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How neural stem cell therapy promotes brain repair after stroke

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SUMMARY

The human brain has a very limited capacity for self-repair, presenting significant challenges in recovery following injuries such as ischemic stroke. Stem cell-based therapies have emerged as promising strategies to enhance post-stroke recovery. Building on a large body of preclinical evidence, clinical trials are currently ongoing to prove the efficacy of stem cell therapy in stroke patients. However, the mechanisms through which stem cell grafts promote neural repair remain incompletely understood. Key questions include whether these effects are primarily driven by (1) the secretion of trophic factors that stimulate endogenous repair processes, (2) direct neural cell replacement, or (3) a combination of both mechanisms. This review explores the latest advancements in neural stem cell therapy for stroke, highlighting research insights in brain repair mechanisms. Deciphering the fundamental mechanisms underlying stem cell-mediated brain regeneration holds the potential to refine therapeutic strategies and advance treatments for a range of neurological disorders.

INTRODUCTION

Repair and regeneration of damaged organs is a fundamental principle for the survival of any organism. Generally, this is accomplished through two interdependent processes: (1) the dead tissue must be replaced by newly generated cells, and then (2) new cells must differentiate and become organized in complex patterns to restore the original structure and function of the injured organ. In humans, the repair properties may vary considerably between different organs. Some tissues, such as skin and liver, have strong endogenous cell replacement and pattern repair capabilities. In contrast, others, including the central nervous system (CNS), show only low regenerative potential (Chen et al., 2022). This is particularly problematic for patients suffering from brain disorders and injuries.

The most common cause of severe brain damage is ischemic stroke, yearly affecting over 13.7 million people and one in four people over age 25 in their lifetime (GBD 2016 Stroke Collaborators, 2019). An ischemic stroke typically occurs when an artery that supplies blood to the brain becomes blocked by a blood clot or plaque. If the blockage cannot be resolved with acute treatment, deficiency of oxygen and nutrients may rapidly cause severe brain damage or death. For each hour that treatment does not occur, the brain loses as many neurons as in 3.6 years of aging (Saver, 2006), and although other cell types within the stroke core are less sensitive to ischemia, they all eventually degenerate within a few hours following the infarct. Surrounding the stroke core, the peri-infarct zone consists of functionally impaired yet still viable tissue. Within the peri-infarct region, microglia become activated, and peripheral immune cells including neutrophils and macrophages are recruited through endothelial cells across the blood-brain barrier (BBB) minutes following the injury. The pro-inflammatory state promotes cytokine release, formation of reactive oxygen species, and extracellular matrix disruption. Astrocytes are activated days following the injury and produce cytokines and proteoglycans, the main component of the glial scar (Weber et al., 2022). These three cell types contribute to the secondary damage but also remodel the extracellular matrix and generate signals for neural repair. Absence of both inflammation and scar-forming processes has been associated with poor stroke recovery in preclinical models (Liddelow and Barres, 2016). In the later phases, within weeks to months, low levels of endogenous remodeling and regenerative processes take place, including angiogenesis, neurogenesis, and axonal sprouting. Primary functional recovery usually occurs within the first 3 months but can continue up to 3 years following stroke (Belagaje, 2017). As time is an extraordinarily critical factor, the primary aim in clinical practice is to restore blood flow as soon as possible through enzymatic or mechanical removal of the blood clot. Currently, the only treatment option of acute ischemic stroke patients is to restore blood flow by reperfusion therapy (Figure 1). The sole authorized drug available for treatment is the recombinant human tissue plasminogen activator alteplase. Although numerous randomized controlled trials and more than 25 years of clinical use have shown that intravenous administration of alteplase reduces disability in patients who experienced an acute ischemic stroke (Emberson et al., 2014), the relatively short treatment window narrows down its application since reperfusion therapies are only efficient until affected neural tissue is lost, and the infarct transits from the acute to the chronic phase (Grøan et al., 2021).

Cell therapy is emerging as a promising and novel treatment paradigm for stroke, which has also been recognized by the Stroke Treatment Academic Industry Roundtable

