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REVIEW

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# Human dental pulp stem cells for spinal cord injury

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**Abstract**

Spinal cord injury (SCI) is a serious neurological disorder that causes loss of mobility, pain, and autonomic dysfunction, resulting in altered sensation and devastating loss of function. Current treatments for SCI mainly focus on surgery and drug therapy to promote neurological recovery. However, there are virtually no effective remedies for irreversible nerve damage that result in a victim's loss of motor function and sensory changes that occur after an injury. With the continuous development of medical technology, stem-cell-based regenerative medicine provides researchers with new treatment ideas. The effectiveness of mesenchymal stem cells and their derivatives from different sources in treating SCI varies. Recent studies have highlighted that dental pulp stem cells (DPSCs) may contribute to anti-inflammatory regulation, anti-apoptotic regulation, and axonal regeneration in the treatment of SCI patients. In addition, the combination of new biomaterials and dental pulp stem cells is promising in the treatment of SCI. This article reviews the role of DPSCs in SCI treatment in recent years, discusses the advantages of DPSCs, explores potential development directions, and looks forward to providing new insights for future research in this critical field.

**Keywords** Spinal cord injury, Stem cells, Dental pulp stem cells, Inflammatory, Axonal regeneration, Apoptosis, Neurorestoration

## Introduction

SCI is classified as a devastating neurological disorder with permanent disability rates exceeding 60% in severe cases [1]. Global SCI incidence spans 10–83 cases per million annually, with developing nations showing 3-fold higher rates than industrialized countries [2, 3]. The current treatments for SCI include medication and surgery. Medications include glucocorticoids, neurotrophic factors, vitamins, trimethoprim hydrochloride [4–6]. Commonly used procedures include decompression surgery, fixation surgery, and nerve repair surgery [7, 8]. These approaches can protect neurons, reduce neuronal damage, and promote neurological recovery. However, they are unable to reverse severe, irreversible neuronal damage caused by SCI [9]. To address this limitation, regenerative medicine technologies—including stem cell therapy,

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tissue engineering, and other advanced strategies—have emerged as promising tools to repair injured tissues and organs [10].

Stem cells possess critical therapeutic properties—including self-renewal capacity, multipotent differentiation potential, paracrine signaling, and immunomodulatory functions—which enable them to promote regeneration and repair when transplanted into SCI lesion sites [11]. Over the past decade, cell-based therapies have emerged as novel treatment options for a wide range of neurological disorders, including Alzheimer's disease, Parkinson's disease, stroke, SCI, and peripheral nerve injury [12–14]. The advantages of dental pulp stem cells include ease of isolation, lack of ethical controversy, low immunogenicity, and low risk of graft rejection [15]. DPSCs are involved in nerve repair in SCI through various pathways, such as anti-inflammatory effects, promotion of axonal regeneration, reduction of apoptosis, and immunomodulation [16]. This review summarizes the advantages of DPSCs over other mesenchymal stem cells and the ways in which DPSCs promote nerve repair in SCI.

## Spinal cord injury

Traumatic spinal cord injury (TSCI) is a spinal cord injury caused by external trauma (such as an accident), and symptoms usually develop suddenly and are severe. Non-traumatic spinal cord injury (NTSCI) is caused by internal factors (such as degenerative spinal disease) and symptoms develop more slowly. Currently, SCI is mainly caused by traumatic factors, and this article deals with this type.

The pathological processes involved in the course of SCI, which lead to a series of secondary impairments culminating in partial or total loss of sensory and motor function, are complex [17]. During SCI, infiltrating inflammatory immune cells produce and release into the extracellular microenvironment excess reactive oxygen species (ROS), which induce oxidative stress, cellular excitotoxicity, and severe inflammatory responses that upregulate the levels of inflammatory cytokines, such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), resulting in neuronal cells, extensive apoptosis of neuroglia and oligodendrocytes [18]. Microglia are the main innate immune cells of the central nervous system (CNS) and play an important role in neuroinflammation [19]. Microglia are rapidly activated after SCI, and excessive microglia activation leads to the overproduction of pro-inflammatory factors (e.g., IL-1β, TNF-α) and ROS, which cause neuronal death and microglial apoptosis, further exacerbating the secondary injury [20, 21]. The inflammatory response also leads to the production of large amounts of free radicals by immune cells such as macrophages and neutrophils, as well as reactive oxygen radicals from ischemia-reperfusion injury and excitotoxicity, which damage the mitochondria of many types of spinal cord cells such as neurons, microglia, astrocytes, and trigger disorders of energy metabolism and apoptosis of the spinal cord cells, which aggravate the secondary injury [22].

