Chapter 8

Neural Stem Cells Application for Central Nervous System Pathologies

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Abstract

The discovery and advent of NSCs[1] in preclinical and clinical studies have led to progress in the alleviation of several pathological conditions. NSCs are rare cell numbers of the CNS[2] and are located in the SVZ[3] and the hippocampal DG[4] in adults and mature rodents. To date, the number of studies related to the application of NSCs in animals and human

1. Neural stem cells
2. Central nervous system
3. Subgranular zone
4. Dentate gyrus

counterparts has increased. It has been suggested that NSCs can efficiently directly commit to neuronal cells to restore function or replace injured neurons or glia. Besides, the release of several cytokines and growth factors facilitates the neuroregeneration following transplantation into the injured sites. Here, in this chapter, the therapeutic properties of NSCs will be discussed under pathological conditions related to neurodegeneration or ischemic diseases. Understanding the molecular mechanisms provided by transplanted NSCs can help us in the development of nascent therapeutic protocols.

Keywords: Neural Stem Cells; Neurodegeneration; Ischemic Changes; Therapy; Regenerative Medicine.

1. NSCs (terminology, origin, and function)

As above-mentioned, NSCs are multipotent cells that can differentiate into neurons, astrocytes, and oligodendrocytes [1]. These cells are important in brain development, homeostasis, and regeneration during both embryonic and adult neurogenesis [2]. It is thought that NSCs originate from the neuroectoderm during embryogenesis and further generate from the primary CNS neural tube. The process of neurulation is stimulated by the invagination of neural plates to form neural tube structures via various mechanisms. Primary NSCs or neuroepithelial cells undergo symmetric division to increase NSC populations. The activity of neuroepithelial cells produces RGCs[1], induces the migration of progenitor cells, and the subsequent formation of scaffolding structures. In the next steps, RGCs form numerous glial cells and neurons during embryonic neurogenesis, and some lineage-restricted progenitors with the potential to commit to oligodendrocytes, astrocytes, and neurons [3]. Around 13.5

[1]. Radial glial cells

to 15.5 days of embryonic phases, a fraction of RGCs can generate dormant pre-B1 cells, which can produce adult NSCs in regions like the SVZ. Calling attention, the temporal and spatial development of the CNS is a multi-step process [4-6]. Compared to embryonic neurogenesis, adult neurogenesis has also confirmed by the confirmation of NSCs in SVZ and SGZ regions [7]. It is believed that the SVZ NSC subpopulation, known also as type A neuroblast, exhibits astroglial properties (B1) with the potential to orient into IPCs[1], and constitute the BBB[2] with the production of astrocytes, and brain ECs[3] (**Figure 1**) [8].

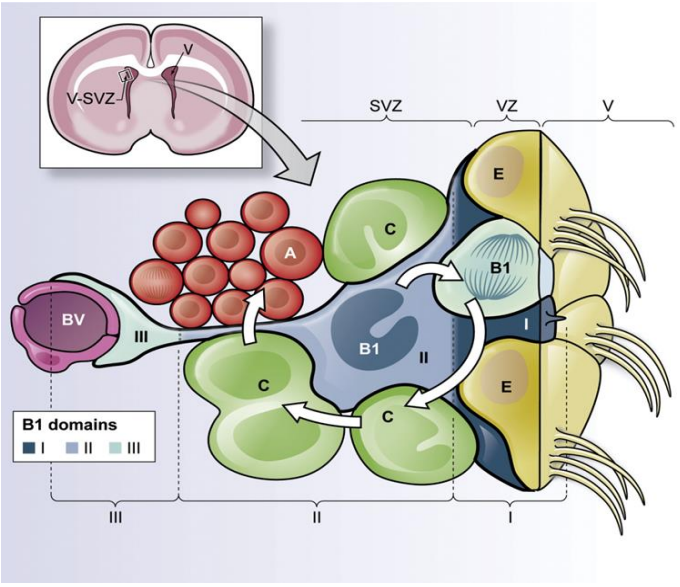

Figure 1. The existence of neurogenesis regions in the adult SVZ. The frontal cross-section of an adult mouse brain (upper left image) is SVZ, which continues neurogenesis of the lateral ventricle walls throughout life. The Lower panel indicates the organization of cells within the SVZ microenvironment. In this region, type B1 NSCs (Blue) are enclosed by multi-ciliated ependymal cells (E), generating a pinwheel appearance. B1 cells transformed to IPCs or C cells (Green), which per se commit into type A neuroblasts (Red). The resting B1 cells with a thin apical process reach the ventricles and BV[4] (Purple). It is thought that