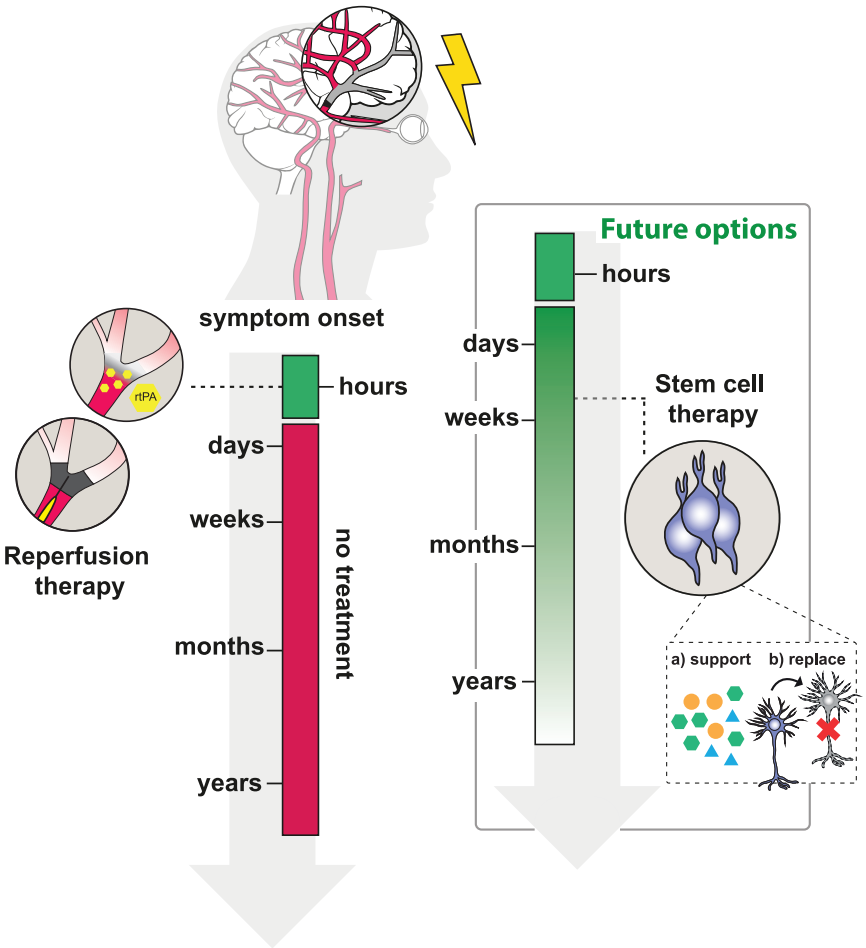

Figure 1. Existing and future options to treat ischemic stroke

Left: currently, stroke treatment is limited to reperfusion therapy, i.e., the mechanical or enzymatical (rtPA) removal of the blood clot. However, this is effective only within a narrow time window after symptom onset. Right: cell-based therapies could offer a promising future option, with the potential to extend the therapeutic window and improve outcomes for patients who fall outside the time frame for reperfusion therapy. rtPA, recombinant human tissue plasminogen activator.

(Liebeskind et al., 2018). Notably, cell therapy in stroke has already reached the translational stage, with 30 (active or completed) clinical trials and therapeutic results in humans (Negoro et al., 2019). The safety of cell therapies in stroke has been demonstrated, further confirming the potential of this approach. However, efficacy of these therapies still needs to be confirmed in human subjects, and more work is needed to optimize stem cell application in clinical practice (Rust and Tackenberg, 2022).

This review compiles evidence from various preclinical studies, focusing on how stem cells, especially neural stem and progenitor cells (NSCs and NPCs), contribute to brain repair after stroke, and examines the mechanisms driving stem cell-based brain regeneration.

CURRENT CLINICAL LANDSCAPE FOR CELL THERAPY FOR STROKE

Previous randomized clinical trials have concentrated predominantly on the use of autologous mesenchymal stem cells (MSCs) due to their high capacity for self-renewal and easy accessibility from various sources (MSCs are naturally available in all mesenchymal tissues, including bone marrow, adipose tissue, umbilical cord, and dental pulp) (Yan et al., 2023).

In various phase 1 and phase 2 clinical trials, MSCs derived from different sources have been explored, consistently proving to be safe and well tolerated (Table 1). Notable examples include the AMASCIS trial (de Celis-Ruiz et al., 2022), a phase 2 randomized, double-blind, placebo-controlled trial evaluating the allogeneic transplantation of adipose tissue-derived MSCs; the MASTERS trial (Hess et al., 2017), which tested the intravenous injection of bone marrow-derived multipotent adult progenitor cells; and the RAINBOW trial (Kawabori et al., 2024), a phase 1/2 open-label study evaluating the safety and tolerability of intracerebral transplantation of autologous mesenchymal stromal cells. While these studies demonstrated encouraging safety profiles, efficacy signals remain inconsistent. To date, only one phase 2/3 trial has been conducted: the TREASURE (Houkin et al., 2024) study, which evaluated

Table 1. Summary of selected clinical trials of stem cells in the treatment of ischemic stroke in adults (2017–today)