The blood-spinal cord barrier (BSCB) is composed of endothelial cells interconnected via tight junction proteins (TJs), supported by interactions with astrocytes, pericytes, and perivascular microglia [23]. The BSCB is similar to the blood-brain barrier (BBB) and plays an important role in directing molecular exchanges between circulating blood and the blood and spinal cord to maintain CNS homeostasis [24]. In SCI, the lesion-induced hypoxic microenvironment reduces TJ protein expression, induces endothelial cell dysfunction, and promotes BSCB destruction, which leads to inflammation and ischemia, and likewise contributes to the progression of spinal cord neural tissue injury [25].

At this stage, the treatment for SCI includes surgery, medication, and rehabilitation. Surgical interventions aim to restore spinal cord anatomy, alleviate compression, and mitigate secondary injuries by removing fluid, blood clots, or decompressing affected areas. Pharmacological approaches utilize glucocorticoids, neurotrophic factors, and anti-edemic agents to improve blood flow, reduce inflammation, and protect neural function. While these conventional therapies partially alleviate damage, their efficacy remains limited, underscoring the critical need for novel treatment strategies [26].

The use of synthetic biomaterials and cells to construct functional tissues and organs has become a new research direction for stem cell-based regenerative medicine technology [27]. In the treatment of neurological diseases, tissue engineering can be used to repair damaged nerve tissues [28]. For example, implanting synthetic scaffolding materials into damaged neural tissues can provide support and guide the regeneration of nerve cells. Compared with traditional therapies, stem cell transplantation for SCI can repair damaged nerves, inhibit apoptosis, and promote nerve regeneration, making them more effective in repairing damaged nerves and restoring nerve function, thus improving patients' quality of life [29]. Chen et al. found through systematic evaluation and meta-analysis that stem cell transplantation for the treatment of spinal cord injuries produced better results than conventional therapies, including improvements in motor, sensory, and quality of life [30]. Over the past decade, dental pulp stem cells have been intensively researched as an autologous stem cell therapy in the field of regenerative medicine and tissue engineering and have shown amazing therapeutic capabilities in animal and human models for oral, neurological, corneal, cardiovascular, hepatic, diabetes, renal diseases, muscular dystrophy

and autoimmune diseases [31]. Therefore, many mesenchymal stem cells have become the subject of research, among which dental pulp stem cells have attracted much attention in stem cell therapy for SCI because of their nerve repair properties [32, 33].

