

REVIEW

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Biomaterials and cell-based therapy post spinal cord injury

Sara Haratizadeh^{1†}, Haitao Liu^{2†}, Hengde Li², Mohsen Adeli^{3,4*}  and Angelo H. All^{2*} 

Abstract

Spinal cord injury (SCI) imposes a significant physical, social, and economic burden on millions of patients and their families worldwide. Although medical and surgical care improvements have decreased mortality rates, sustained recovery remains constrained. Cell-based therapies offer a promising strategy for neuroprotection and neuro-regeneration post-SCI. This article reviews the most promising preclinical approaches, encompassing the transplantation of embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), neural stem cells (NSCs), oligodendrocyte progenitor cells (OPCs), Schwann cells (SCs), and olfactory ensheathing cells (OECs), along with the activation of endogenous pluripotency cell banking strategies. We also outline key ancillary strategies to enhance graft cell viability and differentiation, such as trophic factor assistance, engineered biomaterials for supportive scaffolds, and innovative methods for a synergistic effect in treatment, including promoting neuronal regeneration and reducing glial scars. We highlight the key aspects of SCI pathophysiology, the fundamental biology of cell treatments, and the advantages and limitations of each approach.

Keywords Cell therapy, Stem cells, Biomaterials, Spinal cord injury, Regenerative medicine

Graphical abstract

There are several approaches to treating spinal cord injuries that show great promise: Cellular therapies, which utilize a range of cells such as embryonic, neural, and mesenchymal stem cells, along with astrocytes, Schwann cells, olfactory ensheathing cells, and reprogrammed cells; The use of innovative biomaterials, including hydrogels, collagen, polycaprolactone fibers, and advanced 3D-printing technologies, provides valuable support for tissue repair.

Introduction

Spinal cord injury (SCI) is a progressive injury characterized by the acute phase of primary injury composed of vasculature damage and hemorrhage, inflammation, excitotoxicity, formation of free-radical, ionic, and neurotransmitter imbalances. This is followed by the subacute phase, which includes Wallerian Degeneration and Acute Axonal Demyelination [1, 2], activation of astrocytes, glial scar formation, and formation of hostile microenvironments. The third phase is a long-term chronic phase, which is progressive neurodegeneration, causing further motor, sensory, and autonomic nerve dysfunction. SCI can be classified into six major

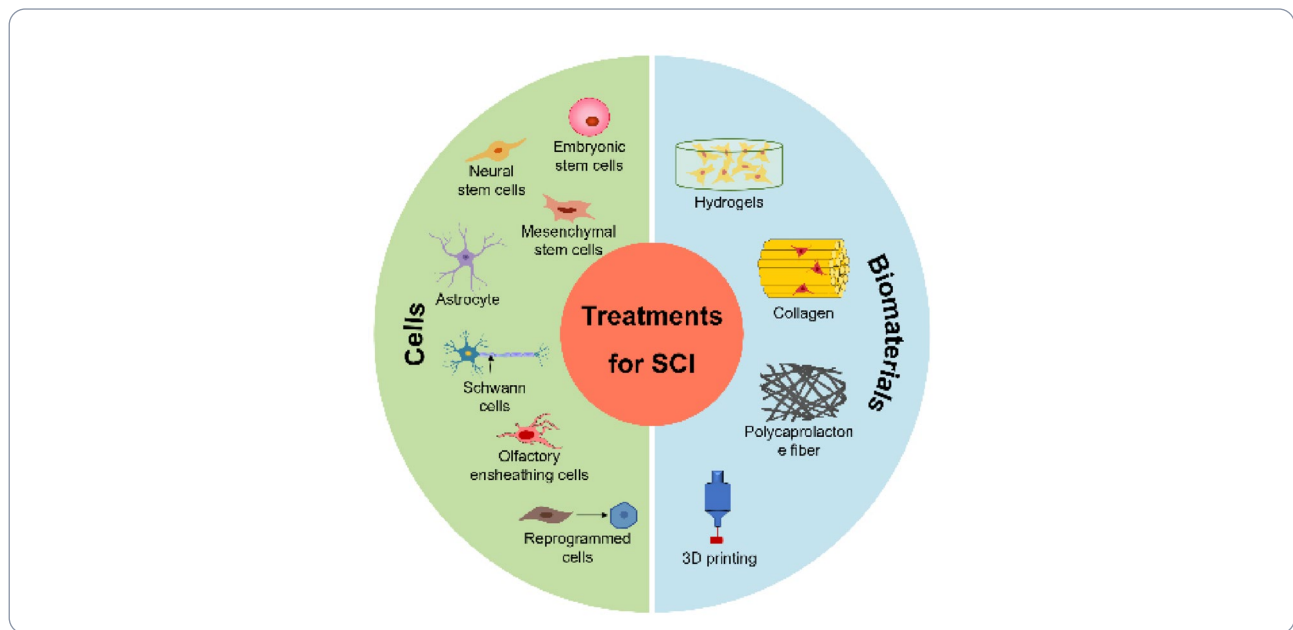
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categories: contusion [3–15], compression [16, 17], focal demyelination [18–22], transection [23–29], ischemic, and infection. Ding et al. [30] reported that globally, the prevalence of SCI varies from 25 to 1300 per million of the population, the incidence is nearly 1 million new cases per year, and the overall healthcare cost per patient is US\$2–4 Million. After mental disorders, the socio-economic cost of SCI is the highest among all neurological diseases. Neuroelectrophysiology is the leading objective functional assessment for SCI. Imaging examinations are used to locate the site and identify the anatomical extension of the injury. Motor behavioral tests are used to identify the neuro-deficits [23, 31]. Unfortunately, there are no gold standards treatments for SCI yet. Among palliatives and potential tentative treatments, stem cell replacement therapy, hypothermia, functional electrical stimulation, rehabilitation, corrective surgery, and pharmaceutical intervention have been investigated extensively and recommended case by case. In this review article, we aim to present recent studies focused on biomaterials and cell therapies post-SCI.

In terms of pharmacological interventions, intravenous methylprednisolone has been commonly administered as an initial response treatment [32]. Additionally, gabapentin is often prescribed to alleviate chronic neuropathic pain associated with SCI. Surgical interventions such as laminectomy, decompression [33], and stabilization surgery have also been employed to address SCI-related complications. Procedures like therapeutic hypothermia have gained attention and have been studied widely as a plausible neuro-protective and treatment approach [34–36]. Rehabilitation therapy significantly contributes to the management of SCI. Techniques such as functional

electrical stimulation [37, 38] and physical exercise, physiotherapy, and rehabilitation are commonly utilized to facilitate recovery and improve functional outcomes. However, the effectiveness of these approaches is often limited by the pathophysiological events associated with SCI. Trauma can cause the disruption of the blood-spinal cord barrier, resulting in hemorrhagic bleeding and ischemia, followed by extensive cell death in the affected area. Subsequently, inflammation, local ischemia, hypoxia, and edema occur, accompanied by the production of reactive oxygen species (ROS), lipid peroxidation, demyelination [39], degeneration of spinal cord axons, gliosis, neuronal apoptosis, and necrosis. Finally, cavities and scars are formed at the site of injury, which among other hurdles, impede the establishment of a favorable microenvironment for neurological repair and regeneration [40]. Consequently, most patients with severe SCI will not be able to achieve major satisfactory recovery [41]. Given this challenge, cell replacement therapy has emerged as a new research focus.

Cell therapy is defined as a therapeutic approach where living cells are transplanted in patients to initiate, promote, or expedite therapeutic effects [42]. Treatments involving the graft of mature cells (e.g., Schwann cells (SCs) [43]) or stem cells (e.g., embryonic stem cells (ESCs) [1], neural stem cells (NSCs), oligodendrocyte progenitor cells (OPCs) [44]) are among the most encouraging approaches for treating SCI. Stem cells are undifferentiated or partially differentiated cells that can proliferate, self-renew, and differentiate into various functioning cells. Numerous treatments have found improved outcomes in animal SCI and hold promise for enhancing recovery at different stages of the injury. Most

animal trials have involved direct cell implantation into the damaged spinal cord. Nevertheless, several clinical studies have offered preliminary indications of the practicality and safety of intraspinal cell transplantation [45]. Previous literature has indicated the advantageous functional effects of cell therapy for SCI [46]. In various experimental settings, different stem cell types, including mesenchymal stem cells (MSCs) [47], ESCs [48], hematopoietic stem cells, NSCs [49], induced pluripotent stem cells (iPSCs) [50] have been utilized for in vivo spinal transplantation. Furthermore, reprogramming cells such as adipocyte-derived dedifferentiated fat cells [51] have also been explored for functional restoration in SCI. The rationales for cell therapy post-SCI include the substitution of impaired neurons and glial cells, releasing trophic factors, controlling gliosis and scar development, hindering cyst formation, and encouraging axon extension [52]. The current interest in cell therapy post-SCI is evident in the annual publication numbers, which displayed a relatively consistent growth trend from 1995 to 2025. Publications from the most recent 4 years contribute to 28.0% of the total. This rapid surge underscores cell therapy's emerging potential and ongoing challenges in SCI (Fig. 1).

Spinal cord injury

Pathophysiological events

SCI commonly arises from traumatic events, leading to vertebral fractures and deformities that cause sudden mechanical disruption and displacement of the vertebral column, along with compression or severing of the spinal cord. Various descending motor tracts, like the corticospinal tract, direct the transmission of motor commands to regulate skeletal muscle movement [53], and ascending sensory tracts, such as the spinothalamic tract for pain

and temperature perception sensations [54], the dorsal column medial lemniscus pathway conveying touch, pressure, and proprioception [55], and the spinocerebellar tract relaying unconscious proprioceptive feedback from muscles, tendons, and joints to the cerebellum [56], may exhibit functional anomalies. Each phase of SCI entails distinctive pathophysiological mechanisms (Fig. 2). Cellular necrosis affects neurons and oligodendrocytes (OLs), leading to the production of cytotoxic agents that initiate robust inflammatory cascades characterized by the secretion of proinflammatory cytokines such as interleukin-1 β (IL-1 β), IL-6, and tumor necrosis factor- α (TNF- α) [57, 58]. In the acute phase post-SCI, the oxidative stress reactions results in lipid peroxidation, generation of ROS and nitric oxide, mitochondrial aberrations [59, 60], inflammatory cytokine release, inflammatory cell infiltration, disruptions in intracellular calcium homeostasis, aberrant protease activity, DNA lesions, microglial activation, and cellular death, including neurons, astrocytes, and OLs [61]. This is followed by the subacute phase and deteriorate event like ongoing ischemia and edema, perturbations in ionic homeostasis, glutamatergic excitotoxicity, free radical generation, further inflammation and ROS formation [62], macrophage/microglial infiltration, demyelination, reactive astrogliosis [63, 64], and further demyelination. Throughout the chronic phase, the acute inflammatory response attenuates, with cystic cavitation, constrained axonal regeneration and growth, Wallerian degeneration of damaged neurons [65], restricted remyelination or demyelination, macrophage infiltration, limited regenerative cell migration [63] and continued glial scar formation [66].

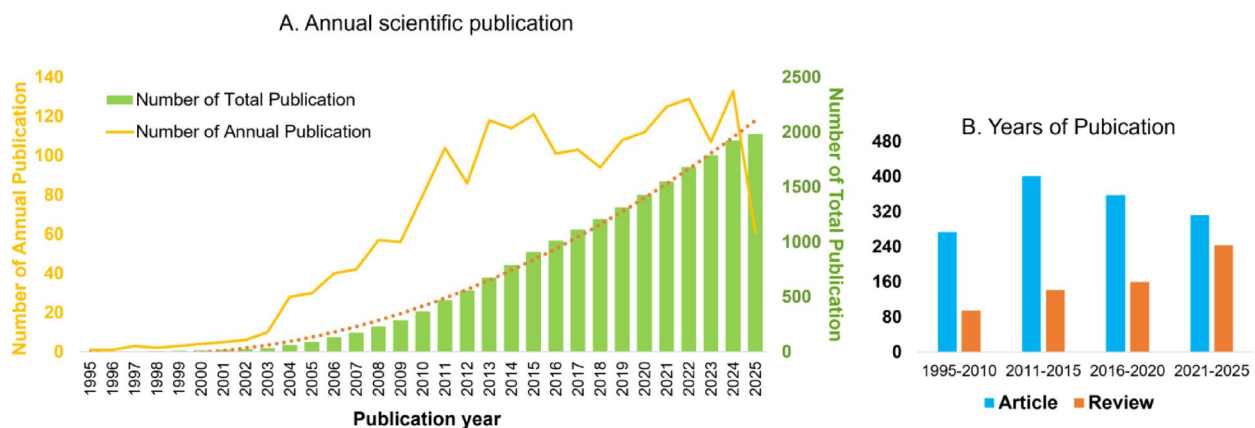


Fig. 1 Number of publications on cell therapy post-SCI since 1995. **A** The annual number of articles, reviews, and proceedings was analyzed. **B** Number of published articles and reviews on cell therapy post-SCI from 1995 to 2025. The data reveals a notable increase in global original research, with 273 articles published between 1995 and 2010, 358 articles from 2016 to 2020, and 312 articles in the recent four years (2021–2025). There has also been a growing interest in cell therapy for SCI, as evidenced by the rising number of reviews, which increased from 95 during 1995–2010 to 160 in 2016–2020 and 243 in 2021–2025

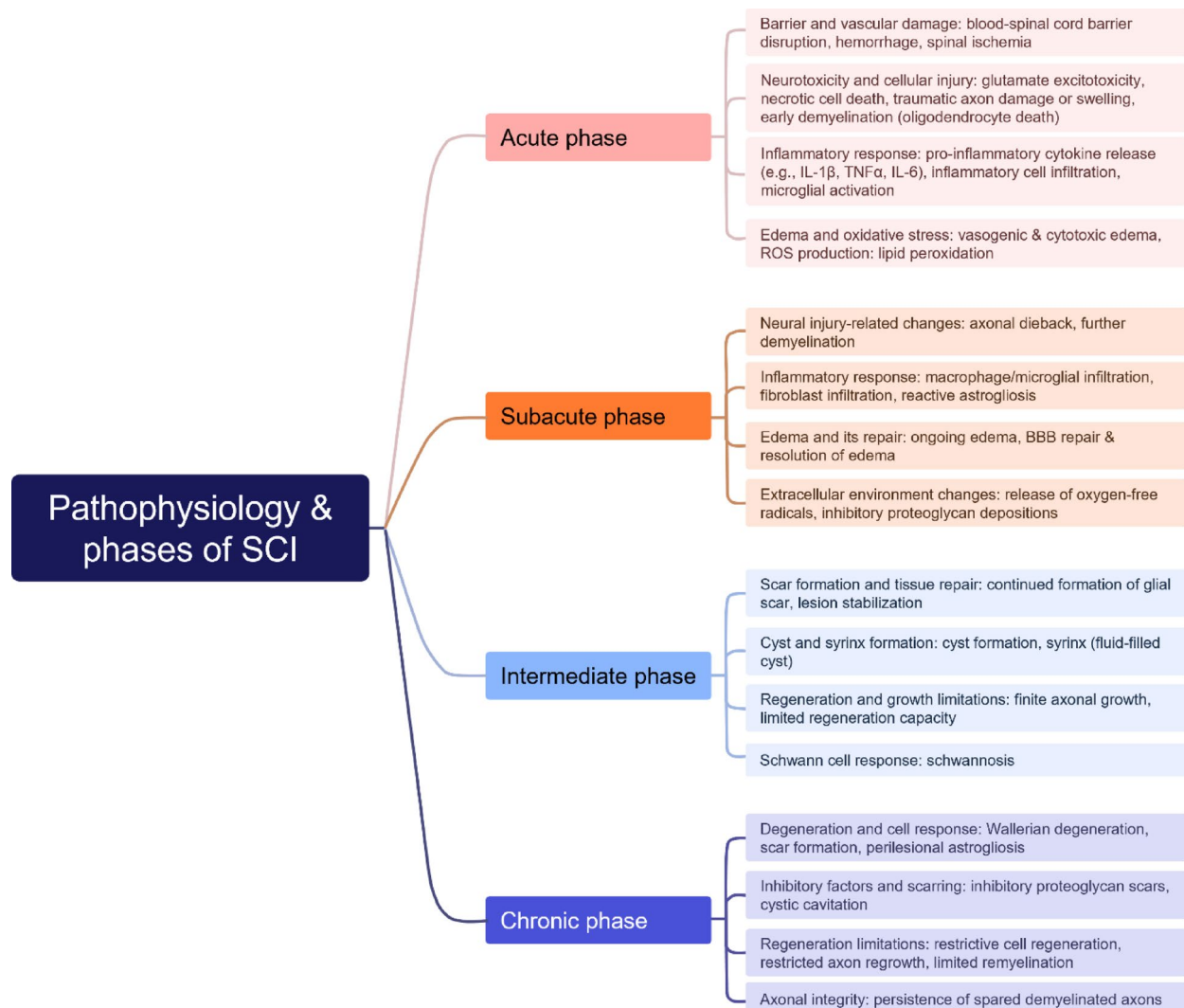


Fig. 2 Phases and pathophysiology of SCI

Treatment strategies

SCI treatment focuses on immediate care and neuroprotection in the acute phase, cellular therapy, pharmacological interventions in the subacute phase, and assessment and rehabilitation strategies in the chronic phase (Table 1).

Cautious transport, minimizing head and spine movement, is imperative [67]. Immediate pharmacological interventions play a pivotal role in mitigating secondary injury, thereby fostering neuronal and axonal survival and reducing the extent of apoptosis or necrosis. While high-dose methylprednisolone therapy was previously considered beneficial, its efficacy, as evidenced by studies like the National Acute Spinal Cord Injury Study (NASCIS I and II), remains moderate to modest, with potential for significant complications [67]. Hypothermia therapy, both systemic and localized, demonstrates promise in reducing tissue metabolic activity, oxygen consumption,

inhibiting inflammatory and apoptotic events [68–70]. Surgical interventions, such as early decompression and stabilization procedures like laminotomy or laminectomy, aim to release pressure on spinal cords or nerve roots, restore spinal stability, prevent secondary injury expansion, and preserve neural tissue [71, 72]. Additionally, there are various form of cell therapy mechanisms including axonal regeneration and neural tissue regeneration, which often involving growth factors, anti-inflammatory cytokines and neurotrophic factor releasing [73]. Electrical stimulation therapy and rehabilitation training further aid in promoting axonal regeneration and nerve function restoration [74].

In the chronic phase, interventions may include nerve bridging, cell therapy, neural rehabilitation training, and pharmacological neural repair therapy [67].

Table 1 Summarized therapeutic interventions

Phase	Treatment methods	Therapeutic aim
Acute phase	Rapid stabilization, life support	Neuroprotection
	Early surgical decompression and stabilization	Immunomodulation
	Neuroprotection; diuretics and steroid therapy	Remyelination
	Hypothermia treatment to reduce metabolic activity	Glial scar degradation
Sub-acute phase	Continued medication, reducing inflammation	Neuroprotection
	Promoting angiogenesis	Immunomodulation
	Promoting axonal regeneration	Glial scar degradation
Inter-mediate phase	Neural regeneration strategies: neurotrophic factor	Stabilization
	Cell therapy	Regeneration
	Functional electrical stimulation	Remyelination
	Rehabilitation	Rehabilitation
Chronic phase	Neurological function assessment, electrophysiology	Stabilization
	Surgical interventions; nerve bridging	Regeneration
	Cell therapies	Remyelination
	Rehabilitation	Rehabilitation

methods. The first is introducing exogenous cells to regenerate injured sites and or provide supportive effects to surviving cells. The second approach involves guiding or enhancing the capabilities of endogenous progenitor cells. The efficacy of this treatment depends on the cell type, timing, and delivery route [75]. These effects involve regulating the immune system, replenishing lost neurons, providing essential neurotrophic factors and extracellular matrix components, and ultimately fostering regeneration [76]. Many studies have investigated the effectiveness of various cell types in pre-clinical and clinical trials for SCI [77, 78]. Researchers have explored a variety of adult and stem cell types, including NSCs, ESCs, MSCs, iPSCs, OPCs [42, 44], olfactory ensheathing cells (OECs), SCs, amniotic epithelial stem cells, and genetically modified cells [76].

Cell Replacement Post-SCI In case of SCI, following transplantation of stem cells, it is expected that these cells facilitate functional enhancements and recovery. The mechanisms of cell replacement therapy post-SCI could be neuroprotection, immune system regulation, axon growth, regeneration, and myelin repair (Fig. 3).