Study completion	Trial number	Cell product	Dosage	Delivery	No. of patients	Phase	Main outcomes	Status
2024	NCT05697718	allogeneic UC-MSCs	3 treatment groups: single dose of 5×10^7/10×10^7/20×10^7 cells/kg	i.v.	18	1	–	recruitment completed
2024	NCT04811651 (UMSIS)	allogeneic UC-MSCs	single dose of 100×10^6 cells/kg	i.v.	156	2		completed, no publications
2024	NCT04093336	allogeneic UC-MSCs	single dose of 2×10^6 cells/kg	i.v.	120	1/2		unknown
2023	NCT05292625	allogeneic UC-MSCs	2 doses of 1.5×10^6 cells/kg with an interval of 3 months	i.v./i.t.	48	1/2	–	completed, no publications
2023	NCT05850208	autologous BM-MSCs	2 doses of 1×10^6 cells/kg, with an interval of 1 week	i.v.	60	1		unknown
2023	NCT03545607 (MASTERS-2)	allogeneic BM multipotent adult progenitor cells	single dose of 1.2×10^9 cells/kg	i.v.	300	3		completed, no publications
2023	NCT02961504 (TREASURE)	allogeneic BM adult progenitor cells	single dose of 1.2×10^9 cells/kg	i.v.	206	2/3	no significant difference in neurological or functional improvement observed among the treatment groups. Demonstrated safety and feasibility	completed, one publication (Houkin et al., 2024)
2023	NCT02178657 (IBIS)	autologous BM-MNCs	single dose ranging from 2 to 5×10^6 cells/kg	i.a.	76	2	no significant difference in neurological or functional improvement observed among the treatment groups. Demonstrated safety and feasibility	completed, one publication (Moniche et al., 2023)
2022	NCT04590118 (ASSIST)	allogeneic MSCs	single dose of $0.5–2 \times 10^6$ cells/kg	i.v.	60	1/2a		unknown
2022	NCT05008588	allogeneic UC-MSCs	single dose of 20×10^6 cells/kg	i.v.	15	1/2a		unknown
2021	NCT04280003	autologous AD-MSCs	single dose of 1×10^6 cells/kg	i.v.	30	2b	patients in the AD-MSC group showed a nonsignificantly lower median NIHSS score. No differences in mRS scores. Demonstrated safety and feasibility	completed, one publication (de Celis-Ruiz et al., 2022)

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Table 1. *Continued*

Study completion	Trial number	Cell product	Dosage	Delivery	No. of patients	Phase	Main outcomes	Status
2018	NCT02813512	autologous AD-SCs	single dose of 1×10^8 cells/kg	i.c.	3	1	significant improvements in NIHSS, Barthel Index, Berg balance scale, and Fugl-Meyer modified sensation. Demonstrated safety and feasibility	completed, one publication (Chiu et al., 2022)
2018	NCT01297413	allogeneic BM-MSCs	single dose ranging from 0.5 to 1.5×10^6 cells/kg	i.v.	38	1/2	significant improvements in Barthel Index scores. Demonstrated safety and feasibility	completed, one publication (Levy et al., 2019)
2017	NCT00875654 (ISIS-HERMES)	autologous BM-MSCs	2 treatment groups: single low dose of 100×10^6 cells/kg and single high dose of 300×10^6 cells/kg	i.v.	31	2	no treatment effects on the Barthel Index, NIHSS, and modified-Rankin scores, significant motor improvements (NIHSS, Fugl-Meyer, and fMRI). Demonstrated safety and feasibility	completed with 2 publications (Hannanu et al., 2020; Jaillard et al., 2020)
2017	NCT03296618	allogeneic fetal NSCs	single dose of $1.2–8 \times 10^7$ cells/kg	i.c.	18	1	significant improvements in Fugl-Meyer Motor Score, the NIHSS and the mRS scores. Demonstrated safety and feasibility	completed, one publication (Zhang et al., 2019)
2017	NCT01678534 (AMASCIS)	allogeneic AD-MSCs	single dose of 1×10^6 cells/kg	i.v.	19	2	patients in the AD-MSC group showed a nonsignificantly lower median NIHSS score. No differences in mRS scores. Demonstrated safety and feasibility	completed, one publication (de Celis-Ruiz et al., 2022)
2017	NCT02580019	allogeneic UC-MSCs	single dose of 2×10^7 cells/kg	i.v.	2	2		completed, no publications
2017	NCT01468064	autologous BM-SCs And EPCs	2 treatment groups: 2 doses of 2.5×10^6 cells/kg, with an interval of 1 week of either BM-MSCs or EPCs	i.v.	20	1/2	no significant difference in neurological or functional improvement observed among the treatment groups, except for the Scandinavia Stroke Scale score at 3 months between the EPC group and placebo-controlled group. Demonstrated safety and feasibility	completed, one publication (Fang et al., 2019)

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Table 1. *Continued*

Study completion	Trial number	Cell product	Dosage	Delivery	No. of patients	Phase	Main outcomes	Status
2017	NCT02117635 (PISCES-II)	allogeneic fetal NSCs	single dose of 20×10^6 cells/kg	i.c.	23	1/2	improvement in the ARAT test (only in patients with residual upper limb movement at baseline). Demonstrated safety and feasibility	completed, one publication (Muir et al., 2020)
2017	NCT02378974	allogeneic UC-MSCs	unknown	i.v.	18	1/2		completed, no publications
2017	NCT01716481	autologous BM-MSCs	single dose of 1×10^6 cells/kg	i.v.	39	3	significant improvements in lower extremity motor function. Demonstrated safety and feasibility	completed, 3 publications (Bang et al., 2022; Chung et al., 2021; Lee et al., 2022)