## Dental pulp stem cells

The pulp is a loose connective tissue located in the root canal, surrounded by highly mineralized dentin, and consists of pulp stem cells, neuronal cells, intercellular matrix, and intercellular fluid, and contains an abundance of neural, vascular, and fibrous tissues, which have mainly formative, trophic, and sensory functions. Pulp stem cells are localized in the perivascular area of pulp tissue and are MSCs of oral and maxillofacial tissue origin. Stem cells can be isolated from a wide variety of oral tissues, such as craniofacial bone, dental pulp, periodontal ligament, dental sacs, dental embryos, root tips, oral mucosa, gingiva, and periosteum. The common method of isolation and culture of DPSCs used for the study was to clean the teeth with 70% ethanol after tooth extraction, extract the pulp using a sterile instrument, and place it in sterile 1X phosphate-buffered saline, and a solution containing trypsin-EDTA and penicillin-streptomycin. Then, single-cell suspensions were obtained by digestion with type I collagenase at 37 °C for 60 min. Next, researchers cultured cells in DPSCs medium containing Dulbecco's Modified Eagle Medium (DMEM) and MCDB-201 with various supplements, and the culture medium was changed every 4 days. When the cells reached 30% confluence, they were passaged using trypsin-EDTA-containing PBS, re-inoculated at a density of 100–150 cells/cm$^2$, and tested for mycoplasma contamination at the 5th and 10th passages to ensure culture purity [34, 35]. The dental compartment harbors multiple resident stem cell populations: DPSCs from permanent teeth, Dental Follicle Stem Cells (DFSCs) isolated from developing tooth germ connective tissue, Stem Cells from Apical Papilla (SCAPs) residing in immature tooth apices, Stem Cells from Human Exfoliated Deciduous teeth (SHEDs) obtained from deciduous tooth pulp, Periodontal Ligament Stem Cells (PDLSCs) from permanent tooth ligaments, and Gingival Mesenchymal Stem Cells (GMSCs) from gingival tissue. SHEDs demonstrate superior proliferative capacity and enhanced neurogenic differentiation potential compared to other dental stem cell types, positioning them as particularly viable candidates for neural regeneration therapies. While PDLSCs have to form periodontal structures; SCAPs are characterized by a high proliferation rate and are more inclined to undergo bone/odontogenic differentiation, making them a superior cell source for tissue regeneration, so they are more commonly used in various studies of SCI; DFPCs are highly differentiated in later stages, are related to stages, and are related to immune regulation; and GMSCs are less studied, but they have the characteristics of self-renewal and multiple or pluripotent [15, 36, 37](Table 1; Fig. 1).

Researchers have studied dental pulp stem cells for a long time, and their understanding of the properties of dental pulp stem cells has gradually increased. In 2000 and 2003, two dental pulp-derived mesenchymal stem cells (MSCs) were identified [38, 39]. DPSCs and SHEDs were successively isolated and identified, and both exhibited typical MSC characteristics, i.e., adherent growth, self-renewal, clonogenicity, and multidirectional differentiation potential, in vitro [40]. In 2011, researchers found that DPSCs could differentiate into other neural crest-derived tissues. DPSCs are expected to be a source of other neural crest-derived tissues [41]. In 2014, researchers provided new insights into the dynamics of tooth organogenesis and growth by combining clonal color-coding techniques with peripheral neuroglial tracking. A large number of mesenchymal stem cells is derived from peripheral nerve-associated neuroglial cells during tooth development, self-renewal, and repair [42]. Mouse incisors are accompanied by growth centered on vascular-neural bundles, and in growing mouse incisors, pulp stem cells are located around small arteries and supported by Gli1+-labeled neurovascular bundles; moreover, pulp stem cells show great advantages and potential for generating blood vessels and becoming nerves [43]. The efficacy of treatment with pulp stem cells

**Table 1** Sources and classification of DPSCs

| Types | DPSC | SHED | PDLSC | SCAP | DFPC | GMSCs |
|---|---|---|---|---|---|---|
| Location | Obstructed third molar and orthodontic teeth, and redundant teeth | Dental pulp of exfoliated deciduous tooth | Periodontal ligament | Apical papilla of an incompletely developed tooth | Connective tissue around the tooth | Gingiva around the tooth |
| Characteristic | • Easy to isolate<br>• Cryopreserve<br>• Potential form ultispectral differentiation | • High proliferation rate<br>• High neural differentiation potential<br>• Painless collection is less invasive | • Potential to form periodontal structures | • High proliferation rate<br>• More inclined to bone/odontogenic differentiation<br>• Superior cell source for tissue regeneration | • Highly differentiated in later stages<br>• Related to immune regulation | • Self-renewal and multi- or pluripotency |

**Abbreviations**: DPSCs, Dental pulp stem cells; DPSC, Permanent tooth pulp stem cells; SHED, Human exfoliated deciduous teeth; PDLSC, Periodontal ligament stem cells; SCAP, Stem cells from apical papilla; DFPC, Dental follicle precursor cells; GMSCs, Gingiva-derived mesenchymal stem cells