[1]. Intermediate progenitors
[2]. Blood-brain-barrier
[3]. Endothelial cells
[4]. Blood vessels

these cells are seen with different microanatomical locations. In the first domain (apical cells indicated by dark blue), these cells are in close contact with CSF[1] to receive factors and signaling molecules from ependymal cells. In the second region, the intermediate cells (indicated by medium blue) interact with IPCs, neuroblasts, and other cells. In the domain III (Basal region), these cells have direct contact with the BV and respond to EC signals. These features help B1 cells integrate different external and endogenous signals during neurogenesis. Copyright. [9]. Cell Stem Cell.

SGZ, a germinal layer of the hippocampus's DG, is located between the GCL[2], containing numerous granule neurons, and the hilus. It has been thought that SGZ is actively involved in adult neurogenesis, which coincides with the proliferation and differentiation of NSCs into granule neurons to integrate the hippocampal circuits. The SGZ is juxtaposed to blood vessels to provide a microenvironment for neurogenesis for a whole lifetime. This region is also involved in cognitive functions, learning, and memory [10-12]. Of note, SGZ NSCs are astroglial cells with numerous radial processes crossing the GCL. These are also named RAs[3], type A neuroblasts, and radial RGCs with the potential to produce DGNs[4] via the activity of IPC1 and IPC2 [13].

1. Cerebrospinal fluid
2. Granule cell layer
3. Radial astrocytes
4. Dentate granule neurons

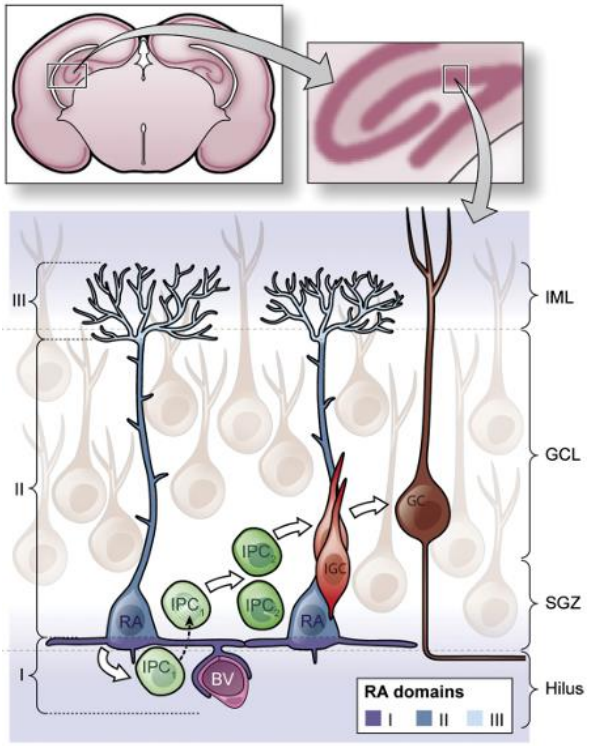

Figure 2. Schematic illustration of SGZ radial astrocytes. Coronal section of the adult mouse brain, indicating the hippocampal DG (upper panel) and SGZ (lower panel). RAs or Type 1 cells (blue) are NSCs and can express glial markers like GFAP[1]. The polarization of Ras leads to numerous primary cilia in the proximal domain I region (Dark blue) with integration with BV (purple) and neighboring RAs. Domain II (intermediate, medium blue) contains the RA body and shaft that facilitate direct interactions with IPCs (Green) and

[1]. Glial Fibrillary Acidic Protein

mature GCs[1] (Brown) to regulate neurogenesis. Finally, in the distal Domain III region (Light blue), these cells have several branches into the IML[2], contacting synaptic terminals and glial cells to sense neural activity. RAs generate IPCs (Type 2a/2b cells), which differentiate into immature GCs (red) and ultimately excitatory GCs. This compartmentalization allows RAs to integrate vascular, cellular, and synaptic cues to maintain adult neurogenesis in the SGZ.[9]

