

Review Article

Innovative approaches in neural stem cell therapy: a comprehensive review of mechanisms and applications

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Abstract: Stem cell therapy is revolutionizing the treatment of neurological disorders, offering innovative approaches for regeneration and repair. This paper explores five distinct mechanisms of stem cell therapy, focusing on their applications and therapeutic potential. Neural stem cells (NSCs) combined with pharmacological agents, such as FTY720, enhance remyelination and neural repair in multiple sclerosis (MS) and spinal cord injuries (SCI). Induced pluripotent stem cells (iPSCs) provide a personalized approach by enabling the generation of patient-specific NSCs for treating conditions like Parkinson's Disease (PD). Gene-editing technologies, such as CRISPR-Cas9, expand the scope of NSC applications by facilitating precise interventions for genetic disorders like SMARD1. Neurotrophic factors derived from NSCs present a cell-free alternative to promote neuronal survival and repair in diseases such as Parkinson's and Huntington's disease. Additionally, NSC-derived extracellular vesicle therapies, such as intranasal delivery methods for AD treatment, offer non-invasive approaches to reduce neuroinflammation and enhance cognitive recovery. While these mechanisms demonstrate remarkable therapeutic potential, challenges such as cost, scalability, and safety remain. This review provides a comprehensive analysis of these mechanisms, highlighting their contributions to the future of regenerative medicine and personalized therapeutic strategies.

Keywords: Stem cell therapy, neurodegenerative, neurological disorders, regenerative, brain, transplant, Parkinson's disease

Background

Stem cells are cells present in multicellular organisms that meet two specific requirements: perpetual self-renewal and the ability to differentiate into specialized cell types. Perpetual self-renewal and the production of progeny that is identical to the originator cell is a trait also met by cancer cells, which divide and grow in an uncontrolled manner. This is why a second requirement, namely the ability to differentiate into specialized cell types that can become part of a healthy organism, is critical in classifying cells as stem cells.

Stem cells can have further classifications, such as "adult" or "embryonic", depending on the stage of development that the organism from which they originated was in. However, these classifications have become outdated as scientific advancements have made it pos-

sible to transition between adult cells and their embryonic forms.

The predominant naming system for stem cells classifies them by their potency, or ability to differentiate into different cells. This ranges from totipotent (most versatile) to pluripotent, multipotent, and unipotent (most restricted). This paper focuses on pluripotent and multipotent cells. Pluripotent stem cells can differentiate into any of the cell types in the body. They are only present for a brief period in the embryo before differentiating into multipotent stem cells. Multipotent stem cells are more limited in terms of the kinds of cells they can become and can differentiate into cells of a particular germ line (endoderm, mesoderm, or ectoderm) or cells of a particular tissue. The typical developmental progression of stem cells follows a hierarchical pattern where totipotent stem cells give rise to pluripotent stem cells, which differ-

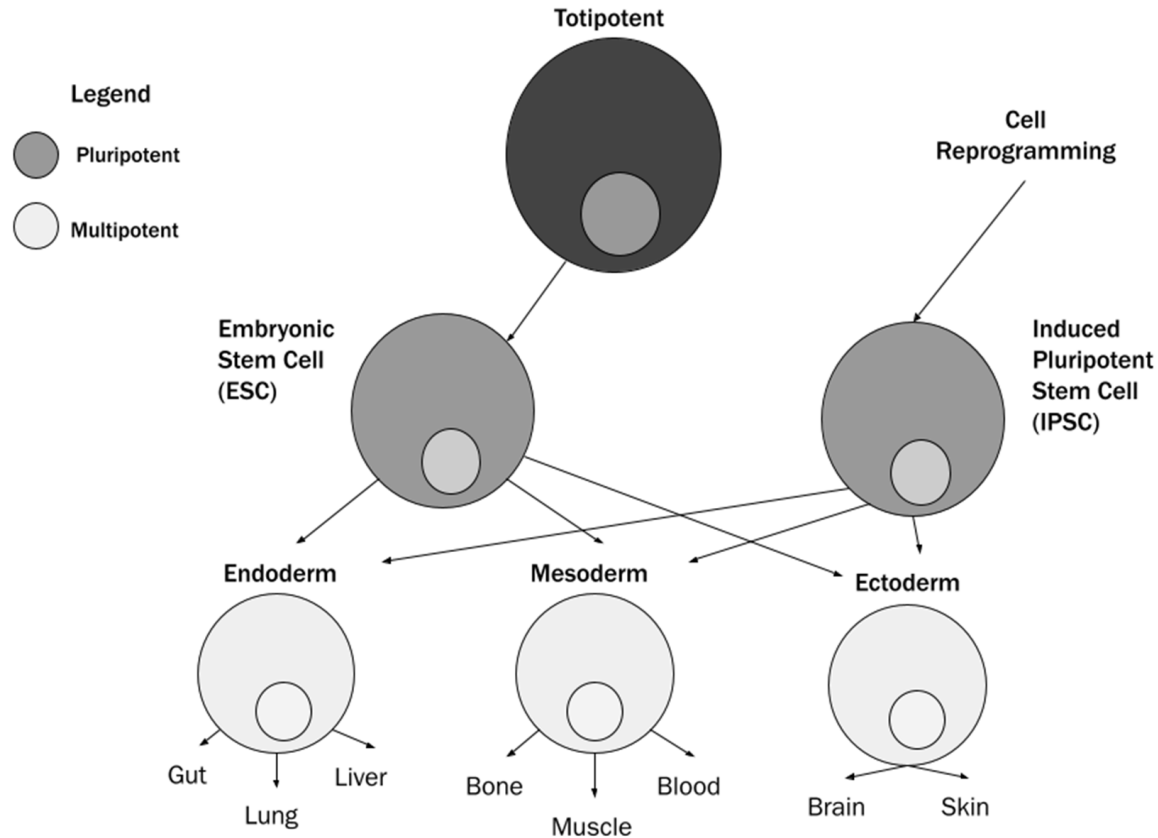


Figure 1. Cell differentiation pathway.

