].

Furthermore, carbon quantum dots can be used to diagnose mild traumatic brain injury (mTBI). Due to the lack of reliable biomarkers and detection methods, the accurate early identification of mTBI is difficult. S100 calcium-binding protein-β (S100-β), which is mainly produced by astrocytes in the CNS, is an essential early biomarker for mTBI. Researchers have used S100$-\beta$ peptide-modified polymer carbon quantum dots (pep-CPDs) for the early detection of molecular markers of mTBI, and the results demonstrated good *in vivo* imaging ability in mice. Compared to traditional detection methods, the pep-CPDs system achieved sensitive and rapid characterization for mTBI diagnosis[181].

4.2.2. Metals and metal oxides

Au nanoparticles, a type of colloidal nanoparticle, are known for their surface plasmon resonance properties and are used for imaging and biosensing. TBI is associated with elevated levels of neuron-specific enolase (NSE) and S100-β protein in the blood. Thus, measuring these factors can facilitate the preliminary diagnosis of TBI. Hollow gold nanoparticles (HAu-nanoparticles) containing highly active groups, such as the redox molecule 4-mercaptobenzoic acid (4-MBA) and Nile blue A (NBA), can bind to antibodies and generate signals. Wang et al.[171] synthesized HAu-nanoparticles@4-MBA and HAu-nanoparticles@NBA as probes, building a more sensitive Raman scattering immunosensor for which the lower detection limits for NSE and S100-β were 0.1 and 0.06 ng/mL, respectively; thus, these probes showed great value in the early diagnosis of TBI. The plasma level of ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) is significantly higher in TBI patients than in healthy individuals; thus, UCH-L1 is a biomarker for TBI diagnosis[173]. Singh et al.[172] leveraged the surface plasmon resonance of Au nanoparticles to develop a rapid method to detect UCH-L1, with a detection limit of 0.5 ng/mL and sensitivity and specificity reaching 100%. This method can serve as a basis for judging the severity of TBI. Zhang proposed gold particles with enzyme-like activity for early intervention in TBI. Ultrasmall gold clusters exhibit a H_2O_2 decomposition rate that is 10 times greater than that of glassy carbon electrodes, thus demonstrating good catalytic activity. Reducing excess superoxide radicals ($O_2^{-\cdot}$) and H_2O_2 in the body alleviates the oxidative stress caused by TBI[182].

mTBI and free radicals: mTBI can lead to abnormal free radical generation related to oxidative stress, secondary injury signaling cascades, and mitochondrial dysfunction. Current antioxidants can clear only single free radicals and are quickly degraded, while more effective antioxidants that maintain activity over time are needed to alleviate the chronic high-level oxidative stress induced by mTBI.

Cerium and cerium oxide nanoparticles (CeO-nanoparticles): Cerium, a lanthanide rare earth element with multiple valence states, provides high redox ability in its oxide form due to "holes" or oxygen defects in the matrix. Regenerable CeO-nanoparticles can sustain the survival of brain cells and mixed neuron-organ cultures and can maintain normal calcium signal transmission. In a rat mTBI model, CeO-nanoparticles preserved the endogenous antioxidant system, reduced free radical damage to macromolecules, and improved cognitive function[183]. Youn et al.[184] studied CeO-nanoparticles of two different shapes: cerium oxide nanorods (Ceria NRs) and cerium oxide nanospheres (Ceria NSs). Both Ceria NRs and Ceria NSs lowered the levels of ROS and reduced the mRNA expression of superoxide dismutase, irrespective of nanoparticle shape. These compounds significantly reduced oxygen free radical levels in SH-SY5Y cells, with Ceria NRs showing stronger anti-inflammatory effects than Ceria NSs, which is likely related to the impact of nanoparticle morphology on cellular uptake.

4.3. Organic nanoparticles for the diagnosis and treatment of TBI

4.3.1. Polymer nanoparticles

Polymer nanoparticles (PNs) consist of natural or synthetic polymers. The main preparation materials include PLGA, proteins, polysaccharides, polycaprolactone, and PEG, among others, all of which exhibit excellent biodegradability. They can encapsulate small-molecule drugs, peptide or protein drugs, gene therapy drugs, etc. Due to the broad selectivity and chemical modification characteristics of polymers, binding to receptors or target molecules can be achieved, allowing targeted delivery to the brain[185]. The BBB is damaged in the acute phase of TBI, leading to increased permeability, and the enhanced permeation and retention effect provides opportunities for the passive accumulation of nanoparticles. To understand the physicochemical properties that facilitate optimal uptake and retention kinetics in TBI, researchers compared the accumulation and retention kinetics of three different-sized nanoparticles with different relaxation characteristics in a CCI mouse model of TBI. They found that 80 nm PLGA nanoparticles injected 3 h after injury had the highest permeation coefficient in TBI, exhibiting greater accumulation kinetics than did the small molecule Gd-DTPA; thus, optimizing the administration time and nanoparticle physicochemical properties can maximize delivery[186]. Additionally, polymer nanosystems with imaging mechanisms have been widely applied in TBI diagnosis. Cruz et al.[187] prepared PEG-PLGA nanoparticles and coupled the amino-terminal groups of PEG to the near-infrared fluorescent dye IRDye-CW800. *In vivo* optical imaging showed that, compared to 800 nm PLGA nanoparticles, smaller 100 nm PLGA nanoparticles exhibited better penetration in a mouse model of TBI. Upon further encapsulating IRDye-CW800

with perfluorocarbon in PLGA nanoparticles, the necrotic area of TBI could be specifically visualized in mice through optical imaging and fluorine magnetic resonance imaging (^{19}F MRI), enabling qualitative optical monitoring of TBI and quantitative 3D information on deeper tissue lesions through MRI[188].

Polyester nanoparticles can deliver drugs to endothelial cells, thus improving neurological function; for example, polyester nanoparticles can deliver drugs that reduce ROS and maintain BBB integrity to protect neural tissue[189]. In a tissue-engineered cerebral endothelial model, the expression of cell adhesion molecules (E-selectin) was upregulated in response to shockwave-induced damage. P-selectin glycoprotein ligand-1 (PSGL-1) is a glycoprotein on endothelial cells that can bind to selectin family members (P-selectin, E-selectin, and L-selectin). Researchers encapsulated poloxamer 188 and the antioxidant *N*-acetylcysteine in poly(lactic-*co*-glycoside) (PLGA) nanoparticles and modified them with PSGL-1 for the specific targeting of damaged tissues; this system successfully repaired tissue structure, functional integrity, and endothelial cell damage[190]. Other researchers prepared carboxylated PLGA (PLGA-COOH) immunomodulatory nanoparticles that bind to monocyte-macrophage receptors, inhibiting monocyte movement to inflammatory sites; this system reduced the proinflammatory polarization of macrophages and alleviated brain tissue edema, thereby increasing the recovery of visual and motor functions in mouse models[191]. One advantage of PNs is their ability to encapsulate large molecular drugs for targeted delivery. Neurotrophic factors regulate neuronal plasticity and promote neuron growth, proliferation, and survival[192]. By encapsulating neurotrophic factors with PNs, problems such as a short half-life and low BBB permeability can be improved. Wang et al.[193] prepared polybutylcyanoacrylate nanoparticles and delivered β-NGF to rat TBI sites. β-NGF was found to maintain the neurotrophic activity that promotes neurite growth, thereby reducing the mortality rate of TBI rats. Cerebrolysin is a neuroprotective peptide that can stimulate neurological recovery to enhance the self-repair ability of the brain, but has drawbacks such as a short half-life and poor stability. Ruozi et al.[194] prepared PLGA nanoparticles carrying cerebrolysin to address these issues, and administering these nanoparticles 4 h after rat brain injury reduced TBI-induced cerebral edema and BBB structural damage, providing neuroprotection. Yuan et al.[195] designed and synthesized composite nanoparticles (PMNT/F@D-nanoparticles) with good biocompatibility by combining the conjugated polythiophene derivatives PMNT and fullerenol, thus integrating inflammation microenvironment regulation and cell differentiation therapy (Fig. 5). On the one hand, the PMNT/F@D-nanoparticles reduced the percentage of apoptotic cells from 63.2% to 26.0% by decreasing cellular ROS levels, thus protecting neurons from ischemic injury; on the other hand, the PMNT/F@D-nanoparticles effectively promoted PC12 cell proliferation and differentiation, providing dual-target protection for brain injury treatment.

siRNA can efficiently and specifically silence the expression of target genes. Li and others[196] prepared PLGA nanoparticles for siRNA delivery and examined the effects of polysorbate 80 (PS 80), poloxamer 188 (Pluronic F-68), DSPE-PEG-glutathione (GSH), and DSPE-PEG-transferrin (Tf) surface coatings on BBB transport. The surface coating of nanoparticles and its density affect nanoparticle uptake by neural cells and the resulting gene silencing efficiency. When administered during the early or late stages of brain injury (corresponding to a physically damaged BBB and an intact, self-repairing BBB), PS 80-modified nanoparticles reduced tau protein expression by 50%, while the accumulation of nanoparticles in the injury site was increased threefold[196]. Macks and others[197] used the cationic amphiphilic copolymer poly(glycolide-*co*-ε-caprolactone)-polyethylenimine (PgP) to deliver siRNA that silences the RhoA/Rho-associated