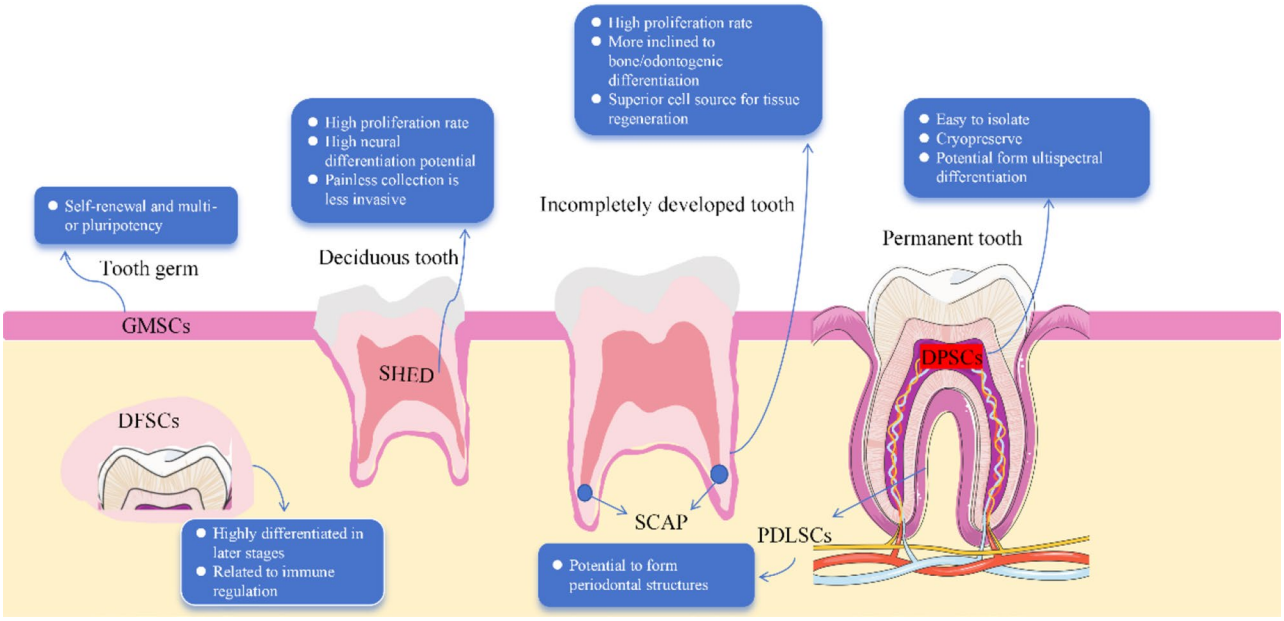


**Fig. 1** DFSCs are derived from the developing tooth germ and can be isolated from the connective tissue surrounding the tooth.SHED is derived from the pulp of deciduous teeth.SCAP is derived from the apical papilla of incompletely developed teeth. In permanent teeth, DPSCs and PDLSCs can be isolated from the pulp and periodontal ligament, and GMSCs are found in the gingiva surrounding the tooth. Abbreviations: DPSCs, Dental pulp stem cells; DPSC, Permanent tooth pulp stem cells; SHED, Human exfoliated deciduous teeth; PDLSC, Periodontal ligament stem cells; SCAP, Stem cells from apical papilla; DFPC, Dental follicle precursor cells; GMSCs, Gingiva-derived mesenchymal stem cells

from different sources also varies; the expression level of CD146 (CD146 is a membrane protein that is a surface marker for a variety of stem cells) is positively correlated with the proliferation, differentiation, and immunomodulatory capacity of dental pulp stem cells, and CD146 can be used as a key functional marker to assess the efficacy of treatment with dental pulp stem cells [44].