The function of NSCs is tightly controlled by intrinsic (*i.e.*, epigenetic factors) and extrinsic mechanisms (*i.e.*, external microenvironmental signals). Endogenous factors such as SOX2[3] and PAX6 are integral to NSC activity via preserving self-renewal and differentiation capacity. Other factors, such as Spy1[4], trigger NSC symmetric division and are also involved in glioblastoma formation [14, 15]. Various signaling pathways, such as Notch, Wnt/β-catenin, and Shh[5], are important determinants in NSC behavior. It is believed that Notch signaling can maintain NSC quiescence via suppression of pro-neural genes by Hes1 and Hes5. In contrast, the Wnt signaling cascade stimulates the proliferation and differentiation of these cells [16-18]. Epigenetic modifications such as DNA methylation, histone acetylation, and ncRNAs[6] such as microRNAs, control chromatin accessibility and gene expression in NSCs [19]. For example, the methylation of RNA via METTL1 stimulates hippocampal neurogenesis in adult mice [20]. Growth factors such as EGF[7], FGF[8], and BMPs[9] control NSC differentiation into different lineages. Notably, the

1. Granule cells
2. Inner molecular layer
3. SRY-box 2
4. RingoA
5. Sonic Hedgehog
6. noncoding RNAs
7. Epidermal growth factor
8. Fibroblast growth factor
9. Bone morphogenetic proteins

existence of a vascular niche helps provide oxygen, micronutrients, and factors such as VEGF[1] to control the activity of NSC (**Figure 3**) [21].

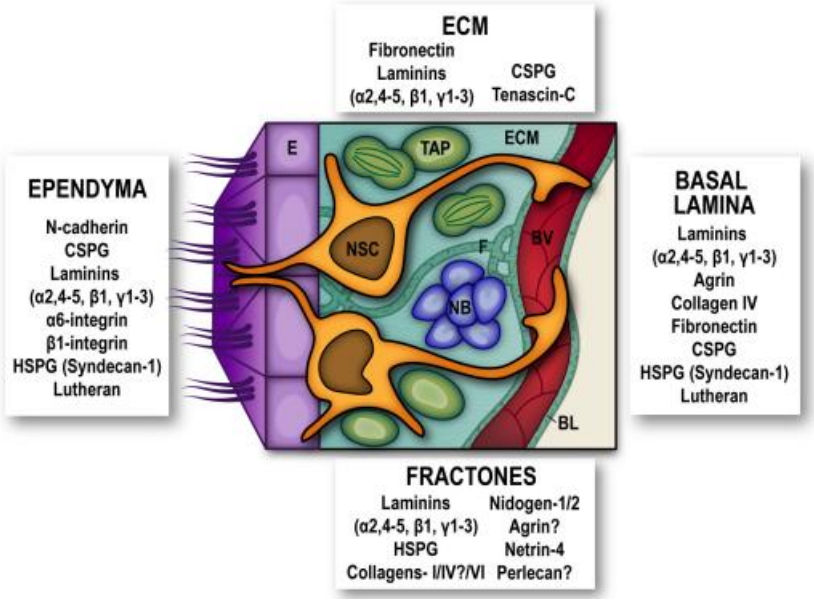

Figure 3. The SEZ[2] niche contains ECM[3] and adhesion molecules with the potential to recognize different cell types, multiciliate ependymal cells, NSCs, transit-amplifying progenitors, neuroblasts, and BV. Distinct proteins and adhesion molecules exist in the ECM, basal lamina, fractones, and ependymal cells of the SEZ, such as HSPG[4] and CSPG[5] [22].

2. NSC-based therapies

The failure of an endogenous reparative system is one of the main aspects of neurodegenerative disorders such as AD[6], PD[7], and HD[8], leading to the lack of successful regeneration and restoration of

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1. Vascular endothelial growth factor
2. Subependymal zone
3. Extracellular matrix
4. Heparan-sulfate proteoglycans
5. Chondroitin sulfate proteoglycans
6. Alzheimer's disease
7. Parkinson's disease
8. Huntington's disease

neurological function [23, 24]. It has been estimated that tens of millions of people suffer from neurodegenerative disorders globally. With the aging of the world population, the physical and socioeconomic burden has increased dramatically in the healthcare system during recent decades [25]. Calling attention, the therapeutic properties of conventional drug treatment are limited due to several side effects and the inability to replace the damaged neural tissue with mature and functional neurons [26]. In line with these descriptions, urgent therapeutic modalities should be developed and examined in preclinical and clinical settings. Both direct differentiation into the neuronal cells and the release of various cytokines have been related to the regenerative function of NSCs [27, 28]. During several neuropathological conditions, these cells produce different neurotrophic factors, leading to activation of endogenous neurogenesis, neuroprotection, immune cell regulation, and neural circuit remodeling [29-32]. Both allogenic and autologous sources of NSCs have been used in different experiments to elucidate their regenerative potential [33]. These cells can be harvested from other cell sources like hESCs[1] and iPSCs[2] under controlled culture systems [34]. Currently, different strategies are used to achieve the regenerative potential of NSCs.