ARAT, action research arm test; AD-MSC, adipose-derived; BM, bone marrow; fMRI, functional magnetic resonance imaging; i.a., intraarterial; i.c., intracranial; i.t., intrathecal; i.v., intravenous; MNCs, mononuclear cells; MSCs, mesenchymal stem cells; mRS, modified Rankin Scale; NIHSS, National Institute of Health Stroke Scale; NSC, neural stem cells; UC, umbilical cord blood.

intravenously injected bone marrow-derived multipotent adult progenitor cells in ischemic stroke patients. Although TREASURE confirmed the safety and tolerability of this approach, it did not yield discernible improvements in clinical outcomes, leaving the therapeutic potential of MSCs and other adult stem and progenitor cells for ischemic stroke unproven. One key hurdle that continues to limit robust therapeutic efficacy in clinical trials is a mismatch between preclinical and clinical settings, where younger, healthier animal models do not reflect the complexity of stroke patients who are typically older and have comorbidities (Cui et al., 2009; Möller et al., 2015; Sandu et al., 2017). Updated guidelines suggest using models that align more closely with the targeted patient population and combining cell-based therapies with standard stroke medications (e.g., antiplatelets, antihypertensives, and statins) (Boltze et al., 2019). Further, delivering cells to the injured brain remains challenging. Intravenous injection is minimally invasive yet yields poor cell homing to the brain (Achón Buil et al., 2023; Chung et al., 2021). Intraarterial delivery offers more precise targeting but raises embolic risks, while direct intracerebral injection bypasses the BBB but is strongly invasive (Achón Buil et al., 2023; Yan et al., 2023). Recent advances, such as overexpressing cell surface receptors (e.g., CXCR1, CCR2, and CXCR4) (Huang et al., 2018; Kim et al., 2011; Yang et al., 2015) that facilitate BBB crossing, or navigating robots (Janiak et al., 2023), may improve these applications. Immune rejection further limits graft survival, though transient immunosuppression or transplants with immune-evasive properties show promise (Achón Buil et al., 2024). Finally, timing is crucial: if cells are administered too early, they might disrupt endogenous repair, whereas waiting too long may miss a critical window for neuroregeneration (Cha et al., 2024; Li et al., 2021). The time point of administration may also be crucial for the survival of the graft as it was recently shown that NPCs transplanted 7 days post stroke survived better compared to transplantation 1 day post stroke (Weber et al., 2025). Thus, defining optimal time window, delivery strategies, and appropriate adjunct treatments will be vital to achieving consistent clinical benefits.

More recently, NSCs have garnered increasing interest as a multimodal therapeutic option for stroke. In addition to producing neuroprotective and regenerative growth factors, NSCs have the unique ability to differentiate into neural cell types, potentially replacing cells lost or damaged during ischemic events (Baker et al., 2017; Rust et al., 2022; Tornero et al., 2013). This dual capability positions NSCs as promising candidates for addressing the multifaceted challenges of stroke recovery.

One of the earliest large-scale clinical trials investigating NSCs in ischemic stroke, the Pilot Investigation of Stem

Cells in Stroke (PISCES) (Muir et al., 2020), provided preliminary data on the feasibility and tolerability of stereotactic intracerebral injection of human NSCs (hNSCs). The trial, which completed phase 1 (NCT01151124) and phase 2 (NCT03629275), demonstrated that hNSCs could be safely delivered into the brain. Notably, some patients with residual upper limb movement showed functional improvement, although the extent of these improvements varied between individuals.

Among the cell types explored to date, NSCs and NPCs hold the greatest promise for stroke therapy. Significant progress has been made in understanding the mechanisms underlying NSC/NPC-mediated tissue recovery, including neuroprotection, neurogenesis, and the modulation of inflammation in preclinical stroke models. However, many questions remain unresolved, particularly regarding the optimization of delivery methods, survival, and functional integration of these cells.

NEURAL STEM/PROGENITOR CELLS

NSCs are a promising cell source for neurorestoration as their primed neural lineage limits the potential of generating undesired non-neural phenotypes. They possess the ability to continually self-renew, initially giving rise to radial glial progenitor cells that, in turn, are responsible for creating both neurons and glial cells (astrocytes and oligodendrocytes) in the CNS during development (Lim and Alvarez-Buylla, 2014). NPCs are the progenitor cells of the CNS that give rise to a wide range of glial and neuronal cell types but do not generate non-neural cells, similar to NSCs. They are usually identified based on morphology, gene expression profile, and temporal distribution and function (Martínez-Cerdeño and Noctor, 2018). In contrast to NSCs, NPCs are considered to be more lineage restricted with a reduced self-renewing capacity (Oikari et al., 2016).

NSCs can be found in human and rodent CNS tissue during development and in adult life—that is the subgranular zone of the dentate gyrus, the subventricular zone of the lateral ventricles, and the ependymal in the spinal cord (Fernández-Muñoz et al., 2020). The limited accessibility of these neurogenic niches, however, restricts the application of primary NSCs, as most often the tissue is collected from elective or spontaneous termination of pregnancies or from adult and/or fetal autopsy specimens (Palmer et al., 2001). Thus, a preferable source of exogenous NSCs and NPCs is by differentiation of pluripotent cells, such as embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). There is a wide variety of different protocols to obtain NSC and NPCs from pluripotent stem cells ESCs and iPSCs (Bohaciakova et al., 2019; Rust et al., 2022; Sugai et al., 2021; Vitillo and Vallier, 2021; Vitillo et al., 2020). They all differ in the conditions and duration required for cultivation and are usually chosen based on the purposes for which the cells are generated. For potential use in clinical trials, NSC/NPCs must be homogeneous, stable, self-renewable cultures with well-defined characteristics and low tumorigenic properties.