Cell replacement prospect

Cell replacement strategy encompasses two primary

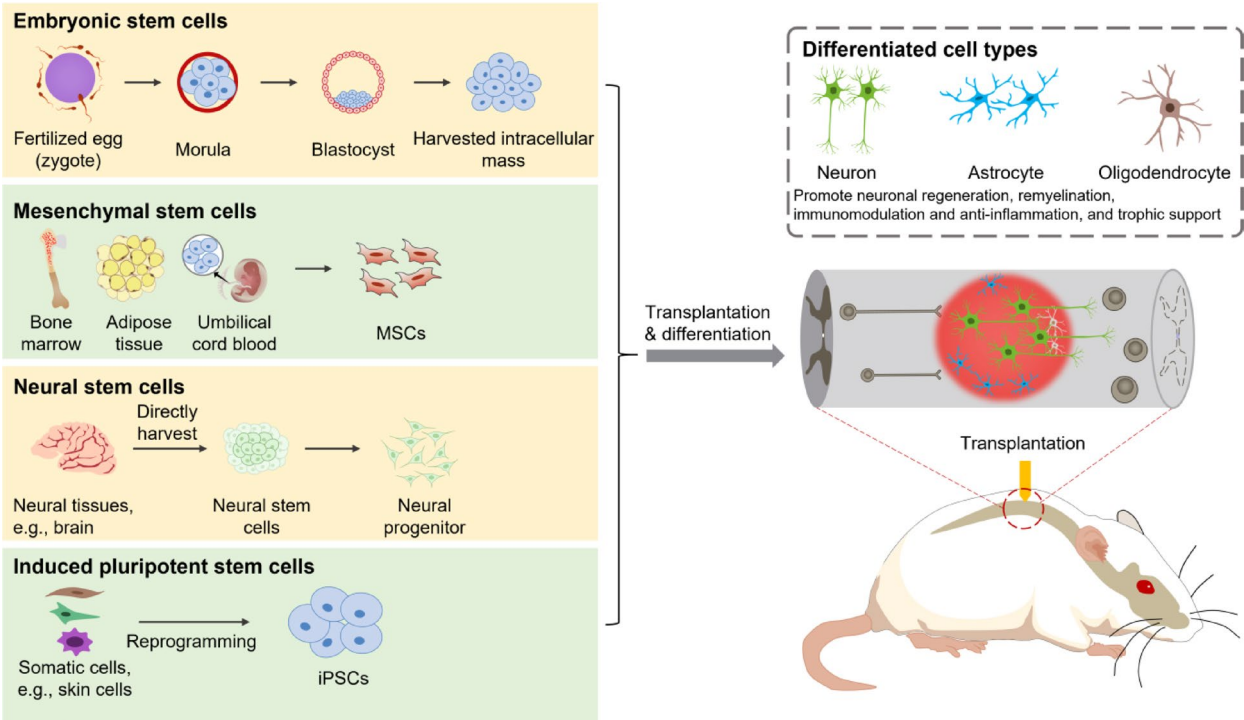


Fig. 3 Schematic representation of stem cell sources and their application in SCI therapy. This figure outlines four main stem cell types for SCI therapy: ESCs, MSCs, neural stem/progenitor cells (NSPCs), and iPSCs. ESCs are sourced from the blastocyst's inner cell mass, while MSCs come from bone marrow and adipose tissue. NSPCs are isolated from neural tissues, and iPSCs are created by reprogramming somatic cells. These stem cells can differentiate into neural lineages at the SCI site, promoting neuronal regeneration and recovery. This illustration highlights the unique attributes and therapeutic potentials of each stem cell type

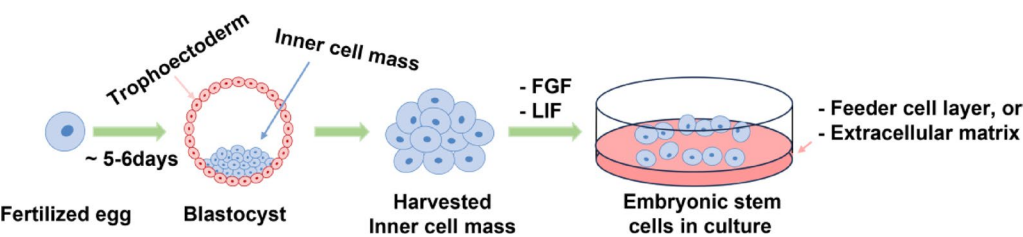


Fig. 4 Process of embryonic stem cell extraction and culture. Embryonic stem cell (ESC) extraction begins with the fertilization of an egg, leading to the formation of a blastocyst around five to six days later [57]. The inner cell mass (ICM) of the blastocyst is isolated to harvest ESCs. In culture, factors such as FGFs are added to maintain the undifferentiated state of the ESCs, while leukemia inhibitory factor (LIF) enhances their proliferation. Additionally, a feeder cell layer or extracellular matrix (ECM) is used to support cell growth and attachment, creating an optimal environment for the maintenance of pluripotent stem cells

Table 2 ESCs used to treat SCI

Cell type	Interven- tion time	Transplantation site	Repair mechanism	Refer- ences
ESC-NSC	1 week	Bilaterally adjacent to the midline dorsal vein, 1 mm rostral and caudal to the clip injury site	OL differentiation, myelin basic protein expression	[85]
ESC-OPCs	2 h	Epicenter, 4 mm rostral and caudal, 1 mm lateral to the clip injury site	Axon remyelination	[44]
	2 h	Epicenter, 4 mm above and 1 mm to the left, and 4 mm below and 1 mm to the right	Axon remyelination, reduce cavitation	[42]
	3 h & 24 h	At depths of 1.0 mm into white matter at T7 and T9, and 1.5 mm into gray matter at the T8 epicenter	Integrate into host spinal cord	[1]
ESC-dl4 GABA neurons	1 week	Right side at the border of T10/T11	Integrate into local neural circuits	[86]

Stem cells

Stem cells are specialized cells capable of self-replication and differentiation into various cell types under specific conditions. They can be classified into several types, notably ESCs, NSCs, MSCs, and iPSCs. ESCs, derived from early embryos, are pluripotent and can differentiate into nearly all cell types; neural stem cells, found in the central nervous system, can become neurons and glial cells; MSCs are multipotent stem cells capable of differentiating into various cell types. Bone marrow MSCs (BMSCs) are located in bone marrow and could give rise to osteoblasts, chondrocytes, and adipocytes. Adipose-derived stem cells are obtained from adipose tissue and are known for their capacity to contribute to diverse cell lineages, including adipocytes, osteoblasts, chondrocytes, myocytes, neurons, and endothelial cells. Amniotic fluid MSCs (AF-MSCs) are sourced from amniotic fluid and possess the potential to develop into multiple cell types. Umbilical cord MSCs (UC-MSCs) are derived from the umbilical cord and can generate various cell types, including adipocytes, osteoblasts, chondrocytes, neurons, myocytes, and endothelial cells. iPSCs are somatic cells that have been genetically reprogrammed to an embryonic-like pluripotent state, allowing them to differentiate into virtually any cell type. In SCI treatment, stem cells show great potential by replacing damaged cells, promoting nerve regeneration, and repairing the injured microenvironment.

ESCs

ESCs are pluripotent stem cells and sourced from the inner cell mass of the blastocyst or early-stage pre-implantation embryos, approximately 5–6 days post-fertilization in humans [79] (Fig. 4). ESCs possess robust self-renewal and differentiation ability, enabling them to differentiate into different cell types [80, 81]. Under correct stimuli, the ESCs would differentiate into neural precursor cells (NPCs) and consequently, into neurons, OLs, and astrocytes, three types of cells often implicated in remyelination, regeneration, and repair of neural tissue [82]. ESCs differentiation strategies have mostly focused on generating neurons, NSCs, and OPCs. These stem cells have fundamental role in improving motor dysfunction, reorganizing neuronal circuits, and facilitating axonal remyelination [83, 84] (Table 2).

Bazley et al. demonstrated that 100 days after transplanting 125,000 cells/ μ l of hESCs-derived OPCs sourced from H1 in immunosuppressed rats (injected 1.5 mm below the surface of the cord) and 2 h post T8 moderate contusive SCI, there were improvements in anatomical structures (assessed by DT-MRI), functional responses (via electrophysiology—somatosensory evoked potential, SSEP), and locomotion (evaluated using Basso, Beattie and Bresnahan (BBB) scoring). Their study confirmed OPC transplantation remyelinated axons and repaired SCI, resulting in functional improvements [44]. All et al. also reported that similar OPCs generated in a similar

SCI model showed significant improvements in neuro-electrophysiological activity (increased SSEP signal amplitude and decreased latency), leading to better nerve impulse conduction. The immunohistochemical (IHC) assay revealed that transplanted OPCs survived and integrated well into the host tissue, further developing into myelin-producing OLs. Additionally, 6 weeks after the SCI, rats that received transplanted OPCs showed a 30% reduction in cavitation and new myelin sheath formation, as detected by scanning electron microscope (SEM). Based on these findings, it could be concluded that the improved function is due to the remyelination of spared nerve fibers by the transplanted hESC-derived OPCs [42].

In addition, Gong et al. transplanted hESC and iPSC-derived spinal dI4 progenitors (10^6 cells/ $3\text{ }\mu\text{l}$) into the right side of the T10/T11 severe SCI contusion rat model (at the depth of 1–1.5 mm) after a week, and by 12 weeks post-transplantation, immunoreactivity assay illustrated that more than 92% of the grafts were identified as Gamma-aminobutyric acid (GABA) neurons, and 51% of these cells had matured into GABA neurons, indicative of successful differentiation within the injured spinal cord parenchyma. Grafted cells form synapses with host motor neurons, migrated and further matured with their axons predominantly traversing the white matter

down to the lumbar cord. Moreover, confocal z stacks microscopy displayed that multiple synaptic terminals of the transplanted cells were scored on the surface of motor neurons. GABA punctae on the surface of the grafted cells compared to each motor neuron in SCI animals, were increased more than 5 times after 3 months post cell transplantation. This suggests that the grafted GABA neurons connect with the host motor neurons. GABA neuron transplantation relieved spasticity-like symptoms, which was measured by a larger reduction of the mean relative amplitude of the Hoffman spinal reflex excitability from 0.1 to 5 Hz by the 6th weeks post-transplantation. This could be attributed to trophic support, modulation of inflammation, and repair of neural circuits. Animals receiving GABA neuron transplantation also exhibited notable improvements in their BBB scores, specifically consistent forelimb-hindlimb coordination at 12 weeks, underscoring motor function recovery post-SCI [86].

However, ESCs have faced some challenges, including ethical issues regarding their extraction, stimulation of immune responses due to their allogenic source, and risk of tumorigenicity [87] (Fig. 5). The safety and efficacy of ESC-based therapies depend on carefully managing these risks. Despite these obstacles, scientists are still looking



Fig. 5 Schematic representation of the therapeutic potential of ESCs in SCI treatment

at ways to use h-ESCs' regenerative potential for various pathologies.

NSCs

NSCs are multipotent, self-renewing cells in the central nervous system that, under specific endogenous stimuli, could migrate, proliferate, and differentiate into OLs, astrocytes, and neurons and foster regeneration of neural parenchyma. The therapeutic potential of NSCs in SCI is realized through a multitude of mechanisms, including differentiation of neuroglial and OLs, enhancing remyelination and replacing damaged or lost neural cells, and their integration into local circuits [88]. NSCs also modulate the inflammation, and facilitate the recruitment of anti-inflammatory M2 macrophages, stimulate the release of regenerative factors, such as neurotrophic factor and nerve growth factor [89, 90], accelerate axonal growth, and form connections among compromised neurons [90] (Fig. 6).

Ceto et al. transplanted NSCs ($2 \mu\text{l} \times 250,000$ cells/ μl , extracted from embryonic spinal cord) in a T12 transection injury mouse model of SCI and determined their efficacy through ex vivo assessments. The ChrimsonR-labeled corticospinal tract axons notably demonstrated extensive regeneration of the spinal NSC grafts by fluorescence microscopy. The graft cells exhibited spontaneous activity in clusters that resembled patterns evoked in the intact spinal cord, insinuating that transplanted cells can respond to and integrate host inputs. Furthermore, using fluorescence tracing, it was demonstrated that the

neuronal clusters within the grafts exhibited temporal correlated calcium activity indicating receipt of similar presynaptic inputs with high level of interconnectivity. The grafts could be organized into functional synaptic networks, with simultaneous activity in discrete neuronal assemblies upon activation of host inputs. Interestingly, the activation of graft neuronal axons could also activate host neurons below the lesion, proving the potential for functional integration of transplanted NSCs into the existing neural network [88].

In 2020, Llorens-Bobadilla et al. demonstrated that ectopic expression of the transcription factor OLIG2 could facilitate the specific differentiation of NSCs into OLs. They performed mild T9 contusive SCI in "olig2 transgenic mice" and investigated the chromatin accessibility of OPC-enriched regions containing OLIG2 binding sites in ependymal cells by scRNA- and scATAC-sequencing assay. Following the injury, OLIG2-expressing ependymal cells rapidly increased the accessibility of these regions, leading to specific oligodendrogenesis, but not at the expense of astrocytes scarring. The IHC and SEM examinations showed these newly generated OLs could myelinate axons, and their extensions were found to be colocalized with the MBP within the axonal myelin wrappings. Moreover, twelve weeks post-SCI and in the presence of the ependymal-derived OLs, the corticospinal tract conductivities showed significant improvements [91].

These results provide compelling evidence of adult NSCs' potential for functional recovery post-SCI through

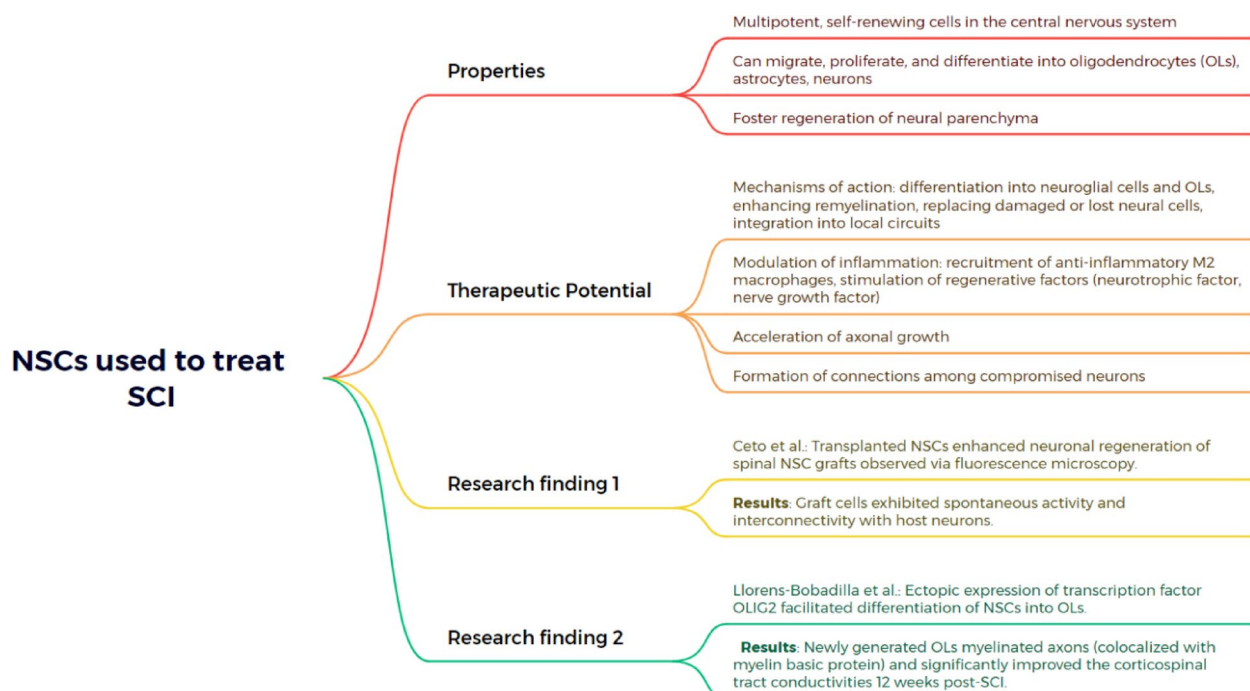


Fig. 6 Schematic representation of the therapeutic potential of NSCs in SCI treatment

repair, regeneration, and establishment of synaptic connections.

MSCs

MSCs, also known as mesenchymal stroma cells, can be found in various tissues such as bone marrow, umbilical cord, dental pulp, amnion, placenta, peripheral blood, and adipose tissue [92] (Fig. 7). They are multilineage cells able to self-renew and differentiate in many types of cells, such as osteoblast, chondrocytes, fibroblast, adipocytes [93], glial cells, and neurons when exposed to different inductive agents (e.g., cytokines and growth factors). They have low immunogenicity, high viability, and are easy to isolate from healthy donors, expand in vitro, and preserve in cell regenerative medicine [94]. Transplantation of MSCs to treat SCI are mainly through replacing the damaged cells and enhancing anti-inflammation, remyelination, axonal growth, neurotrophic factors and cytokines secretion, and angiogenesis. They can differentiate into neurons and glial cells (e.g., OLs), allowing the synapse formation with host neural circuits [94], and supporting the recovery processes. They may reduce inflammation post-SCI mainly by releasing

exosomes through paracrine effects. Exosomes, small membrane vesicles from MSCs, can target inflammatory areas and release anti-inflammatory molecules [95]. MSCs also enhance macrophage differentiation into the anti-inflammatory M2 subtype and regulate intracellular inflammatory pathways, e.g., downregulating TLR4 and NF- κ B, Jagged1/Notch, and inflammasome pathways. MSCs improve the axonal regeneration potentials by the secretion of bioactive factors (e.g., brain-derived neurotrophic factor (BDNF) and β nerve growth factor), activation of neuronal growth and differentiation (e.g., PI3K/AKT/mTOR pathway, and Wnt/ β -catenin pathway) and inhibition of astrogliosis (e.g., TGF- β /Smads pathway) [96]. They can also rescue the death of OLs caused by SCI, enhance MBP and oligodendrocyte protein (CNPase) expression, and promote axonal remyelination [97]. MSCs also secrete neurotrophic factors to maintain neuron survival and generation, and decrease cell death e.g., BDNF, glial cell line-derived neurotrophic factor (GDNF), nerve growth factor (NGF), NT-1, NT-3, CNTF, and basic fibroblast growth factor (bFGF) [98]. Moreover, MSCs could activate angiogenesis by secreting angiogenic factors e.g., vascular endothelial growth

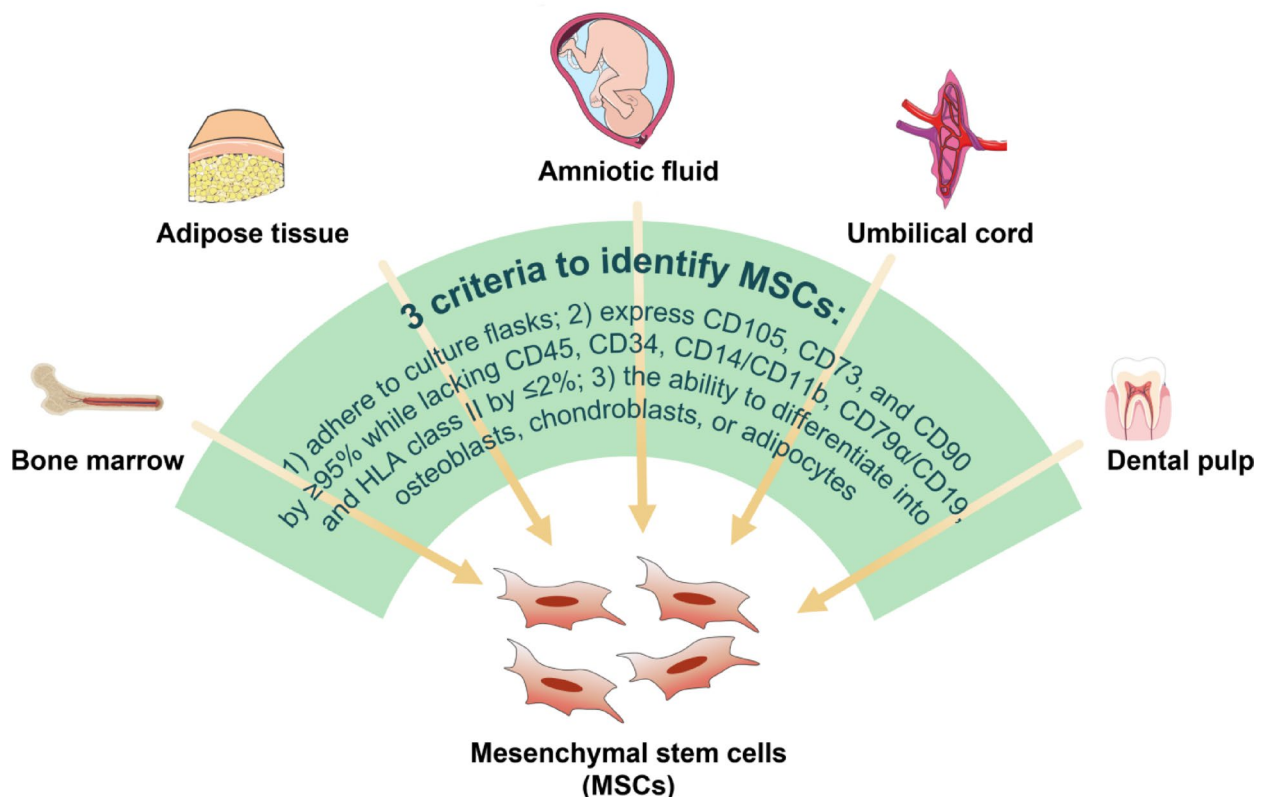


Fig. 7 Various sources of mesenchymal stem cells. These cells are primarily derived from bone marrow, adipose tissue, amniotic fluid, umbilical cord, and dental pulp. To be classified as MSCs, these cells must adhere to culture flasks, demonstrate the ability to differentiate into osteoblasts, chondroblasts, or adipocytes, and express the surface markers CD105, CD73, and CD90 at levels of $\geq 95\%$. Additionally, they should lack the expression of CD45, CD34, CD14/CD11b, CD79a/CD19, and HLA class II at levels of $\leq 2\%$ [69]

factor (VEGF), insulin growth factor-1, fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF) [99], regulate the expression of cell adhesion molecules and integrins, like modulation of cell adhesion molecules (ICAM-1) and vascular cell adhesion molecule-1, aiding endothelial cell recruitment and migration to the injury site [100].