2.1. Direct transplantation

The implantation of NSCs aims to restore function by replenishing lost neurons and glial cells [35]. This approach has demonstrated therapeutic potential in preclinical studies of SCI[3], where transplanted NSCs improved motor function, reduced inflammation, and promoted remyelination in rodents [36, 37]. Studies showed NSC grafts implanted in adult brains form

[1]. Human embryonic stem cells
[2]. Induced pluripotent stem cells
[3]. Spinal cord injury

clusters near the injection site with limited migration ability and therapeutic efficacy [38]. Therefore, achieving functional recovery in CNS injuries requires transplanted cells to migrate either widely across affected regions or at least within critical injury zones. These challenges can be appropriately addressed through strategies like hypoxic preconditioning to enhance migration-related gene expression (e.g., HIF-1α[1] and CXCR4[2]). A close interaction between transplanted NSCs and host glia is critical in scar remodeling, inflammatory responses, and neural circuit reconstruction [38, 39]. Liu et al. transplanted human NSCs into seven cynomolgus monkeys using a stereotactic device four days after IS[3] induced by photothrombotic occlusion. It was demonstrated that human NSC transplantation controlled infarcted lesion volume without causing remarkable side effects. They found a stimulated GABAergic[4]- and glutamatergic-based neurogenesis in the ischemic region with enhanced mitochondrial function. qRT-PCR[5] data and immunohistochemical staining confirmed that NSCs promoted neurogenesis and inhibited post-infarct inflammation via regulation of glia and recruitment of peripheral immune cell infiltration [40]. Of note, transplantation of NSCs into the dorsal putamen in 8 patients with moderate PD has no side effects or immune system reaction after 1 to 4 years. PET[6] imaging showed the activation of dopaminergic neurons in the midbrain without any effects on patients' neuropsychological scores. Except for one patient, in other cases, motor functions were improved and responded to L-dopa[7] [41]. Curtis and

1. Hypoxia-inducible factor 1-alpha
2. C-X-C chemokine receptor type 4
3. Ischemic stroke
4. Gamma aminobutyric acid
5. Quantitative real-time reverse transcription
6. Positron emission tomography
7. l-3,4-dihydroxyphenylalanine (levodopa)

colleagues assessed the feasibility and safety of transplanting human spinal cord NSCs (also known as NSI-566) for treating chronic SCI [42]. In this scenario, 4 participants with SCI at the thoracic region (T2-T12) underwent laminectomy, durotomy, and received NSI-566 cells in six separate stereotactic injections in the midline bilateral region. Interestingly, patients showed no side effects even after 18 to 27 months after cell transplantation. Based on the ISNCSCI classification, three patients had improved sensory and motor scores, which necessitate further investigations [42]. In a phase I clinical trial led by Leone and colleagues, allogeneic human NSCs were used in the alleviation of secondary progressive 15 MS[1] patients over one year. Their data indicated that intracerebroventricular injection of these cells with concomitant immunosuppressive therapy had no side effects or mortality. More interestingly, MRI[2] revealed non-significant changes in lesion volume, brain, and function. In biochemical analysis, the contents of acyl-carnitines and fatty acids were elevated in CSF[3], indicating the potency of NSCs in the reduction of neuronal degeneration and reliable translation into human medicine [43]. Although the application of medications targeting immune cell function coincided with neuroprotection can yield better regeneration outcomes in progressive MS; however, numerous studies are needed to elucidate the underlying mechanisms. The transplantation of NSCs in different animal models of MS alleviates the pathological conditions via simultaneous neuroprotection and remyelination via paracrine activity [44, 45]. In a non-randomized and single-dose-finding phase 1 clinical study (NCT03269071), the safety and tolerability of intrathecal transplantation of