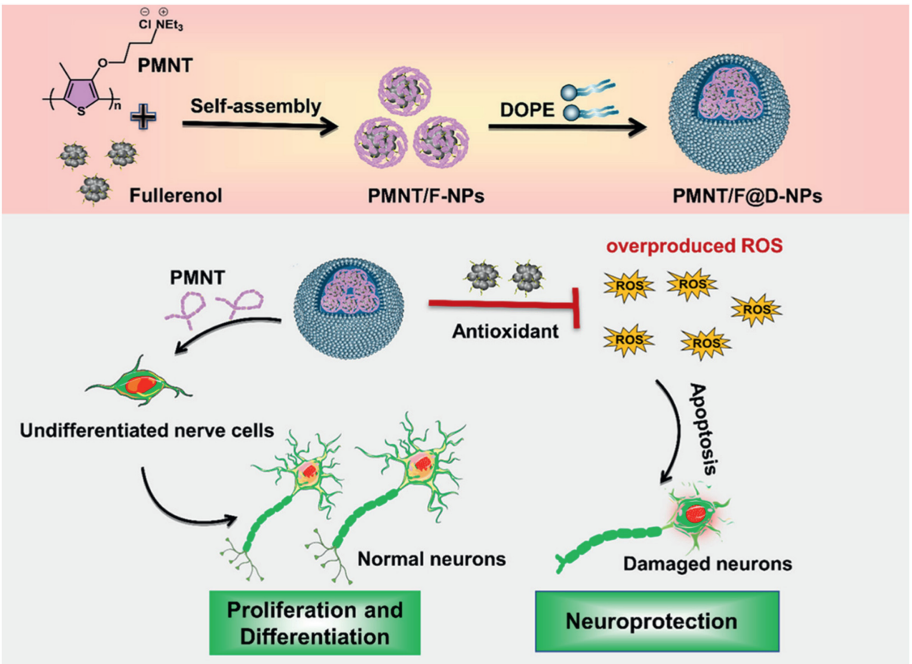

Figure 5 PMNT/F@D-nanoparticles reduced reactive ROS accumulation in the cellular microenvironment, inhibited cell apoptosis, and simultaneously promoted PC12 cell proliferation and differentiation, thereby improving the microenvironment of the injured area to protect nerve cells from ischemic damage (Reprinted with the permission from Ref. 195. Copyright © 2022, J Am Chem Soc).

protein kinase signaling pathway, thereby reducing RhoA expression in a rat TBI model. PgP also reduced astroglial proliferation and inflammation, promoted axonal regeneration and retention, increased the neuronal survival rate in the ipsilateral cortex, and decreased secondary damage[197]. In addition, PgP can be used to deliver the phosphodiesterase inhibitor rolipram to brain injury sites, restoring the local levels of cyclic adenosine monophosphate to near the levels in the control group. This system reduced injury site volume, neuroinflammation, and cell apoptosis and improved motor function 7 days after TBI, effectively treating acute mild TBI[198].

4.3.2. Protein nanoparticles

Nanoparticles based on natural proteins such as albumin and lipoproteins exhibit high biocompatibility and drug loading capabilities. Drugs can be covalently bound within the spatial structure of the protein or adsorbed onto the matrix structure through physical interactions. Lipoproteins are natural particles formed by the aggregation of triglycerides, phospholipids, and cholesterol esters. Chen et al.[199] designed TBI-targeting lipoprotein nanoparticles loaded with cyclosporine A and modified them with the cell-penetrating peptide MMP-9. This system can accumulate at MMP-9-overexpressing TBI lesion sites, effectively entering target cells, binding to the mitochondrial membrane, and aggregating at TBI lesion sites while reducing neuronal injury, alleviating neuroinflammation, and improving memory deficits. In addition to lipoproteins, nanomedicine based on albumin has been found to be successful in clinical applications. Albumin nanoparticles prepared through chemical crosslinking or the reduction of albumin at high temperature can deliver metal ions, insoluble small molecules, and nutrients to tissues in organs including the brain[200]. In the treatment of TBI, the intracerebral differentiation and migration of NSCs are vital for various physiological and pathological processes in the CNS. SDF-1α is a chemotactic factor that can recruit NSCs, and albumin nanoparticles carrying SDF-1 increase the bioactivity of SDF-1α, promoting NSC migration and thus improving the pathological condition of TBI[201]. Furthermore, Wu et al.[202] encapsulated the *N*-methyl-D-aspartate receptor inhibitor Tat-NR2B9c in CCAQK peptide-modified albumin nanoparticles, which significantly reduced Tat-NR2B9c cytotoxicity and increased CNS drug transport, increasing the transport efficiency across the BBB from 10% to 30%. Recombinant human erythropoietin (rh-EPO) can exert neuroprotective effects by inhibiting cell apoptosis, promoting vascular regeneration, and reducing cerebral edema. Xue et al.[203] prepared rh-EPO-loaded albumin nanoparticles using electrostatic spraying technology and modified them with Tween 80, which promoted carrier passage through the BBB to the brain injury site *via* low-density lipoprotein receptor-mediated endocytosis. At a concentration of 5000 IU/kg, the water content in the area of brain edema was reduced by nearly 10% compared to that in the control group.

4.3.3. Lipid nanoparticles

Lipid nanoparticles (LNPs), including liposomes, solid LNPs, nanostructured lipid carriers, and cationic lipid-nucleic acid complexes, are among the most widely used nanoparticle platforms. They can transport hydrophobic or hydrophilic molecules, proteins, and large nucleic acids[204]. For the treatment of brain injuries, liposomes can be targeted to inflammatory sites through surface ligand modification, thus increasing the local drug concentration while reducing toxicity. Researchers studied the intravenous injection of coumarin 153 and rhodamine B dual-fluorescently labeled liposomes. In experimental mice, the ipsilateral cortex fluorescence signal increased 1.2- to 1.9-fold compared to that in the contralateral cortex, and heightened fluorescence was also observed in the damaged hippocampal tissue. Liposomes accumulated at the injury site in a CCI injury mouse model[205].

Mitochondrial dysfunction is critical for secondary injury in TBI, and excessive ROS production leads to mitochondrial oxidative stress, membrane potential changes, and reduced ATP production, resulting in mitochondrial structural and functional damage that lead to apoptosis. Targeted delivery of drugs to protect mitochondrial function is an effective method for preventing further cascading events and cell apoptosis. To scavenge and deactivate ROS, Tarudji et al.[206] developed antioxidant thioether-crosslinked LNPs, which reduced the secondary spread of injury in a mouse model of mild CCI and improved spatial memory and learning ability in CCI mice. In brain injury sites, excessive ROS production and Ca^{2+} influx are vital factors leading to secondary injury in TBI. Researchers designed a lipid polymer modified with a cysteine−alanine−glutamine−lysine (CAQK) peptide to deliver nimodipine to the brain. This modification increased nimodipine accumulation at the injury site, which inhibited Ca^{2+} influx in neurons, reduced ROS-mediated neurodegeneration in surrounding tissues, and effectively prevented the spread of secondary injury in a mouse TBI model[207].

4.3.4. Dendritic macromolecules

Polyamidoamine (PAMAM) dendrimers are large-molecule polymers with 3D hyperbranched structures. They consist of three main structural domains: a core, dendritic branch units, and surface-active groups. With their branched structure, dendritic polymers have smaller particle sizes (1−10 nm) than linear polymers (10−500 nm), and they are highly flexible in terms of modifications, enabling broad application in gene therapy, drug delivery, imaging and diagnosis[208].

Neutral hydroxyl-terminated PAMAM dendrimers (PAMAM−OH) show promise as candidates for drug delivery applications due to their low cytotoxicity and good biocompatibility. Without the attachment of targeting ligands, 4th- and 6th-generation PAMAM−OH can effectively cross the damaged BBB, specifically targeting sites of brain injury-related inflammation when administered systemically[209]. PAMAM can transport anti-inflammatory drugs to the brain, where they activate microglia and astrocytes while reducing dose-dependent side effects and improving the pharmacokinetic characteristics of the drugs. Moreover, surface amine-functionalized G4-polyamide nanoparticles injected into mouse ventricles can penetrate brain tissue, selectively localize to neurons, and inhibit the activation of microglia in the M1 cortex in mice injected with lipopolysaccharides, thereby reducing the central toxicity of PAMAM[210].