entiate into multipotent stem cells, before finally developing into unipotent progenitors that produce fully specialized cells.

Multipotent stem cells can follow four distinct developmental paths: quiescence, symmetric self-renewal (**Figure 1**), asymmetric self-renewal, or symmetric differentiation. During quiescence, the stem cell remains dormant, refraining from division or differentiation, which causes no change in the organism's stem cell pool. With symmetric self-renewal, a stem cell will undergo cell division to produce two identical stem cell progeny, increasing the size of the stem cell pool. In asymmetrical self-renewal, the stem cell divides into two progeny - one identical stem cell and one more specialized cell (somatic or progenitor cell) - while simultaneously maintaining the stem cell pool while generating cells that contribute to tissue function. Finally, symmetric division occurs when the stem cell will divide into two specialized cells, resulting in a decrease in the stem cell pool.

Therapeutically, multipotent stem cells from bone marrow have been used clinically since the 1960s for treating leukemia and other blood disorders [1]. Research has expanded to identify additional sources with greater plasticity, including neural progenitor cells, placental cells, and umbilical cord blood. Despite their promise, stem cell therapies face significant challenges, including immune rejection, tumor formation risks, ethical concerns with embryonic sources, and high costs associated with isolation, expansion, and quality control. These limitations have driven interest in alternative approaches that might capture the therapeutic benefits of stem cells while avoiding their inherent complications - particularly in the field of neurological disorders, where neural stem cell transplantation presents unique delivery and safety challenges.

Introduction

Neural stem cells (NSCs) have emerged as a transformative therapeutic approach for treat-

ing neurological disorders through their unique capacity to differentiate into neurons, astrocytes, and oligodendrocytes. NSC-based therapies offer the potential to address neurological conditions while promoting neural regeneration, reducing inflammation, and restoring functional connectivity. In research and clinical applications, stem cells are cultivated by obtaining samples from patients [2]. NSCs can be derived from various sources, including embryonic tissue, induced pluripotent stem cells (iPSCs), and adult neural tissue. Once isolated and cultured under specialized conditions with appropriate growth factors and media, these cells can be expanded and directed toward specific neural phenotypes to enhance their therapeutic potential. The cultivation process requires carefully controlled environments with regulated pH levels and oxygen concentrations to maintain their neurogenic properties and viability.

Once cultured, stem cells can be genetically engineered or guided to differentiate into specific functional phenotypes to optimize their therapeutic potential. Predominant strategies for utilizing stem cells in clinical applications, as outlined in this report, include: the use of neural stem cells (NSCs) in combination with standard pharmacological treatments; NSC replacement therapies employing gene editing techniques; the application of induced pluripotent stem cells (iPSCs); and the deployment of NSC-derived neurotrophic factors and extracellular vesicles for therapeutic purposes. Due to the complexity of stem cells and the intricate processes involved in their extraction, purification, cultivation, and application, stem cell treatments are often costly. The price varies significantly based on the condition being treated and the specific type of stem cell therapy utilized [3]. Additionally, the high costs associated with research and development, coupled with the challenges of complex manufacturing and processing, further drive up expenses. All these factors lead to the issue of making this a widely accessible and universal strategy for patient therapies.

Stem cell therapy is groundbreaking due to its unique ability to regenerate and repair by differentiating into various cell types. This makes them a promising approach for treating diseases that currently have no effective treatment.

Another key advantage of stem cell therapy is its ability to be personalized, using the body's healthy cells to repair damaged or missing tissue is a novel concept. Lastly, stem cells can be used with technologies like CRISPR to address specific genetic disorders or diseases that can have long-term, life-altering effects on individuals. This paper examines these five distinct NSC therapeutic mechanisms, analyzing their specific applications, clinical efficacy, and potential for addressing the complex pathophysiology of neurological disorders that currently lack effective treatments.

Combination therapy between medication and neural stem cells

Transplantation of NSCs has demonstrated the capability to repair myelin, reduce axonal damage, and improve neurological function. While clinical research behind the complementary relationship between NSCs and medication is low, the popularity of applying this method to future trials steadily grows due to research over the last decade. In 2019, Ikhsan et al. investigated the processes of combined therapy with drug classes commonly prescribed to geriatric patients, aiming to elucidate potential effects on NSC proliferation and differentiation [4]. The analysis consisted of 5,954 publications, of which 214 met the inclusion criteria, and only 62 studies provided completed datasets suitable for meta-analysis. Among the drug classes, antidepressants were found to stimulate NSC proliferation under psychological conditions and further enhance proliferation in the context of stress [4]. With the majority (65%) of publications analyzed in the study showing that antidepressants stimulate proliferation, this leads us to believe that one of the major obstacles of stem cell therapy efficacy to be alleviated. We expect the usage of antidepressants to mitigate the low stem cell survival and integration since the antidepressant stimulated proliferation would give rise to a larger population of viable cells. Additionally, enhanced proliferation under stress is critical to future applications as there is considerable stress and inflammation at the site of any operation. The increased proliferation under stress would allow larger populations of NSC to integrate and survive the hostile environment during an immune response. Nevertheless, these drugs did not significantly influence NSC differentia-