BMSCs

BMSCs are a type of multi-directional lineage that can be isolated from bone marrow tissue. Among other pre-clinical applications, BMSCs have also been implicated in SCI treatments and shown to promote recovery by anti-inflammation, promoting axonal regeneration, releasing neurotrophic factors, reduction of the inflammatory response, and glial scarring [101] (Fig. 8). The therapeutic efficacy of BMSCs was presented by Lv et al. The study involved transplanting BMSCs (10 μ l, 5×10^7 cells/mL, extracted from rat tibia) through subarachnoid injection in Allen's model of rat moderate contusion, 30 min after T10 SCI. The results of western blot analysis showed that BMSCs could mitigate the overexpression of TGF- β 1, Smad2, and phosphorylated Smad2, which are typically induced early (one week) post-SCI. The TGF- β /Smads signals play a major role in hypertrophic scar formation, leading to fibrosis and diminishing the proliferation potential of astrocytes in and around the SCI site. Reducing TGF- β /Smads signals resulted in improved rat's motor behavior 5 weeks after BMSCs transplantation [102].

In another study, Li et al. genetically modified BMSCs (isolated from rat's femur) to express CD157, a cell surface molecule implicated in regulating mitochondrial transfer. These engineered BMSCs (10^6 cells/10 μ l) were

co-cultured with ventral spinal cord motor neuron cells 4.1 (VSC4.1) and transplanted into the injury epicenter (T10) after moderate contusion SCI in rats. The immunofluorescent assay and Mito Tracker red staining showed that modified BMSCs enhanced the delivery of extracellular mitochondrial particles to motor neurons through upregulating CD157 in BMSCs. TUNEL assay confirmed that CD157 reduced cellular apoptosis *in vitro*, and behavior assessment demonstrated locomotion improvements *in vivo*. The immunofluorescent assay confirmed that transplantation of BMSCs expressing CD157 at the SCI site significantly improved axonal regeneration and reduced neuronal apoptosis via down-regulating GRP78 and NF- κ B and up-regulating Bcl-xl and p-Akt. Such BMSCs treatment is also associated with hypertrophic scar inhibition in contusive-SCI [103].

Various studies have convincingly demonstrated that early transplantation of BMSC after contusive SCI can improve motor function and promote neuron viability and axonal myelination.

Adipose-derived MSCs

Adipose-derived MSCs (AD-MSCs), obtained from adipose tissue, have the potential to avert the progression of SCI, through neuronal differentiation, neurotrophic factors secretion, immunomodulation, promotion of nerve regeneration, and inhibition of apoptosis [104] (Fig. 9). AD-MSCs offer several advantages, including their abundance, low immunogenicity, good proliferative capacity, and the ability to be obtained through minimally invasive procedures [105].

Vialle et al. [106] harvested human AD-MSCs (10^6 cells/100 μ l) and administered them directly into the dural sac of rats (immediately after T10 contusion SCI),

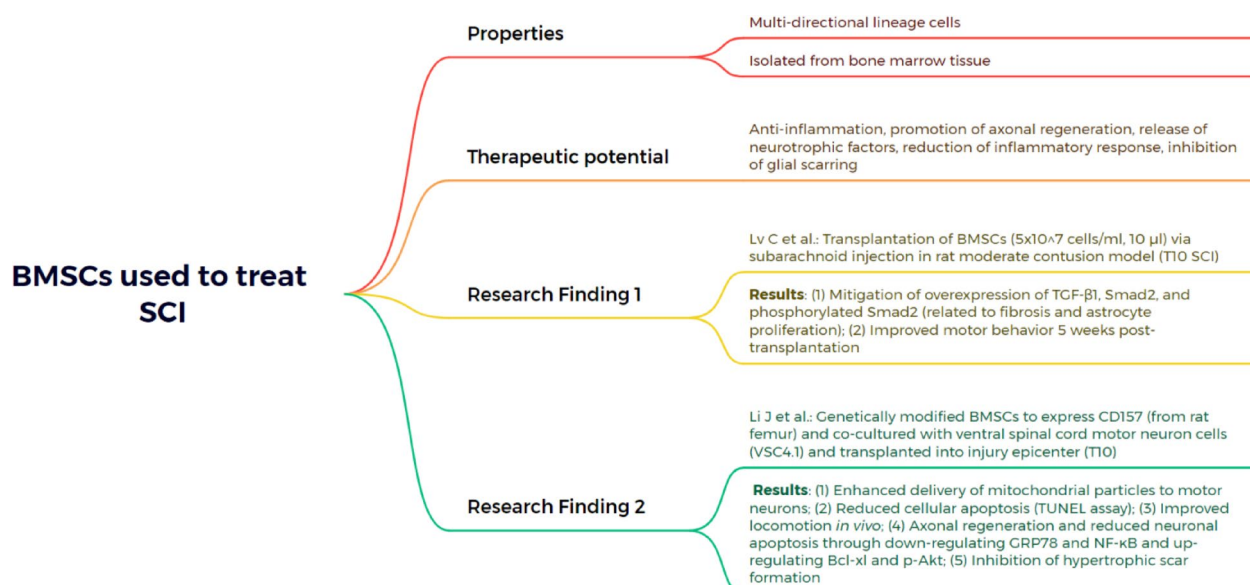


Fig. 8 Schematic representation of the therapeutic potential of BMSCs in SCI treatment

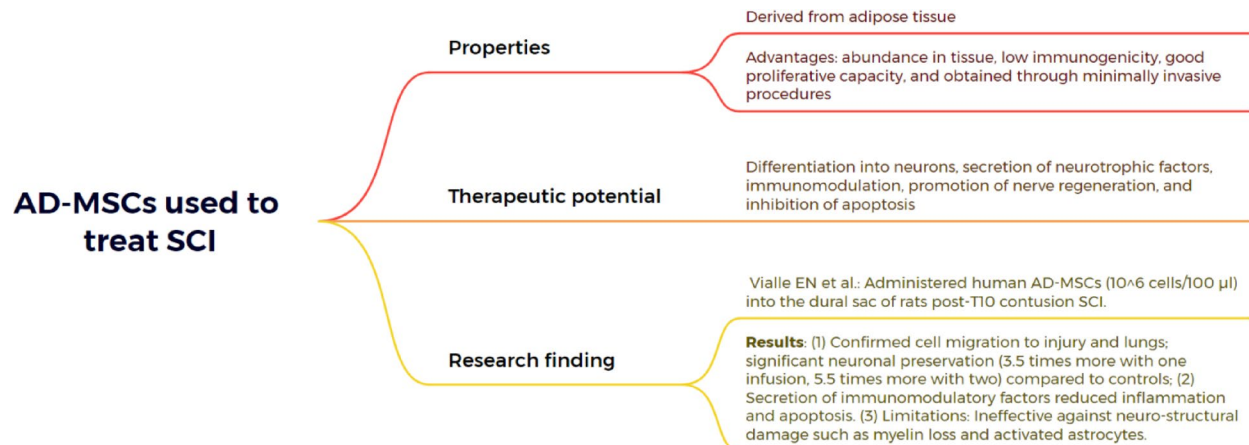


Fig. 9 Schematic representation of the therapeutic potential of AD-MSCs in SCI treatment

either once or twice weekly to assess their migratory capacity. Injecting cells into the dural sac (away from the injury site) allowed a larger volume of AD-MSCs to be delivered safely. They also incorporated luciferase genes into the cells, enabling them to perform non-invasive in vivo monitoring by bioluminescence. AD-MSCs survived and were detected at the injection site and, after 48 h, within the injury site and lung, confirming cell migration potential. The bioluminescence signal intensity significantly decreased in rats with one AD-MSCs infusion on day 7 and with two infusions on days 7 and 9. Interestingly, seven days after infusion, no AD-MSCs were detectable in all rats. However, forty days post-SCI, the histomorphology examination revealed that the AD-MSCs triggered significant reductions in neuronal loss. Specifically, the one infusion of AD-MSCs preserved approximately 3.5 times (193.5 vs. 56.7), and the two infusions 5.5 times (187.4 vs. 38) more neurons in the injury site when compared to one and two medium infusions in the control group, respectively. These results suggested that AD-MSCs infusion(s) substantially reduced neuronal loss by secreting immunomodulatory factors that reduce inflammation, inhibit apoptosis, and protect neurons. Nevertheless, the morphometric and IHC assays demonstrated that AD-MSCs were ineffective in protecting neuro-structural damages caused by myelin loss or activated astrocytes [106].

Amniotic MSCs

Amniotic MSCs (AMSCs), derived from the amnion after delivery, possess multipotent differentiation capacity, strong self-renewal capability, trophic factor secretion, absence of tumorigenicity, and minimal immunogenicity [107]. Interestingly, when used to treat SCI, the AMSCs demonstrated noticeable promises to promote axonal regeneration, angiogenesis, anti-inflammation, and anti-apoptosis [108] (Fig. 10).

Zhou et al. intravenously (*i.v.*) administered human AMSCs (4×10^6 cells/500 μ l) 2 h after T9 severe SCI

contusion in rats. One week later, the presence of hAM-SCs at the SCI site was confirmed through antihuman-specific nuclear antigen (hNu) immunofluorescence staining, showing that the cells had survived, though in small numbers. This confirmed that hAMSCs could safely circulate in the blood and reach the SCI site. Immunofluorescent staining demonstrated that hAM-SCs reduced the number of ED1 positive microphage/microglia, caspase3 positive cells, and the inflammatory cytokines, such as TNF- α , IL-6, and IL-1 β (using ELISA assay) in the lesion area. Additionally, by the end of two weeks, the motor function (BBB scores) began to show clear improvement in SCI rats treated with hAMSCs. At four weeks post-SCI, immuno-histological examinations showed that rats treated with hAMSCs exhibited significantly increased von Willebrand factor in blood vessels, as well as a higher presence of 5-hydroxytryptamine receptor (5-HT) on neuronal fibers and growth-associated protein 43 (GAP43) fibers, indicating enhanced angiogenesis and axonal regeneration in the SCI rats treated with *i.v.* AMSCs [108].

Lale Ataei M conducted a study showing that the immediate transplantation of human AF-MSCs (5×10^5 cells/5 μ l) into the injury site following moderate T10 SCI in rats resulted in an increase in neuroblast marker, neurogenesis marker doublecortin (DCX), and a decrease in glial fibrillary acidic protein (GFAP) levels. ELISA confirmed AF-MSCs could enhance the secretion of neurotrophic factors such as BDNF, IL-1 β , and IL-6. Transcriptomic analysis revealed that genes like PAK5, ST8SIA3, and NRXN1 were positively correlated with DCX expression, suggesting their involvement in neuro-active ligand-receptor interactions [109].

These findings suggest that AMSCs can migrate into the SCI site and improve angiogenesis and axon remodeling. They can also facilitate neural tissue regeneration and repair by promoting neurogenesis and reducing astrogliosis.

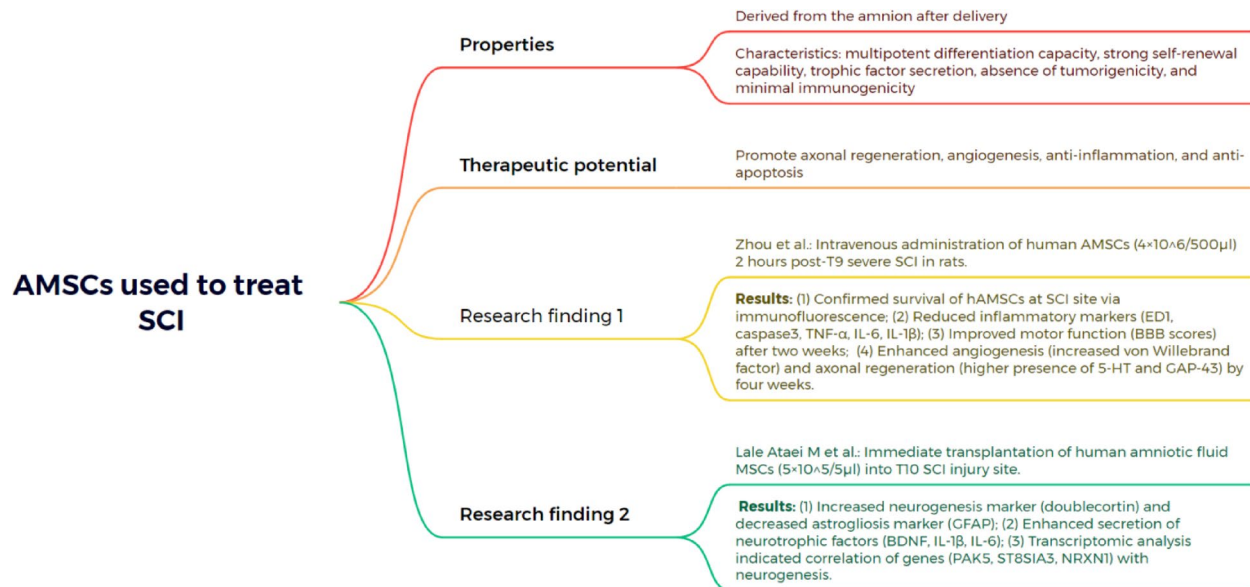


Fig. 10 Schematic representation of the therapeutic potential of AMSCs in SCI treatment

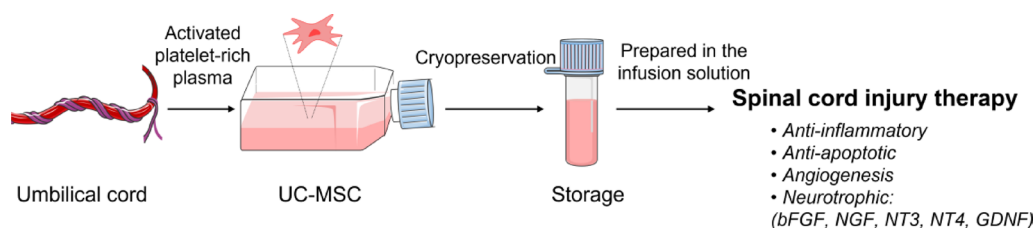


Fig. 11 Isolation and culture of mesenchymal stem cells from the umbilical cord (UC-MSC) using activated platelet-rich plasma for potential treatment of SCI. Mechanisms of action include anti-inflammatory effects, anti-apoptotic properties, promotion of angiogenesis, and neurotrophic support via factors such as bFGF, NGF, NT3, NT4, and GDNF. For long-term storage, cryopreservation techniques are employed. Prepared UC-MSCs are resuspended in various infusion solutions before administration, including 0.9% saline, 5% dextrose, dextrose and sodium chloride injection, Plasma-Lyte A, and 1% or 5% human serum albumin

UC-MSCs

UC-MSCs are a valuable source of stem cells. UC-MSCs contain hematopoietic stem cells and small embryonic-like stem cells [110], that could contribute to SCI recovery by providing neurotrophic, anti-inflammatory, anti-apoptotic, and pro-angiogenic effects. These stem cells can be obtained through non-invasive collection methods, such as cord blood banking. The advantages of UC-MSCs include being easy to expand in vitro and having low immunogenicity. Studies have shown that UC-MSCs can differentiate into neurons, OLs, and astrocytes [111, 112]. They also secrete neurotrophic hormones, such as bFGF, NGF, neurotrophin 3 (NT3), neurotrophin 4 (NT4), and GDNF [113], and inhibit the secretion of pro-inflammatory factors such as TNF- α , IL-8, and IL-1 β [114] (Fig. 11). These factors play crucial roles in promoting axonal remyelination and enhancing functionality post-SCI, leading to improvements in locomotion (Fig. 12).

Yang et al. conducted balloon compression model of SCI on rabbits (T6/T7), and 24 h later, transplanted

hUC-MSCs intravenously either once (6×10^6 cells/mL) or three times (2×10^6 cells/mL) on days +1, +7, and +14. They found that hUC-MSCs predominantly differentiate into OLs (>54%) two months post-transplantation. SSEP monitoring showed increased amplitude and reduced latency in treated rabbits, suggesting the remyelination of intact axons and restoring their conductivity. These cells also exhibited anti-inflammatory properties by inhibiting microglial/macrophage infiltration, reducing astrogliosis as indicated by lower levels of GFAP, providing anti-apoptotic benefits, and contributing to the preservation of axons, evidenced by the maintenance of neurofilaments. Furthermore, Reuter's score and modified Rivlin's test showed enhanced muscle tone, better motor and sensory functions in the hindlimbs, and improved spinal reflexes. Likewise, hNu immunofluorescent staining illustrated that the migration of hUC-MSCs was greater in 3-day repeated transplantation [115].

Yao et al. demonstrated that treatment with UC-MSCs (3×10^5 cells/ $2 \mu\text{l}$) can attenuate crushed model (five seconds compression of T10 by forceps) of mice SCI

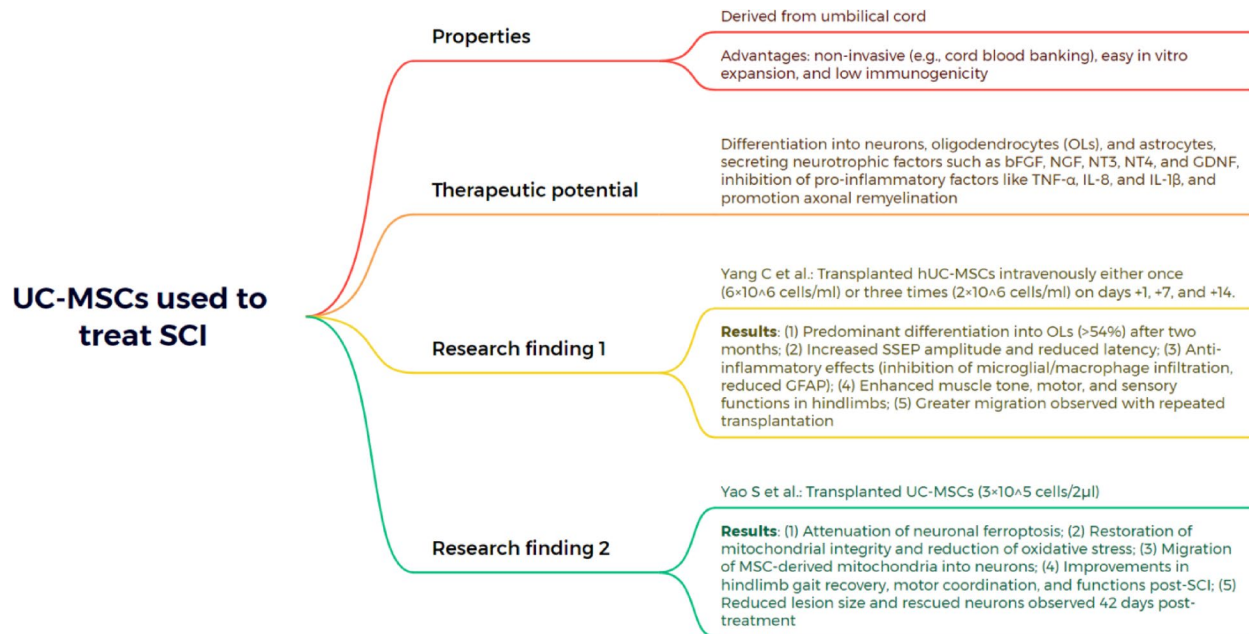


Fig. 12 Schematic representation of the therapeutic potential of UC-MSCs in SCI treatment

by counteracting neuronal ferroptosis, a mitochondrial quality control-related cell death. Ferroptosis, driven by iron-dependent oxidative stress, contributes significantly to neuronal loss following SCI. Since mitochondrial integrity is disrupted during SCI, it increases ROS production and subsequent oxidative damage. Intracellular Iron Colorimetric Assay demonstrated that the SCI group showed a surge in ferroptosis markers, including heightened expression of ferroptosis drivers and a depletion of protective suppressors. Soon after the injury, neurons exhibited mitochondrial shrinkage with dense inner membranes and reduced cristae. An increase in transferrin receptors, crucial for iron uptake, was detected in SCI neurons, along with a rise in 4-HNE, a lipid peroxidation marker, signaling ferroptosis. UC-MSC transplantation mitigated this neuronal ferroptosis and fostered recovery, supported by mitochondrial transfer. Time-dependent observations confirmed the migration of MSC-derived mitochondria into neuronal cells, significantly reducing intracellular and lipid ROS in ferroptosis neurons. Flow cytometry and Mitotracker assays revealed that after SCI, these MSC-derived mitochondria were also found within spinal neurons in vivo, showcasing the MSCs' capability to transfer mitochondria to injured neurons. The UC-MSCs reduced the expression of ferroptosis markers and restored mitochondrial structure, leading to an increased survival rate of neurons. The Footprint test, Basso mouse scale, and motor evoked potential (MEP) assessments affirmed that this mitochondrial rescue would be translated into tangible benefits, with improvements in hindlimb gait recovery, motor coordination, and functions post-SCI. Moreover, 42 days post-SCI and MSCs

treatment, reduced lesion size and rescued neurons were well-noticeable [116].