TBI can lead to mitochondrial damage, resulting in oxidative stress, apoptosis, and reduced energy production, thereby impairing neural function[211]. Sharma et al.[212] developed a mitochondria-targeting dendritic molecule modified with triphenylphosphine (TPP), TPP-D-NAC, for a rabbit TBI model. After intravenous administration, TPP-D-NAC showed significantly greater colocalization with mitochondria than unmodified TPP dendrimers did. The overall level of cellular uptake remained unchanged, confirming the ability of TPP-D-NAC to cross the BBB and target glial cells. TPP-D-NAC administration reduced TBI-induced oxidative stress-induced neuroglial cell death; when TPP-D-NAC was administered at a dose of 10 μg/mL, the

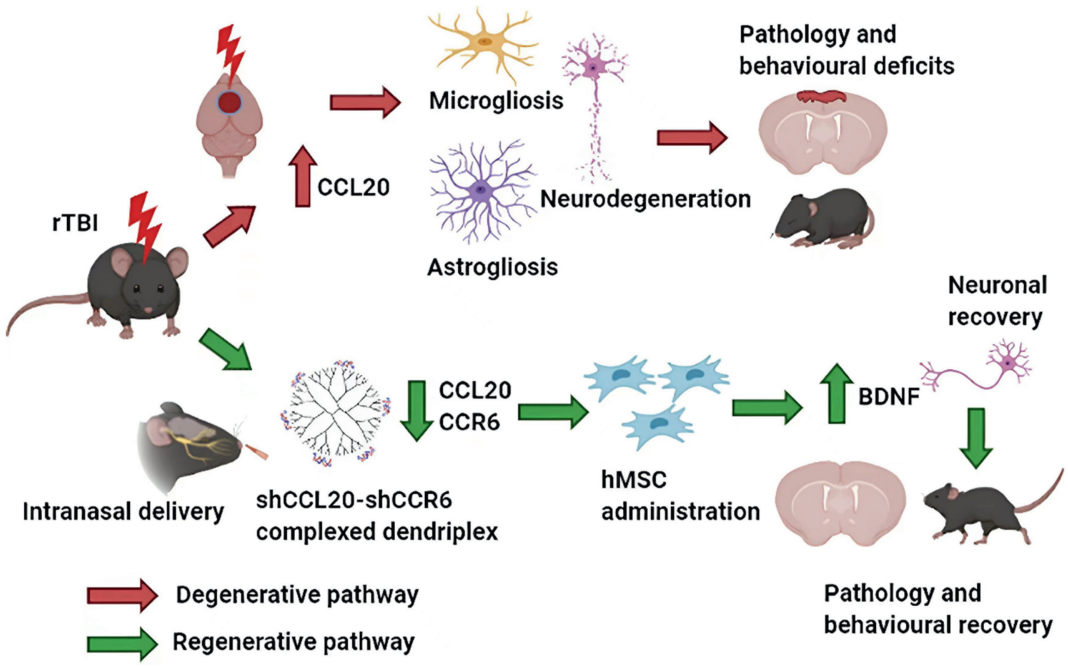

Figure 6 Schematic illustration of the manufacturing and *in vivo* experimental process of dendritic polymers. PAMAM nanodimers effectively delivered plasmid DNA encoding CCL20 and/or CCR6 to the brain and spleen, and treatment with shCCL20−CCR6 nanodimers reduced rTBI-induced neuroinflammation; combined treatment with shCCL20−CCR6 nanodimers and hMSCs increased BDNF expression and more effectively reduced TBI-related damage (Reprinted with the permission from Ref. 213. Copyright © 2020, Nanomedicine).

mitochondrial superoxide levels were only 10% greater than those in normal cells.

Sharma et al.[209] constructed a potent anti-inflammatory and antioxidant drug delivery carrier using sinomenine with 4th generation PAMAM dendrimers (D-Sino), which alleviated early/acute inflammation by inhibiting proinflammatory cytokines (TNF-α, IL-1β, CCL-3, and IL-6) and had a more pronounced effect on NF-κB activation and nuclear transcription than did free drugs. Dendritic macromolecules can also be combined with stem cell therapy to improve the efficacy of MSCs transplantation after brain injury. Mayilsamy et al.[213] used dendritic molecules for the targeted delivery of shRNAs that silence the inflammatory chemokine receptor CCR6, thereby building stable dendritic complexes that enhance gene transfection efficiency through a "proton sponge effect" in lysosomes (Fig. 6). This effect alleviated inflammation at TBI sites, and when combined with subsequent hMSCs transplantation, this system downregulated the expression of brain cortex inflammation markers, increased BDNF expression, and improved neurogenesis and behavioral defects caused by brain injury.

4.3.5. Biomimetic nanoparticles

Brain-targeted nanomedicine still presents many key problems that urgently need to be addressed, such as preventing the clearance of exogenous nanoparticles by the MPS *in vivo*, increasing the efficiency of targeting through the BBB, and increasing the efficiency of targeting to specific brain regions (Fig. 7)[7]. Bionic nanomedicine systems are a type of drug delivery system designed to mimic or utilize endogenous biological materials, including cells, EVs, and hybrids made from engineered cell membranes and exogenous carrier materials. These carriers have good biological membrane transport characteristics, high biocompatibility, and specific targeting mechanisms. By mimicking the physiological characteristics of biological structural units within living tissues and through structural modification and transformation of the carriers, these systems not only retain the physicochemical properties of nanomaterials but also inherit the advantageous functions of the source cells. These advantages allow the delivery of diagnostic agents, small-molecule drugs, nucleic acids, and genes to the target site, thereby achieving good targeting effects[214]. Therefore, biomimetic nanoparticles wrapped by cell membranes can be designed, and biomimetic nanoparticles can be prepared by including cell membrane layers or cell-derived nanovesicles or by simulating the physical properties of cells. These biomimetic nanoparticles can then penetrate the BBB to travel to the injury site for TBI treatment[215]. For example, Sun attached the C3 peptide, which specifically targets neuron surfaces, and the SS31 peptide, which targets the inner mitochondrial membrane, to the surface of red blood cell membranes and loaded the PARP inhibitor olaparib (Ola) into a nanolipid carrier to create a composite biomimetic nanomedicine (C3/SS31-RBCNLCs-Ola) to target brain neuron mitochondria. The results showed that the biomimetic carrier delivered high concentrations of Ola to brain mitochondria, effectively improving mitochondrial function, reducing the percentage of apoptotic cells from 54.6% to 17.45% *in vitro*, and reducing the area of brain injury from 22.8% to 8.35% *in vivo*, thereby preventing neuronal cell death caused by excessive activation of mitochondrial PARP (mt-PARP) due to injury[216]. In addition to red blood cell membranes, leukocyte membranes are also commonly used to wrap nanoparticles for use as biomimetic carriers. Leukocytes have been shown to home to inflammatory sites and adhere to inflamed endothelial cells, making them an effective tool for targeted drug delivery to TBI, where increased expression of vascular adhesion molecules and chemokines lead to early leukocyte recruitment[215]. Assaf Zinger extracted membrane proteins from leukocytes and integrated them

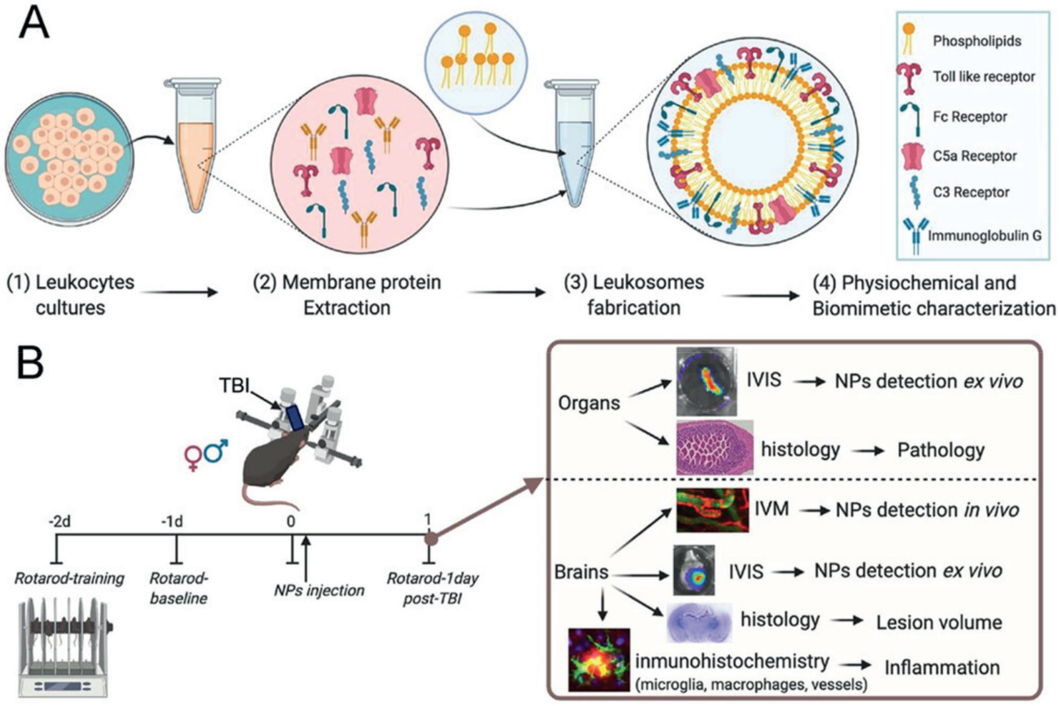

Figure 7 Schematic of the manufacturing and characterization of leukocyte nanoparticles and *in vitro* and *in vivo* experiments. Leukos were prepared by extracting membrane proteins from cultured leukocytes, characterized for their physicochemical and biomimetic properties, and then evaluated for targeting in a TBI mouse model through an *in vivo* imaging system and intravital microscopy as well as immunohistochemical techniques (Reprinted with the permission from Ref. 7. Copyright © 2021, Adv Funct Mater).

into liposomal membranes to obtain engineered leukocyte bodies (Leukos) for use as therapeutic diagnostic tools in TBI. By targeting endothelial cells with CD11b and preventing uptake by the MPS through CD47, Leukos achieved 24-h circulation *in vivo* and increased accumulation in brain tissue by 1.5 times compared to that of unmodified liposomes, thus facilitating diagnosis and reducing lesion size[7]. Further optimizing the ratio of lipids to leukocyte membrane proteins showed that the accumulation of leukocytes with a membrane protein:lipid ratio of 1:20 at inflammatory sites was increased 2.7-fold[217]. Nano-drug delivery systems for the diagnosis and treatment of TBI are listed in Table 3[7,166,179,182-184,188,194,197,199,201,207,209,212,213,216,217]. In TBI, the interaction between neutrophils and endothelial cells in brain blood vessels mediates the excessive production of oxygen free radicals, playing a significant role in ischemia/reperfusion injury[218]. Fang et al.[219] took advantage of the inflammatory recruitment of neutrophils by preparing neutrophil membrane-coated mesoporous Prussian blue nanoenzymes. By clearing ROS, they reduced neutrophil recruitment, altered microglial cell polarization, and reduced neuronal apoptosis, showing potential for targeted treatment of ischemic brain injury.