tion and the limited availability of comprehensive data for other drug classes precluded robust conclusions regarding their interactions with NSCs. We anticipate that further studies into drug supplementation and interactions will subvert the obstacles of NSC transplantation, including poor cell survival, differentiation, and integration, especially in a hostile inflammatory environment.

However, subsequent studies have provided significant results displaying the enhancements of NSC transplantation survival when combined with cognitive-enhancing and immunosuppressive drugs, biasing them towards glial differentiation and promoting functional myelination. These integrated therapies have been demonstrated through *in vitro* and *in vivo* trials to enhance improvements for patients with multiple sclerosis (MS) and spinal cord injuries (SCI).

MS is a chronic autoimmune disease characterized by the demyelination of neurons in the CNS. In comparison to either treatment alone, research by Zhang et al. prompted the supplementary properties of Fingolimod (FTY720), a common MS medication and lipophilic sphingosine 1-phosphate (S1P) analog, and how it improved remyelination, decreased inflammation, and restored neurological function when combined with transplanted NSCs [5]. By utilizing organotypic cerebellar slice cultures from the forebrain of C57/Bl6 mouse pups, analysis demonstrates an increase in myelin basic protein expression treated with this combination, indicating robust myelination. Myelin ensures efficient transmission of action potentials down axons. When myelin is absent or damaged, conduction velocities are dramatically reduced, and action potentials may fail to propagate entirely, leading to weaker and less reliable signals the further they travel. The increased myelination shown from FTY720 would allow for slowing the progression of MS as well as increasing the survival and integration of transplanted NSCs. The NSCs which could differentiate into oligodendrocytes to promote remyelination would be expected to have greater effect due to the increased myelin protein expression. Additionally, results indicate that FTY720 might be involved in guiding NSC lineage specification due to higher rates of oligodendrocyte differentiation. *In vivo*, shiver mice, who were also treat-

ed with the combined therapy, manifested a notable reduction in seizure frequency and improved survival rates alongside their improved functional recovery (**Figure 1**) [5]. Future clinical trials are needed to conclude long term efficacy of FTY720, but current studies show promise for increased remyelination in MS phenotype. These findings have laid the foundation for future synergistic mechanisms between neurological medication and NSCs, operating beyond immunomodulation.

SCI is notable for its incurable and disabling properties, accompanied by complex inflammation-related pathological processes of the damaged spinal cord. In a recent 2024 study, Qi et al. assessed the therapeutic potential of an injectable hydrogel system loaded with carbon dots (CDs) and FTY720, combined with NSCs, for SCI treatment. *In vivo* trials of SCI rat models exhibited an increased effect of cavity reduction and myelin repair with the composite hydrogel compared to the control groups [6]. FTY720 combined therapy with NSCs having projected an increase in myelination exhibits potential for treatment of many complications, SCI especially. SCI typically presents with nerve damage leading to impaired neural signaling, which is why the combination therapy of FTY720 with NSCs in the hydrogel system shows such crucial therapeutic potential. The study demonstrates increased remyelination rates compared to treatment without the combination, meaning that damaged neural connections are able to repair more effectively than with conventional treatments alone. Additionally, *in vitro* trials indicated that the composite not only promoted NSC differentiation into neonatal neurons, but also dramatically bolstered proliferation, promoting neural regeneration and hence lowering the cavity area [6]. Replacement or repair of damaged neural cells is crucial for recovery and the transplanted cells along with the increased proliferation by FTY720 provides the cells needed to restore the damaged neural connection. Histological analyses further confirmed enhanced neural regeneration and remyelination, along with reduced glial scar formation in the treatment group.

Both MS and SCI share critical pathological features, including demyelination, neuroinflammation, and impaired neural regeneration. Insights

that have guided SCI research towards incorporating similar MS combination therapies can be attributable to past research linking SCI to an increased risk of developing MS. In 2015, data from the Taiwan National Health Insurance Research Database was analyzed by Lin et al. and revealed that immune system dysregulation following SCI can potentially trigger the demyelination processes of MS [7]. Overall, the translational pipeline from MS to SCI research exemplifies how foundational knowledge in one domain can catalyze breakthroughs in another.

FTY720 elicits a synergistic effect exceeding the efficacy of either treatment individually by enhancing neural stem cell (NSC) function and modulating the immune response through direct mechanisms. The translation of these findings into clinical applications remains imperative as ongoing research seeks to elucidate the underlying mechanisms of these interactions, offering potential for improved therapeutic outcomes in multiple sclerosis (MS) and spinal cord injury (SCI) patients.