DPSCs, first isolated from adult human third molars by Gronthos et al. [38]are a unique population of mesenchymal stem cells (MSCs) originating from the embryonic neural crest. This origin endows them with distinct neurotrophic properties and a predisposition toward neuronal differentiation. Unlike bone marrow-derived MSCs (BM-MSCs), DPSCs express a combination of MSC surface markers (e.g., STRO-1, CD73, CD90, CD105) while lacking hematopoietic markers (CD34, CD45), confirming their mesenchymal lineage [40]. Notably, DPSCs exhibit higher proliferative rates and clonogenic potential compared to BM-MSCs, attributed to their telomerase activity and expression of pluripotency factors like OCT4 and NANOG [45]. Their multipotency extends beyond trilineage differentiation (osteogenic, adipogenic, chondrogenic) to include neurogenic differentiation. DPSCs secrete neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), which promote neurite outgrowth and neuronal survival in vitro [46, 47]. Additionally, their immunomodulatory properties—mediated via prostaglandin E2 and TGF-β secretion—enable them to suppress T-cell proliferation, making them promising for allogeneic applications [47]. Clinically, DPSCs demonstrate regenerative potential in preclinical models of SCI, peripheral nerve repair, and neurodegenerative diseases, partly due to their ability to migrate to injury sites and integrate into host tissues [48]. Their minimally invasive isolation from extracted teeth and lack of ethical controversies further enhance their translational appeal compared to embryonic stem cells.

## Mechanisms of DPSCs in the treatment of SCI

Given the superiority of dental pulp stem cells over other stem cells, they have been used in the study of many diseases, such as Alzheimer's disease, tooth repair and regeneration, optic nerve damage, bone tissue regeneration, and SCI repair [14, 49–51]. Next, we will specifically present the research progress on the mechanism of dental pulp stem cells in the treatment of SCI (Fig. 2).

### Anti-inflammatory effects

Near the site of injury, SCI is a traumatic disorder of the central nervous system, resulting in cellular responses such as inflammation and apoptosis due to an augmented release of biomolecules like interleukin (IL-6)and tumor necrosis factor (TNF-α) [52, 53]. DPSCs, capable of distinguishing morphogenetic cells from osteoblasts, chondrocytes, adipocytes, endothelial cells, and neurons, also have the ability to control a broad array of immune cells such as T- and B-lymphocytes, dendritic cells(DCs)and natural killer(NK)cells - these cells inhibit