[1]. Multiple sclerosis
[2]. Magnetic resonance imaging
[3]. Cerebrospinal fluid

human fetal NSCs were assessed in 12 progressive MS patients. Their data indicated the lack of any side effects in patients after two years. The higher doses of NSCs can efficiently prolong brain atrophy and increase the CSF levels of neuroprotective and anti-inflammatory cytokines, indicating the applicability of further clinical trials with higher NSC doses in large populations [46]. In another phase 1 clinical study, it was suggested that intranasal administration of ANGE-S003 NSCs in 18 patients with progressive PD, with three different doses, 1.5, 5, and 15 × 10^6 cells show safety with improved function over 6 months [47]. Despite the existence of the therapeutic efficiency of NSCs in different animal models, it has not been found that these features can be obtained in human counterparts [48, 49]. Although the number of studies related to NSC application has increased, some problems limit their bulk application in the clinical setting. For example, NSCs should be transplanted in high numbers, which necessitates ex vivo manipulation and expansion [49]. Besides, the lack of suitable characterization markers for isolated cells makes the application of NSCs problematic for clinical purposes [50]. The in vitro expansion protocols have a prolonged process that may affect the stemness of NSCs [51-53]. Therefore, standardizing the culture protocols and isolation process can help obtain relatively uniform NSCs in terms of function and regenerative potential.

2.2. NSCs paracrine activity

NSC paracrine effects may be safer and more effective compared to direct cell-based therapies [54]. Neurotrophic factors such as BDNF[1] [55], NGF[2],

[1]. Brain-derived neurotrophic factor
[2]. Nerve growth factor

GDNF[1], CNTF[2], and VEGF were found in the NSC secretome with the potential to influence the survival and activity of endogenous neurons and stimulate axonal/dendritic connections [56]. The administration of human CNSC-SE[3] in AD mice via the nasal cavity. Data confirmed enhanced neuronal cell activity and dendritic process formation. *In vivo* data revealed that CNSC-SE-treated AD mice had fewer Aβ[4] plaque deposits and better memory function [57]. Direct injury and stress to the ER[5] are the main causes of neuronal damage after TBI[6], leading to progressive and secondary pathologies [58]. The injection of NSC secretome in TBI rats contributes to the improvement of CNS function and behavior. Ultrastructural images and proteomic analysis revealed the restoration of the ER because of the activation of the PERK/eIF2α axis and the inhibition of apoptosis. Using the LC-MS/MS/MS[7], calumenin was found in the NSC secretome with a protective role against ER stress via the stimulation of ubiquitination [59]. In a study conducted by our research group, it was found that the NSC secretome can alleviate AD-related changes via the up-regulation of genes such as PI3K, Akt, MAPK, and ERK, and the reduction of GSK3β belonging to the Wnt/β-catenin signaling pathway. Along with these changes, the number of $BrdU^+/Nestin^+$ and $BrdU^+/NeuN^+$ NSCs was increased after the injection of NSC secretome, resulting in reduced neurotoxicity and enhanced neuronal function [60]. Data have revealed the existence of nanosized EVs[8] in NSC secretome, leading to numerous therapeutic outcomes [61]. These particles are actively involved in intercellular communication between the close and

1. Glial-cell-line-derived neurotrophic factor
2. Ciliary neurotrophic factor
3. iPSC-derived cortical NSC secretome
4. Amyloid beta
5. Endoplasmic reticulm
6. Traumatic brain injury
7. Liquid chromatography-tandem mass spectrometry
8. Extracellular vesicles

remote cells [62]. In general, EVs encompass a heterogeneous population including Exos[1] (40–100 nm), MVs[2] (100–1000 nm), and apoptotic bodies (1000-5000 nm) harboring different signaling factors [63, 64]. Due to their nano-sized properties, it is suggested that vesicles can transfer the BBB from blood to CSF and *vice versa* [64, 65]. Direct fusion with the plasma membrane, ligand-receptor interaction, endocytosis, micropinocytosis, and lipid rafts are non-invasive internalization routes for EVs, making them valid bioshuttles in regenerative medicine [66, 67]. Different regenerative outcomes, such as neurogenesis, synaptic plasticity, and anti-inflammatory response, make NSC EVs therapeutic tools in neurodegenerative medicine [68]. As expected, the transfer of signaling biomolecules between the donor and acceptor cells can restore, change, and regulate several signaling cascades [69]. The application of NSC EVs instead of parent cells can address some limitations related to immunogenic response and cell expansion problems, while the load of drugs and various factors can be delivered into the injured site via these particles [70, 71]. In IS mice induced by MCAO[3], the administration of adhesive NSC Exo-loaded HA[4] led to expression of angiogenesis-related factors and neurological improvement, especially motor function. Besides, the necrotic zone volume and inflammation were reduced in the presence of HA hydrogel containing NSC Exos [72]. To increase the on-target delivery of NSC Exos, several studies have been conducted to date. For example, Tian et al. produced engineered NSC EVs with a recombinant fusion protein consisting of RGD-4C peptide (ACDCRGDCFC) attached to PS[5]-binding domains of lactadherin. The