5. Bioscaffolds for TBI treatment

Tissue engineering, proposed by Langer and Vancanti[220], refers to the use of bioactive substances to reconstruct or repair organs and tissues through *in vitro* cultivation or construction techniques. Through multidisciplinary and multifaceted research, researchers have developed a series of innovative tissue engineering strategies, including the integration of biomaterials with stem cells, the engineering of cell scaffolds using physical or chemical methods to mimic the growth-supporting cellular microenvironment, and collaboration between cells and bioactive molecules[221]. The basic method of biomaterial scaffolding is *in vitro* culture of high concentrations of tissue cells on biocompatible biomaterials to obtain implants that can be placed at defect sites, where they are gradually degraded and absorbed to repair defects and restore functions. The combination of cell therapy and biomaterial scaffolding can provide physical support for transplanted cells, and the scaffolds can provide a microenvironment conducive to cell infiltration and differentiation. These scaffolds not only support surrounding nerve tissue but also serve as the matrix for cell growth, neurite formation, and axon regeneration. Scaffolds can also carry bioactive molecules that create a relatively stable, permeable, and nutrient-rich environment for regeneration[222]. Differences in the charge, hardness, and surface morphology of the biomaterials used for cell culture result in varying effects on cell adhesion, differentiation, and growth.

Biomarkers are biological indicators that can be measured to assess physiological or pathological processes or responses to therapeutic interventions. In the last decade, numerous biomarkers have been identified for accurately gauging the severity of neural injury and neurodegenerative progression, including proteomic biomarkers[223], EVs[224], miRNAs[225], cellular metabolites[226] and so on. In the context of bioscaffold-based treatments, biomarkers are crucial for monitoring the efficacy and progression of tissue engineering and regenerative medicine strategies. Based on the literature, several potential biomarkers can be utilized for this purpose. The potential biomarkers for monitoring the efficacy of bioscaffold-based treatments are listed in Table 4[227-239].

Table 3 Nano-drug delivery systems for the diagnosis and treatment of TBI.

Type of nano drug delivery systems	Loading agent	Recipient	Administration	Function & mechanism	Ref.
Carbon quantum dots	−	Rat model of TBI	Tail vein injection	Was transported across the BBB by the glucose transporter and accumulated in neurons	166
Carbon nanotube	−	Rat model of TBI	Tail vein injection	Ameliorated the reduction of cerebral blood flow associated with TBI, reduced oxidative stress and inflammatory responses in mice, quenched superoxide anions, and scavenged ROS	179
Gold nanoparticles	−	Mouse model of TBI	Tail vein injection	Reduced excessive superoxide radicals ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) in the body and reduced oxidative stress caused by TBI	182
Cerium oxide nanoparticles	−	Rat model of TBI	Tail vein injection	In the aftermath of mild to moderate TBI, lessened cell death and damage	183,184
PEG-PLGA nanoparticles	IRDye 800CW	Mouse model of TBI	Tail vein injection	Using optical imaging and ^{19}F MRI, the necrotic region of the TBI location was specifically identified	188
PgP nanoparticles	siRNA	Mouse model of TBI	Intraparenchymal injection	Silencing of the RhoA/Rho-associated protein kinase signaling pathway by siRNA reduced RhoA expression, reduced astrocyte proliferation and inflammation, and promoted axonal regeneration and retention	197
PLGA nanoparticles	Cerebrolysin	Rat model of TBI	Tail vein injection	Improved the neuroprotective effect of cerebrolysin on TBI	194
Lipoprotein nanoparticles	Cyclosporine A	Mouse model of TBI	Tail vein injection	Successfully decreased neuronal damage and inflammation, corrected mitochondrial dysfunction *in vitro*, and restored memory impairments	199
Albumin nanoparticles	Stromal cell-derived factor-1 (SDF-1)	Rat model of TBI	Tail vein injection	Maintained SDF-1α activity and improved NSC migration	201
CL-PPS nanoparticles	Nimodipine	Mouse model of TBI	Tail vein injection	Inhibition of Ca^{2+} influx in neurons reduced ROS-mediated neurodegeneration in surrounding tissues	207
PAMAM-OH	shRNA	Mouse model of TBI	Intraparenchymal, intraventricular and subarachnoid injection	In the M1 cortex of mice injected with lipopolysaccharide, selective localization of neurons by endosomal uptake and suppression of microglial activation were observed	213
G4-PAMAM	Sinomenine	Rabbit model of TBI	Intravenous injection	Inhibition of pro-inflammatory cytokines (TNF-α, IL-1β, CCL-3 and IL-6) reduced early/acute inflammation	209
TPP-PAMAM	*N*-Acetyl cysteine	Rabbit model of TBI	Intravenous injection	Reduced TBI-induced oxidative stress-induced glial cell death	212
Erythrocyte membrane biomimetic nanoparticles	Olaparib	Mouse model of TBI	Tail vein injection	Minimized degenerative alterations of cortical and hippocampal neurons and prevented neuronal cell death brought on by damage-induced mt-PARP overactivation was prevented both *in vitro* and *in vivo*	216
Leukocyte biomimetic nanoparticles	−	Mouse model of TBI	Retroorbital injection	Leuko nanoparticles contributed to the diagnosis of inflammation at the site of TBI and reduced the size of the lesion	7,217

−, not applicable. ROS, reactive oxygen species; PEG-PLGA, polyethylene glycol−poly lactic acid-*co*-glycolic acid; PAMAM-OH, hydroxyl terminal poly(amidoamine) dendrimer; TPP, triphenyl-phosphonium; G4-PAMAM, generation 4 of polyamidoamine dendrimer; CCL-3, chemokine (C−C motif) ligand 3.

Table 4 The potential biomarkers for monitoring the efficacy of bioscaffold-based treatments.

Category	Biomarker	Biological function	Indication of scaffold	Ref.
Inflammatory markers	CRP	CRP serves as an indicator of various infectious and non-infectious inflammations and is a non-specific marker of systemic inflammation	Indicates the degree of inflammation and the body's immune response to the scaffold implantation	227
	Interleukins (*e.g.*, IL-6, IL-10)	IL-10 is an anti-inflammatory cytokine. IL-6 is a pro-inflammatory cytokine and one of the earliest markers to elevate during the onset of inflammation		228-230
Angiogenesis markers	VEGF	VEGF is a highly specific pro-angiogenic growth factor that promotes the migration, proliferation, and formation of vascular endothelial cells.	Demonstrates the scaffold's ability to promote vascular regeneration	231
	CD31 (PECAM-1)	CD31 is expressed in endothelial cells, playing a crucial role in the regulation of angiogenesis, vascular permeability, and cellular responsiveness.		232,233
Cellular markers	Stem cell markers (*e.g.*, CD34, CD73, CD90, CD105)	CD90, CD105, and CD73 are positive markers for MSCs, while CD34 is a negative marker. CD105 plays a role in cell migration. CD90 is widely expressed on the surface of various stem cells and is involved in cell adhesion and migration.	Demonstrates the proliferation and differentiation capability of stem cells within the scaffold, as well as the cell survival within the scaffold.	234
	Proliferation markers (*e.g.*, Ki-67, PCNA)	Ki67 can serve as a marker for cell proliferation. PCNA plays a role in the initiation of cell proliferation and is an indicator of the proliferative state of cells.		235
ECM components	Collagen types (*e.g.*, collagen I, collagen II, collagen III)	Collagen can initiate and maintain interactions between cells and ECM. Collagen can initiate and maintain interactions between cells and ECM	Indicates the scaffold's ability to promote tissue regeneration and repair post-implantation	236,237
Metabolic activity markers	LDH	LDH is an oxidoreductase enzyme involved in the glycolytic pathway, catalyzing the reversible conversion of lactate to pyruvate.	Indicates the cellular metabolic activity and proliferation rate within the scaffold	238
	Glucose consumption and lactate production	Glucose plays a crucial role in organismal carbohydrate metabolism. Lactic acid is a metabolic byproduct generated during cellular energy metabolism processes.		239

CRP, C-reactive protein; VEGF, vascular endothelial growth factor; MSCs, mesenchymal stem cells; PCNA, proliferating cell nuclear antigen; ECM, the extracellular matrix; LDH, lactate dehydrogenase.

5.1. Effects of the physical/chemical properties of biomaterials on cell behavior

Biomaterial scaffolds and biological scaffolds are the two main types of scaffold materials used in CNS disease applications; both possess a 3D topological structure that closely mimics the natural ECM environment. Biomaterial scaffolds are composed of natural or synthetic polymers, while biological scaffolds are typically decellularized mammalian tissues. Hydrogels and electrospun nanofibers are two common types of biomaterial scaffolds. A hydrogel is a hydrophilic network polymer structure colloid. Hydrogels offer superior fluidity compared to other solid systems like nanofibers, which require molding into specific shapes prior to implantation, thereby potentially reducing the need for extensive surgical incisions[240]. Moreover, hydrogels can be modified to mimic the mechanical properties of brain tissue, enhancing their histocompatibility[241]. The polymer materials that form hydrogels are divided into natural and synthetic materials[242]. Natural materials such as hyaluronic acid (HA), silk, chitosan, alginate salts, and collagen possess advantages such as biodegradability, high biocompatibility, and the presence of specific cell adhesion molecules[243]. In contrast, synthetic biomaterials such as PLGA and polycaprolactone (PCL) offer greater stability and regulatory control. However, they often exhibit challenges with low biocompatibility and limited capacity to induce tissue regeneration. These drawbacks can be addressed by modifying them with various functional molecules, such as angiogenic or neurotrophic factors[244]. Electrospun nanofiber scaffolds, which are prepared using the electrospinning method and consist of interwoven nanofibers with a high surface area-to-volume ratio, are also suitable for use as tissue regeneration scaffolding materials[245]. Electrospun nanofibers offer advantages such as simple preparation and adjustable mechanical properties[246]. They can mimic the hierarchical fibrillar arrangement found in collagen, laminin, and other fibrils of the ECM[247].