Gene-edited neural stem cell therapy with respiratory distress

Neural cell replacement therapy utilizing gene-edited stem cells involves employing techniques such as CRISPR-Cas9 to precisely correct targeted genetic mutations. Pluripotent stem cells are induced to differentiate into neural stem cells (NSCs), which are subsequently cultured in specialized media and subjected to targeted genetic modifications to confer desired phenotypic traits. Large-scale cultivation of a specific NSC lineage enables cryopreservation for subsequent therapeutic applications. These mechanisms facilitate targeted interventions for specific genetic abnormalities. Such therapies are particularly suitable for disorders driven by defined genotype mutations or abnormalities.

Spinal muscular atrophy with respiratory distress type 1 (SMARD1) is an autosomal recessive motor neuron disease that presents as symmetric, predominantly distal, muscular weakness and progressive muscular atrophy [8, 9]. A mutation in the IGHMBP2 gene results in decreased production of IGHMBP2 protein characterized by reduced motor neuron survival

due to the protein's role in expected neuronal functions [10]. This disease is currently incurable; only multidisciplinary care to improve the quality of life for patients has been clinically implemented. However, gene therapy shows promise in preventing or ceasing neurodegenerative disease in SMARD1 patients. Specifically, stem cell transplantation can display positive results by both replacing cells and providing support to endogenous motor neurons. Due to this approach's ability to replace damaged neural cells, the therapeutic window is expanded from the presymptomatic stages to the symptomatic stage 8. The therapeutic window is the time frame in which administration of treatments can provide a clinical benefit to the patients. We expect that this increase in the therapeutic window will allow for further research and innovation of techniques to drastically improve outcomes for SMARD1 patients. Rodent (murine) models, particularly B6.BKS lghmbp2nmd-2 J mice are commonly used. This model has a conserved lghmbp2 region localized on chromosome 19 with a spontaneous homozygous mutation in the lghmbp2 gene that results in phenotypic expression similar to that of human SMARD1. The mutation is a substitution mutation from an A to a G in intron 4, which causes an 80% decrease in the production of functional lghmbp2 transcripts [8]. iPSC-derived NSCs used to treat these mice were able to preserve endogenous motor neurons and improve/rectify the phenotype of the animal model. The iPSCs used were derived from a healthy subject and then reprogrammed. The iPSCs were then maintained with a specific medium and differentiated towards an NSC line. Finally, the iPSC-derived NSCs were selected for the expression of certain genes. The relative ease with which iPSCs can be cultivated gives us reason to believe that the implementation of iPSCs to treat neurodegenerative diseases on a large scale. The NSCs derived from these iPSCs are preferred in treatment, and since they can also be easily procured using standard protocols, we have reason to believe that this method of cultivation can become a widespread source of NSCs for the treatment of neurodegenerative diseases.

The selected NSCs were pretreated with trophic factors and inhibitors that optimized cell survival and function. NOD/SCID (immunodeficient) mice were used for preliminary testing to

assess the migration and engraftment of the NSCs.

The stem cells were injected intrathecally (in CSF) into the model mice. The model mice were either homozygous for *nmd* (neuromuscular degeneration) or WT (wild type). A study by Forotti et al. shows that the *nmd* mice presented a serious phenotype with a low survival time of approximately 13.4 days $\pm$ 2.8 days [8]. Data on survival, weight, and muscle function and ability (evaluated by the hindlimb splay) were monitored weekly.

The *nmd* mice were sacrificed on postnatal days 3 and 10, and tissues of interest, such as the spinal cord, diaphragm, and gastrocnemius, were isolated and preserved to be analyzed [8, 10]. Following analysis revealed improved morphology and cross-sectional area of myofibers. The higher cross-sectional area of myofibers indicates an increased number of contractile proteins (actin and myosin) being present. This means that the mice in the treatment group were able to retain more of their muscular strength, which indicates that the treatment is effective in slowing muscular atrophy. Neuron connections to muscles in the NMJs are crucial for the ability of muscles to receive signals and contract or relax accordingly. The *nmd* mice display higher levels of denervated NMJs, which leads to the characteristic muscular atrophy that is observed. Upon treatment, fewer denervated NMJs (neuromuscular junctions) were observed in the specimens. This leads us to maintain that the gene-edited neural stem cell therapy can diminish the effects of the neuromuscular degeneration in mice caused by the mutation in chromosome 19. Due to the similarity between SMARD1 in humans and the mutation/phenotype of the tested mice, we conclude that this treatment can be applied to SMARD1 in an effective manner. The cells showed tremendous migration capacity and engraftment, as they were visible in the gray matter of the anterior horns of the lumbar spine of the NOD mice. The high level of migration displayed by the NSCs leads us to believe that this method of treatment can be implemented with higher efficacy in diseases that cause neural degeneration in a widespread manner. Directed cell migration would be able to further increase the efficacy of this type of stem cell treatment, as

the stem cells would be able to localize to the area of interest and further increase the efficacy of the treatment [11]. The data suggests that rather than the NSCs differentiating into neurons, the donor NSCs exerted paracrine actions on the endogenous motor neurons. The treatment significantly improved lifespans in the *nmd* mice (22.7 $\pm$ 5.8 days) [8]. We expect that this drastic improvement in the lifespan of the mice models is indicative of the validity of this form of stem cell therapy. With results such as these, it can be reasonably inferred that the application and testing of gene-edited stem cell therapy is a promising mechanism for future neurological treatments. Overall, the treatments result in the improved pathological phenotype of treated mice in terms of survival, growth, and muscular function. Although the results of these studies show promise in treating diseases caused by genotype mutations/abnormalities, further testing is required to determine long-term efficacy as well as safety.