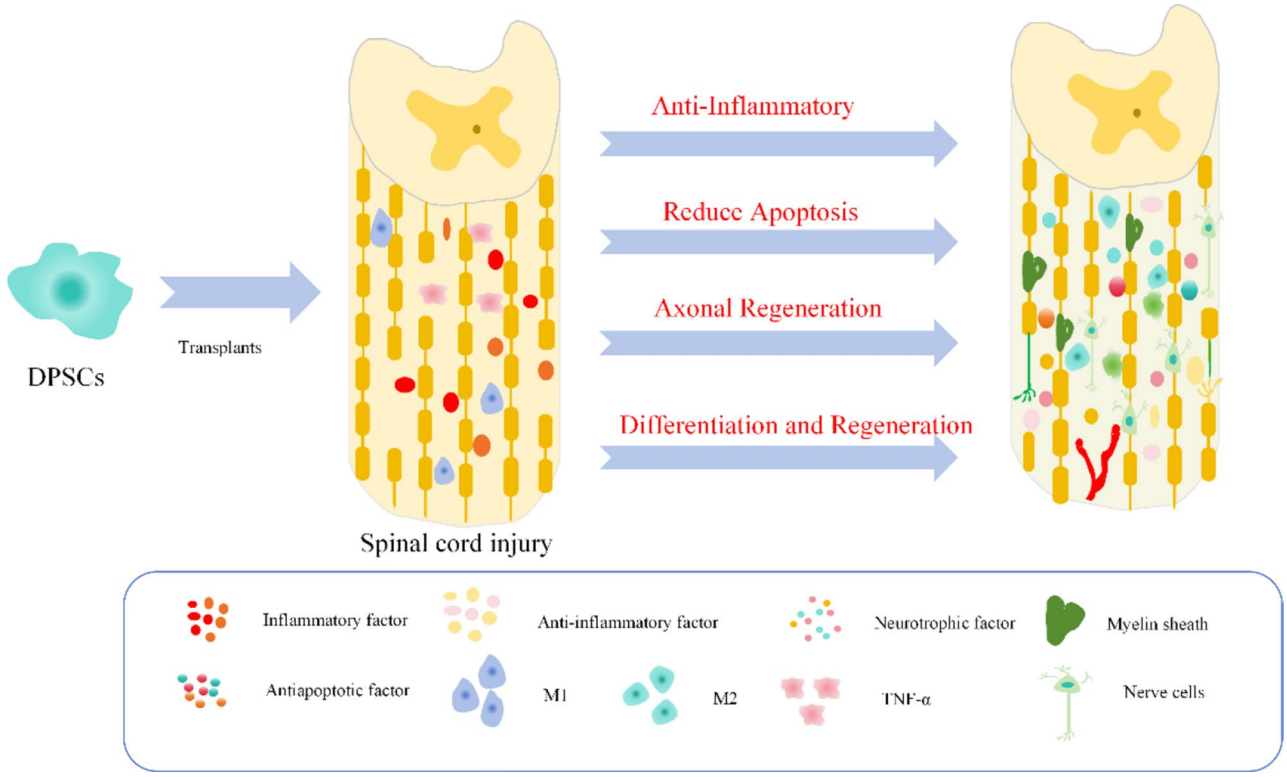


**Fig. 2** DPSCs transplantation treats SCI through four main pathways, anti-inflammatory, axonal regeneration, reduction of apoptosis, differentiation and regeneration. Abbreviations: DPSCs, dental pulp stem cells; SCI, spinal cord injury

proinflammatory processes while stimulating anti-inflammatory mechanisms [54, 55]. M1 macrophages digest debris and participate in inflammation, whereas M2 macrophages play a role in reducing inflammation. M1 macrophages contribute to inflammation through the release of proinflammatory cytokines and nitric oxide. Additionally, M1 macrophages are crucial in recruiting and activating astrocytes. In contrast, M2 macrophages induce tissue repair by releasing anti-inflammatory cytokines [56, 57]. Qiao and Prateeksha reported that DPSC-derived exosomes promote proliferation, migration, and osteogenesis of PDLSCs in vitro while exerting anti-inflammatory effects through inhibition of the IL-6/JAK2/STAT3 signaling pathway and promoting macrophage polarisation from the M1 phenotype to the M2 phenotype, thereby modulating the immune microenvironment, which reflects the anti-inflammatory effects of DPSCs [58, 59].

Matsubara's SCI Treatment Research revealed a new set of M2 inducers, namely monocyte chemoattractant protein-1(MCP-1)and sialic acid-binding Ig-like lectin-9(ED-Siglec-9) when SHEDs were intrathecally injected into the injured spinal cord of rats. These substances induced macrophages to adopt the M2 phenotype, as well as enhanced anti-inflammatory effects and suppressed proinflammatory responses [60]. Albashari et al. investigated the effect of DPSCs on SCI and reported that DPSCs express IL-6, IL-8, and TGFβ via Toll-like receptors (TLRs) during the neuroinflammatory phase of CNS disease treatment. In SCI, IL-8 expression in DPSCs is increased due to TLR4 expression, which is associated with the maintenance of neuronal cell integrity and reduced lesions. T cells were encouraged to release inflammatory factors by DPSCs, such as intracellular adhesion molecule-1, vascular adhesion molecule-1, human leukocyte antigen-G, intracellular adhesion molecule-1, IL-10 and hepatocyte growth factor(HGF), while suppressing proinflammatory elements like IL-6, IL-7, IL-17 A, IL-12 and TNF-α [61, 62]. Therefore, pulp stem cells have strong anti-inflammatory effects and can inhibit the progression of inflammation after SCI, attenuate nerve damage, and promote regeneration of injured central nervous system tissues(Fig. 3; Table 2).

### Axonal regeneration

A century ago, Ramón y Cajal, often hailed as one of the pioneers of contemporary neuroscience, declared that neurons in the mature central nervous system (CNS) were incapable of regenerating. Yet, in more recent times, there has been a significant advancement in comprehending the molecular mechanisms that govern axonal regeneration following injury to the CNS. It is believed that nerve repair and axonal regeneration can be facilitated by stem cell regeneration therapy after nervous system