These findings demonstrate that UC-MSCs are effective in promoting neurological function recovery, making them a promising treatment for SCI. Their ability to release trophic factors, facilitate nerve regeneration and motor recovery, suppress inflammation and apoptosis, and integrate into the neural pathways of the damaged spinal cord, positions them as valuable candidates for cell-based therapies aimed at treating SCI.

Glial cells

Glial cells, including astrocytes, SCs, and OECs, provide structural support, maintain neural homeostasis, and facilitate intercellular communication. Astrocytes provide metabolic support and form a glial scar that can inhibit axonal regeneration but also stabilize the blood-spinal cord barrier and promote neuronal survival. SCs, from the peripheral nervous system, facilitate axonal regeneration through myelination and growth factor secretion, while OECs support neuronal growth and reduce inflammation. Together, these glial cell types offer significant therapeutic potential for enhancing recovery after SCI by modulating inflammation and promoting regeneration (Table 3).

Astrocytes

Astrocytes are a type of specialized glial cells that make up most of the cells in the human CNS. They perform metabolic and homeostatic functions and support anatomical structures, synapse formation, and the overall microenvironment.

Table 3 Glial cells used to treat SCI

Cell type	Intervention time	Transplantation site	Repair mechanism	References
Astrocytes (A2 type)	Immediately	Epicenter	Reduce neuronal apoptosis, decrease cavity area, minimize microglial accumulation and astrocyte proliferation, enhance neuronal survival, and promote myelin preservation	[117]
SC-GDNF	1 week	Epicenter	Promote integration between SCs and astrocytes, axonal regeneration and remyelination	[118]
SCs	1 week	Intrathecal injection	Reduce genes and proteins of inflammasome components, increase neuronal survival and myelination	[119]
OECs	1 day	<i>i.v</i>	Secrete IL-1Ra, reduce microglia activation and the pro-inflammatory factors, reduce neuroinflammation and glial scar	[120]
OECs	Immediately	Epicenter	APOE/TREM2/NF-κB pathway, promote microglia M2 polarization and nerve fiber continuity	[121]

The glial scar formation is a complex process involving depositing extracellular matrix proteins, including chondroitin sulfate proteoglycans, laminin, collagen, and fibronectin [122]. Their neuroprotective tasks include stabilizing the blood–brain barrier and regulating neurotransmitters. After SCI, astrocytes undergo reactive gliosis, becoming activated and forming a glial scar [123], which is a block of extracellular matrix composed of chondroitin sulfate proteoglycans, laminin, collagen, and fibronectin. The glial scar surrounds the injury site to contain the inflammatory response but also presents a barrier to axonal regeneration. Astrocyte stem cells have the potential to regulate the composition of the extracellular matrix, secrete various growth factors that promote neuronal survival and axonal growth/regeneration, and reduce glial scar formation [124] (Table 3).

Chang et al. transplanted 10⁵ cells/5 µl of A2 astrocytes (sourced from IL-4-activated microglial differentiation) directly into mice spinal cord with T10 moderate contusion immediately after injury. Hematoxylin and eosin (H&E), LFB, and SEM assays were performed for myelination evaluation and immunohistopathological examinations. The results showed higher myelin preservation and reduced lesion area in A2 astrocyte transplantation at 4 weeks after injury. A2 astrocytes also decreased the Iba-1 and GFAP markers and inhibited microglia accumulation and astrogliosis. Moreover, NF-200 neuronal cell markers increased in the injury site, and both Foot-print and swimming tests displayed significant functional recovery, confirming the neuroprotective potential of A2 astrocyte transplantation [117].

Tamaru et al. extracted astrocytes from mice cortices and injected 10⁵ cells into the dorsal surface of T9 immediately after mild contusion SCI. They also isolated astrocytes from glial scars at different acute, sub-acute, and chronic post-SCI phases for evaluating gene expression. The qPCR results showed upregulation of SOX9 expression (a critical factor in glial cell differentiation) and downregulation CSPG genes (produced by the glial scar) in the chronic phase compared to the sub-acute phase. They demonstrated that scar-forming astrocytes are

affected by SCI microenvironment and their phenotype is altered, though IHC assay showed no phenotype changes of collagen-I-grown scar-forming astrocytes (Col-I-SAs), even after the effects of integrin or N-cadherin were depleted (two elements of glial scar formation). Remarkably, the transplanted Col-I-SAs transformed residential astrocytes into scar-forming astrocytes. Yet, the anti-β1-integrin antibody blocked the scar-forming astrocytes transformation and reduced the glial scar size in the chronic phase, evolving the glial scars characterization over time following SCI [125].

Transplantation of the A2 astrocytes has the potential to protect neurons, promote neurofilament formation, and improve motor function post-SCI. Nonetheless, the glial scar remains present long after injury and represents a potential target for therapeutic intervention. Evidently, further research is necessary to optimize transplantation protocols, elucidate the mechanisms by which astrocytes remodel tissue, and assess the long-term therapeutic effects.

SCs

SCs are another type of glial cells in the peripheral nervous system. SCs wrap axons and form myelin sheath around them. They can easily be harvested from a patient’s peripheral nerves (like the sural nerve), cultured/maintained in laboratory, and prepared for transplantation [126]. SCs secrete nerve growth factors and support axonal recovery [127]. SCs exhibit remarkable plasticity following injury, undergoing dedifferentiation, proliferation, and migration [128]. Such superb adaptive response is crucial for their safe and effective transplantation in combination with other cell types for treating SCI [129, 130] (Table 3).

The therapeutic mechanisms of SCs in SCI involve several aspects. Firstly, SCs facilitate axon regeneration by secretion of growth factors and extracellular matrix components [131]. Secondly, SCs can provide ensheathment and myelination to newly regenerated and demyelinated axons, which enhances their conduction efficiency and protects them from degeneration [132]. This process can

help reduce the lesion size and improve axonal integration into the SCs implants. However, there are also some limitations to SCs therapy for SCI. One challenge is the need for multiple quality control steps to ensure the identification, purification, viability, stability, and sterility of the transplanted SCs before use. Additionally, defining the biological activity of the cellular product can be challenging. Standardized isolation and culture protocols and quality control guarantee the safety and efficacy of SCs therapy for SCI [126, 132]. Despite these limitations, SCs hold great potential for SCI treatment due to their inherent regenerative and myelination-promoting characteristics.

Du et al. transplanted 10^5 cells/ μ l SCs overexpressing GDNF into rat's T11 a week after transection SCI (at 0.5 & 1.0 mm caudal to the edge of the lesion and at 0.5 mm lateral to the midline and 1.0 mm ventral to the dorsal surface of the spinal cord). GDNF promoted the integration of SCs and astrocytes and attenuated the glial scar formation response. Morphologically, IHC showed thin processes with lower GFAP expression. Ten weeks after SCs transplantation, the anterograde tracing presented propriospinal tract regeneration. The immunoassay of myelin protein zero, synaptophysin expression, and SEM analysis confirmed improved myelination and newly established synapses. Likewise, the BBB scoring revealed locomotion recovery [118].

Kharazinejad et al. transplanted 3×10^5 cells/ μ l (i) SCs derived from rat's sciatic nerve or (ii) Wharton's jelly MSCs (WJ-MSCs) into the injury epicenter at T10 seven days after compression SCI in rats and compared their efficacy. The western blotting, q-PCR, and ELISA demonstrated that 21 days post-injury, SCs modulate immune activity by reducing genes and proteins of inflammasome components, including AIM2, apoptosis-associated speck-like protein, caspase-1, IL-1 β , and IL-18. Histopathological assessment (Nissl and LFB staining) illustrated that both SCs and WJ-MSCs could increase neuronal survival and myelination in the injured parenchyma. The weekly BBB scoring showed significant locomotion recovery improvements only in the SCs and WJ-MSCs transplantation groups. They finally concluded that SCs transplantation had more effectiveness in addressing AIM2 inflammasome activation and its related inflammatory pathways in the SCI model [119].

Although the transplantation of SCs improved inflammation, reduced neuronal loss, and promoted remyelination, further research and development efforts are required to optimize SCs transplantation protocols and address challenges related to defining cellular biological activity.

OECs

OECs are a type of glial cells found along the olfactory nerve and the outer nerve layer of the olfactory bulb. OECs serve several neuroprotective functions in the CNS. They could reduce inflammation by clearing debris at the injury site by engulfing of debris mediated by p38MAPK signaling [133], and producing anti-inflammatory factors (IL-4, IL-10, IL-13, and TGF- β), improving the hostile microenvironment. Additionally, OECs secrete various growth-promoting factors (NGF, bFGF, and GDNF) that enhance neuronal survival, axonal growth, and axonal regeneration by mediating the Wnt3/ β -catenin pathway, interacting microphage/microglia and inhibiting JAK/STAT pathway. Therefore, OECs are considered excellent candidates for cell treatment post-SCI [134] (Table 3).

In 2021, Zhang et al. performed T12 hemisection SCI in a rat model and 24 h later, infused 10^6 cells/mL OECs (sourced from 6–7 weeks young male adult rats) via a tail vein. Then, they performed protein microarrays and ELISA assays to assess differential proteins in spinal cord parenchyma. The results showed that OECs could release anti-inflammatory factors at the lesion site under the stimulation of activated microglia. They secrete interleukin-1 receptor antagonists to reduce microglia activation and the proinflammatory factors (IL-1 α and IL-1 β) secreted by microglia and Notch-1 and Notch-2. Thereby reducing neuroinflammation and glial scars after SCI. IHC results showed a decreased expression of mitochondrial translocator protein (TSPO), which is an indicator of microglial activation and Iba-1 (microglial marker). They also reported an increased level of IL-1Ra, indicating reduced neuro-inflammation and microglial activation at 7-days and 14-days post-transplantation in the glial scar. The ex vivo autoradiography and PET-CT analysis showed that OECs migrated from the blood circulation to the SCI site, proving the low immunogenicity potential of OECs [120].

In another study, Jiang et al. treated OECs (10^5 cells/2 μ l, sourced from 2 to 3 months old rat) with 1 μ M curcumin for 7–10 days and generated curcumin-activated OECs (aOECs). They used aOECs to examine their effect on activation of microglial after moderate T9 SCI compression. They isolated microglia from rat's brains and treated with 100 ng/mL lipopolysaccharide (LPS) inducer for 24 h. These shifted M1 phenotype microglia were treated with condition medium (CM) of aOECs and OECs for 48 h. Their study group included (i) control, (ii) LPS, (iii) LPS + OECs-CM, and (iv) LPS + aOECs-CM. Western blotting and immunofluorescence staining confirmed that aOECs regulate the polarization of microglia to an anti-inflammatory M2 phenotype through the APOE/TREM2/NF- κ B signaling pathway. They also reported nerve fibers with better continuity

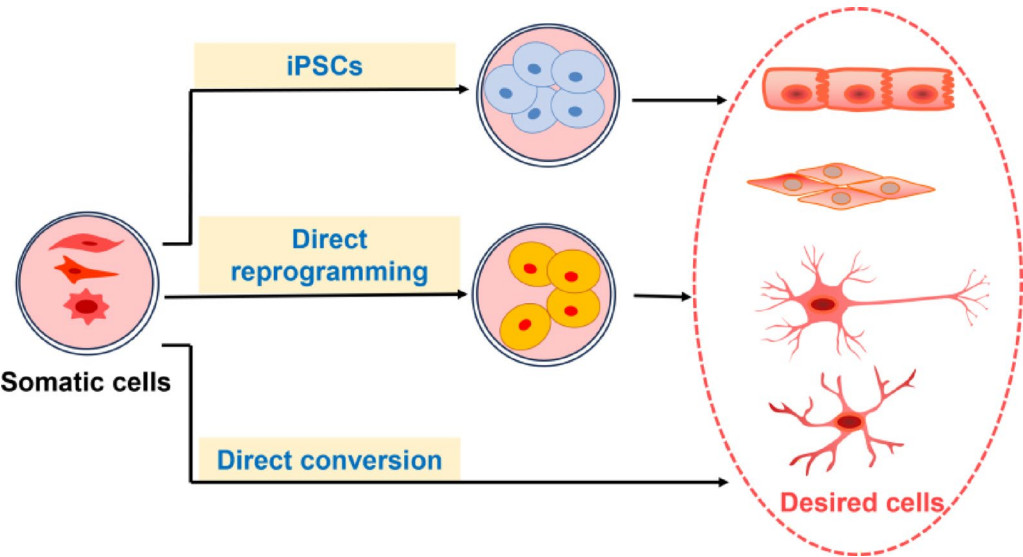


Fig. 13 Overview of three reprogramming methods: iPSCs, direct reprogramming, and direct conversion

Table 4 iPSCs used to treat SCI

Cell type	Intervention time	Transplantation site	Repair mechanism	References
iPSC-OPCs	1 day	Epicenter	Mature into OLs, remyelination, decrease glial scarring and cavity size	[140]
iPSC-NSPCs	9 days	Epicenter	Differentiate into neurons, establish synapses, and prevent spinal cord atrophy	[141]
iPSC-NSCs	immediately	1 mm rostral to the epicenter	Replace lost neurons, mitigate fibrosis, suppress glial scar formation, and reduce inflammation	[142]
iPSC-NSCs	2 days	Epicenter	Differentiate into neurons and glial cells, and form connections with host cells	[143]

and rostral-caudal directionality. The weekly BBB scores showed motor behavior improvements and significant functional recovery post-SCI [121].

These studies confirmed that the intraparenchymal transplantation of OECs not only suppresses the neuro-inflammatory responses and reduces the glial scar, but also produces IL-1Ra when activated by the inflammatory microenvironment post-SCI. They are also capable of transforming the M1 phenotype of microglial cells to the M2 phenotype. Early treatment with OECs improves motor function following SCI.

Reprogrammed cells

Reprogramming cell technologies include three methods: iPSCs, direct reprogramming, and direct conversion (Fig. 13). iPSCs are generated by reprogramming factors that transform somatic cells into a pluripotent state, allowing them to differentiate into various cell types, such as OLs and neurons, under appropriate conditions. This approach is widely used in the treatment of SCI to promote remyelination and neuroprotection. Direct reprogramming involves the transient expression of specific transcription factors, which leads cells into a potential pluripotent or multipotent state, allowing them to subsequently convert into other cell types. Direct conversion, on the other hand, rapidly generates target cell

types through the forced expression of specific transcription factors, for instance, to further facilitating the reconstruction of neural networks.

iPSCs

iPSCs are created by reprogramming adult somatic cells into a pluripotent state [135]. This reprogramming was first achieved in 2006 by introducing the four Yamanaka factors, Oct4 (Pou5f1), Sox2, Klf4, cMyc [136], specific genes encoding transcription factors, into the cells. In this reprogramming technique, the nucleosome is displaced during cell division, providing access for transcription factors to bind to the cis-regulatory regions. iPSCs can propagate indefinitely in culture but retain their potential to differentiate into any cell type (including neurons) [50, 137]. Yamanaka (2012 Nobel Prize in Physiology or Medicine) and colleagues’ groundbreaking method of generating pluripotent cells opened new possibilities for personalized regenerative medicine and bypassed ethical concerns associated with ESCs [138].

iPSC-derived NSCs can produce neuroprotective factors (GDNF, BDNF and NT3), promote angiogenesis, enhance remyelination, and ultimately contribute to functional recovery post-SCI [139, 140] (Table 4). Researchers have successfully generated iPSCs from various cell sources, including skin cells, peripheral

blood monocytes, MSCs, and fibroblast cell lines. These iPSCs can then be differentiated into specific cell types relevant to SCI, such as OPCs and NSPCs, by expressing nestin, RIP, O1, and O4 [140] (Fig. 14).

Kawai et al. transplanted 5×10^5 cells/2 μ l iPSC-derived NSPCs into the epicenter of injury at T10 level nine days after a mild SCI contusion and activated them using clozapine N-oxide as a chemogenetic stimulator. Kinematic analysis of locomotion, utilizing rotating rod and treadmill gait tests, showed functional improvements in mice six weeks post-injury. The histopathological examinations confirmed that iPSC-derived NSPCs prevented spinal cord atrophy. RNA sequencing and IHC demonstrated that expression of synapse-related genes and proteins, such as Syn1, vGLUT1, and vGAT, was enhanced in the lesion area two weeks after transplantation. Moreover, synaptic transmission imaging was used to assess the Ca^{2+} levels of the cells, a facilitator of neurotransmitter release, to confirm neuron-to-neuron interaction enhancement, particularly in mice receiving activated cells [141].

Kong et al. demonstrated the efficacy of transplanting 10^5 cells/ μ l human iPSCs-derived NSCs immediately post-severe contusive SCI, 1 mm rostral to the lesion epicenter at T10 in facilitating the functional recovery in mice. The inflammatory cell counts and the fibrotic to healthy areas ratio were calculated using H&E and Masson trichrome staining, respectively. The immediate transplantation of hiPSC-derived NSCs resulted in reduced infiltration of inflammatory cells and necrotic tissue area at 28 days. Furthermore, IHC demonstrated their differentiation into GFAP-positive astrocytes, TUJ1-positive neurons, and SOX2-positive NSCs in two weeks. Remarkably, ELISA showed decreased serum

cytokine levels of IL-6 and VEGF, indicating decreases in inflammation. Interestingly, they reported no major muscle atrophy in treated mice post-SCI and likewise, the weekly BBB scoring also indicated locomotion improvements. They also performed integrated optimal density to assess the astrogliosis and qualify the stain density of GFAP in the lesion area. The results showed that the GFAP in the transplanted group decreased on days 7 and 14, implying that astrocytes facilitate axonal regeneration by not creating a strong barrier and blocking their path [142].

Early transplantation of the hiPSC-derived NSCs led to significant functional improvements post-SCI, including prevention of parenchyma collapse, decreased infiltration of inflammatory cells, reduced necrotic tissue area, diminished fibrotic area, decreased presence of GFAP-positive glial scars. hiPSC treatment could offer many therapeutic implications post-SCI.

Direct reprogramming cells

Direct reprogramming, also known as cell lineage conversion, is a cellular reprogramming approach that directly transforms one cell type into another without going through an intermediate pluripotent stage [144]. Unlike iPSCs reprogramming, which requires the expression of pluripotency-associated genes for an extended period, direct reprogramming involves transient expression of specific transcription factors, miRNAs, or other chemical or physical signaling molecules (SOX2, SOX10, ASCL1, LHX3 and etc.) to initiate the conversion process [144]. The process of direct reprogramming begins with the activation of specific transcription factors or signaling pathways that are associated with the desired target cell lineage. These factors are introduced into the

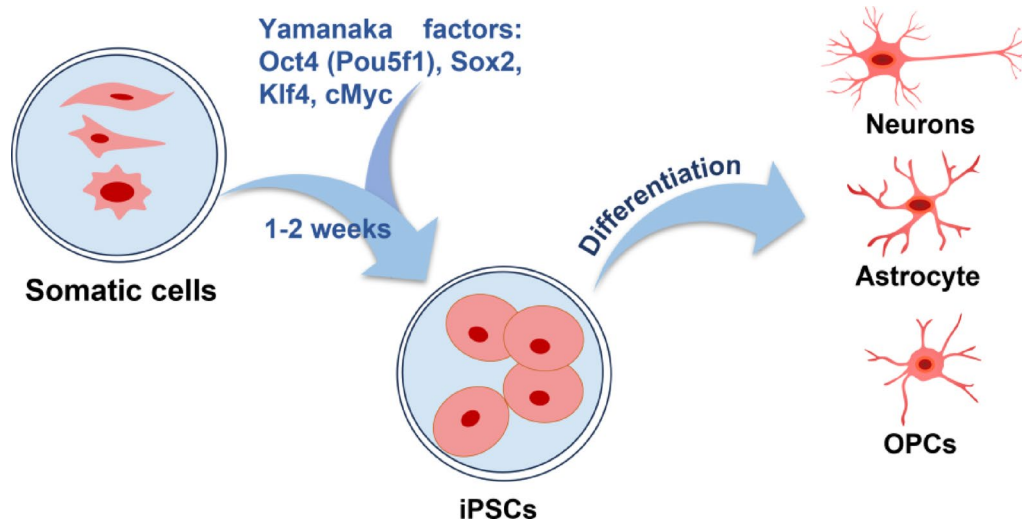


Fig. 14 Reprogramming somatic cells into iPSCs using Yamanaka factors requires an induction time of 1–2 weeks. The reprogramming process of iPSCs involves introducing Yamanaka factors: Oct4 (Pou5f1), Sox2, Klf4, and cMyc. These iPSCs can subsequently differentiate into various cell types, including neurons, astrocytes, and OPCs (in approximately 2 weeks), highlighting their potential for neurological regenerative research

cells of interest, typically through viral vectors or other delivery methods. The transient expression of these factors induces a cellular state that is epigenetically “plastic” or receptive to lineage conversion [145]. During the initial phase of direct reprogramming, the cells undergo a period of instability where they lose their original cell identity and acquire a more malleable state. Specific signaling conditions, including growth factors or modulation of signaling pathways, are often employed to facilitate and guide the conversion process [146].