BRAIN REPAIR MECHANISMS OF HUMAN NEURAL STEM AND PROGENITOR CELLS

A central question that continues to elicit debate is whether human NPCs/NSCs primarily exert their beneficial effect through paracrine signaling ("bystander effect") or via direct cell replacement, or a combination of both (Figure 2). Preclinical studies have provided evidence that protective factors secreted from NSCs/NPCs contribute to brain regeneration after stroke. However, state-of-the-art research modalities revealed new insights into how grafted cells actively also contribute to neural circuit reconstruction in animal models of stroke. In general, the relative contribution of paracrine signaling versus direct cell replacement may shift over time following ischemic stroke. In the acute to sub-acute phase (days to a few weeks), transplanted cells predominantly exert paracrine effects, modulating inflammation, protecting vulnerable tissue, restoring vasculature, and promoting endogenous repair processes. As the tissue environment stabilizes and inflammation subsides, the mechanism may gradually shift toward direct cell replacement. Studies in rodent models indicate that NPCs begin expressing early differentiation markers (e.g., βIII-tubulin and GFAP) a couple of weeks after transplantation (Daadi et al., 2008; Rust et al., 2022), but it typically takes 3–7 weeks for these cells to adopt more mature neuronal phenotypes and integrate into host neural circuits. Fully functional engraftment requires synaptic connectivity, sufficient trophic support, and a well-vascularized microenvironment and often correlates with behavioral or functional improvements at later time points (Palma-Tortosa et al., 2020; Weber et al., 2024a). In the following chapters, we discuss in detail how these mechanisms unfold across each stage of stroke recovery.

Paracrine effects

A preclinical observation supporting the "bystander" hypothesis is that functional improvements can be detected before grafted cells fully differentiate into specific neural cells, suggesting that the early therapeutic benefits may arise from factors secreted by the transplanted NSCs/NPCs rather than their integration into injured tissue. Indeed, studies in various animal models of ischemic stroke have repeatedly cemented bystander effects as a fundamental mechanism of stem cell therapy

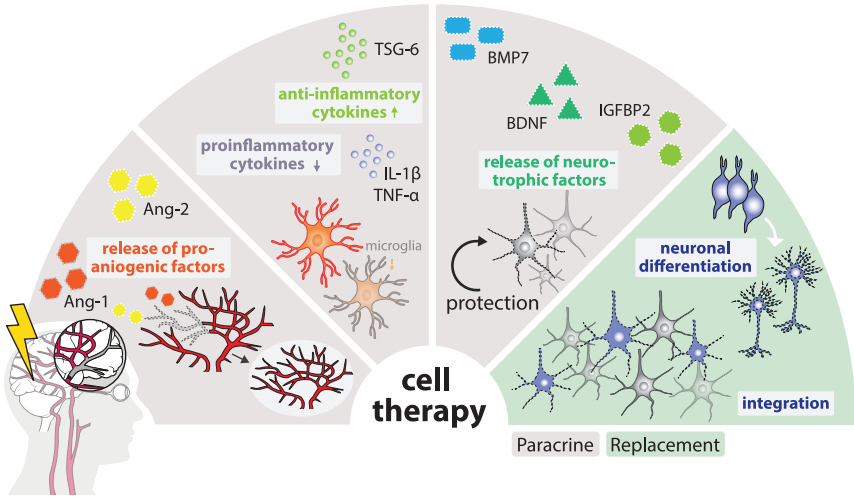

Figure 2. Potential mechanisms of neural stem cell-based brain regeneration

Neuronal degeneration, increased apoptosis, activation of astrocytes and microglia, as well as vascular remodeling are involved in the pathophysiology of ischemic stroke. Cell therapy may reverse these processes through several paracrine mechanisms or by direct cell replacement. (1) Stem cells may release trophic factors, e.g., BMP6 or BDNF, to prevent neuronal cell death, (2) stem cells may inhibit the inflammatory response through the release of anti-inflammatory cytokines, e.g., TSG-6, and the suppression of proinflammatory cytokines, e.g., IL-1β or TNF-α, (3) stem cells can promote angiogenesis through the release of proangiogenic factors and the upregulation of e.g., Ang-1/-2, and (4) stem cells may differentiate into neurons to replace damaged cells and support reconstruction of neural circuits. Ang-1/-2, angiopoietin 1/2; TSG-6, tumor necrosis factor-inducible gene 6 protein; IL-1β, interleukin-1 beta; TNF-α: tumor necrosis factor; IGFBP2, insulin-like growth factor-binding protein 2; BMP7, bone morphogenetic protein 7; BDNF, brain-derived neurotrophic factor.