Induced pluripotent and neural stem cells

Induced pluripotent stem cells (iPSCs) represent another significant method in stem cell therapy, offering potential treatments for a variety of diseases. These cells are typically derived from adult skin or blood samples, which are then reprogrammed to an embryonic-like state, allowing them to differentiate into the specific cell types required for healing [2, 12, 13]. Research has been conducted on the application of iPSCs in treating conditions such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), hematological disorders, and kidney disease [12-14].

Induced pluripotent stem cells (iPSCs) are cultivated by first obtaining somatic cells from the patient or donor, typically from blood or skin [2]. These cells are then reprogrammed using specific transcription factors to return them to an embryonic-like state. The reprogrammed cells are cultured in a specialized medium to promote growth. Although not all cells will become iPSCs, those that do are selected and tested for pluripotency through surface markers and gene expression. Once confirmed as true iPSCs, they are differentiated into the required cell type, expanded, and purified to remove any undifferentiated cells, reducing the risk of

tumor formation or rejection by the host. Finally, the iPSC-derived cells are administered back to the patient via injection, infusion, or surgical grafting, depending on the treatment needed.

Parkinson's disease is a progressive neurodegenerative disorder that affects the dopaminergic neurons in the substantia nigra pars compacta of the basal ganglia. Misfolded alpha-synuclein accumulates in the neurons causing the formation of Lewy bodies which then impair normal cellular function and cause vesicular disruption. This then leads to neuroinflammation, excitotoxicity and impaired dopamine homeostasis. Autologous induced pluripotent stem cells have been used in recent research to generate midbrain dopaminergic progenitor cells from a patient's personal iPSCs and inserted back into the putamen region of the brain [15]. The procedure did not require immunosuppressive therapy, due to the use of autologous cells. Typically, cell transplants would require immunosuppressive therapy to ensure that the newly transplanted cells are integrated properly not targeted by the hosts immune system. Cells transplanted from iPSCs provide the unique advantage of being cultivated from the host, avoiding any rejection of foreign cells. While this allows for higher integration of cells, this also leads to less inflammation and stress post procedure and would not compromise the immune system lowering risk of infection. Post-implantation assessment with positron-emission tomography (PET) scans using fluorine-18-L-dihydroxyphenylalanine assists in determining graft survival [15]. Evaluations displayed an improvement in PD symptoms after 18 to 24 months [15].

Another attractive addition to this highly personalized mechanism is the quick integration of induced neural stem cells (iNSCs). These cells are induced somatic cells that are directly reprogrammed into neural stem cells without first becoming iPSCs [16]. They are generated through the overexpression of neural-specific transcription factors, enabling direct lineage conversion that bypasses the pluripotent intermediate stage. This approach decreases the procedural complexity and reduces the cultivation duration typically required for induced pluripotent stem cells (iPSCs). Human neural stem cells (hNSCs) were directly reprogrammed from

peripheral blood mononuclear cells to achieve complete functional integration into the adult murine brain. The study demonstrates the successful conversion of somatic blood cells into induced neural stem cells (iNSCs) via transient overexpression of specific transcription factors. These reprogrammed cells were subsequently transplanted into the hippocampus and striatum of adult immunodeficient mice to assess engraftment and functional integration. The results showed functional integration capabilities 12 weeks post transplantation [17]. The iNSC-derived neurons exhibited markers to show neuronal differentiation, as well as cellular extensions of the transplanted cells in surrounding brain tissue. Electrophysiological assessments were performed to ensure these neurons developed functional properties, which were used to determine successful functional integration [17]. Overall, this study demonstrates successful functional integration of iNSCs derived from human blood cells into mice.

Neurotrophic factor-based treatment

NSC therapy has long been considered a promising approach for treating many neurological disorders. However, its clinical application has been hindered by the cost and significant concerns regarding the safety of grafting neural stem cells [18]. These challenges have shifted research focus toward exploring alternative approaches, particularly the therapeutic potential of the neuronal secretome; the collection of protein and molecules secreted by NSCs. Neurotrophic factors are one of the many components secreted that make up the secretome and have sparked growing interest as a viable alternative for therapeutic applications. Unlike traditional cell replacement therapies, neurotrophic factors can be administered through less invasive methods, are relatively inexpensive, and can be efficiently cultivated in laboratory settings.

In fact, neurotrophic factor-based therapies have already demonstrated safety and tolerability in clinical trials. For instance, a Phase I study involving 57 ALS patients highlighted the safe application of neurotrophic factor treatments [19]. These findings reinforce the advantages of neurotrophic factors as a viable, cell-free therapeutic option with reduced risks compared to classical cell transplants.

Neurotrophic factors have been extensively studied for their critical roles in supporting neuronal survival, proliferation, and maturation. They have shown therapeutic potential across a wide range of neurological disorders, including hypoxic-ischemic brain injury, ALS, PD, HD, and AD [20]. Preclinical models and clinical trials have demonstrated the efficacy of neurotrophic factor-based treatments, with the added benefit of scalability for large-scale production.