In the processes of neural direct reprogramming, various cell types, including astrocytes and fibroblasts, have been successfully converted. Transcription factors, such as SOX2 and ASCL1, have been utilized to drive the conversion process and initiate the neuronal lineage program [147] (Fig. 15).

Somatic cell reprogramming mainly involves converting readily accessible or expansively proliferating somatic cells, such as fibroblasts or reactive astrocytes post-nerve injury, into desired cell types like neurons. This process can be easily performed in vitro but also within a controlled in vivo environment [148]. For example, fibroblasts and astrocytes can be converted into motor neurons in vitro by several key transcriptional factors, miRNA, or small molecules (human Sonic Hedgehog, fibroblast growth factor-8b, Wnt1, Wnt5a, BDNF and GDNF). In in vivo settings, neurons that have undergone reprogramming demonstrate the capacity to establish synaptic linkages with host neuronal networks. This intricate synaptic integration is instrumental in restoring neurological functions. Direct cell reprogramming can be harnessed through two promising strategies in SCI treatment: endogenous (autologous) and exogenous (allogeneic) cell reprogramming [149, 150].

Zarei-Kheirabadi et al. employed the zinc-finger transcription factor (Zfp521) strategy to transform astrocytes from adult rat brains into induced NSCs. They infused 2×10^5 cells/ μ l Zfp521-transfected astrocytes 2 mm rostral to the injury epicenter in rats a week after T10 severe

contusive SCI. Six weeks later, the result of qPCR and IHC showed that Zfp521 could reprogram resident astrocytes to neuronal lineage via an intermediary progenitor phase through the expression of nestin, DCX, MAP2, and Tuj1 markers at the level of gene and protein. Accordingly, the GFAP marker and GFAP fluorescent intensity were also decreased around the injured site. The expression of GAP43 (a protein typically expressed in growing axons during development and after injury in adults) was increased, indicating the presence of regenerating axons. Furthermore, this reprogramming has been associated with electrophysiological enhancements (MEP signals showed decrease in latency and increase in amplitude signals), indicative of functional improvements. Equally, the seven weeks of behavioral examinations (BBB scoring) confirmed locomotion improvements in treated rats post-SCI [151].

Lee used two transcriptional regulators, OCT4 and LHX3, to convert human fibroblasts into induced motor neurons (iMNs). This process involved a temporary phase of proliferative iMN-intermediate cells. They injected 10^6 cells/ μ l into rats one week after T9 moderate compression SCI. Immunostaining confirmed that most of the engrafted iMNs expressed Tuj1 and MBP and were surrounded by the host's MBP-positive OLs. H&E staining showed a significant reduction in cavity formation, and BBB scores indicated noticeable motor function recovery eight weeks post-SCI. In a safety control group, the potential for tumor growth was assessed by implanting iMNs under the skin of immune-deficient mice. The 12-month study reported no tumor formation [152].

In a study by Ataei in 2023, it was found that Sox2 is present in the hAF-MSCs CM, which transformed exogenous human astrocytes into neuroblasts. The study involved injecting 5×10^5 cells/ 5μ l hAF-MSCs and human astrocytes into rats with T10 moderate SCI contusion at the injury site. Additionally, the rats received intraperitoneal injections of 500 μ l hAF-MSCs CM for one week. The WB results showed an increased release of

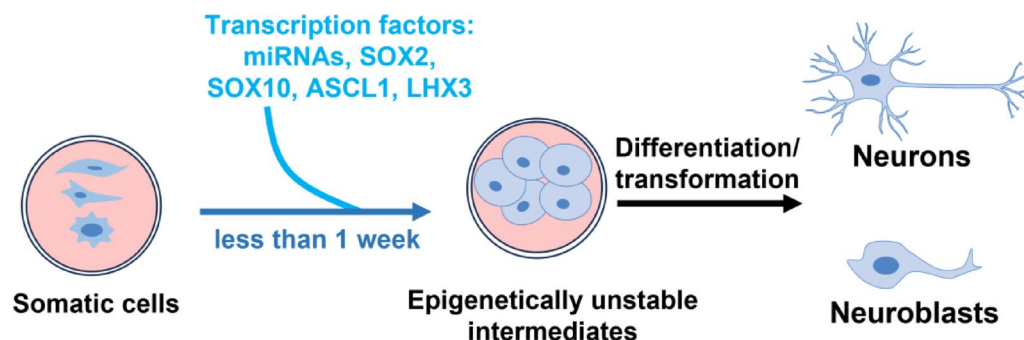


Fig. 15 Direct reprogramming processes and further cell differentiation. The direct reprogramming process involves adding transcription, including miRNAs, SOX2, SOX10, ASCL1, and LHX3, with an induction time of less than 1 week. These factors lead to the formation of epigenetically unstable intermediates, which can subsequently differentiate into various cell types, such as fibroblasts and astrocytes, etc.

Sox2 protein, which played a critical role in transforming astrocytes into DCX-positive neuroblasts. This conversion was found to occur through juxtacrine and paracrine mechanisms. Two weeks post-SCI, there was an increase in neuron cells (DCX expression) and a decrease in the number of GFAP⁺ cells. The conditioned medium also led to improved weekly locomotion BBB scores. They concluded that the secretome of hAF-MSCs facilitates the transformation of astrocytes into neuroblasts, thereby enhancing functional recovery [153].

Direct reprogramming has several advantages over iPSC generation. These include a shorter time frame, avoiding pluripotency-related risks, and the potential for more efficient generation of mature and functional cells. It offers a more direct and efficient approach to generating specific cell types by bypassing the pluripotent state and reducing the risk of teratoma formation associated with iPSCs. However, further studies are needed to optimize the protocols, improve reprogramming efficiency and cell commitment, and ensure the functionality and stability of the resulting cells [146] (Fig. 16).

Direct conversion cells

Direct conversion is a process of cell fate conversion without going through pluripotency. It entails the forced expression of specific transcription factors that are characteristic of the desired target cell lineage. The discovery of “master control genes” or “pioneer factors” has been instrumental in enabling direct conversion. These factors can bind specific sites in the genome that are typically inaccessible to other factors. By displacing nucleosomes,

they initiate transcriptional and epigenetic changes that drive cell fate transition. During direct conversion, the ectopically expressed transcription factors drive the lineage conversion process. They initiate a cascade of molecular events that cause the activation of lineage-specific gene expression, and the suppression of genes associated with the original cell type. Besides transcription factors, chromatin modifiers and other regulatory agents are recruited to remodel chromatin structure and enhance the gene expression of the target cell type [146, 154] (Fig. 17).

Recent advancements in genetic conversion have focused on reprogramming glial cells into neurons to limit glial scar formation and restore the balance between astroglial and neuronal cells. Genetic conversion encompasses transcription factor-mediated approaches, utilizing factors such as NeuroD1 [155], Ascl1 [156], and Neurogenin2 [157, 158], as well as small molecule-mediated and chemically induced reprogramming [159]. These approaches take advantage of the intrinsic plasticity of the CNS to promote neuronal replacement and circuit restoration by activating neuronal genes, silencing glial genes (e.g., GFAP of astrocytes), or modulating signaling pathways and gene expression (such as Notch, Wnt, and MAPK/ERK), thereby inducing astrocytes and microglial cells to acquire neuronal phenotypes [160]. The benefits of genetic conversion reprogramming include reduced risks of immune rejection and tumorigenesis associated with exogenous cell transplantation [160]. Following injury, astrocytes undergo morphological and functional activation, becoming reactive astrocytes capable of

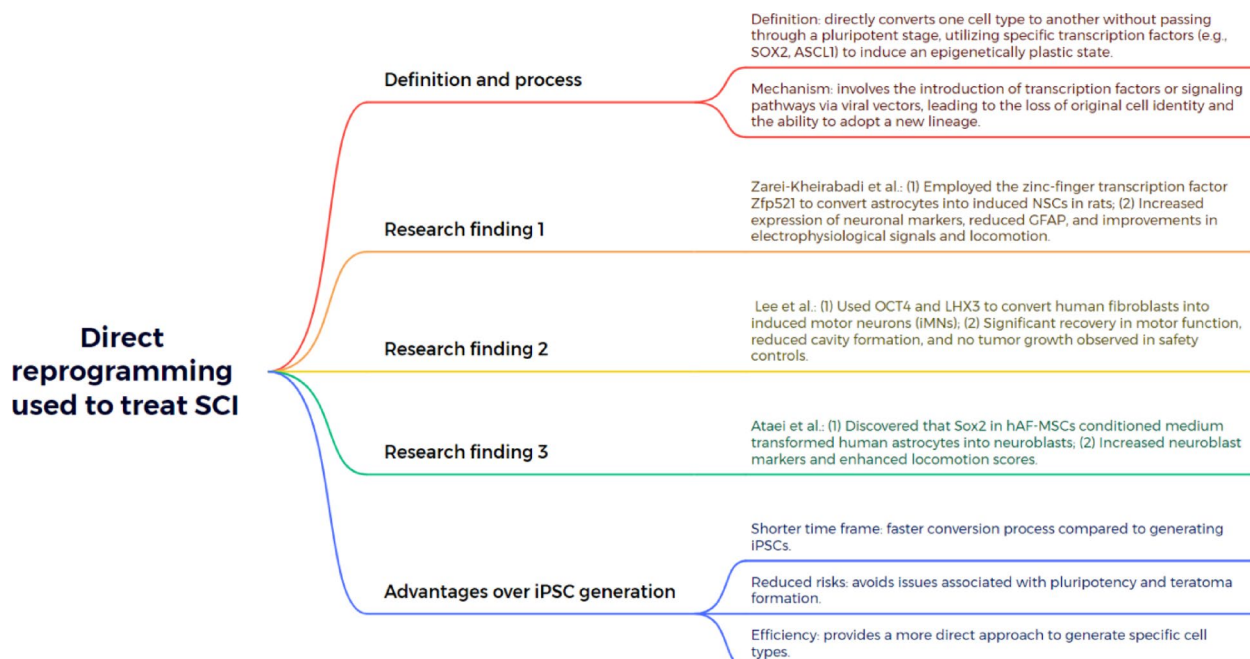


Fig. 16 Schematic representation of the therapeutic potential of direct reprogramming cells in SCI treatment

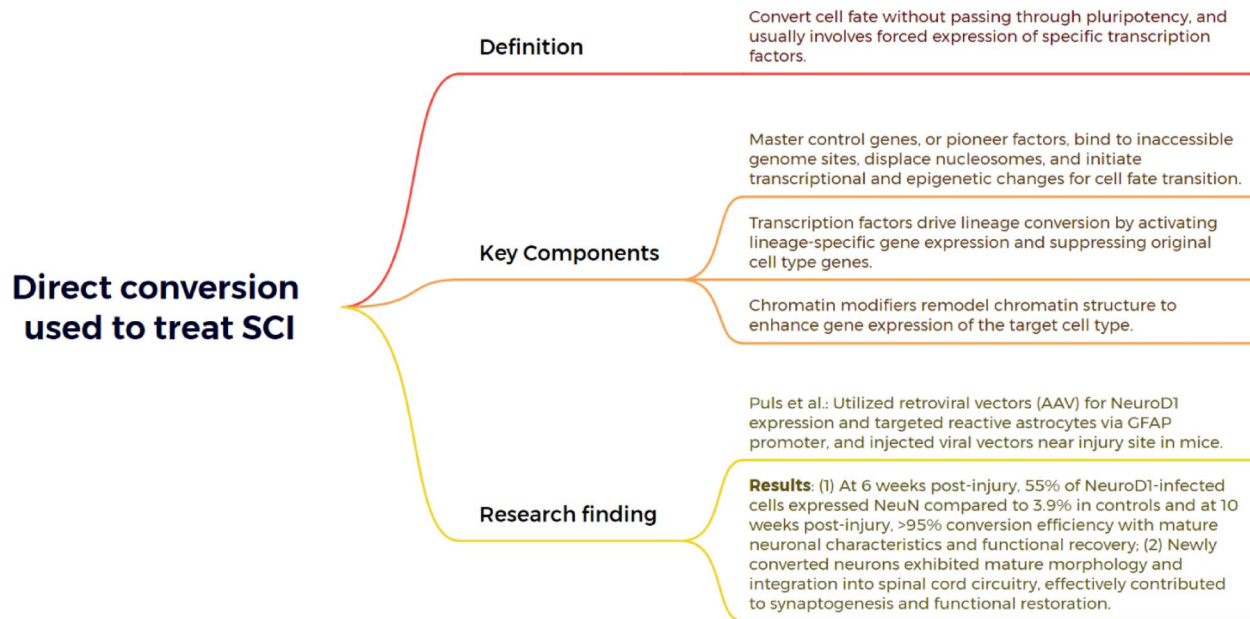


Fig. 17 Schematic representation of the therapeutic potential of direct reprogramming cells in SCI treatment

rapidly compensating for neuronal loss [158]. Harnessing endogenous cells for reprogramming eliminates the need for cell transplantation. Additionally, this approach can alleviate inflammatory responses, glial scar formation [161], and promote long-term circuit reconstruction.

Puls et al. performed neural conversion to reprogram neurons by expressing neurogenic transcription factors from glial cells by mediating a single neural transcription factor, NeuroD1. They produced retroviral vectors (adeno-associated virus) expressing Neuro-D1 for gene delivery and a CreFlex system (it includes two adeno-associated viruses, one carrying the GFAP-Cre gen and the other carrying the transgene in reverse orientation) with GFAP promoter to target reactive astrocytes specifically. They then injected 10^{10} Genome Copy/mL, 1 mm away from injury site in depth of 0.4–0.8 mm at T11/T12 at 10 days and 4 months after mild contusive SCI in mice as acute and chronic injury manners, respectively. Interestingly, IHC examinations confirmed that NeuroD1-infected cells expressed a higher number of NeuN markers and the lower number of GFAP marker than control group after 6 weeks post-viral injection, demonstrating successful neural conversion. In fact, they found approximately 2000 cells in the core areas surrounding the injury site had converted into neurons in NeuroD1-infected cells (about 55% NeuN⁺ cells) in comparison with control group (about 3.9% NeuN⁺ cells) after acute injection. In the chronic experiment, the tissues were extracted at 10 weeks post viral infection. In the control group, they detected S100b⁺ astrocytes and no NeuN⁺ cells, but conversion efficiency reached >95% in the NeuroD1 infected cells after 10 weeks post viral

injection. They observed mature neural morphology and c-Fos⁺ cells within the neurons, suggesting integration into the spinal cord circuitry. Tix3 marker expression was also detected in the dorsal horn of spinal cord, which exhibited glutaminergic neurons after 10 weeks post viral injection. The newly converted neurons were well-integrated into the local neural network, producing/promoting synaptogenesis. The electrophysiological tests also demonstrated noticeable functional restoration post-SCI [162].

Direct conversion is a promising approach for generating specific cell types in regenerative medicine. The goals are to optimize conversion protocols, improve efficiency, and expand the repertoire of cell types that can be directly converted.

Combined cell therapies

Combined cell therapy, involving the co-transplanting of stem cells such as MSCs, OECs, SCs, iPSCs, etc., holds great potential for improving functional restoration, tissue repair, and reducing inflammation as a post-SCI treatment. This approach harnesses the synergistic effects of the cells to address pathological barriers and takes advantage of the combined effects of its diverse components [163] (Fig. 18).

In 2024, Du et al. conducted a study to assess the safety and effectiveness of a combined cell therapy involving the transplantation of hAC-MSCs and iPSCs-derived NSCs (M&N) in adult mice following severe contusive SCI. They transplanted 1×10^5 of each cell/ μ l into the epicenter of T10 immediately post-injury. Four weeks later, results from ELISA and qPCR tests confirmed the

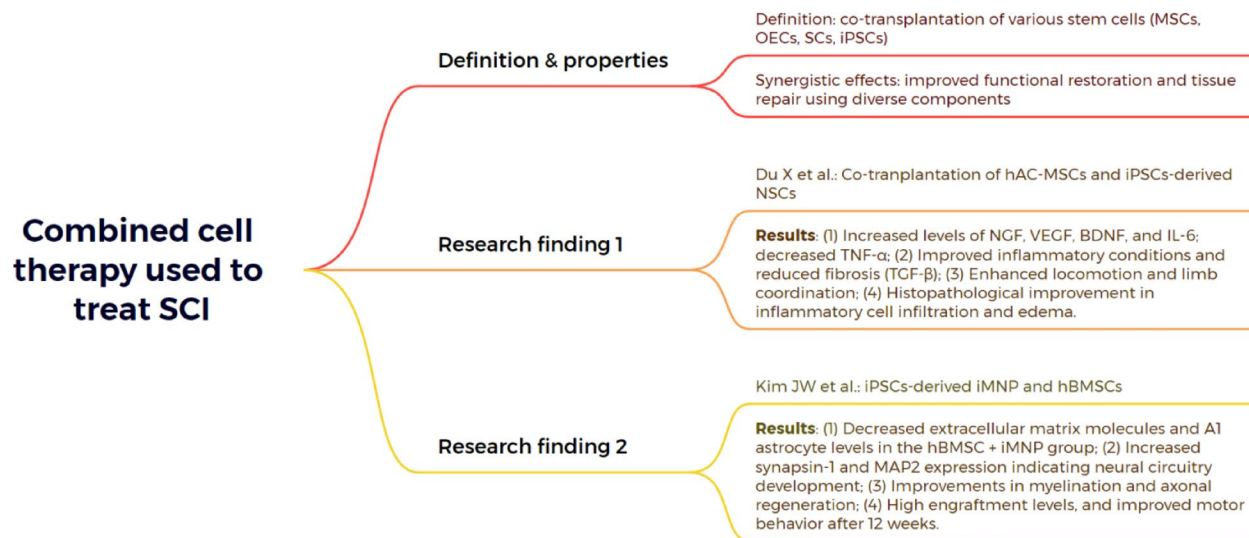


Fig. 18 Schematic representation of the therapeutic potential of combined cell therapy in SCI treatment

immunoregulatory and neuroprotective effects of the M&N treatment. The levels of NGF, VEGF, BDNF, and IL-6 increased, while the levels of TNF- α in both serum and spinal cord tissue decreased significantly, without any observed toxicity or increase in spleen size. Additionally, Western Blot analysis showed a weekly decrease in TGF- β levels, a marker of fibrosis. Histopathological examinations (H&E and Masson staining) confirmed that inflammatory cell infiltration and fibrosis were improved. The mice also showed significant improvements in locomotion indicating better limb coordination. Transmission electron microscopy (TEM) revealed a significant reduction in intermediate filaments between proliferating astrocytes and reduced neural edema and demyelination. They concluded that M&N combined cell transplantation therapy improves inflammatory conditions and provides neuroprotective effects [164].

In another study, iPSC-derived motor neuron progenitor cells (iMNPs), generated from peripheral blood mononuclear cells, were combined with hBMSCs to be tested for treating rats with acute and chronic T9 severe contused SCI. Their experimental groups included: (i) chronic SCI + iMNP (injection of 10^6 cells/10 μ l into T9, 6-weeks post-SCI), (ii) acute SCI + hBMSCs (injection of 10^6 cells/100 μ l into the tail vein 1-day and 7-days post-SCI), and (iii) chronic SCI + hBMSC and iMNP (injection of 10^6 hBMSCs/100 μ l into the tail vein at 1-day and 7 days post-SCI and 10^6 iMNP/10 μ l into T9 at 6-weeks after hBMSCs transplantation). The fluorescence intensity at the lesion site indicated decreased levels of Neurocan (extracellular matrix molecules assessment at the lesion site) and C3 (A1 astrocyte type) markers in the hBMSC + iMNP group. Furthermore, it was observed that transplanted hBMSCs did not remain at the site of injury in two groups of hBMSCs and hBMSC + iMNP

rats, while transplanted iMNPs were still present. The immunostaining results indicated neural circuitry development through an increase in synapsin-1 expression and MAP2 (an axonal sprouting marker) in the iMNP and hBMSC + iMNP groups. Additionally, TEM analysis detected improvements in myelination and axonal regeneration in the cell combinatory group. They also reported a high level of engraftment of the transplanted cells into the spinal cord parenchyma and improved motor behavior compared to the other groups after 12 weeks [165].

Overall, combined cell therapy has been proven to be both safe and effective. It can improve demyelination, neuroinflammation, and motor function deficits in the spinal cord through its synergistic effects. This innovative approach shows promise in repairing pathological barriers and enhancing recovery in SCI therapy. However, further research is needed to determine the best cell types, dosages, and timing for administration.

Combined application of cell therapy and biomaterials

Biomaterials have emerged as promising therapeutic options for treating SCI. They offer innovative solutions to enhance neuron regeneration and functional restoration. These advanced materials have great potential in providing structural support, delivering therapeutic agents, and creating conducive environments for neural repair due to their unique characteristics and biocompatibility [63, 166].