[1]. Exosomes
[2]. Microvesicles
[3]. Middle cerebral artery occlusion
[4]. Hyaluronic acid
[5]. Phosphatidylserine

systemic injection of RGD-C1C2 led to local accumulation of engineered EVs inside the ischemic region, resulting in the inflammatory response. Molecular analyses revealed several miRNAs with anti-inflammatory properties [73]. In another study, the intranasal administration of hiPSC-NSC-EVs[1] in transgenic 5xFAD mice resulted in their uptake by microglia and astrocytes, leading to the reduction of microglia NLRP3 inflammasome and INF-γ[2] signaling [74]. Of note, these EVs yield relatively similar *in vivo* outcomes in Aβ oligomer-exposed microglia in the culture system. After two-month culture time, the cluster of microglia, inflammasome, and Aβ deposits was reduced [74]. Zhang et al. found that hiPSC-NSC-EVs can alleviate the inflammation and oxidative stress in IS mice coincided with accelerated differentiation of NSCs into mature neurons. The simultaneous application of NSCs and EVs yielded higher regenerative outcomes via the reduction of infarct volume, cell death, and scar formation [75]. Other cell death types can be controlled by NSC EVs. In this regard, Peng et al. demonstrated that NSC EVs alleviated rat IS changes by the reduction of NLRP3 inflammasomes and thereby neuronal pyroptosis. These effects were associated with the existence of YBX1[3], leading to stability of GPR30[4] and interaction with IGF2BP1. It is thought that factor GPR30 can promote NLRP3 ubiquitination via interaction with SPOP[5] [76]. Unfortunately, most EV experiments are associated with preclinical studies; just a few clinical trials have been done in IS patients.

2.3. Biomaterial-based transplantation of NSCs

The simultaneous use of hydrogels and scaffolds can stimulate NSC viability, differentiation rate, and on-target delivery [77]. An experiment

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1. human-iPSC-derived NSC EVs
2. Interferon-1
3. Y box binding protein
4. m6A-modified G protein-coupled receptor 30
5. Speckle-type POZ protein

showed that NSC-loaded CSPG hydrogel triggers a regenerative potential of microglia/macrophage lineage following IS in mice [78]. Data confirmed the increase of PPAR-γ[1], MCP-1[2] , and IL-10 in encapsulated cells compared to the NSC alone group. Along with these changes, post-stroke depression-like behavior was reduced. More interestingly, microglia/macrophages encapsulated in CSPG hydrogel had superior angiogenesis behavior in *in vitro* conditions [78]. In an interesting study, it was determined that nanofibrous hydrogels with bioactive IGF-1[3] molecules activate and reduce NSC apoptosis, and improve their orientation toward mature neurons and oligodendrocytes (**Figure 4**). Inside the SCI sites, the neurite outgrowth formation and myelin generation were significantly stimulated by hydrogel-containing NSCs. Likewise, NSC EV-loaded IGF-1 hydrogel promotes the number of differentiated neurons with an expression of miRNAs regulating inflammation and myelin formation. The study confirmed that EVs are successfully internalized by endogenous NSCs [79].

[1]. Peroxisome proliferator-activated receptor gamma
[2]. Monocyte chemoattractant protein-1
[3]. Insulin-like growth factor-1