Interest in neurotrophic factor-based therapies has developed over decades of research, which revealed their potential in treating cognitive disorders. Neurotrophic factors can be organized into three families: the neurotrophins, glial cell line-derived neurotrophic factor (GDNF) family ligands (GFLs), and the ciliary neurotrophic factor (CNTF) family of cytokines. Neurotrophins, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), play a significant role in the survival of peripheral neurons (including autonomic, sensory, and motor neurons) as well as central nervous system neurons. GDNF family ligands are crucial for the development and maintenance of spinal motor neurons, dopaminergic midbrain neurons, and cerebellar neurons, while CNTFs exhibit neuroprotective effects across various regions of the nervous system. Each of these families of neurotrophic factors have been studied for their unique functionalities and applications for neural disorders [21]. Given the distinct attributes of each family and the diverse approaches to therapeutic implementation, it is anticipated that the majority of neurological conditions can be addressed or, at minimum, their symptomatic manifestations can be mitigated.

Among these families, neurotrophins have been particularly well-studied for their roles in synaptic plasticity, neurogenesis, and neuronal differentiation. This family, which includes NGF and BDNF, has shown therapeutic applications in neurodegenerative diseases such as AD and PD [20]. Although NGF and BDNF share similar functions and mechanisms, they are expressed in different regions of the nervous system. NGF supports the survival of cholinergic neurons in the basal forebrain, whereas BDNF promotes the survival of dopaminergic neurons in the substantia nigra [22]. Both neurotrophins sig-

nal through receptor tyrosine kinases (*Ntrk1* for NGF and *Ntrk2* for BDNF) [21]. Studies by Nordvall et al. have shown that ectopic BDNF expression yields therapeutic effects comparable to transplanted stem cells [23]. Furthermore, BDNF promotes the survival of dopaminergic neurons in animal models of PD and has been explored as a potential therapy for stimulating neuroplasticity and cognitive function in AD [22, 24, 25].

Beyond neurodegenerative disorders, neurotrophins have demonstrated promising therapeutic potential in peripheral nervous system applications, particularly in ophthalmology. NGF plays a crucial role in the development, maintenance, and function of sensory neurons innervating the eye [26]. NGF has shown remarkable efficacy in treating noninfectious corneal ulcers resulting from various etiologies such as chemical burns and neurotrophic keratopathy [27]. In a clinical trial involving 12 patients with corneal ulcers of diverse origins, topical NGF administration promoted epithelial healing, reduced inflammation, and improved corneal innervation [27]. More recent research has extended these findings to pediatric populations, where perturbations in NGF signaling have been implicated in various pediatric ophthalmic disorders, including congenital corneal anesthesia, neurotrophic keratitis, and congenital glaucoma [26]. This application is particularly significant considering the cornea is one of the most densely innervated tissues in the body, with numerous sensory nerve endings essential for maintaining ocular surface integrity. The neurotrophic properties of NGF not only facilitate corneal wound healing but also restore the neurological feedback mechanisms crucial for tear film production and corneal homeostasis, addressing the underlying pathophysiology rather than merely treating symptoms. These findings further demonstrate the versatility of neurotrophins as therapeutic agents across different regions of the nervous system and across age groups.

The GDNF family ligands (GFLs) have also attracted significant interest for their ability to support spinal motor neurons and dopaminergic midbrain neurons. First discovered in the early 1990s, GDNF was identified as a powerful supporter of dopaminergic neurons, which are critically affected in Parkinson's disease [28].

Like other neurotrophic factors, GDNF works by binding to specific receptors on neurons, triggering pathways that help neurons survive, migrate, and maintain their specialized functions [28].

Research into GDNF as a potential treatment for Parkinson's disease has shown promising results, particularly in animal studies [28]. However, translating these findings to effective human treatments has faced challenges. While safety hasn't been a major concern, researchers are still working to determine the best ways to deliver GDNF to the affected brain regions and establish consistent benefits [28]. Recent animal studies have explored innovative delivery methods, such as using macrophages (immune cells) to transport GDNF, which has shown improvements in preserving neural tissue and recovering motor function [28]. Studies in non-human primates have also demonstrated better movement and dopaminergic neuron survival after delivering GDNF using viral vectors [28]. Neurturin (NRTN), a GFL member, has shown promising results in Phase II clinical trials for treating PD [29]. These positive findings have spurred the development of GFL mimetics for a range of conditions, including ischemic stroke, amyotrophic lateral sclerosis, PD, and Alzheimer's disease.

The CNTF family has been investigated for its applications in HD, a progressive neurodegenerative disorder causing motor deficits and cognitive impairment. Mittoux et al. demonstrated the efficacy of CNTF in HD primate models, where semipermeable capsules containing baby hamster kidney (BHK) cells engineered to produce human CNTF were implanted into the striata of primates. The study showed that CNTF prevented neuronal degeneration and restored neostriatal function [30].

These studies highlight the benefits of neurotrophic factor-based therapeutic approaches, including the circumvention of health risks linked to conventional cell transplantation and the facilitation of cost-effective, scalable manufacturing processes. Neurotrophic factors constitute a promising, cell-free modality for the treatment of neurodegenerative diseases. Ongoing research aimed at optimizing delivery methods and augmenting therapeutic efficacy is crucial to fully realize their potential in managing neurological disorders.

Neural stem cell extracellular derived vesicles and neuroinflammation

Recent research has shown the therapeutic potential of stem cells due to their ability to secrete extracellular vesicles (EVs) [31]. EVs are a fundamental component of the cell-to-cell communication process, capable of modulating immune responses and inflammation. The ability of EVs to transition from inflammation to tissue repair is currently being studied with the aim of finding a credible substitute for traditional cell therapies to treat inflammation-mediated conditions [31].