(Baker et al., 2017; Eckert et al., 2015; Ha et al., 2022; Lee et al., 2017; Li et al., 2023a). These paracrine mechanisms involve the release of bioactive molecules, such as growth factors, cytokines, and extracellular vesicles, which modulate inflammation, promote neuroprotection, enhance angiogenesis, and stimulate endogenous repair processes in the injured brain.

Promotion of angiogenesis and restoration of vascular integrity

Angiogenesis plays a crucial role in neural regeneration and functional recovery following ischemic stroke (Rust, 2020; Rust et al., 2019). Angiogenesis of cerebral microvasculature promotes blood flow and nutrient supply to the damaged brain regions, and numerous factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 govern angiogenesis and vascular maturation following cerebral ischemia. Local transplantations of several cell sources, both neural and non-neural, have shown the ability to restore vascular integrity and improve BBB function in mice (Rust et al., 2025; Weber et al., 2024a). NSC/NPC grafting results in elevated VEGF immunoreactivity in astrocytic endfeet and vessel walls and increased growth of proliferating vessels adjacent to the ischemic tissue (Chau et al., 2014; Oki et al., 2012; Tatarishvili et al., 2014; Weber et al., 2024a). Other pro-angiogenic factors, such as angiopoietin (Ang)-1 and Ang-2, have been demonstrated to be released from stem cells, resulting in angiogenesis-mediated neovascularization (Casas et al., 2018; Chou and Modo, 2016; Rezaie et al., 2018; Xue et al., 2018). NSC-derived small extracellular vesicles promoted cerebral angiogenesis along with neurological functions after middle cerebral artery occlusion (MCAO) in mice, although the underlying mechanisms remained unclear (Gu et al., 2023; Li et al., 2023b).

It is widely acknowledged that enhanced angiogenesis correlates strongly with improved neurological and functional outcomes post stroke, but only if simultaneous restoration of the neurovascular unit and the BBB can be guaranteed (Zhang et al., 2023b). Earlier preclinical transplantation experiments showed that NPC grafts induce acute postischemic neuroprotection by stabilizing the BBB. Engrafted NPCs can reduce stroke-induced elevated matrix metalloproteinase (MMP)-9 levels and prevent zona occludens (ZO-1) degradation in ischemic animals (Huang et al., 2014). Additional studies on NPC-derived extracellular vesicles (EVs) suggest that the application of EVs inhibits the nuclear factor κB (NF-κB) pathway, resulting in reduced ABCB1 and MMP-9 activation. The latter eventually induces basal membrane disruption (Zhang et al., 2021).

Modulation of inflammatory responses

The rapid activation of resident immune cells by pro-inflammatory signals accelerates the infiltration of inflammatory cells (including neutrophils, monocytes/macrophages, distinct T cell subtypes, etc.) into the injured region, thereby exacerbating brain damage (Achón Buil et al., 2023). It is hypothesized that stem cells participate in the inflammatory response and immune modulation after ischemia and can modulate these pathophysiological changes in an indirect manner. Immunocytochemistry data of an MCAO model found augmented infiltration of $ED1^{+}$ cells, a marker for activated macrophages in the ischemic area in NPC-grafted animals as far as 8 weeks post transplantation (Chang et al., 2013; Lee et al., 2017). At the same time, NPC-receiving animals exhibited significant improvement in grip strength and paretic forelimb activity (Lee et al., 2017). Further research found a decrease

in the numbers of activated microglia in the ischemic area accompanied by improved neurological functions after intraparenchymal NPC administration in stroked compared to non-treated animals (Eckert et al., 2015; Weber et al., 2024a). Real-time PCR revealed significant downregulation of pro-inflammatory cytokines (tumor necrosis factor alpha [TNF-α], interleukin [IL]-6, IL-1β) and factors that mediate the infiltration process of immune cells (MCP-1 and MIP-1α) (Eckert et al., 2015).

NSC-derived EVs have also been demonstrated to modulate microglia activation and phenotype polarization after brain injury. EVs were found to selectively accumulate in ischemic brain regions after intravenous injection and reduce inflammatory response of microglia, leading to decreased production of pro-inflammatory cytokines such as TNF-α or IL-1β (Tian et al., 2021). And when injected together with NSCs, NSC-derived exosomes reduced the expression of inflammatory cytokines TNF-α and IL-β while increasing the expression of anti-inflammatory cytokine IL-10 after MCAO in mice (Zhang et al., 2023a).