5.1.1. Effect of charge on cell behavior

The charge characteristics of material surfaces affect cell adhesion. The hydrophilic oligosaccharide chains of fucose and sialic acid, which carry negative charges at their branch ends, envelop the exterior of the mammalian cell membrane, resulting in unevenly distributed negative charges[248]. The electrostatic interaction between the positively charged material surface and negatively charged cells facilitates cell adhesion, whereas negatively charged materials are unfavorable for adhesion due to electrostatic repulsion. Schneider et al.[249] found that when

osteoblasts and fibroblasts were cultured on positively charged 2-methacryloxyethyl trimethyl ammonium chloride-grafted hydroxyethyl methacrylate (HEMA) hydrogels, the repelling HEMA hydrogel exhibited maximum cell attachment and spreading, while cell attachment was reduced in negatively charged sodium methacrylate-grafted HEMA hydrogels and even further reduced in neutrally charged HEMA hydrogels. However, Hu et al.[250] cultured rat hippocampal neurons on positively charged ethylenediamine-functionalized carbon nanotubes (EN-CNTs), negatively charged carboxylated carbon nanotubes (carboxylated-CNTs), and amphoteric polyaminobenzene sulfonic acid-functionalized carbon nanotubes (PABS-CNTs). Different charge properties resulted in no difference in synapse number, but neurons cultured on positively charged EN-CNTs had longer average lengths. Neurons wrapped in alginate beads modified with fibronectin or HA demonstrated a trend toward neural differentiation when grown in alginate or alginate-HA. A comparative study using collagen-based, polylactic acid-hydroxyethyl acid copolymer, and cationic chitosan 3D scaffolds to maintain the stemness of mouse ESCs revealed greater cell proliferation in the cationic chitosan-based 3D scaffold than in the collagen-based and polylactic acid-hydroxyethyl acid copolymer-based 3D scaffolds. Positively charged materials seem to promote cell proliferation, while negatively charged biomaterials tend to promote cell differentiation[251,252].

5.1.2. Effect of stiffness on cell behavior

The rigidity of the matrix material also regulates cell adhesion and differentiation. Stem cells perceive mechanical signals and transduce them into biological signals, triggering a series of biological reactions and simultaneously influencing stem cell function and differentiation[253]. Engler et al.[254] studied the effect of chemically cross-linked polyacrylamide gel coatings with different elasticities on the differentiation of BMSCs. They found that at lower stiffnesses (0.1−1 kPa), BMSCs differentiated into neurons; at medium stiffnesses (8−17 kPa), BMSCs differentiated into muscle cells; and at higher stiffnesses (25−40 kPa), BMSCs differentiated into osteoblasts. When cells are encapsulated within materials, mechanical strength is essential for controlling cell behavior, such as vitality, metabolite production, and growth factor secretion. Researchers compared the survival rate and antibody production of hybridoma cells in solid and liquid alginate−agarose bead gels with different mechanical strengths. The results showed that the hybridoma cells exhibited a better growth pattern in the liquefied gel than in the solid gel, increasing cell vitality and antibody production[255]. The overall mechanical performance of scaffold materials may play a decisive role in signaling pathways involved in stem cell differentiation. *In vitro* cells are sensitive to mechanical stimulation and show rapid responses, presumably because mechanical stimuli activate cell surface receptors, thereby triggering intracellular signaling cascades, activating specific genes and secreting the corresponding ECM[256].

5.1.3. Effect of morphology on cell behavior

Cell growth on material surfaces is regulated by the morphology of the material surface, and various aspects of this interaction have been studied. The roughness of the material can influence cell behavior. For example, Olivares-Navarrete et al.[257] found that sandblasted titanium plates, whose surface roughness was increased from 0.8 to 3.22 μm, promoted the differentiation of BMSCs into osteoblasts. Different fiber diameters can also affect cell differentiation. Takahashi et al.[258] studied polyethylene terephthalate nonwoven fabrics of various diameters and reported that a 9 μm diameter and lower porosity were more favorable for alkaline phosphatase and osteocalcin secretion by BMSCs, encouraging their differentiation into osteoblasts. In recent years, the micropatterning of material surfaces has gained attention. Micropatterned environments with linear patterns of width/spacing (W/S) of 40/30 μm can guide neural lineage differentiation[259], whereas a W/S of 20/40 μm has been reported to guide myogenic lineage differentiation[260]. In addition to linear patterns, different sizes of square patterns also affect cell growth. Cells can proliferate and differentiate on 300 μm × 300 μm, 200-μm spacing square patterns, 5 μm × 5 μm, 5-μm spacing square patterns but not on 50 μm × 50 μm, 50-μm spacing patterns. This is because the largest square pattern has enough space for cell attachment, while the smallest pattern allows cells to extend their filopodia on a small square pattern[261]. Cell penetration can be controlled by the porosity of 3D scaffolds. For example, Khayyatan et al.[262] controlled the porosity of collagen scaffolds by adjusting the freezing temperature. The permeation rate of human induced pluripotent stem cell-derived neural precursor cells (hiPSCs-nanoparticles) was the highest in collagen scaffolds prepared at −196 °C and with smaller pores (<50 μm). A smaller pore diameter results in a larger internal surface area, providing more adhesion space for cells. In summary, the manipulation of material morphology is primarily applicable to cell differentiation. Constraining cells within the space dictated by surface morphology enables control over cell shape.

In the context of tissue engineering, suitable matrix environments for cell culture and differentiation can be designed. This includes designing matrices that increase cell attachment to substrates based on cell repulsion, maintaining the dryness of the cell bank or guiding cell differentiation into specific therapeutic lineages.

5.2. Physical supporting effect of bioscaffolds on TBI lesion site

TBI causes severe tissue damage in the brain, leading to swelling. Once the swelling subsides, structural support for the damaged area is needed to prevent tissue collapse. Biomaterials that can provide sufficient physical support and can be easily implanted, such as electrospun nanofiber scaffolds and various hydrogels, are candidate materials for physical support. Among these materials, electrospun nanofiber scaffolds are made by electrospinning and consist of intertwined nanofibers with a high surface area-to-volume ratio[263]. Since the arrangement, diameter, and interfacial distance of electrospun nanofibers can be adjusted to form surfaces suitable for neural stem cell adhesion and axon support, electrospun nanofibers are superior to hydrogels for simulating the 3D environment of neural tissue[264]. Tang et al.[265] functionalized PLGA with sphingosine *N*-deacylase (SCDase)-hydrolyzed lysoganglioside (LysoGM1) to form oriented fiber networks by electrospinning, creating an oriented biofunctional scaffold (aPLGA-LysoGM1). Here, gangliosides were shown to be necessary for membrane extension during axon and dendrite growth, and the functionalized LysoGM1 promoted neuron growth and regeneration of damaged brain tissue (Fig. 8). Therefore, aligned biofunctional scaffolds can increase neuronal activity, axonal growth, and synapse formation and protect neurons from stress-related damage. However, the sheet-like structure of electrospun nanofibers prevents them from making sufficient contact with surrounding tissues in morphologically diverse lesion

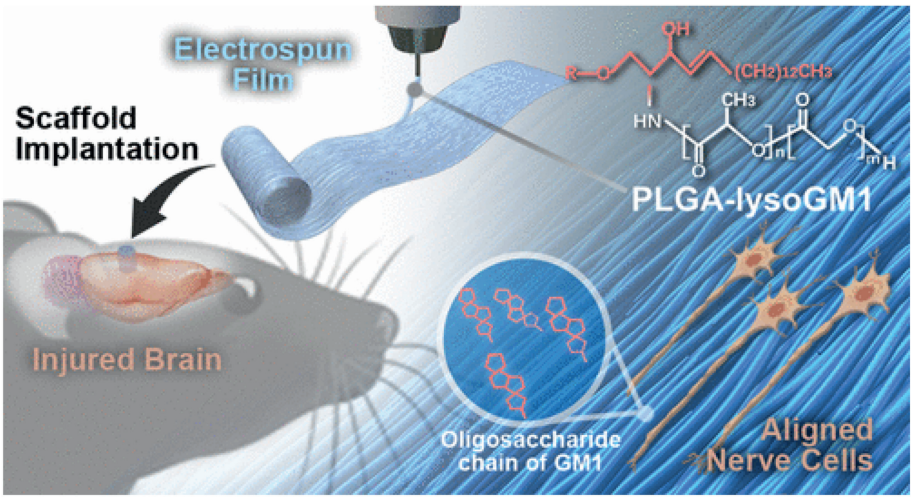

Figure 8 Bioscaffolds that provide physical support for the lesion site. Electrospun PLGA-functionalized sphingomyelin ceramide LysoGM1 formed a directional fiber network. The scaffold supported the growth of neuronal axons and protected neurons from pressure-related damage. (Reprinted with the permission from Ref. 265. Copyright © 2020, Acs Biomater Sci Eng).

tissues[266]. Injectable hydrogels can achieve this physical contact; their flowability gives them the ability to adequately fill lesion sites, thereby preventing further tissue collapse after injury. Hydrogels possess high biocompatibility because their polymer network structure can swell in water, and they can retain a large amount of water. Cui et al.[267] synthesized a poly[*N*-(2-hydroxypropyl) methacrylamide] (PHPMA) hydrogel bearing an axon growth-promoting sequence of an immobilized laminin-derived peptide (IKVAV). This hydrogel exhibited an interconnected porous structure; in a rat model of brain injury, it supported axon growth and promoted tissue regeneration. Polymer hydrogels provide structure and maintain 3D continuity across defects and can serve as a scaffold for the reorganization of repair cells, angiogenesis, and axon growth.