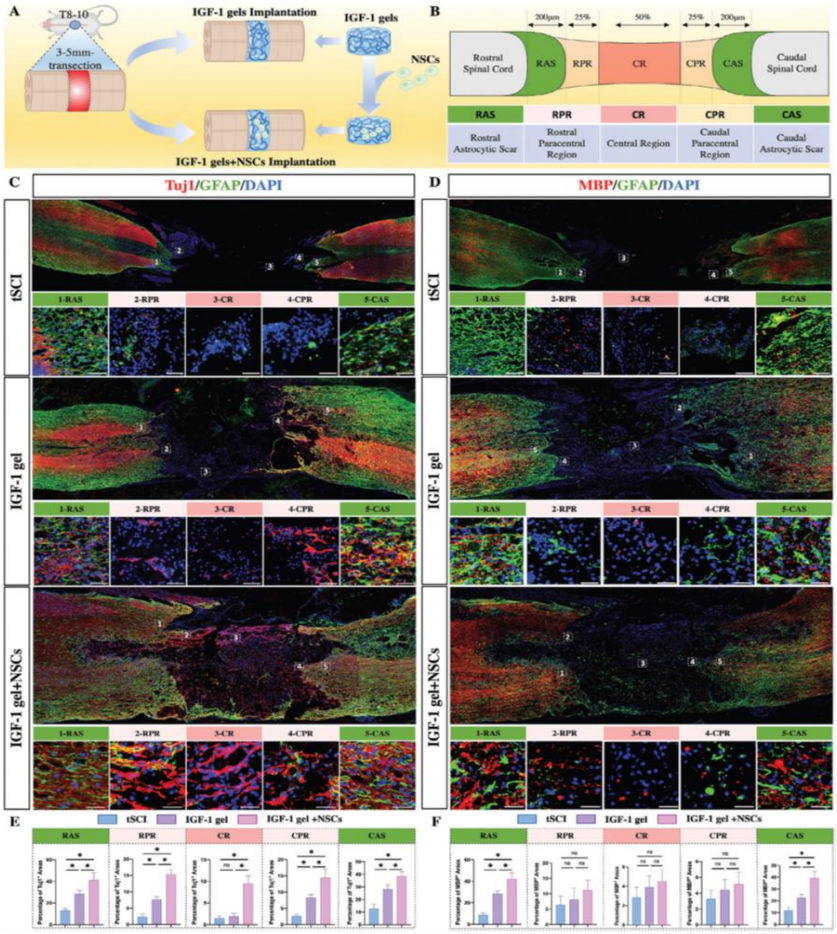

Figure 4. Transplantation of IGF-1-enriched CSPG hydrogel with NSCs for the alleviation of SCI (**A-F**). Data confirmed stimulated neurite outgrowth and regeneration of the myelin sheath. Schematic illustration of the tSCI[1] site and the transplantation of IGF-1-loaded hydrogel (**A**). Different histological regions were examined following tSCI (**B**). Tuj1[2] and GFAP[3] immunostaining was performed in the tSCI in different groups, including IGF-1 and IGF-1 + NSC groups, after week 6 following tSCI (**C** and **E**). The transplantation of NSC-loaded IGF-1 increased the number of $Tuj1^+$ cells and prolonged the outgrowth of neuronal fibers inside the lesion site (n = 5; scale bar = 100 μm). MBP[4] and GFAP double immunostaining indicated the production of myelin sheaths within the astrocytic boundary

[1]. Transected SCI
[2]. Class III β-tubulin
[3]. Glial fibrillary acidic protein
[4]. Myelin Basic Protein

after implantation of NSC-loaded IGF-1 gels (n = 5; scale bar, 100 μm) (**D** and **F**). Copyright. [79]. Advanced Science. 2024.

In a study conducted by Chen et al., the possible impact of CSF flow was assessed on NSC transplantation using hydrogels after TBI[1] induction in rats. It was indicated that GelMA-Alg[2] can promote NSC attachment, growth, and differentiation properties [77]. In addition to increased retention time in the presence of CSF flow. Along with these features, the number of microglia, astrogliosis, and injured neurons was significantly diminished, coinciding with neurogenesis and neurological recovery [77]. Supporting hydrogels can support the physiological activity of NSCs. In this regard, Kim and co-workers used a spheroid-based culture system for the expansion of murine NSCs with simultaneous expression of SOX2, PAX6, Olig2, nestin, and DCX[3]. The transplantation of hydrogel-based NSC spheroid sheets into TBI animals increased the viability of transplanted cells and promoted the number of Tuj-1 expressing cells and angiogenesis potential, leading to improved cognitive function [80]. In an ionic cross-linked Alg-based hydrogel containing rat NSCs, the viability and phenotype acquisition were stimulated within the polymeric network. Transplantation of NSC-loaded Alg hydrogel transected SCI rats preserved about one-third of transplanted cells with significant differentiation toward neurons, astrocytes, and oligodendrocytes. The growth of axons and synaptogenesis was stimulated and extended into grey and white matter, resulting in improved successful electrophysiological conductivity in the hindlimb [81]. The transplantation

[1]. Traumatic brain injury
[2]. Gelatin methacrylate-sodium alginate
[3]. Doublecortin

of chemically incorporated CDs[1] and FTY720[2] GelMA hydrogel with NSCs into SCI animals stimulated neurogenesis, synaptogenesis, and conductive regeneration via simultaneous reduction of ROS[3], leading to the reduction of cavity size [82]. Comini and colleagues used a neurotrophin-enriched Col[4] hydrogel to increase the survival and maturation of iPSC-DAPs[5] in PD rats. Tyrosine hydroxylase immunostaining indicated better regenerative outcomes in the presence of GDNF and BDNF with enhanced dopaminergic differentiation [83].