While systemically injected stem cells haven been shown to promote brain regeneration, their mode of action is still in debate. A recent hypothesis suggests that systemically applied cells modulate and reprogram host immune cells in peripheral organs rather than directly in the brain ("bioreactor hypothesis") (Savitz and Cox, 2023). Evidence from preclinical studies in animal models of CNS injury supports the idea that peripheral immunomodulation, through the release of cytokines and the increased efflux of regulatory T cells, could prevent activated immune cells from crossing the BBB into the brain parenchyma, thereby attenuating the propagation of microglial activation and expansion of tissue loss (Mays and Savitz, 2018; Savitz and Cox, 2023). In a mouse model of multiple sclerosis, intravenous administration of NPCs reduced tissue injury and CNS inflammation, while NPCs did not migrate to the brain but were instead detected in lymph nodes. Here, the NPCs suppressed the activation and proliferation of pro-inflammatory T cells (Einstein et al., 2007). And after intracerebral hemorrhage in rats, transplanted NSCs were found in the spleen and decreased TNF-a, IL-6, and NF-κB levels (Lee et al., 2008).

Astrocyte activation is another feature of neuroinflammation. This process plays a dual role: astrogliosis is essential for repair processes; however, the non-permissive scar tissue impedes with neurogenesis and functional recovery in the chronic phase of ischemia (Iadecola et al., 2020). Interestingly, this non-permissive barrier seems to harbor many important structural and chemical cues that are preserved upon surface transplantation, resulting in superior outcomes in terms of graft integration and functional recovery (Sekiya et al., 2015). More recently, NPCs transplanted into CNS lesions have been shown to restrict inflammation by adapting a wound repair astroglia phenotype with transcriptional and morphological features similar to newly proliferated host astrocytes that surround ischemic lesions (O'Shea et al., 2022). Exposing the cell transplant to a mix of environmental cues from inflammatory/fibrotic cells in the stroke core and neural cells outside the ischemic area can modulate grafted NPC transcription and subsequent differentiation, thereby contributing to preserving healthy neuronal tissue from fibrotic and inflammatory cells.

Neuroprotection

Numerous preclinical studies have demonstrated that stem cells exhibit neuroprotective effects through upregulation of pro-survival factors, facilitating endogenous neurogenesis and synaptic remodeling (Lu et al., 2023; Zhang et al., 2020). *In vitro* stimulation of mouse NPCs transduced with an optochemogenetic fusion protein increased the expression of pro-survival and pro-regenerative genes, including brain-derived neurotrophic factor (BDNF) and nerve growth factor (Yu et al., 2019). Cell transplantation showed superior functional recoveries in NPC-treated young and aged mice after cortical stroke. BDNF is an activity-dependent factor known to promote axonal sprouting/extension (Mamounas et al., 2000). It may be possible that trophic support, such as increased release of BDNF, can augment functional recovery after injury (van Velthoven et al., 2013). Cytokine array analysis of the culture supernatant from an iPSC-NPC/OGD-cortical-cell co-culture system revealed enriched expression levels of factors involved in neuroprotection and neurogenesis such as insulin-like growth factor-binding protein 2 (IGFBP2) and bone morphogenetic protein 7 (BMP7) (Lee et al., 2017). IGFBP2 is expressed during early postnatal neurogenesis in the mouse hippocampus and is upregulated in the hippocampus after murine stroke, while BMP7 promotes neural regeneration and motor recovery in rodent stroke models (Chang et al., 2003). A more recent study found upregulated IGFBP5 levels in the infarct core of ischemic mice (Weber et al., 2024b), a protein suggested to be essential for regulating reparative angiogenesis (Song et al., 2024). In a rat MCAO model of stroke, NPC-transplanted groups showed elevated numbers of BrdU-DCX double-positive cells on the ipsilateral side that were unrelated to the cell graft, suggesting that transplanted NPCs contribute to the proliferation of endogenous NSCs in the subventricular zone, as well as to their migration into the infarcted area (Chang et al., 2013). Similarly, NPC transplantation into the cortex of a photothrombotic stroke mouse model increased the number of EdU/NeuN-positive cells within the ischemic border zone, which only minimally colocalized with human nuclear co-staining, suggesting increased endogenous neurogenesis (Weber et al., 2024a). The role of endogenous neurogenesis in stroke recovery has been

discussed extensively; however, whether these newly formed neurons are relevant for functional recovery remains to be elucidated.

Cell replacement

Research into transplantation-based cortical repair has come a long way since scientists first investigated and confirmed the anatomical and functional integration of fetal cortical tissue into healthy adult rats (Girman and Golovina, 1990). Since then, multiple animal studies have explored the potential of donor cells to form connections with the host tissue and integrate into existing neuronal circuits. In the stroked-damaged cortex of mice and rats, transplanted NSCs fully differentiated into mature neurons and extended their axons even into the contralateral hemisphere at 10 weeks post injury. These graft-derived neurons were able to respond electrophysiologically to peripheral stimulation confirming the host circuitry integration (Oki et al., 2012). However, while the authors observed improved forelimb movements, they emphasize that behavioral recovery was initiated earlier, before any functional neurons could have developed from the cell graft, and thus most likely attributable to mechanisms other than cell replacement.