Decellularized mammalian tissue prepared as a bioscaffold has high biocompatibility and provides physical support at injury sites. Clinical applications have shown that ECM scaffolds from both allogeneic and xenogeneic sources can promote functional reconstruction of damaged tissue, enhance new tissue formation, and reduce scar tissue deposition[268,269]. Chaplin et al.[270] removed the epidermis and all dermal cells from the pig skin without damaging the collagen matrix, thus creating a material with low immunogenicity and resistance to calcification. When the material was compared with autologous porcine dura as a dura mater substitute in pig models, both grafts performed well without inflammation or cerebrospinal fluid leakage; therefore, this decellularized dermal allograft is a near-ideal dura mater substitute. Additionally, Cobb et al.[271] found that xenogenic porcine small intestine submucosa (SIS) is feasible as a dura mater substitute.

5.3. Bioscaffolds for cellular tissue regeneration

Stem cell therapy has the potential to replace lost brain cells and provide neurotrophic factors, holding great potential for TBI treatment[272]. The effectiveness of stem cell therapy for brain injury is closely related to the survival rate of transplanted stem cells, and the use of hydrogels as carriers of stem cells can enhance cell survival and implantation[273]. Liang et al.[274] reported the use of injectable HA hydrogels to improve the survival rate of transplanted mouse C17.2 cells, human neural progenitor cells (NPCs), and human glial-restricted precursor cells (GRPs), and the results showed that the hydrogel protected allogeneic transplant cells, extending the survival time of C17.2 cells implanted in immunologically active mice to 12 days and significantly increasing the survival rate of NPCs and GRPs implanted in the brains of immunologically active or immune-deficient mice (5 and 7 days, respectively). ECM proteins, such as fibronectin and laminin, participate in neural development and can provide adhesive support for donor cells and mediate subsequent cell signaling. Tate et al.[275] transplanted NSCs into the brains of injured mice, and cells delivered within laminin or fibronectin scaffolds showed more widespread distribution in damaged brain tissue than cells delivered in culture medium. Eight weeks after transplantation, cells transplanted in scaffolds displayed higher survival rates than cells transplanted in culture medium, and cells transplanted in laminin scaffolds showed higher survival rates than those transplanted in fibronectin scaffolds. Guan et al.[276] used type I collagen mixed with hMSCs to treat brain trauma and reported that the optimal biological distribution of hMSCs occurred when the cells were cultured in collagen scaffolds. Researchers have shown that hMSCs transplanted in the presence of collagen showed greater differentiation into neurons (4.6%), oligodendrocytes (1.8%), and astrocytes (0.8%) than hMSCs transplanted alone, suggesting that collagen protein scaffolds can effectively increase the survival of cells *in vivo* and neurite growth, thus improving neural function. Combining NSCs, scaffolds, and growth factors in neural tissue engineering may increase the ability to repair injured neural tissue. Researchers prepared a conductive fiber scaffold by seeding human umbilical cord MSCs on a 3D scaffold made of polylactic acid coated with alginate and gelatin and featuring a MWNT coating, with the goal of providing a suitable neural regeneration microenvironment. This system showed greater than 80% porosity, promoted cell adhesion and increased MSCs differentiation into neuron-like cells, as indicated by continuous expression of nestin, Map2, and NSE after 21 days of incubation with 1 mmol/L valproic acid[277]. The physical and chemical properties of bioscaffolds can be designed to promote the differentiation of stem cells into specific cell types. Cooper et al.[278] used chitosan-based fibrous matrices with diameters of

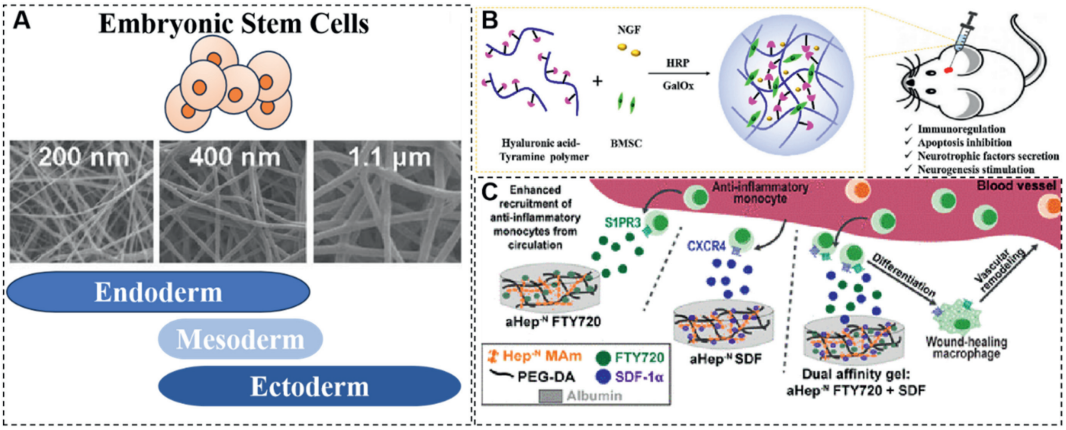

Figure 9 Mechanisms by which implantable scaffolds exert therapeutic effects on TBI. (A) Bioscaffolds for cellular tissue regeneration. A chitosan-based fiber matrix supported morphologically mediated hESC differentiation; substrates made up of 400 nm- and 1.1 μm-diameter fibers increased the expression of neural cell markers, while substrates composed of 200 nm-diameter fibers increased the expression of bone and liver markers. (Reprinted with the permission from Ref. 278. Copyright ©2012, Macromol Biosci). (B) Bioscaffolds for TBI inflammation treatment. *In situ* injection of a HA hydrogel with galactose oxidase and horseradish peroxidase bioenzymes encapsulating BMSCs and NGF promoted the survival and proliferation of endogenous neural cells through the release of neurotrophic factors and modulation of neuroinflammation. (Reprinted with the permission from Ref. 228. Copyright © 2021, Mater Today Bio). (C) Bioscaffolds for vascular remodeling. A dual-affinity hydrogel with Hep-N cells with growth factor affinity and the lipid companion activity of albumin showed the potential to repair brain vessels. (Reprinted with the permission from Ref. 296. Copyright © 2018, Acs Biomater Sci Eng).

200 nm, 400 nm, and 1.1 μm to simulate the ECM structure and elucidate the matrix-mediated differentiation of human embryonic stem cells (hESCs); their results showed that the fibrous matrices supported morphology-mediated hESCs differentiation, that matrices composed of 400 nm and 1.1 μm fibers increased neural cell marker expression, and that matrices composed of 200 nm fibers increased osteogenic and hepatic marker expression (Fig. 9A). The majority of TBI studies have been performed in rodent models[279]. Since the brain structures of rodents and humans are very different[280], it is better to choose dogs, which have a brain structure more similar to that of humans, as models. Jiang et al.[281] used canine TBI as a model and implanted a complex scaffold fabricated from collagen/silk fibroin and human umbilical cord MSCs to promote cerebral cortex repair and motor functional recovery.

Stem cell therapy has received extensive attention for its potential to replace lost neurons after TBI[282]; however, the low retention of stem cells at the lesion site and ethical concerns limit their application. In recent years, stem cell-derived Exos have been considered as alternatives to exogenous stem cells to promote extensive tissue regeneration due to their multiple biological properties, such as the ability to avoiding attack by T and NK cells and the ability to act as paracrine messengers to regulate the microenvironment to induce tissue regeneration and promote angiogenesis[283,284]. Inspired by exosomal alterations caused by cells in different external microenvironments, Liu's team[285] designed hypoxia-preconditioned Exos derived from HUCMSCs and proposed them as an alternative treatment to promote nerve regeneration after TBI. The team[286] also proposed a new strategy of incorporating stem cell-derived Exos (BMEs) into hyaluronan-collagen hydrogel (DHC-BMEs) to mimic the brain matrix and stably release Exos for brain injury repair. *In vitro* experiments have proven that BMEs and DHC can synergistically promote synapse formation and even brain structure reconstruction, ultimately promoting the recovery of neural function after TBI. 3D-printed scaffolds combined with BMEs have been used to promote the repair and regeneration of damaged areas in animal models of TBI[287,288]. However, traditional 3D printing techniques may impair the bioactivity of Exos. Therefore, Liu et al.[289] used low-temperature 3D-printing to maintain the bioactivity of Exos in the resulting scaffolds. Researchers have also sought to reduce inflammation *via* the use of scaffolds made from biological materials such as hydrogels. Wang et al.[290] decellularized, freeze-dried, and rehydrated pig bladder to form a biological hydrogel and cultured NSCs in the hydrogel before transplantation into rat brains. The authors found that the biological hydrogel matrix supported NSC proliferation and differentiation while also reducing inflammation.