These tiny membrane-bound structures enveloping bioactive molecules such as proteins, lipids, and miRNAs (microRNAs) can transfer molecules to other cells and impose changes in their functions. Most essentially, RNA contents within EVs impose variability in the expression of genes and can regulate the recipient cells in critical cellular signaling pathways. The capacity of EVs to modulate cellular fate makes them a potential therapy for neurological disorders, including Alzheimer's disease (AD). Currently, AD is the most common type of dementia and leading cause of death for people aged sixty-five and above [32]. Neuroinflammation, chronic activation of glial cells, and accumulation of plaques and proteins are hallmarks of AD, which results in an unremitting cascade of neurodegeneration and cognitive decline. To delay or alter the course of AD, other recent studies have evaluated methylated EVs obtained from NSCs as a potential therapeutic [31, 33, 34]. Investigations of the properties of NSC-derived EVs have shown their potential therapeutic effects for AD treatment.

In one study, NSC derived from hiPSCs were cultured in differentiation media to produce EVs, which were subsequently isolated by a combination of anion exchange chromatography (AEC) and size exclusion chromatography (SEC) [31]. For characterization based on their size, concentration, and protein composition, the EVs went through NP (nanoparticle) tracking analysis, Western blotting, and electron microscopy [31]. The other study focused on intranasal NSC-derived EVs in the animal model for AD [34]. The EVs were sprayed via a nasal route so that cells involved in chronic neuroinflammation could be targeted. The researchers

tracked the EVs by their incorporation into microglia, brain immune cells associated with inflammatory processes of AD [33, 34].

Both studies emphasized the therapeutic promise of NSC-derived EVs in the treatment of Alzheimer's Disease. From the first study, the EVs were shown to contain characteristic vesicular markers and contained miRNAs involved in CNS processes, suggesting that those NSC-derived EVs carry molecules capable of influencing the brain [31]. The second study showed that intranasally applied EVs reduced inflammation and plaque accumulation in an animal model of AD [34]. In these studies, the microscopic environment led to the incorporation of EVs by microglia, leading to the degradation of toxic proteins while maintaining their ability to clear amyloid plaques [34].

Thus, this approach supports the hypothesis that neural stem cell-derived extracellular vesicles (NSC-EVs) may contribute to the mitigation of neurodegeneration associated with Alzheimer's disease. These findings underscore a multifaceted perspective, suggesting that NSC-EVs could play a role in targeting inflammatory and proteopathic mechanisms underlying neuronal damage in Alzheimer's pathology. Furthermore, intranasal delivery offers a non-invasive administration route that may be particularly advantageous for patients with cognitive impairments, who might exhibit reduced compliance or tolerance to invasive procedures. Intranasal administration confers distinct pharmacokinetic benefits over systemic delivery, including circumventing the blood-brain barrier, facilitating rapid transport to the central nervous system, and minimizing systemic side effects. Additionally, its simplicity and non-invasiveness render it suitable for long-term therapeutic regimens, which are often necessary in managing neurodegenerative conditions such as Alzheimer's disease. These attributes collectively highlight intranasal administration as an efficacious modality for delivering NSC-derived EVs.

General discussion

Stem cell research has emerged as a promising frontier in neurotherapeutics owing to its potential to target previously intractable neurological disorders. This paper analyzes discrete mechanisms for the deployment of stem cells in the

therapeutic management of neurological diseases.

NSC transplantation combined with pharmacological intervention. In multiple sclerosis (MS), Zhang et al. demonstrates that combining NSCs with Fingolimod (FTY720) significantly enhanced remyelination, reduced inflammation, and improved neurological function beyond what either treatment could achieve alone [5]. The synergistic effect promoted oligodendrocyte differentiation and functional recovery in experimental models [4]. Similar outcomes were observed in spinal cord injuries (SCI) models done by Qi et al., where an injectable hydrogel system loaded with carbon dots and FTY720, combined with NSCs, created a favorable microenvironment for neural regeneration [6]. The parallel findings between MS and SCI studies, conditions sharing similar pathological features, suggests that this combinatorial approach holds significant promise for clinical translation.

Gene-edited neural stem cells (NSCs) provide targeted therapeutic interventions for genetic neurological disorders. This strategy demonstrates significant efficacy in conditions characterized by specific genetic mutations, such as spinal muscular atrophy with respiratory distress type 1 (SMARD1). Forotti et al. demonstrated that iPSC-derived NSCs could significantly improve the pathological phenotype in SMARD1 mouse models. The mice treated showed enhanced survival rates, improved growth, and better muscular function. Importantly, the NSCs appeared to exert their therapeutic effects primarily through paracrine actions on endogenous motor neurons rather than direct neuronal differentiation. This mechanism allows for scalable production of therapeutically modified stem cells that can address the common genetic basis of these conditions across patients.