In a similar setting, single-nucleus profiling and RNA sequencing of donor and cortical host tissue revealed that NPC grafts in a stroke mouse model primarily differentiated into GABAergic neurons and communicated with host cells through regeneration-associated neurexin, neuregulin, neural cell adhesion molecule, and SLIT signaling pathways, resulting in anatomical and functional long-term recovery (Weber et al., 2024a). Six months after intracortical transplantation, monosynaptic tracing revealed that transplanted iPSC-derived NPCs formed functional efferent synaptic input with host neurons in the uninjured contralateral somatosensory cortex in brains of rats with ischemic cortical stroke (Palma-Tortosa et al., 2020). Along with this finding, stroke-induced asymmetry in the cylinder test had been significantly reduced by the transplant compared to nontreated groups. It was shown, however, that inhibition of grafted neurons did not reverse this effect, arguing against neuronal replacement as central mechanism in graft-induced behavior recovery (Palma-Tortosa et al., 2020). In spite of that, another study also provided evidence that activity of grafted cortical neurons may contribute to the maintenance of motor function through transcallosal connections to the corresponding cortical area in the contralateral hemisphere (Tornero et al., 2013). Inevitably, functional integration of grafted neurons/neural progenitors can potentially lead to the reconstruction of cortical neural circuitry. As for which neural circuits contribute to behavioral improvement after stroke, this remains an open question. Transplantation of predifferentiated GABAergic neurons leads to accelerated motor improvements in rats compared to undifferentiated human NSC injection (Abeysinghe et al., 2015). However, the early effects, 1 week post transplantation, suggest regenerative mechanisms beyond cell replacement. While direct neuronal replacement and circuit reconstruction may drive later-stage, long-term improvements, initial functional gains often reflect paracrine support, enhanced plasticity, or the reactivation of dormant host circuits (Benowitz and Carmichael, 2010; Cramer, 2008) rather than immediate integration of the transplanted cells.

More recent studies suggest that effective neural replacement therapy may require the use of a neural substrate that reproduces the structural and functional complexity of the cortex. Developments in cell culture and biomaterial technologies have advanced therapeutical applications of iPSC-derived 3D human brain organoids for CNS repair. Such organoids consist of a complex microenvironment with abundant neural and non-neural cell types at different stages, including NSCs, neurons, astrocytes, and oligodendrocytes (Dong et al., 2021; Revah et al., 2022). Brain organoids grafted onto mouse cortex lesions show increased cell survival and differentiation compared to NSC transplantation. Human iPSC-derived cortical organoids grafted into the somatosensory cortex of athymic rats contained a large number of mature neurons that integrate into sensory and motivation-related circuits (Revah et al., 2022). Further, iPSC- and ESC-derived cerebral organoids that were transplanted into the junction of the infarct core and the peri-infarct zone of a photothrombotic stroke model showed neuronal differentiation, axonal projection, and integration into host neuronal circuits thereby eliminating sensorimotor defects (Cao et al., 2023). In sum, cerebral organoids may offer a new strategy for reconstructing/replacing infarcted tissue; however, several challenges remain in the application of organoids for regenerative medicine (Song et al., 2021). The lack of vascularization restricts nutrient and oxygen supply leading to necrosis in the organoid core and reduced survival (Shariati et al., 2021). To address this, efforts are being made to engineer brain organoids with functional vascular systems (Cakir et al., 2019).

Another challenge is the limited maturation of brain organoids *in vitro* (Song et al., 2021). However, it was shown that *in vivo* transplantation enhances maturation and electrophysiological activity of the grafted organoids (Revah et al., 2022). Additionally, heterogeneity between organoids and across various protocols for organoid generation presents a further obstacle. To improve reproducibility, researchers need to implement rigorous experimental designs, transparent methodologies, and data-sharing practices (Paşca et al., 2025), which will significantly enhance the future applicability of organoids in regenerative medicine.

CONCLUSION AND FUTURE DIRECTIONS

The CNS exhibits limited regenerative potential, posing significant challenges for patients afflicted by ischemic stroke. Yet, despite the vast potential, cell therapy for stroke comes along with a history of clinical trials that did not prove efficacy. However, focusing on NSC types such as NPCs and NSCs instead of mesenchymal or other adult stem cells may be more promising.

We further believe that understanding the precise mechanisms underlying stem cell-based brain recovery can result in better cell therapy products and higher translational success, as important parameters such as the best cell type, ideal application route, or timing of transplantations can be identified for the respective disease. Accordingly, differences in these parameters will certainly have contributed to the inconsistent outcomes in recent clinical trials. Over the years, numerous studies involving the transplantation of different cell types into various models of ischemia have demonstrated mechanistic insights into brain recovery. While several studies have primarily focused on bystander effects, more recent work using NPC and NSC transplantation has shown the generation of specific synaptic connections between host and graft tissue and the exchange of information. However, whether this functional integration really contributes to brain regeneration will need further proof. We argue that further investigation into the yet unidentified mechanisms of cell-based brain regeneration will uncover the ideal stem cell type for therapy and is required before advancing to larger clinical trials.

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AUTHOR CONTRIBUTIONS

All authors contributed to the conceptualization, writing and editing of the manuscript, and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used ChatGPT V o1 (OpenAI) for grammar, syntax, and typo correction. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication. No content generation or data analysis was conducted using AI.

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