5.4. *Bioscaffolds for treatment of TBI-related inflammation*

TBI can damage the BBB structure. The entry of blood, fibroblasts, and other inflammatory factors into the brain can cause a complex inflammatory response, and chronic encephalitis is a key obstacle to neural repair after TBI[291]. The 3D structure of electrospun nanofibers can modulate the biological properties of astrocytes, offering the potential to alter the phenotype of astrocytes after injury[292]. Nisbet et al.[266] implanted randomly arranged electrospun PC scaffolds into the caudate nucleus of rats, and the number of microglia and astrocytes was significantly lower than that in the metal wire control group, indicating that there was no severe inflammatory response from astrocytes or microglia. On this basis, Maclean et al.[293] synthesized a novel poly(L-lysine)-lactide for the layer-by-layer functionalization of PCL nanofibers, covalently linking the galactose portion to the surface of the nanofiber scaffold. They found that, compared to control PCL nanofibers, galactose-linked PCL nanofibers could produce cultured astrocytes in a milder inflammatory state and increase the survival rate of neurons after injury. These studies indicate that nanofiber scaffolds have value for alleviating brain inflammation and promoting cell growth. Functionalized hydrogels can also reduce the inflammatory response. Jeong et al.[294] studied the effects of dexamethasone-conjugated hyaluronic acid (HA-DXM)

bound to a photopolymerized polyethylene glycol bis(acryloyloxyethyl ether) hydrogel (PEG-bi-AA) on the inflammatory response, cell apoptosis, and functional recovery in a CCI rat model. Compared to those in the control group, the rats in the PEG-bi-AA/HA-DXM treatment group exhibited significant reductions in inflammation, cell apoptosis, and brain injury-induced cavity volume and displayed a higher neuron survival rate. Wang et al.[228] synthesized and optimized an HA hydrogel with both galactose oxidase and horseradish peroxidase for *in situ* injection and studied its therapeutic effect on BMSCs and NGF in TBI mice (Fig. 9B). Tyramine-modified HA hydrogels and NGF-loaded hydrogels both exhibited good biocompatibility. More importantly, hydrogels loaded with BMSCs and NGF might promote the survival and proliferation of endogenous neural cells through the release of neurotrophic factors and the regulation of neural inflammation, thereby accelerating the repair process and thus the recovery of neural function in mice with brain trauma.

5.5. *Bioscaffolds for vascular remodeling in TBI*

TBI can lead to extensive microvascular collapse, perivascular edema and microthrombus formation, resulting in microvascular ischemia, local hypoxia and selective neuronal loss[295]. The immune properties of biomaterial scaffolds can regulate functional effects, such as angiogenesis and transplantation integration/survival. The host material response is designed to optimize the recruitment of pro-regenerative leukocyte subsets, which then mature into macrophages to promote wound healing. Ly6Clow monocytes exhibit high expression of the bioactive lipid receptor sphingosine-1-phosphate receptor 3 (S1PR3) and the matrix-derived factor-1α (SDF-1α) receptor CXCR4. Ogle et al.[296] designed a dual-affinity hydrogel with heparin derivative (Hep-N) growth factor affinity and albumin lipid chaperone activity by using the mechanical synergy between signal axes to promote the recruitment of endogenous proregenerative monocytes (Fig. 9C). The sphingosine analogs FTY720 and SDF-1α were coloaded into and coreleased from a Hep-N-functionalized PEG hydrogel. The hydrogel was implanted into mouse skin wounds, and the results showed increased early accumulation of differentiated CD206+ macrophages in the tissues around the hydrogel and sustained vascular network expansion. Therefore, this system has the potential to repair cerebral vessels. Somaa et al.[297] prepared an IKVAV self-assembled hydrogel scaffold. After focal cerebral ischemia, the group of rats transplanted with scaffold-bearing cells showed gradual improvement in brain motor function compared with the rats in the cell-only and scaffold-only control groups. Cortical atrophy was significantly reduced in the animals that received the peptide scaffold with transplanted cells, neuronal differentiation was significantly greater, and the electrophysiological characteristics were significantly improved, indicating that the neurons were fully integrated. More importantly, host-derived angiogenesis was observed in the graft. Deguchi et al.[298] adsorbed basic fibroblast growth factor (bFGF) and EGF onto a porous block scaffold of gelatin and 3-(glycidylpropyl)trimethoxysilane and implanted it into a cerebral cortex defect. The scaffold was retained in the rat brain tissue for 60 days, where it maintained the integrity of the brain morphology and attached well to the surrounding brain tissue. The marginal cavity of the scaffold was occupied by new brain tissue, and the number of new cells was proportional to the dose of bFGF and EGF. A 2-fold increase in vascular endothelial cells, astrocytes and microglia was observed in the presence of bFGF/EGF compared with the numbers in the presence of growth factor-free scaffolds. In addition to growth factors, Exos secreted by cells can directly bind to scaffolds. Exos can affect the response of cells to diseases such as trauma and infection. The scaffold can prolong the retention of Exos at the injured site and maintain the transmission of Exos[299]. Thus, Zhang et al.[300] found that in a mouse model of brain injury, intravenous delivery of hMSCs Exos cultured in 3D collagen scaffolds promoted endogenous angiogenesis and neurogenesis, reduced neuroinflammation, and improved functional recovery after TBI. Electrospun fibers have been widely studied and used for tissue vascular repair due to their high porosity and similar structure to natural ECM. Madhavan et al.[301] prepared a scaffold system composed of polyurea, poly(serine hexamethylene urea) (PSHU), polyester and PCL by electrospinning. The polymer ratio and electrospinning flow rate were directly related to the mechanical properties. The elasticity of the scaffold with a low PSHU content was close to that of the natural artery of mammals, while increasing the electrospinning flow rate increased the elasticity of the matrix. The optimal elasticity was conducive to the adhesion, growth and maintenance of vascular smooth muscle cells. In addition, the Gly−Arg−Gly−Asp−Ser (RGD) peptide-modified polyurea main chain significantly increased the clotting time, thereby reducing the formation of surface thrombosis and preventing intimal hyperplasia. The results showed that these electrospun fibers are a suitable material for vascular transplantation applications. However, vascular graft scaffolds fabricated by electrospinning usually have relatively small pores and low porosity, which limits the penetration of cells into the scaffold and hinders the regeneration and remodeling of the graft. Thus, Tan et al.[302] prepared heparin-functionalized scaffolds composed of PCL, gelatin and polyvinyl alcohol (PVA) by co-electrospinning. PVA fibers produced electrospun scaffolds with high porosity, which significantly enhanced cell proliferation and infiltration, and were rapidly degraded *in vivo*; heparin functionalization had a good antithrombotic effect and promoted the growth of endothelial cells. The bioscaffolds for TBI treatment are listed in Table 5[228,265−267,270,271,274,293,294,297,301,302]).

6. Risks and side effects

Therapeutic strategies for TBI involving biomaterials and stem cells/EVs hold promise, but safety concerns remain significant. Stem cell transplantation has emerged as a novel therapy for neurological diseases; however, it is associated with side effects and faces several clinical development challenges, including immune rejection, genomic instability, and risks of tumorigenesis. Exogenous stem cells are recognized by the body as foreign entities, triggering an immune response that can lead to transplant failure or other adverse reactions[303]. The formation of tumors in patients due to transplanted cells represents a significant risk associated with stem cell therapy[304]. The pluripotency of stem cells is a critical factor contributing to the risk of tumor formation. Furthermore, the tumorigenic potential of stem cells is influenced by various intrinsic and extrinsic factors, such as the site of administration, conditions of *in vitro* expansion and culture, and genetic modification or reprogramming[305].

The site of administration of hESCs in immunodeficient mice has been shown to significantly impact the rate of teratoma formation[306]. Cell expansion *in vitro* can induce genetic instability and increase tumorigenic potential, thereby limiting their clinical application[307]. During *in vitro* experiments, pluripotent stem cells

Table 5 The bioscaffolds for TBI treatment.

Bioscaffold	Material	Function & mechanism	Loading agent	Ref.
Electrospun nanofibers	PLGA	Promoted neuronal viability, neurite outgrowth, and synapse formation, and protected neurons from pressure-related injury	LysoGM1	265
	PCL	Reduced severe inflammatory response from astrocytes or microglia	−	266
	PCL and poly(L-lysine)-lactobonic acid	Maintained an attenuated inflammatory profile of astrocytes in culture and increased the survival of neurons after traumatic injury	−	293
	PSHU and PCL	As a vascular graft, it enabled vascular smooth muscle cells to adhere, grow, and maintain a smooth muscle cell phenotype	RGD	301
	Polycaprolactone, gelatin, and PVA	As a high-porosity vascular graft, it significantly enhanced cell proliferation and infiltration	Heparin	302
Hydrogels	PHPMA	Provided structured three-dimensional continuity across the defect and promoted reorganization of local wound-repair cells, angiogenesis, and axonal growth into the hydrogel scaffold	IKVAV	267
	HA	Increased the survival of engrafted NSCs	Mouse C17.2 cells, human NPCs, and human GRPs	274
	HA and poly (ethylene) glycol-bis-(acryloyloxy acetate)	Increased the survival of neuronal cells and improved motor functional recovery	Dexamethasone	294
	HA modified by galactose oxidase and horseradish peroxidase	Improved neurological function recovery and accelerated repair in a C57BL/6 TBI mouse model	BMSCs and NGF	228
	IKVAV	Facilitated the survival and maturation of implanted neurons and promoted substantial axonal outgrowth.	Cortical progenitors	297
Organism Materials	Decellularized pig skin	Used as a dural substitute material	−	270
	Xenogeneic porcine SIS	Used as dural substitute material	−	271