Hydrogel

Hydrogel is a biocompatible polymeric material that can replicate the natural extracellular matrix of the spinal cord. Its structure is a waterlogged 3D system that acts as a mechanical support for transplanted cells. This helps

Table 5 Hydrogel used to treat SCI

Hydrogel components	Loaded cells	Intervention time	Mechanisms	References
Hyaluronic acid	hESC-NSCs	1 week	Neurogenesis, oligodendrogenesis, myelination, inhibit scar formation and cavity	[168]
Fibrinogen & thrombin	iNSCs	2 weeks	Differentiation into neurons, astrocytes and OLs, axon extension, new synapses formation with host neurons, myelination	[143]
Hyaluronic acid	hESC-NPCs	Immediately	Angiogenesis, macrophage M2 polarization, remyelination, neural differentiation, inhibit scar formation, inflammation, apoptosis	[169]
Hyperbranched polymer & methacrylate hyaluronic acid	BMSCs	Immediately	Neurogenesis, axon regeneration, inhibit oxidative damage, inflammation, apoptosis, fibrotic and glial scar	[170]
Acryloyl chloride & β -cyclodextrin	ADSCs	Immediately	Neurogenesis, axon regeneration, remyelination, macrophage M2 polarization, inhibit inflammation, apoptosis, cystic cavity	[105]

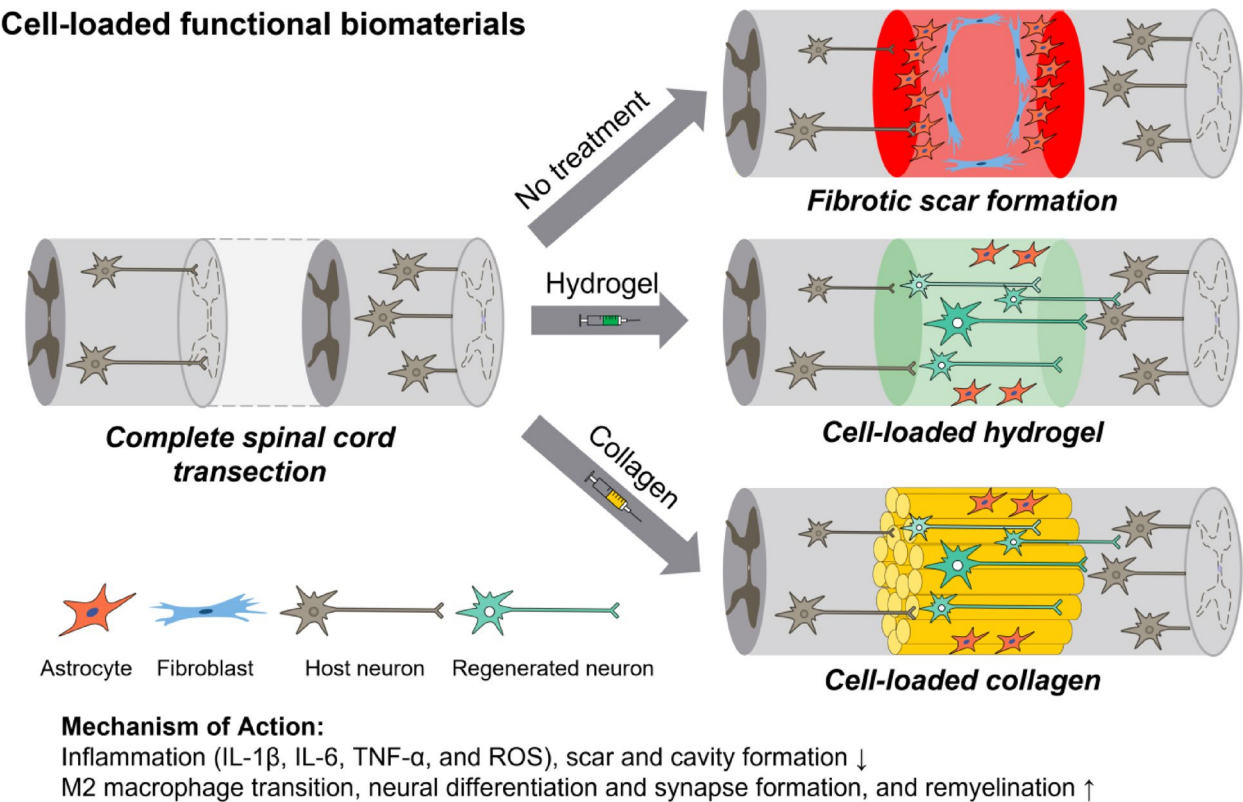


Fig. 19 Hydrogel and collagen scaffold-mediated repair in a complete spinal cord transection model. This diagram illustrates using cell-loaded hydrogels and collagen scaffolds in spinal cord transection. In the untreated group, fibrotic scar formation and excessive astrocyte activation lead to glial cavities. In contrast, cell-loaded hydrogels and collagen scaffolds significantly reduce scarring and cavity size. These biomaterials promote M2 macrophage polarization, aiding tissue restoration and anti-inflammation, while enhancing neural differentiation, synaptic reconstruction, and remyelination

in fulfilling the damaged area, improving implanted cells proliferation and migration, and enhancing axonal regeneration (Table 5). Implanting suitable hydrogels loaded with desired cells at the site of SCI creates a supportive microenvironment, increases cell survival, and improves their function [167] (Fig. 19).

Hyaluronic acid (HA) is an anionic, non-sulfated glycosaminoglycan and is a natural component of the extracellular matrix. It has unique viscoelastic and moisturizing properties and exhibits anti-inflammatory

effects. In the extracellular matrix of the brain and spinal cord, there are high levels of HA, making it a suitable option for treating CNS injuries. Additionally, the high molecular weight of HA plays a critical role in the treatment of SCIs by providing non-immunogenic and biocompatible properties. This impacts astrocyte proliferation, enhances neural tissue regeneration, modulates the immune response, activates macrophages, and provides a supportive scaffold during the SCI treatment [168, 171].

Zarei-Kheirabadi et al. transplanted ESC-NSCs encapsulated in HA hydrogel (10^6 cells/25 μ l prepared hydrogel, cell/HA group) in rats one week after severe T10/T11 SCI. Immunofluorescence analysis showed that the transplanted cells differentiated into neurons, astrocytes, OPCs, and OLs at the lesion site, as indicated by the expression of specific markers such as GFAP, PDGFR- α , O4, MBP, and Tuj1 at seven weeks after injury. Interestingly, the cell/HA group showed more OPCs and OLs, but fewer astrocytes compared to the cell-only group. The presence of HA improved the survival rate of the transplanted cells, as demonstrated by the MTS assay. Motor functions were assessed using MEP monitoring and BBB scoring. Both the cell-only and cell/HA groups exhibited significant improvements in locomotion abilities, including plantar placement of the paw and weight support. In addition, the rats that received cell/HA treatment showed a higher rate of survival. After seven weeks, the groups that received HA, cell, and cell/HA transplantation demonstrated a significant reduction in glial scar areas as observed through IHC, and the size of the cavity decreased in the cell and cell/HA groups as shown by H&E staining. The analysis of axon myelination using Fluoro-Myelin red staining revealed improved myelination in the cell/HA group, indicating integrated white matter. Encapsulating ESC-NSCs in HA led to a decrease in glial scar deposition, enhanced myelination, and improved motor function. It was found that HA could mimic the spinal cord microenvironment at the site of injury and promote oligodendrogenesis [168].

Li et al. reported that combination with biomaterials enhanced the survival and function of ES-cells post-SCI. They encapsulated BMSCs (8×10^6 cells/mL sourced from rat tibia) into an ROS-responsive and scavenging hydrogel involving a crosslinked thioketal-containing hyperbranched polymer with methacrylate hyaluronic acid, neural-specific CQAASIKVAV peptides, epidermal growth factor, and bFGF molecules. They used a rat SCI model and transplanted cells and hydrogel into a 2 mm gap immediately after the T10 complete transection. The ELISA assay results confirmed that levels of IL-1 β , IL-6, and TNF- α were reduced in the cell/hydrogel and hydrogel groups at 7 days post-SCI. Immunofluorescence results showed an increase in the ratio of M2/M1 macrophages. H&E staining indicated a decrease in inflammatory leukocyte infiltration in the cell/hydrogel and hydrogel groups, suggesting the anti-inflammatory potential of the hydrogel during the acute phase of SCI. Nissl staining confirmed the presence of more Nissl bodies in the cell/hydrogel and hydrogel groups, indicating improved neuron morphology. Masson and immunofluorescence staining were also used to assess the expression of PDGFR- β , a fibrotic scar marker, and GFAP, which represents glial scar formation. These stains revealed

reduced scar formation and collagen deposition, and better preservation of neural fibers in the cell/hydrogel group at one week and in both the cell/hydrogel and hydrogel groups at eight weeks post-SCI. After 56 days, the BBB locomotion improved in the cell/hydrogel group, with 66.7% of rats scoring $>7+$. Similarly, the expression of MAP2 and Tuj-1 increased in the cell/hydrogel group, indicating the neurogenesis potential of the cell/hydrogel treatment. More spared parenchyma and axonal regeneration were observed in the hydrogel groups with or without BMSCs. The expression of the NF-200 marker increased in the cell/hydrogel-treated rats. Immunofluorescence using CD31 and alpha-smooth muscle actin revealed no significant vascularization in any group. Thus, it was concluded that protecting BMSCs from oxidative environments is neuroprotective and improves their ability to support the regrowth of nerve fibers and motor function recovery post-SCI. Additionally, a hydrogel that scavenges ROS, with or without BMSCs, can substantially reduce fibrotic scarring and the clustering of glial cells at the injury site [170].

In another study, Son et al. generated induced NSC (iNSCs) directly from human urine cells (hUCs) and fibroblasts by using synthetic mRNAs containing OCT4, KLF4, SOX2, GLIS1, and B18R. They transplanted 1.5×10^6 cells/10 μ l into the T3 region of rats two weeks after a moderate compression SCI. The iNSCs were supported by fibrin hydrogel. Immunohistochemistry showed the ability of iNSCs to differentiate into neurons, astrocytes, and OLs. Eight weeks later, the transplanted iNSCs-derived axons sprouted and formed new synapses. Immunolabeling confirmed that the iNSCs extensively differentiated into astrocytes and OLs, promoting connectivity and myelination, and improving locomotion in rats with the BBB scores >8 . In comparison, the rat groups without iNSC transplantation scored <5 . These findings provide compelling evidence for the potential therapeutic use of iNSCs in SCI. Notably, the use of hUCs and synthetic mRNA presents a promising approach for generating patient-specific iNSCs for future clinical applications [143].

Roh et al. demonstrated the therapeutic potential of composite scaffolds in SCI repair. These scaffolds are made up of hyaluronic acid hydrogel (20 mg/mL), decellularized brain matrix (DBM, 5 mg/mL), and bioactive agents such as polydeoxyribonucleotide (PDRN, 200 mg/mL), TNF- α /interferon- γ primed MSC-derived extracellular vesicles (TI-EVs, 10^8 particles), and embryonic ESC-derived NPCs (1000 cells/ μ l). They exhibited remarkable regenerative potentials including angiogenesis, anti-inflammatory, anti-apoptotic, and neural differentiation properties. Significant regeneration in spinal cord was detected when 20 μ l of the combined hydrogel was implanted in rats immediately after complete T10

transection. The inclusion of rat brain-derived DBM in the hydrogel provided a specialized microenvironment for embedded NPCs. In vitro assays showed adding DBM, PDRN, and TI-EVs in hydrogels notably increased NPC viability by 3.14 folds after 24 h, when compared to treatment with the hydrogel alone. PDRN, a powerful A2a receptor agonist, accelerated tube formation by human umbilical vein endothelial cells, demonstrating robust pro-angiogenic activity. Additionally, Western blot analysis showed that incorporating bioactive molecules effectively reduced neuroinflammation induced by LPS, as shown by decreased expression of pro-inflammatory cytokines (IL-1 β and IL-6) and downregulated MAPK/caspase-3 pathways, ultimately enhancing the expression of the anti-cell death protein BCL-2. Still, 4 weeks post-SCI, immunofluorescence and quantitative PCR results showed that the composite scaffold accelerated the growth of TUJ1 and MAP2 nerve cell proteins (203.1% longer nerve extensions compared to gel-only treatment) while promoting a shift from M1 to M2 macrophage polarization, thus reducing inflammation. The scaffold had a significant impact on pro-inflammatory responses. It reversed the overexpression of inflammatory markers such as CD68, Iba-1, and HMGB1. Additionally, there was a downregulation of apoptotic genes, including caspase-3, and an upregulation of CD206. Enhanced angiogenesis was indicated by increased expression of CD31 and von Willebrand factor. Furthermore, there was an upregulation of the tight junction-related protein occludin, elevated levels of IL-10 (which signifies anti-inflammatory activity), and increased expression of neurotrophins like BDNF and NGF, which are indicative of neuronal differentiation and growth.

The composite scaffold DBM/PDRN/TI-EV/NPC@Gel increased the expression of important markers for neuronal differentiation and growth. Specifically, it led to a significant increase in the expression of the following markers: (i) TUJ-1, an early neuronal development marker (10.045% vs. 1.471%); (ii) vesicular GABA transporter, associated with the formation of GABAergic and glycinergic neurons and synaptic connectivity (11.342% vs. 3.288%); (iii) neurofilament, a neuronal marker (9.005% vs. 2.457%); and (iv) neuronal nuclear protein, a marker for mature neuronal identification and function (16.574% vs. 2.607%). Moreover, the scaffold had a positive impact on remyelination by regulating cellular dynamics. It decreased scar tissue formation, as shown by the reduced levels of laminin within the scarred area (ratio: 12.021% vs. 26.025%). Concurrently, there was a significant decrease in the differentiation of astrocytes, as indicated by reduced levels of GFAP. This effectively reversed the highly agglomerated tissue characteristic of SCI 28 days post-injury (relative ratio of GFAP area: 8.468% vs. 21.145%). Morphologically, tissue treated

with DBM/PDRN/TI-EV/NPC@Gel exhibited a cellular arrangement similar to that observed in sham tissue. It displayed intact morphology and a greater area of myelination, measured at 47.740% compared to 37.285% in the SCI group. Additionally, the scaffold promoted the transcription of OLIG2 by 2.16-fold compared to the SCI group, indicating enhanced OL proliferation and maturation. Furthermore, the expression of NG2, a marker for OL precursor cells, was significantly higher in the scaffold-treated groups. The scaffold promoted the expression of MBP in the SCI group, indicating improved myelination processes. Functional assessments showed a significant improvement in motor function, with the composite scaffold-treated group exhibiting a notable increase in the BBB score 28 days post-SCI.

In summary, the composite scaffold DBM/PDRN/TI-EV/NPC@Gel is a promising therapeutic approach for SCI repair, exhibiting multifaceted regenerative effects including angiogenesis, anti-inflammation, neuronal differentiation, and remyelination [169].

Yuan et al. pioneered a cell-adaptive neurogenic (CaNeu) hydrogel designed specifically as a conduit to deliver Adipose-Derived Stem Cells (ADSCs) and tested it on a T9-10 complete transection rat model. They demonstrated that early implementation of CaNeu hydrogel enhanced axonal growth, leading to improved motor function post-SCI. Developing such hydrogel is a complex process, which involves dissolving β -cyclodextrin (β -CD) in N, N-dimethylformamide (DMF), then reacting it with acryloyl chloride and triethanolamine, and finally precipitating it in acetone to produce Ac- β -CD. After that, gelatin is modified with methacrylic anhydride, leading to the formation of the hydrogel network through UV-mediated polymerization of Ac- β -CD. The resulting CaNeu hydrogel, after dialysis and lyophilization, mimics the structural and mechanical characteristics of neural tissues, creating a favorable environment for the neural differentiation and axonal growth of encapsulated ADSCs, thereby promoting nerve regeneration. ADSCs derived from rat epididymal fat tissue were loaded into the CaNeu hydrogel at a concentration of 10^7 cells/mL, and 10 μ l of CaNeu/ADSCs combination transplanted at the injury site immediately post-SCI. The qPCR confirmed that the CaNeu hydrogel effectively preserved the stemness of the ADSCs as indicated by the expression of nestin and SOX2, in the expansion medium. Immunofluorescence results on day-7 showed upregulation of MAP2, suggesting that CaNeu hydrogel promotes the differentiation of ADSCs and their adoption of neuronal morphology in a neuronal differentiation medium. This also boosted motor functional recovery. Rats in the CaNeu/ADSCs group exhibited significant functional improvement (mean BBB score of 9.2 ± 2.0) compared to rats in the cell-free CaNeu hydrogel group

(BBB 5.0 ± 2.8) or ADSCs cell alone group (BBB 4.5 ± 3.2). Concordant with the BBB scores, the electrophysiological assessments showed increased MEP signals amplitude. They concluded that the CaNeu/ADSCs construct promotes neurogenesis and axonal regeneration and spares (saves) more healthy parenchyma with smaller size cystic cavities post-SCI. Such a 'hydrogel/cells construct' significantly increased the proportion of TUBB3⁺ neuronal structures within the epicenter of the lesion ($47.64\% \pm 3.65\%$), compared to the treatment with only the hydrogel CaNeu group ($43.59\% \pm 1.27\%$), only the ADSCs cell group ($32.80\% \pm 1.41\%$), or only the SCI without treatment group ($18.21\% \pm 1.86\%$). Sixty days after SCI, the immunofluorescence and SEM assays revealed that this treatment regimen increased the expression of MBP and NF-200. This led to neuronal regeneration and myelination with functional axons, as well as the growth of host serotonergic axons and myelin sheath at the injury site. Notably, the CaNeu hydrogel induced a favorable shift in the polarization of recruited macrophages toward the pro-regenerative M2 phenotype, thus mitigating neuroinflammation and apoptosis. Western Blot analysis showed that 7 days post-injury, the CaNeu/ADSCs group exhibited reduced levels of cleaved caspase-3 and Bax (markers of apoptosis), as well as diminished expression of Iba1 coupled with increased levels of the anti-apoptotic protein Bcl-2. The CaNeu/ADSCs group predominantly showed the expression of CD163, a marker of M2 macrophages, indicating the presence of a substantial population of M2 macrophages at both one- and two-weeks post-injury. Similarly, during the first week post-injury, the CaNeu/ADSCs group showed increased expression of another M2 macrophage marker, CD206, and decreased pAkt levels. This emphasizes the role of CaNeu hydrogel along with ADSCs in reducing neuroinflammation through the PI3K/Akt pathway. This process promotes axonal regeneration, myelination, and motor function recovery.

In summary, CaNeu/ADSCs improved locomotor function by promoting axonal regeneration, myelination, anti-inflammation, and anti-apoptosis [105].

Collagen scaffold

Collagen, the primary structural protein found in the ECM, is integral for tissue repair and regeneration. Due

to its unique properties of biocompatibility and biodegradability, collagen is commonly used in scaffold structures. These scaffolds provide a three-dimensional framework of collagen fibers that support the growth and proliferation of regenerative cells and allow for the efficient diffusion of nutrients and waste products. This optimal environment facilitates the regeneration process by promoting cell attachment, proliferation, and differentiation [172] (Fig. 19, Table 6).

Xu et al. produced collagen with an aligned porous structure derived from depilated bovine skin and utilized in T8-T9 complete transection rat model. They transplanted three types of human fetal brain-derived NSPCs (BNSPCs), spinal cord-derived NSPCs (SCNSPCs), and H9 embryonic stem-cell derived NSPCs (H9-NSPCs) with the collagen scaffold into the transected gap immediately post-SCI. They performed RNA sequencing and qPCR in order to prepare a comprehensive transcriptome of these cells in vitro. The cells expressed approximately 15,479 genes. Among these genes, some were closely related to nerve functions such as neuron—achaete-scute family bHLH transcription factor 1 (ASCL1) and DAB1, OLs differentiation, myelination, synapse organization—Wnt family member 7A, leucine-rich repeat kinase 2, and neurotransmitter secretion—glutamate decarboxylase 1 [173]. These genes were mostly expressed by SCNSPCs. SCNSPCs exhibited an increased level of POU3F2, a transcription factor important for controlling myelination in Schwann cells (SCs). They also showed elevated expression of KCNJ10 (a crucial gene for OLs' function and myelination) and DHH (a gene involved in SCs' regulation of nerve perineurium function). One month after SCI, rats were sacrificed for analysis. The immunohistochemistry analysis revealed that the survival rate of SCNSPCs (GFP⁺ cells) was $40.2\% \pm 6.9\%$, and their differentiation rates were higher compared to other transplanted cells. There was a significant increase in the presence of mature neurons that were both NeuN and GFP double-positive at the injury site where the SCNSPCs + scaffold group was implanted (9.1%), in contrast to the BNSPCs + scaffold group (4.5%) or the H9-NSPCs + scaffold group (~0%). The group treated with SCNSPCs + scaffold showed improved electrophysiological and locomotion recovery. Further examination of myelination and axon ensheathment using IHC showed

Table 6 Collagen scaffold used to treat SCI

Collagen origin	Loaded cells	Intervention time	Mechanisms	References
Bovine skin	NSPCs	Immediately	Neurogenesis, remyelination, synaptic formation	[173]
Bovine skin	DV-SC	Immediately	Synaptic connections, neuronal maturation,	[174]
Bovine aponeurosis	iPSCs-NSCs	Immediately	Neuron regeneration, synapse formation, microenvironment, remyelination, inhibit macrophage accumulation and scar formation	[175]

that the expression of S100 β was notably higher in the SCNSPCs + scaffold group compared to the other groups. This indicates that the SCNSPCs cells were wrapped and myelinated by SCs. Additionally, the expression of MBP was significantly higher in the SCNSPCs + scaffold group too. They also analyzed the expression of myelin protein zero and myelin proteolipid protein at the SCI site. The results showed that all three groups could be myelinated by OLs. The markers of synapse formation, SYP (a pre-synaptic marker) and PSD95 (a post-synaptic marker), were distinctly elevated in the SCNSPCs + scaffold group. In the case of neurotransmitter secretion, they found higher expression of GABA⁺ inhibitory neurotransmitters, GAD65/67⁺ inhibitory neurotransmitters, and VGLUT1⁺ excitatory neurotransmitters after the transplantation of SCNSPCs + scaffold. Overall, SCNSPCs showed promising potential for repairing SCI, particularly in promoting neuronal differentiation, myelination, and functional recovery [173].