3. Challenges in clinical translation

Considering the therapeutic potential of NSCs in different preclinical studies, translation of these findings to a clinical setting can provide effective, safe, and feasible therapeutic options. However, this modality encounters some critical challenges that should be addressed by further studies. For example, it is mandatory to purify NSCs and use some biomarkers for their immunophenotyping. Some markers, such as PAX6, SOX1, and SOX2, should be applied for confirmation of the sameness of isolated cells [84, 85]. To date, several administration routes have been introduced in terms of NSC transplantation, such as direct, intravenous, subarachnoid space, and local injury site injection, which are commonly used approaches for NSCs. Calling attention, each administration route has varied therapeutic outcomes. For instance, systemically injected cells or introduction via the subarachnoid space have less on-target capacity as few cells can successfully reach the injured sites, colonize, and exert their therapeutic outcomes [86, 87]. Transient central or local pain,

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[1]. Carbon dots
[2]. Fingolimod
[3]. Reactive oxygen species
[4]. Collagen
[5]. iPSC-derived dopaminergic progenitors

immunosuppression, and neurological impairment are possible side effects after NSC transplantation. Currently, there is no standard protocol for the transplantation of NSCs in terms of time and dose [27, 88]. As a common belief, allogeneic NSCs predispose the activation of alloreactive immune cell function [89]. However, uncontrolled proliferation or inefficient differentiation increases the possibility of tumor formation, especially in circumstances where NSCs are isolated from embryonic sources [90]. The occurrence of functional recovery is closely related to the successful integration of NSC grafts into existing neural networks [89]. In terms of NSC EV therapies, large-scale production, delivery approaches, heterogeneity, and other issues should be standardized [91].

4. Ethical and technical considerations

There are ethical concerns, such as moral, legal, and social issues related to the origin of NSCs and their application in clinics [92]. Commonly, NSCs are isolated from fetal tissue or produced via human ESCs, iPSCs, and adult NSCs. Each of these sources presents different ethical issues [93]. The harvesting of neuroectodermal stem cells from fetal tissues generates ethical controversies regarding the moral status of the fetus and the ethics associated with miscarriages for research purposes. These challenges raise ethical concerns about informed consent from the donor, the commodification of fetal tissues, and the social implications of fetal tissue research [61, 62]. Besides, the most controversial source of NSCs is ESCs, which are obtained from embryos, typically those that were created through IVF[1] but not implanted masses. The processing of these embryos raises ethical concerns, as some believe embryos deserve the same moral standing as individuals. This creates

[1]. *in vitro* infertilization

moral dilemmas related to preserving life and whether there is a trade-off between the advancement of medicine and respect for life [63]. Some countries completely prohibit this practice because of ethical issues. In terms of iPSCs, the genetic manipulation of somatic cells leads to iPSC formation, which raises concerns about genetic alterations and the potential tumorigenicity [64]. For adult NSC isolation, the safety of donors, the surgical process, and ex vivo expansion are the main problems [92, 94].

Compared to NSC preparation and isolation, the administration of these cells needs precise follow-up, informed consent, and monitoring of patient safety. For example, the risk of tumor formation, long-term side effects, and misunderstanding of volunteers who assume that these approaches are a cure-all [95, 96]. It is mandatory to inform the patients about the possible risks and alternative approaches before transplantation of NSCs, and vulnerable candidates should not be included in stem cell therapy programs [97].

5. Conclusions

The clinical use of NSCs in CNS disorders is growing rapidly with great potential. As discussed in this chapter, NSCs with great differentiation capacity and paracrine capacity are valid cells in the restoration of damaged neuron function. The activities of NSCs are regulated by a combination of intrinsic transcriptional programs and signals from specialized areas, which makes it possible to apply NSCs in neurodegeneration, demyelination, and inflammation. In the future, the incorporation of NSC therapy into conventional treatment regimens can have a significant impact on CNS disorders that are currently untreatable.

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