−, not applicable. PLGA, poly(lactic-*co*-glycoside); LysoGM1, lyso-ganglioside; PCL, polycaprolactone; PSHU, poly(serine hexamethylene urea); RGD, the Gly−Arg−Gly−Asp−Ser; PVA, gelatin and polyvinyl alcohol; PHPMA, poly[*N*-(2-hydroxypropyl) methacrylamide]; IKVAV, laminin-derived peptide; HA, hyaluronic acid; NPCs, neural progenitor cells; GRPs, glial-restricted precursor cells; BMSCs, bone marrow mesenchymal stem cells; NGF, nerve growth factor; SIS, small-intestinal submucosa.

may undergo genetic alterations, such as chromosome abnormalities, variations in copy number, and single nucleotide mutations. The accumulation of mutations in tumor-related genes further heightens the risk of tumor development[308]. These findings underscore the importance of closely monitoring the chromosomal status and genome of *in vitro* expanded and cultured stem cells used for therapy to prevent potentially fatal side effects such as tumor formation. Additionally, concerns have been raised about the genetic modification of retroviruses and lentiviruses used in generating iPSCs, which may increase the carcinogenic potential of reprogramming factors and induce DNA damage. These factors also heighten the risk of cancer and immunogenicity associated with iPSCs[309]. Moreover, there is a theoretical risk that stem cells transplanted into the human body may undergo undesired differentiation[310]. It is important to note that the number of transplanted stem cells is critical for optimal tissue function. Insufficient numbers can lead to dysfunction, whereas an excess may promote tumorigenesis[311]. Therefore, the risks and potential complications associated with stem cells must be thoroughly evaluated before widespread clinical application. The challenges in accurately characterizing and quantifying the cargo of Exos, as well as the inability to target them to specific receptors, contribute to off-target problems[312]. Furthermore, the effects of different EVs subtypes on the pathophysiology of TBI vary significantly and necessitate precise differentiation and evaluation[313].

NPs and biological scaffolds also pose specific risks and challenges, primarily determined by their material composition. The primary risks associated with NPs include neurotoxicity and pro-inflammatory responses. Inorganic nanomaterials are often recognized by the immune system as foreign substances, leading to inflammation, oxidative damage, and fibrosis, which compromise their biocompatibility. Additionally, after intravenous injection, these materials are captured by the reticuloendothelial system, resulting in slow and inefficient degradation. Furthermore, standardization, neurotoxicity, and safety considerations remain paramount. Iron oxide nanoparticles are considered one of the most biocompatible magnetic materials suitable for biomedical applications and have been utilized in various CNS applications[314]. Nevertheless, excessive iron accumulation can lead to oxidative stress and protein aggregation, ultimately resulting in apoptosis and damage to nerve tissue[315]. Additionally, PLGA NPs can cause complications due to their limited biocompatibility with brain tissue, leading to pro-inflammatory responses[316]. While many studies have emphasized the high compatibility of biomaterials, predicting the inflammation or oxidative stress induced by their application remains challenging. Moreover, factors such as diffusion into non-specific neural regions may trigger unexpected biochemical reactions that are difficult to anticipate[317].

Biomaterials used to construct bioscaffolds can be classified into natural and synthetic materials. Natural materials, while

advantageous for their biocompatibility, may harbor pathogens that could potentially trigger immune responses *in vivo*[318,319]. At the same time, it is important to note that their interaction with living tissue may still pose a risk of cancer development[320]. Moreover, the degradation process of biological scaffolds requires careful consideration. The decomposition products may have negative effects on the body or alter the local microenvironment. For example, PLGA scaffolds produce a more acidic medium during degradation compared to gelatin scaffolds, which can adversely affect the survival of neurons[321]. Additionally, certain properties of bioscaffolds can exacerbate existing injuries. For instance, hydrogels have high swelling capacity, which may elevate local tissue pressure and contribute to secondary brain injury following trauma. Rigid scaffolds with low plasticity, when implanted into soft and fragile brain tissue, could potentially exacerbate damage to the host tissue[322]. Therefore, the selection of biological materials and the rigorous monitoring and evaluation of safety are crucial. Standardized management procedures must be implemented before clinical application.

7. Conclusions and perspectives

The variable pathophysiological responses, prognoses, and psychosocial impacts make TBI a complex condition. Multimodal TBI intervention not only produces effective clinical treatment but also offers a comprehensive strategy for achieving favorable prognoses. This approach requires extensive collaboration among neurosurgeons, neurologists, psychologists, and rehabilitation experts to ensure positive therapeutic outcomes. Imaging and neuromonitoring have proven instrumental in guiding multimodal TBI treatment, integrating physiological data from these modalities to form a pathophysiological assessment prior to administering targeted pharmacological and/or non-pharmacological interventions[323]. The combined imaging techniques, such as MRI and PET, are capable of integrating structural and functional information of CNS diseases, including TBI, correlating abnormal anatomical regions with disruptions in CNS function[324,325]. Multimodal neuromonitoring (MMM) requires the measurement and integration of multiple biological parameters to better understand a patient's pathophysiological state and guide treatment decisions. The parameters include intracranial pressure, cerebral perfusion pressure, and cerebrovascular autoregulation[326]. Equally important, neuropsychologists play a vital role in the assessment, diagnosis, and treatment of patients with TBI. There is an urgent need to address the high proportion of patients who continue to experience significant mental health and neurobehavioral symptoms after completing outpatient TBI treatment[327]. Conducting neuropsychological tests on TBI patients assesses all aspects of brain function and identifies cognitive, emotional, and behavioral deficits. These assessments help clinicians understand the patient's condition, develop appropriate treatment strategies, and track the effects of these interventions over time[328]. In addition, TBI patients should be treated with a rehabilitation perspective. Providing early and specialized rehabilitation can help patients recover and improve brain function, regain performance, and enhance their quality of life.

This review delves into cutting-edge technologies that show promise for revolutionizing the treatment of TBI. Nanoparticles offer a solution to the longstanding challenges of drug stability and targeted delivery to the brain. Specific targeting peptides, antibodies, or proteins that engage with receptors on brain capillary endothelial cells are often employed for this purpose[329,330]. Unpacking underlying mechanisms such as adsorption-mediated and carrier/receptor-mediated phagocytosis may herald pivotal advancements in TBI therapy. Beyond therapeutic applications, inorganic nanomaterials offer diagnostic potential, with unique photochemical properties suited for MRI, CT imaging, and photothermal therapy, among others[331]. Despite the potential of these materials, there are no ongoing clinical trials focused on nanoparticle formulations for TBI treatment. On the other hand, stem cell technologies present a frontier for neural regeneration, although significant questions about the underlying mechanism and optimization of this process remain. These include the optimal timing, path, and quantity of cell transplantation, as well as the mechanistic basis of its efficacy. Despite these challenges, the technology shows promise for enhancing neurological recovery in TBI patients and holds substantial market potential. With research advances in neuroscience and interdisciplinary fields, stem cell transplantation could become a pivotal tool for an increasingly broad population of TBI patients. In the physiological environment, implanted stem cells must not only stabilize the tissue microenvironment but also integrate, proliferate, and differentiate into neurons, forming neural networks[332]. The therapeutic effect of these cells has been partially attributed to their secretion of growth factors and proteins, resulting in a paracrine mechanisms[333]. Immune modulation, including the inhibition of dendritic cell maturation and control of inflammation, has also been observed[334]. Although stem cells implantation is promising, the potential for side effects such as genetic instability and tumorigenicity due to *in vitro* expansion raises significant concerns[307].

Advances in bioscaffolds technologies as key enablers for cell transplantation in neurosurgical interventions, particularly in TBI treatment, are emerging. These scaffolds serve as matrices for cell anchoring and are optimized to mimic both the biochemical and biophysical conditions of brain tissues, including the ECM[291,335]. Scaffolding technologies are not only crucial for cell anchoring but also instrumental in modulating cellular interactions and biochemical environments. Advanced bioengineering methods, including 3D printing, have enabled the creation of scaffolds that closely emulate natural brain structures. These scaffolds optimize cellular adhesion, proliferation, migration, and differentiation. By pairing these 3D-printed bioscaffolds with stem cells increases the potential for developing efficacious TBI treatments. Recent research has demonstrated that bioscaffolds can be utilized as platforms for drug delivery, enabling a synergistic approach that combines precise drug delivery with effective tissue regeneration. Looking ahead, the gap between experimental data and clinical application is a pressing concern. Clinical trials are necessary to validate the efficacy and safety of these emerging technologies. The intersection of materials science, bioengineering, and neuroscience holds great promise for developing innovative, clinically applicable solutions for TBI treatment in the future.

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Author contributions

Wenya Chi: Writing − original draft. Yingying He: Writing − original draft. Shuisheng Chen: Writing − original draft. Lingyi Guo: Writing − original draft. Yan Yuan: Writing − original draft. Rongjie Li: Writing − original draft. Ruiyao Liu: Writing − original draft. Dairan Zhou: Visualization. Jianzhong Du: Writing − review & editing, Conceptualization. Tao Xu: Writing − review & editing, Conceptualization. Yuan Yu: Writing − review & editing, Funding acquisition, Conceptualization.

Conflicts of interest

The authors declare no conflicts of interest.

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