In another study, Xu et al. used SD rats and rhesus monkeys to create T8-T9 complete transection SCI. Shortly after injury, rats were given collagen with 10^6 cells, and monkeys were given collagen with 10^7 cells. Human spinal cord neural progenitor cells (hscNPCs), derived from 7-week-old human embryo tissue, were induced to differentiate into various types of dorsal and ventral neuronal cells on a collagen scaffold, resulting in engineered human embryonic spinal cord-like tissues with both dorsal and ventral neuronal characteristics (DV-SC). When DV-SC was transplanted into this SCI models in rats and monkeys, it demonstrated better therapeutic effects compared to undifferentiated hscNPCs, including improved cell survival and maturation. DV-SC has been used to establish targeted connections with host ascending and descending axons, which partially restored interrupted neural circuits and improved MEP and hindlimb functions. An aligned porous collagen scaffold derived from depilated bovine skin was used for NPC culture. Immunohistochemistry results showed positive cells for BRN3A (10.5%), TLX3 (7.8%), and LMX1 (17.1%) at day 30 of dorsal differentiation in vitro. Additionally, after 30 days of ventral differentiation in vitro, the cells showed positive expression for the following markers: LHX1 (ventral interneuron, 22.5%), FOXP2 (V1 interneurons, 45.6%), HB9 (motor neuron, 9.1%), ISL 1 (motor neuron, 13.1%), and OLIG2 (motor neuron progenitor, 8.4%). In SCI rats, approximately 5.8×10^5 grafted cells were found to survive in the scaffold + DV-SC group at 2 weeks post-injury, and about 2.4×10^5 grafted cells survived at 2 months post-injury (mpi). Similarly, 2 mpi, 39.1% of grafted cells were positive for TUJ1 and 16.9% were positive for NeuN with scaffold + DV-SC treatment. The cells also expressed BRN3A (9.1%), TLX3 (6.7%), LMX1B (11.3%), HB9 (7.1%), and ISL 1 (2.8%) in the

lesion area. Additionally, in the scaffold + DV-SC group, grafted cells made connections with host CGRP⁺ axons. 18.3% of these connections were PSD95⁺, indicating synaptic connections between DV-SC and host axons at 2 mpi. MEP showed significantly higher hindlimb amplitude in the scaffold + DV-SC group than in the scaffold-only group. The joints and limbs movements showed significant improvement, with a BBB score of 6.7 ± 0.9 in the scaffold + DV-SC group, which was notably higher than the scaffold-only group. At 2 mpi, a significant survival rate of grafted cells was noted in SCI rhesus monkeys, with about 6.1×10^6 cells persisting in the scaffold + DV-SC treated group. Furthermore, 30.5% of the grafted cells exhibited TUJ1 expression within the injury area. However, by 6 mpi, the number of surviving grafted cells decreased to approximately 2.6×10^6 , and the proportion of TUJ1⁺ cells had reduced to 12.8%. Conversely, there was an increase in the percentage of transplanted NeuN⁺ mature neurons from 2 to 6 mpi. Specifically, the proportion of NeuN-positive grafted cells increased from 15.1% at 2 mpi to 65.2% at 6 mpi within the injured area. A notable fraction of grafted cells within this group exhibited differentiation into mature NeuN⁺ neurons. At 6 mpi, observations revealed 13.3% BRN3A⁺ and 16.7% TLX3⁺ grafted cells within the injury site. Likewise, 13.6% of grafted cells displayed HB9⁺ expression, and 3.6% showed ISL1⁺ motor neuron markers. Also, 11.1% of the grafted spinal cord ventral neurons exhibited FOXP2⁺ V1 interneuron markers, with an additional 2.3% expressing SIM1⁺ V3 interneuron markers. At 6 mpi, 35.3% of grafted cells were observed to form PSD95⁺ synapses with host CGRP⁺ axons. Moreover, 5-HT⁺ nerve fibers were identified at the lesion site, forming SYP⁺ synapses with grafted cells at a rate of 18.5%. The MEP signals from two rhesus monkeys indicated functional recovery post-SCI at 6 mpi. One subject showed an amplitude similar to the baseline, while the second monkey displayed an amplitude of 41 μ V. Furthermore, the scaffold + DV-SC group showed partial restoration of hindlimb muscle strength, with one monkey able to alternately lift the left and right knee joints multiple times [174].

Wu et al. found that a functional neural network tissue, constructed from TrkC-modified iPSC-derived NSCs and CBD-NT3-modified linear-ordered collagen scaffolds (LOCS), shows great potential for SCI repair. In a rat model of complete T8-T9 transection SCI, iPSCs derived from the human umbilical cord were induced to differentiate into NSCs. These NSCs were then seeded (5×10^5 cells) onto LOCS along with TrkC modification. Transplantation of this engineered tissue led to the regeneration of neurons and synapses, as indicated by the expression of neuronal markers ChAT, VGLUT1, MAP2, SYN, and PSD95, with minimal astrocytic differentiation. The addition of CBD-NT3 to LOCS enhanced cell

differentiation and neurite outgrowth. RNA sequencing confirmed increased axonogenesis (increased expression of SYN1, SYN3, and PSD95), synaptogenesis, and neurogenesis (increased expression of ChAT, VGLUT1, and MAP2) along with enriched extracellular matrix organization and synaptic pathways. Moreover, immunofluorescence analysis revealed widespread distribution and integration of grafted cells, reduced inflammation (fewer CD68 positive cells), and scar formation (almost no NG2 expression) in the SCI area, and improved microenvironment via increased laminin deposition and integrin $\beta 1$ expression. Functional neural network tissue transplantation significantly promoted neuronal regeneration, differentiation, and synaptic connectivity, as evidenced by an increased Tuj-1 to GFAP ratio and the presence and connection of 5-HT⁺ fibers. Functional improvements were observed in hindlimb, as assessed by the BBB scale (9.25 ± 3.59 vs. 1.75 ± 1.39 in control), with rats in the LOCS+NT3+NSC-TrkC group exhibiting significant voluntary movements 8 weeks after injury. MEP showed enhanced signal conduction through the SCI area (higher amplitude and shorter latency: 4.18 ± 0.13 ms in treated subjects), along with improved hindlimb weight-bearing and movement during walking, indicative of functional recovery.

In summary, the transplantation of functional neural network tissue comprising TrkC-modified iPSC-derived NSCs, and CBD-NT3-modified LOCS holds promise for SCI repair. It offers substantial improvements in locomotor function and neural connectivity [175].

Polycaprolactone fiber (PCL)

Fibers with unique surface characteristics have been developed through alterations in surface chemistry, polymer combinations, or surface modification aimed at boosting the regenerative capacity of nerves. Incorporating proteins or peptide sequences into biodegradable and synthetic polyesters prior to electrospinning allows for integrating these biological agents within the fibers. PCL, a type of biodegradable aliphatic polyester, has recently become a significant agent in biomedical areas. PCL is FDA-approved as a commercial material, biodegradable, and biocompatible with an extensive array of additional polymers. PCL scaffolds also showed unique porous structures in three dimensions and minimal host reactions, suggesting an alternative choice in regenerative medicine [176, 177].

Wang et al. developed Nap-E7-YIGSR peptide-coated PCL fibers and loaded them with spermatogonial stem cells (SSCs) isolated from the seminiferous tubules of 23–25 days old SD rats. They injected 10^6 cells/ $10 \mu\text{l}$ into the epicenter of rats T8 one day after spinal cord compression (Fig. 20). The immunofluorescence analysis showed that the PCL fibers loaded with cells expressed

more GFR α -1 (the adhesion marker of SSCs onto the surface of PCL filaments), so that 28.10% of cells after 2 h, 35.30% after 4 h, 60.35% after 6 h, and 83.24% after 24 h attached on PCL fibers. Additionally, the loaded PCL fibers showed a higher count of neurons (Tuj-1, $29.3\% \pm 6.5$ vs. $5.1\% \pm 3.1$ in the control group). The neurons that were derived from SSCs showed a longitudinal extension of neuronal axons along the PCL fibers. The average axon length of the differentiated neurons in the Nap-E7-YIGSR group ($45.10 \pm 6.1 \mu\text{m}$) was longer than that of both the Nap-E7 ($22.02 \pm 5.8 \mu\text{m}$) and control ($19.90 \pm 3.2 \mu\text{m}$) groups. The neural differentiation capacity of the cell-loaded PCL fibers was improved by activating the integrin $\beta 1$ /GSK3 β / β -catenin pathway, and this was confirmed by motor (ChAT⁺) and sensory (CGRP⁺) neurons using Western blot assay. After seven weeks of transplanting the SSC-derived neurons, the H&E staining showed a decrease in the cavity area ($0.36 \pm 0.03 \text{ mm}^2$ compared to $1.61 \pm 0.22 \text{ mm}^2$ in the control group) and a reduction in muscle atrophy, along with an increase in the weight of the gastrocnemius muscle. Additionally, the IHC analysis revealed increased expression of Tuj-1 (301 ± 23 per visual field compared to 194 ± 17 in the control group), Syn, and MBP (60 ± 18 compared to 3 ± 1 in the control group) markers after 7 weeks. This indicates improved neural differentiation, synapse formation, and myelination in the lesion area. Furthermore, there was a reduction in the formation of glial scars (GFAP expression, 146 ± 15 per visual field vs. 483 ± 25 in the control group) after the transplantation of SSC-derived neurons. They assessed weekly functional recovery using electrophysiology (latency: 8.67 ± 0.24 vs. 13.26 ± 0.21 ms in the control group and amplitudes: 0.11 ± 0.03 mV vs. 1.80 ± 0.25 mV in the sham group), rump-height index assay, and BBB scoring over 7 weeks, which demonstrated significant improvements in motor function. Besides, axon tract-tracing using biotinylated dextran amine confirmed the re-establishment of neural circuits on the caudal side of the SCI site in the group treated with SSC-derived cells. They concluded that cell-loaded PCL fibers contributed to the development of new relay circuits, myelination, and synapse formation [178].

3D printing

The advancing technology of 3D printing has the potential to accurately replicate tissue with the help of a computer guide. 3D printing involves the deposition of biomaterial ink onto a solid or gel substrate. For SCI, combining 3D printing with 3D images allows researchers to customize the shape of the scaffold based on the individual anatomical characteristics of different SCI patients. In other words, it can create a personalized fabrication approach. Additionally, 3D printing provides guidance for axon regeneration, aiding in directing

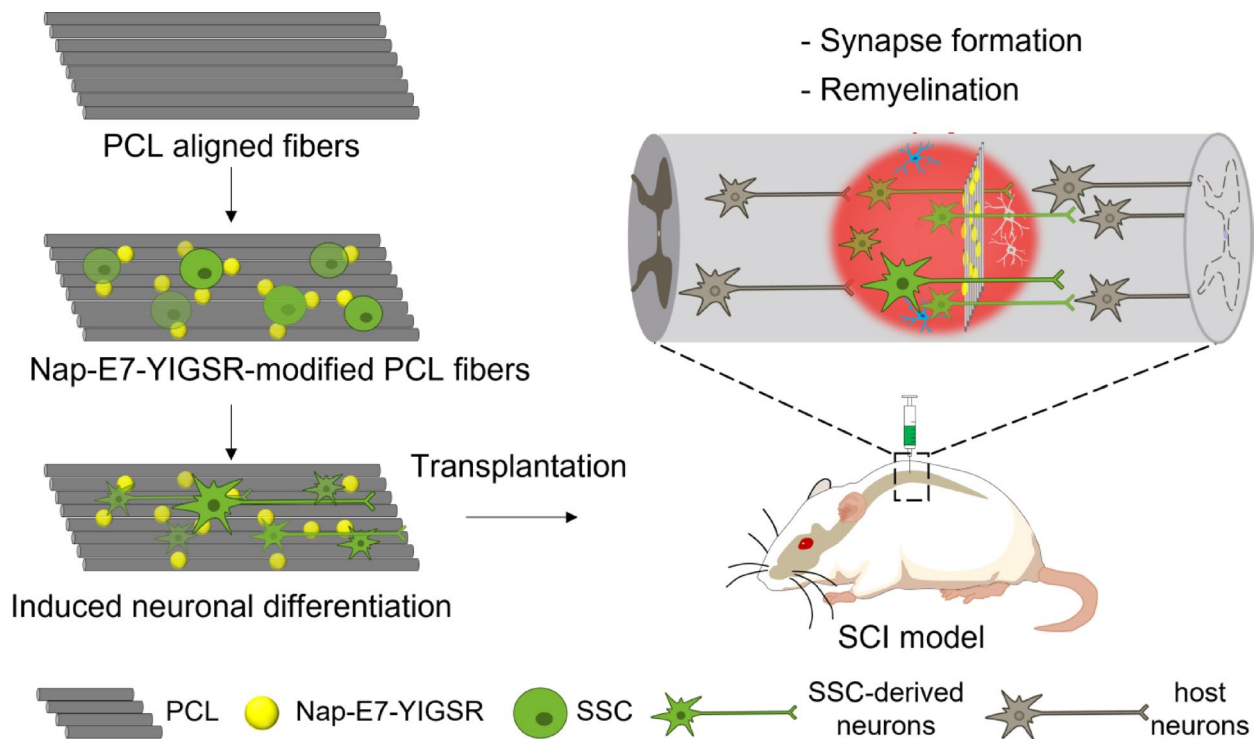


Fig. 20 Cell-loaded PCL fibers-mediated repair in a spinal cord injury model. Nap-E7-YIGSR enhanced cell adhesion to PCL fibers via its peptide sequence (EPLQLKM) and promoted differentiation into SSC neurons through the integrin $\beta 1$ /GSK3 β / β -catenin signaling pathway. Transplanting these induced neurons into SCI lesion sites in rats facilitated the formation of new relay circuits, myelination, and synapses, ultimately improving locomotion recovery. Reproduced with permission under a Creative Commons License from ref [138]. CC BY Attribution 4.0 International 4.0 DEED, Copyright 2022 the Authors, published by Ivyspring International Publisher

regrowth and reconstructing neural pathways within the spinal cord [179, 180] (Fig. 21).

In 2023, Yang et al. conducted a study using a 3D-printed hydrogel loaded with NSCs in a rat model of complete T9-T10 transection. They removed a 4 mm-long segment of the T9-T10 and immediately placed a printed scaffold in the area. The hydrogel, made from gelatin and methacrylic anhydride (GelMA) and hyaluronic acid and methacrylic anhydride (HAMA), served as a platform for delivering NSCs. The NSCs, which were derived from E14 rat embryos, were mixed with GelMA/HAMA ink and printed into the scaffold. The transplantation of the 3D-printed NSC-loaded hydrogel demonstrated strong anti-inflammatory effects by reducing the activation of CD68-positive macrophages/microglia and increasing M2 macrophage polarization (M2 ratio, $53.83 \pm 5.78\%$). This promoted a shift from a pro-inflammatory to an anti-inflammatory state one week after SCI. Such treatment resulted in a pattern of astrocytes that helped connect the host spinal cord and implants, while also preventing scar formation. Immunostaining showed increased regeneration of neurons (Tuj-1) and blood vessels (rat endothelial cell antigen-1) in the group that received both the scaffold and NSCs. Additionally, there was aligned growth of axons (GAP43) and reduced scar

formation compared to the SCI group. Confocal imaging revealed well-regenerated blood vessels around new neurons, suggesting improved support for sustained neuronal function. Functionally active neurons wrapped in myelin were found in higher numbers in the scaffold-NSCs group compared to the scaffold-only group and the SCI control group ($20,225 \pm 2747$ nerves/ mm^2 in scaffold-NSCs, 9092 ± 3228 nerves/ mm^2 in scaffold only, vs. 2918 ± 656 nerves/ mm^2 in SCI control). Additionally, there was evidence of synapse formation (Syn I, a presynaptic marker) and axonal projections (ChAT and tyrosine hydroxylase) across the lesion site, validating the effectiveness of the scaffold-NSCs in rebuilding neural circuits, as indicated by an Immunofluorescence assay conducted 12 weeks post SCI. After 12 weeks, analysis using toluidine blue staining, TEM imaging, and Immunofluorescence (MBP expression) showed that the 3D-printed NSC-loaded hydrogel group had improved myelinated nerve fibers. The MEP assessment, BBB score, and foot printing indicated improved coordination and movement. This integrated approach accelerates SCI repair, highlighting the importance of interactions between neural cells and scaffolds in achieving functional recovery [179].

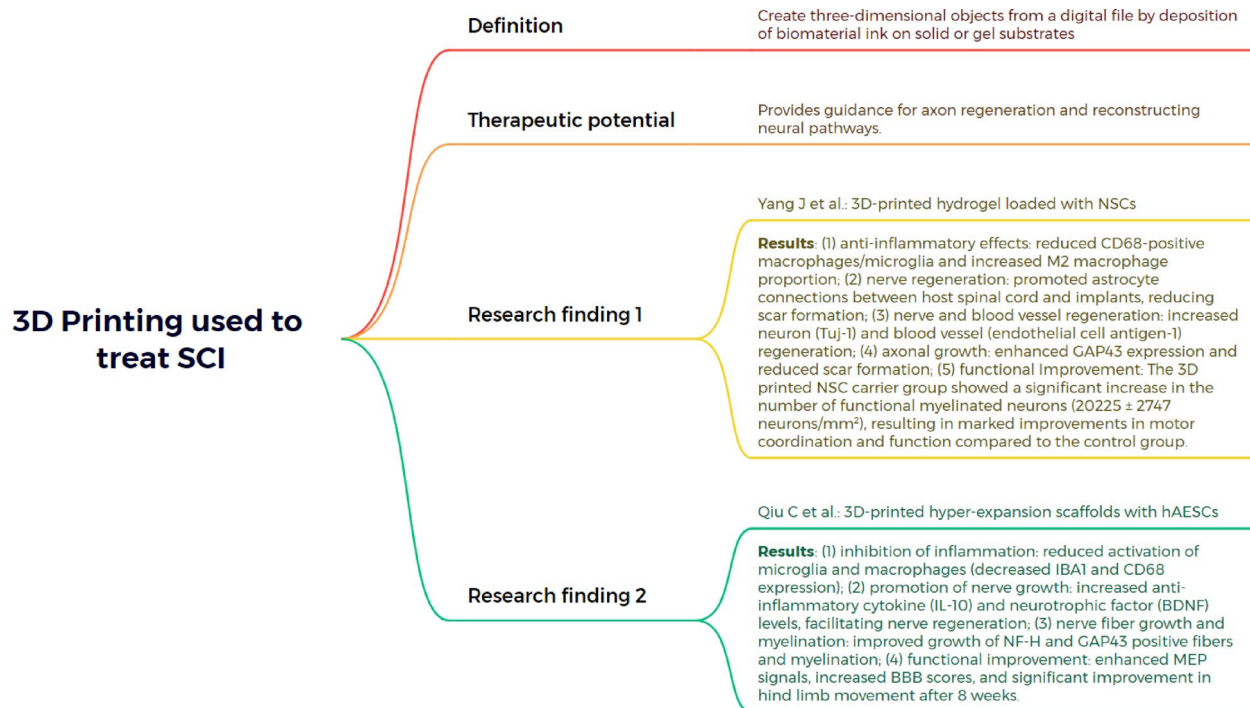


Fig. 21 Schematic representation of the therapeutic potential of 3D printing in SCI treatment

Qiu et al. developed dehydrated Gelatinmethacryloyl (GelMA) scaffolds, known as hyper-expansion scaffolds (HES), using 3D printing technology to facilitate high cell loading. The HES demonstrated a rapid expansion of 80-fold in size within 2 min, driven by macroscopic swelling forces and microscale capillary action, effectively accommodating 2.6 million human amniotic epithelial stem cells (hAESC). The transplantation of HES-hAESC (2×10^5 cells/200 μ l suspension) was performed immediately after a T10 complete transection in a rat model (Fig. 22). Immunofluorescence analysis at 2, 4, 6, 10, 14, and 28 days after SCI showed that treatment with HES-hAESC suppressed the activation of microglia and macrophages. This was indicated by reduced expression of IBA1 and CD68. Additionally, it resulted in decreased levels of the pro-inflammatory cytokines IL-6 and IL-1 β , and increased levels of the anti-inflammatory cytokine IL-10 and the neurotrophic factor BDNF. This promoted nerve growth, as confirmed by qPCR analysis 7 days after SCI. Those treated with HES-hAESC showed no signs of atrophy in the lesion sites. Notably, HES-hAESC treatment led to improved growth and myelination of nerve fibers (NF-H⁺ and GAP43⁺ fibers), which were assessed by IF assay and TEM. Moreover, there were improved MEP signals in terms of amplitude, frequency, and shortened latency. Additionally, there was a higher BBB score and significant improvements in hindlimb locomotion after 8 weeks. Essentially, HES-hAESC transplantation enhanced axonal extension, preserved myelination,

reduced neuroinflammation, and increased neurotrophic factor levels near the injury site [181].