For conditions requiring more personalized approaches, autologous iPSC-derived stem cells provide a promising alternative. Parkinson's disease (PD) studies have shown that midbrain dopaminergic progenitor cells generated from a patient's own iPSCs and implanted into the putamen can improve PD symptoms within 18-24 months. This approach eliminates the need for immunosuppressive therapy and offers highly tailored treatment. Further

streamlining this process, direct reprogramming of somatic cells into induced neural stem cells (iNSCs) has been demonstrated by Berg et al., who showed successful integration of these cells into adult mouse brains [17]. The iNSC-derived neurons exhibited appropriate markers of neuronal differentiation and developed functional electrophysiological properties, suggesting potential for clinical applications in humans. Being able to take native patient cells and reprogram them for neural cell therapies would decrease the immune response and stress on the patient, whilst also ensuring that the stem cells utilized will be integrated into the surrounding tissue.

Despite the therapeutic promise of direct cell replacement therapies, financial and practical limitations have spurred interest in cell-free alternatives [35]. Neurotrophic factors secreted by NSCs offer a more cost-effective and less invasive approach. Neurological disorders such as MS and have been prime targets for development of stem cell therapies as increasing the myelination in the nervous system would greatly improve the prognosis. Whilst the most direct way to increase the myelin is to replace the damaged myelin producing cells, indirect routes are also expected to have similar effects. Neurotrophic factors secreted by cells can induce higher myelin production in the native cells, potentially restoring functionality of myelin producing cells. These factors, which include neurotrophins, glial cell line-derived neurotrophic factor family ligands, and ciliary neurotrophic factor family cytokines, have demonstrated efficacy in treating various neurological disorders. Clinical trials have already established the safety and tolerability of neurotrophic factor-based treatments, particularly in amyotrophic lateral sclerosis (ALS) patients. Their roles in supporting neuronal survival, proliferation, and maturation make them versatile therapeutic agents for conditions ranging from neurodegenerative diseases to peripheral nervous system disorders.

Finally, stem cell-derived extracellular vesicles (EVs) represent an emerging therapeutic strategy, particularly for neurodegenerative conditions like Alzheimer's disease (AD). Studies by Upadhyay et al. demonstrated that intranasally administered NSC-derived EVs effectively reduced inflammation and plaque accumula-

tion in AD animal models. These membrane-bound structures, containing bioactive molecules such as proteins, lipids, and miRNAs, can modulate immune responses and facilitate the transition from inflammation to tissue repair. The non-invasive intranasal delivery method offers distinct advantages, including bypassing the blood-brain barrier and reduced systemic side effects, making it well-suited for long-term treatment of neurodegenerative disorders.

Each of these mechanisms constitutes a distinct strategy for exploiting the regenerative capabilities of stem cells in neurological therapy. While direct cell replacement methods provide extensive solutions for particular pathologies, cell-free modalities that leverage secreted bioactive factors offer more practical and scalable alternatives. As scientific understanding advances, the integration of these complementary approaches may be strategically employed to target the complex, multifaceted pathophysiology of neurological disorders, potentially transforming therapeutic paradigms for previously intractable conditions.

Conclusion

Neural stem cell research constitutes a shift in the therapeutic approach to neurological disorders, providing a promising framework for exploring diverse mechanisms that may enhance their regenerative capabilities. The investigation into the potential functionalities of various mechanisms has yielded significant advancements, building upon prior research and discoveries that support the therapeutic potential of neural stem cells (NSCs).

Combination therapies, such as NSCs with FTY720, have demonstrated the synergistic effect of when both medicines coalesce, producing a greater effect than either therapy alone. The proposed mechanism indicates the promotion of neural repair while enhancing remyelination and reducing inflammatory responses. The few clinical trials available show promise of this therapeutic treatment and we expect more trials moving forward to study the long-term effects of combination therapies of classical NSC transplants with drugs. We hope that these drug interactions will be able to settle the concerns regarding the efficacy of stem cell therapies. Furthermore, CRISPR-Cas9 technology has also widened the applications of

NSCs, specifically SMARD1, which enables precise correction of genetic mutation in pluripotent stem cells. The complications of pluripotent stem cells being differentiated for neurological disorders have been minimized due to SMARD1, promoting neuronal survival and function.

iPSCs offer another alternative, since they have been reprogrammed, to tackle AD pathology. The use of neurotrophic factors such as BDNF and CNTF, is another less invasive but cell-free alternative for enhancing neuronal growth and survival in disorders such as PD and HD offering personalized, immune-compatible cell therapy. This immune-compatible cell therapy has demonstrated graft survival, functional integration, and neuronal activity restoration, further highlighting the potential of autologous iPSC-derived dopaminergic progenitor cells and directly reprogrammed iNSCs as approaches for PD and HD treatment.

Research with NSC-derived EVs highlights their capacity to modulate immune responses and reduce inflammation. Intranasal delivery of EVs provides a non-invasive route for targeting neurodegenerative diseases, such as AD, with the potential to bypass the blood-brain barrier and avoid systemic side effects. With a less invasive route of delivery, stem cell therapies could be more accessible, less costly, and expect to have a faster recovery rate. With one of the main concerns for stem cell therapies being the invasive and costly nature, the development of alternative delivery methods through novel stem cell application brings stem cell therapy back to the forefront of treatment for neurological conditions.

These approaches highlight the tremendous potential of NSCs in developing treatments for an array of complicated disorders affecting the nervous system. However, some challenges including high costs, health considerations, and technical barriers remain. Further clinical research will be required for the development of these NSC mechanisms. To optimize these therapies and make them more widely available, interventions will play an essential role in paving the way for a new era in personalized medicine.

Disclosure of conflict of interest

None.

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