Cell-derived extracellular therapeutics

The extracellular components produced by cells are known as paracrine molecules of stem cells, and they can replicate the regenerative abilities of these cells. These products have been used in preclinical trials to treat various tissue injuries by transferring their bioactive content to other cells and regulating their functions. Some examples of cell-derived extracellular components found in the conditioned medium of stem cells include exosomes and vesicles [182]. Exosomes are small membrane-bound vesicles, typically 30–100 nm in diameter, that contain various molecules such as mRNAs, miRNAs, and proteins. They play a crucial role in communication between cells by transferring information. This process is facilitated by their ability to attach to specific target cells using cell-surface ligands and then release their contents. This activity can modulate immune responses and promote angiogenesis [183] (Fig. 23). Recently, exosome products derived from hBMSC and hUC-MSC, such as CD271, CD56, and mir-138, have been studied for their potential in treating SCI (Fig. 24).

Sun et al. isolated CD271⁺CD56⁺ exosomes from hBMSC. They completely transected the spinal cord at T8 in C57BL/6 mice and removed 1 mm of the parenchyma. Then, they promptly infused 10 μ l of CD271⁺CD56⁺ BMSC-Exos suspension (100 μ g/ μ l) and hydrogel (20 mg/

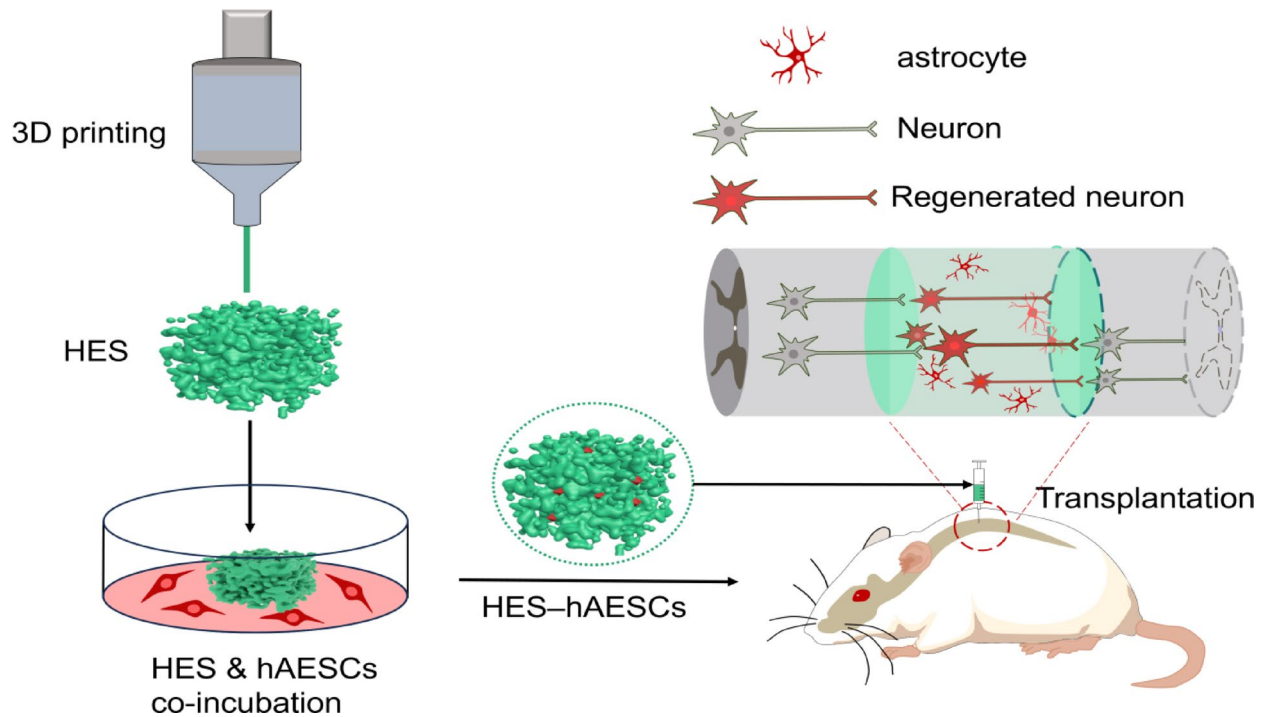


Fig. 22 Strategy proposal for using 3D printed hyper expansion scaffold (HES) as delivery platforms for hAESC in repairing spinal cord injuries

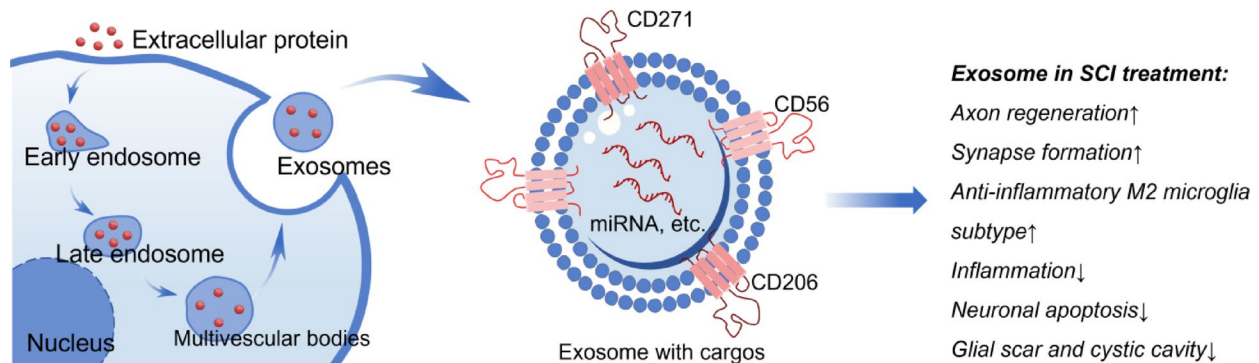


Fig. 23 Schematic representation of the therapeutic potential of exosomes in SCI treatment

mL). The uptake of exosomes by neurons was assessed using PKH26 and 1,1-octadecyl-3,3,3-tetramethylindole tricarboxyl cyanine iodide over 28 days post-injury. Additionally, they used dorsal root ganglion as an in vitro model and assessed axons by NF marker expression for immunofluorescence examination. Their findings indicated that the CD271⁺CD56⁺ BMSC-Exos group exhibited longer maximum extension distances, and a higher number of axon branches compared to the BMSC-Exos group, suggesting that CD271⁺CD56⁺ BMSC-Exos have a greater potential for promoting axon regeneration. Over the eight weeks following SCI, the expression of NF and Syn markers, which evaluate synapse formation of regenerated axons, increased in the group treated with CD271⁺CD56⁺BMSC-Exos hydrogel. The BMS score and MEP showed that this treatment improved motor

functions compared to the SCI group. Additionally, tissue continuity and cavity area were improved in the CD271⁺CD56⁺BMSC-Exos hydrogel group, leading to a reduction in cavity size as observed using H&E staining. Furthermore, electromyography analysis indicated that mice treated with CD271⁺CD56⁺BMSC-Exos hydrogel exhibited higher amplitude signals. They also studied the impact of the miR-431-3p/Repulsive Guidance Molecule Family Member A (RGMA) axis on axon regeneration and neural function. This involved implanting a hydrogel with miR-431-3p inhibitor-loaded CD271⁺CD56⁺ BMSC-Exos. RGMA is a glycosylphosphatidylinositol-linked glycoprotein that binds to its receptor and inhibits neurite growth. The study found that the expression of RGMA increased in the miR-431-3p inhibitor-loaded CD271⁺CD56⁺ BMSC-Exos group, indicating



Fig. 24 Sources of exosome and its functions in SCI treatment. Exosome generation involves a double invagination of the cell membrane, followed by the formation of early and late endosomes, multivesicular bodies, and the release of exosomes into the cytoplasmic environment. Markers such as CD271, CD56, and CD206 can be used to identify exosomes. In SCI treatment, exosomes primarily facilitate axonal regeneration, promote synapse formation, modulate immunity, and reduce glial scar formation and cavity development

the inhibitory role of miR-431-3p on RGMA expression and its potential to improve axon regeneration in the CD271⁺CD56⁺ BMSC-Exos hydrogel group [95].

Xiao et al. loaded miR-138-5p into exosomes derived from human UC-MSCs (hUC-MSCs). They then injected a hydrogel loaded with the miR-138-derived hUC-MSCs exosomes encapsulated in a polymer of polyglycolic acid copolymer and polyethylene glycol (PLGA-PEG-PLGA) into the T10 of rats with compression SCI (10⁹ particles/150 µl). They conducted an experiment using an LPS-induced inflammatory model on BV-2 microglia and found that miR-138-exosome derived from hUC-MSCs (Exos-138) decreased the levels of IL-1β, IL-18, and IL-6 mRNA through the NLRP3-caspase1 signaling pathway, as measured by qPCR analysis. They also observed a reduction in neuronal apoptosis through the Nrf2-keap1 signaling pathway, as determined by western blot analysis. Furthermore, they analyzed the shift from M1 to M2 microglia using an immunofluorescence assay to detect iNOS and CD206 markers. Exosomes, specifically Exos-138 treatment, resulted in a decrease in the level of iNOS and an increase in the level of CD206 compared to the LPS group. This reveals Exos-138's ability to shift microglial polarization towards the M2 phenotype in vitro. ELISA assay demonstrated a reduction in the levels of proinflammatory factors, including TNF-α, IL-1β, and IL-6, in the Exos-138 hydrogel treatment in

the lesion area after seven days. Over the 28 days post-SCI, functional recovery was assessed and the BBB score showed improvements in the Exos-138 hydrogel group. The footprint test also showed enhanced hindlimb locomotor activity, stride length, and width after Exos-138 hydrogel injection. Additionally, H&E and tar purple staining confirmed that the injection of Exos-138 hydrogel decreased the cavity size and increased the number of Nissl bodies. The expression of GFAP and NF200 by IF staining showed less glial scarring and more neurofilament intensity in the lesion site in the Exos-138 hydrogel group. Exos-138 could promote a more advantageous environment for tissue repair following SCI [184].

The findings underscore the significant role of cell-derived extra-cellular products in enhancing neuroprotection, fostering axon regeneration, and promoting synapse formation. This intriguing discovery has sparked further interest in the potential of these products in neural function recovery, inflammation reduction, and prevention of neural apoptosis in the subject animals.

Current limitations and future directions

Cell therapy for SCI holds great potential, but several limitations need to be addressed to improve its efficacy and safety. These limitations include:

1. Lack of standardized procedures: The absence of standardized protocols for cell preparation, delivery, and monitoring hinders comparisons between studies and the establishment of uniform criteria for patient selection and follow-up.
2. Heterogeneity of patient populations: The response to cell therapy may vary among different patient groups, such as those with acute versus chronic injuries or complete versus incomplete injuries. Understanding these variations and tailoring treatments accordingly is crucial [154].
3. Safety and ethical concerns for ESCs: The use of ESCs in SCI treatment raises ethical considerations due to the destruction of embryos. ESCs also carry a risk of tumor formation and teratoma development if they differentiate inappropriately [185]. Allogeneic-derived ESCs can trigger severe immune rejection in the host [186].
4. Challenges with NSCs: NSCs are primarily obtained from embryonic tissues or derived from iPSCs. These approaches have both ethical and low production efficiency issues, as well as some potential for tumorigenicity. Differentiating NSCs into the desired neuronal and glial lineages while avoiding glial scar formation and inflammatory microenvironments is also challenging.
5. Limited clinical data for cell therapy: Many data on cell therapy for SCI come from preclinical tests, and there is a need for more high-quality clinical data to validate these findings [90]. Animal studies usually are performed through standardized operation protocol and specific timings, which are inimitable in human clinical settings. The severity of injury, level of injury sites, and timing after SCI might influence the effects of cell therapy.
6. Unclear mechanism of action for MSCs: The therapeutic effects of MSCs on SCI recovery are not fully understood, and animal studies may not fully reflect the conditions in human clinical settings.
7. Safety concerns with iPSCs: Viral transfection methods for generating iPSCs raise safety concerns, and non-viral reprogramming methods have limitations in terms of poor reproducibility. Besides, epigenetic memory retention and the potential for tumorigenicity are also considerations with iPSCs [146].
8. Limitations of direct reprogramming and direct conversion: Direct reprogramming faces challenges such as low conversion efficiency, immature reprogramming procedures, limited initial cell numbers, and lack of precise differentiation methods. The converted cells may retain cellular age and be affected by transgene load and unfavorable epigenetic modifications [154].

Discussion and conclusion

SCI is a devastating condition with no established standard treatment at present. The injury begins with immediate structural damage, followed by a series of secondary injuries, hemorrhage, swelling, loss of myelin, and necrosis of axons and neurons. Treating SCI remains a significant challenge due to its complex nature. While there are numerous potential strategies, no single solution exists. Therefore, it is important to consider multiple steps at different stages of the acute, sub-acute, and chronic phases. For example, in most contusive SCIs, the first line of treatment is often a surgical procedure to address the primary cause and correct the anatomical structures of the vertebrae column and spinal cord [187]. During the acute phase, hypothermia has been shown to be one of the most effective neuroprotective treatments, with minimal risks if administered correctly. Long-term treatment often involves physical therapy and rehabilitation combined with functional electrical stimulation [34]. In recent years, various cell replacement therapies have emerged as efficient treatments, although optimizing the choice of therapeutic cell(s) and managing the hostile microenvironment, as well as the timing and method of cell implantation, are still areas of ongoing research. Nonetheless, cell transplantation shows promise as a therapeutic strategy, with well-designed translational and clinical studies reporting encouraging outcomes.

In recent clinical trials (phase I and II, but not phase III), two types of cells, namely bone marrow stromal cells (BMSCs) and OECs, have been primarily used in the treatment of moderate to severe contusive SCI during its chronic stages. In nearly half of the cases, the cells were delivered via surgical procedures directly into the spinal cord tissue, while in one-third of the cases, the cells were injected intrathecally, sub-meninge, and or directly into the spinal parenchyma surrounding the injury site. Similarly, support cells, like OECs and SCs, had a major role in improving the outcome of cell replacement therapies in nearly half of the SCI patients. These patients showed almost 40% improvement in their ASIA grades (beyond any possible endogenous recovery) within the first year post traumatic SCI. Timely transplantation of stem cells isolated from peripheral blood and neuronal stem cells showed significant effectiveness in patients' recovery during the early chronic stages. In recent years, and based on numerous clinical studies, it has been confirmed that personalized rehabilitation combined with cell treatment will undoubtedly contribute to neurological functional recoveries. The improvement was even more notable in case of personalized medicine when the rehabilitation was combined specifically with a customized FES [188].

The results from clinical trials involving more than 2400 patients showed that stem cell transplantation improves the ASIA score and urinary, sexual, and gastrointestinal

systems in almost half of the SCI patients. Although some side effects such as neuropathic pain, spasms, and urinary tract infections are inevitable, no severe complications such as tumor formation, which is a key concern in stem cell therapy, were a dominant factor among the patients, especially in recent years. Therefore, stem cell treatment's overall safety and effectiveness among patients with contusive SCI could be considered adequate and is encouraging [189].

It is important to note that when combined with stem cell treatment, biomaterials like hyaluronic acid, collagen, and hydrogels can enhance the effects of cell therapy by ameliorating the hostile post-SCI microenvironment. Scaffolds can provide structural support and serve as conduits for transplanted cells, aiding in their integration and promoting axonal survival, growth, and function [190]. Additionally, nanomaterials such as nanoparticles or nanofibers can be safely used to deliver therapeutic agents, enhance cellular uptake, and provide targeted drug delivery. These nanomaterial-based approaches have the potential to improve the effectiveness and specificity of cell therapy, creating new possibilities for promoting tissue regeneration, functional recovery, and enhancing the quality of life for SCI patients [191].

Additionally, advanced treatment approaches for SCI involve combining cell therapy with pharmacotherapy, physiotherapy, exercise, rehabilitation, electrical stimulation, and magnetic stimulation. The use of MSCs along with electroacupuncture has been found to effectively regulate microglia and astrocyte activation, leading to reduced inflammation and glial scarring. This combination also suppresses the ErbB1/STAT3 signaling pathway, promoting axonal regeneration after SCI [192]. Furthermore, the combined effects of stem cells and electroacupuncture have been shown to enhance axonal recovery and remyelination of the corticospinal tract [193]. Moreover, the combination of exercise and cell therapy has demonstrated significant outcomes, including reduced glial scarring and improved motor function through the PI3K/AKT/mTOR pathway [194].

Furthermore, the incorporation of broader contextual elements—including assistive devices (such as cervical collars [195] and exoskeletons [196]), adjunctive surgical interventions (for example, early tracheostomy, which has been associated with reduced duration of mechanical ventilation, shorter hospital and ICU stays, and a lower incidence of tracheostomy-related complications [197]), social determinants of health, and secondary complications like neurogenic lower urinary tract dysfunction (NLUTD)—is essential for the successful and equitable translation of cell therapies into clinical practice. The effectiveness of cell-based interventions for SCI is closely intertwined with socioeconomic and environmental factors, including economic stability, educational

attainment, healthcare accessibility, and social support, particularly in low- and middle-income settings, where these variables can significantly influence treatment availability, patient adherence, and long-term outcomes [198]. For instance, community-driven educational initiatives may enhance adherence to immunosuppressive protocols following transplantation, whereas policy measures aimed at subsidizing cell therapies could help overcome financial barriers in resource-limited regions. Additionally, men with SCI and NLUTD are at heightened risk for complications such as infections, incontinence, vesicoureteral reflux, nephrolithiasis, and renal failure. Cell-based therapies represent a promising paradigm shift by directly targeting neural repair mechanisms, with the potential to restore bladder innervation and function [199].

In conclusion, stem cells demonstrate both neurodegenerative and neuroprotective properties in the context of SCI transplantation with different targets and responses to stimuli. Overcoming limitations through standardized protocols, a better understanding of patient variations, ethical considerations, improved differentiation methods, and enhanced safety measures will help advance cell therapy for SCI. Combining cell therapy with other treatment modalities and conducting further clinical trials will help optimize outcomes. As research continues to uncover the molecular intricacies of cell regenerative properties, we are getting closer to realizing the full potential of biomaterials and cell therapy in transforming the lives of individuals with SCI, offering them hope for improved quality of life and recovery.

Abbreviations

5-HT	5-hydroxytryptamine receptor
β-CD	β-cyclodextrin
AD-MSCs	Adipose-derived MSCs
ADSCs	Adipose-derived stem cells
AF-MSCs	Amniotic fluid MSCs
AMSCs	Amniotic MSCs
BDNF	Brain-derived neurotrophic factor
bFGF	Basic fibroblast growth factor
BMS	Basso mouse scale
BMSCs	Bone marrow mesenchymal stem cells
BNSPCs	Brain-derived NSPCs
DBM	Decellularized brain matrix
DCX	Doublecortin
DV-SC	Embryonic spinal cord-like tissues with dorsal and ventral neuronal characteristics
ESCs	Embryonic stem cells
FGF	Fibroblast growth factor
GABA	Gamma-aminobutyric acid
GAP43	Growth-associated protein 43
GDNF	Glial cell line-derived neurotrophic factor
GFAP	Glial fibrillary acidic protein
H9-NSPCs	H9 embryonic stem-cell derived NSPCs
H&E	Hematoxylin and eosin staining
HA	Hyaluronic acid
i.v.	Intravenous
IHC	Immunohistochemistry
iMNs	Induced motor neurons
iMNP	IPSC-derived motor neuron progenitor cells

iNSCs	Induced NSCs
iPSCs	Induced pluripotent stem cells
IL-1Ra	Interleukin-1 receptor antagonists
IL-1 β	Interleukin-1 β
MBP	Myelin basic protein
MEP	Motor evoked potential
MSCs	Mesenchymal stem cells
NGF	Nerve growth factor
NPCs	Neural progenitor cells
NSCs	Neural stem cells
NSPCs	Neural stem progenitor cells
OECs	Olfactory ensheathing cells
OLs	Oligodendrocytes
OPCs	Oligodendrocyte progenitor cells
PDGF	Platelet-derived growth factor
PDRN	Polydeoxyribonucleotide
ROS	Reactive oxygen species
SCs	Schwann cells
SC-GDNF	Schwann cells overexpressing GDNF
SCI	Spinal cord injury
SCNSPCs	Spinal cord-derived NSPCs
SEM	Scanning electron microscope
SSEP	Somatosensory evoked potential
T	Thoracic
TEM	Transmission electron microscopy
TI-EVs	TNF- α /interferon- γ primed MSC-derived extracellular vesicles
TNF- α	Tumor necrosis factor- α
VEGF	Vascular endothelial growth factor

Author contributions

Angelo ALL (AA), Sara Haratizadeh (SH), and Haitao Liu (HL) contributed to the conception and writing of the original draft. Hengde Li (HLi), Mohsen Adeli (MA), and HL performed the review, editing, and literature search. HL and HLi developed all figures and graphics. MA and AA provided critical revisions, supervised the process, and finalized the manuscript. AA secured funding to support this article. All authors have read and agreed to the final version of the manuscript.

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Data availability

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors declare their approval and consent for the publication of this review article.

Competing interests

The authors declare that they have no competing interests.

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