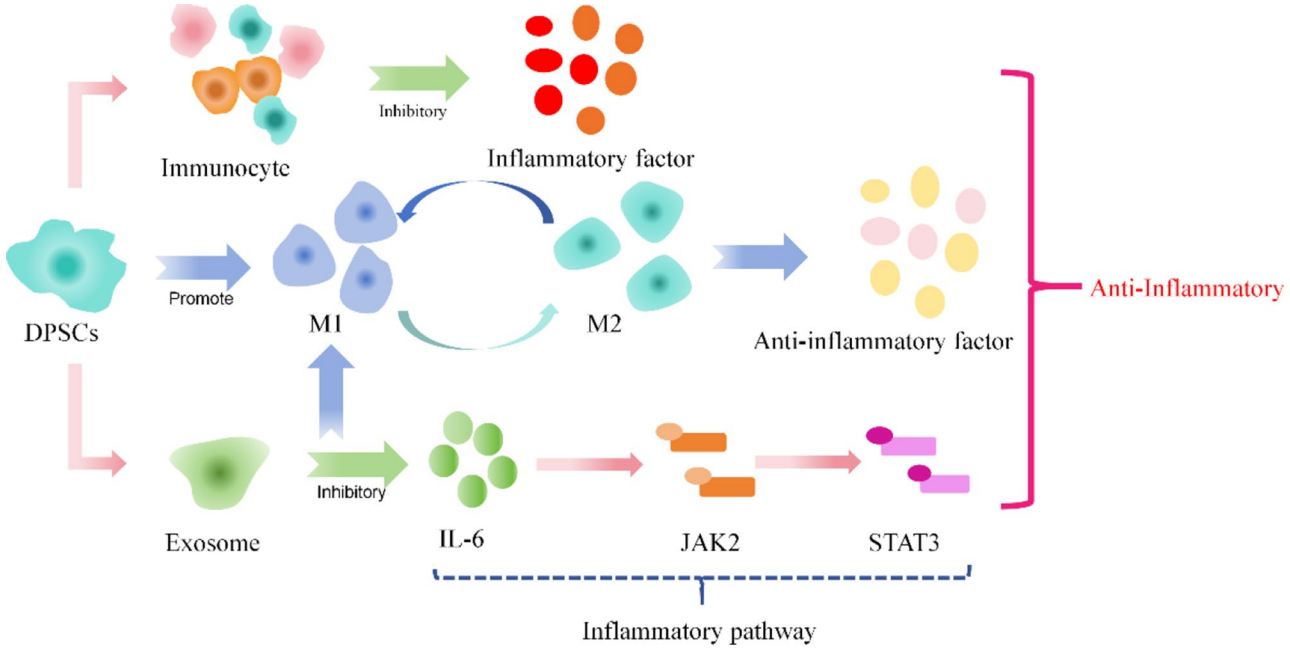


**Fig. 3** In addition to secreting exosomes to inhibit the IL-6/JAK2/STAT3 inflammatory pathway to attenuate the inflammatory response, DPSCs also inhibit the release of inflammatory factors by regulating immune cells to promote the conversion of M1 to M2 (a process that is reversible and regulated by a variety of cytokines and environmental signals), and promote the release of anti-inflammatory factors, thereby attenuating the inflammatory response in SCI. Abbreviations: DPSCs, dental pulp stem cells; SCI, spinal cord injury; M1, macrophages1; M2, macrophages2; IL-6, interleukin-6; JAK2, tyrosine protein kinase 2; STAT3, signal transducer and activator of transcript 3

injury [63, 64]. SCI typically results in the loss of neurons and glial cells, as well as limited axonal regeneration after injury, leading to ongoing dysfunction [16]. Within the vicinity of SCI, reactive astrocytes and oligodendrocytes generate chondroitin sulfate proteoglycans (CSPGs), and various myelin proteins like myelin-associated glycoprotein (MAG), Nogo, oligodendrocyte myelin glycoprotein (OMgp), netrin, semaphorin, and ephrin. These external compounds function as inhibitors of axon growth (AGIs) by activating the Rho GTPase signaling pathway intracellularly [65]. Sakai et al. transplanted SHEDs and DPSCs into mice via spinal cord transection and reported that SHEDS promoted axonal regeneration by inhibiting SCI-transection-induced Rho GTPase activity. SHEDs and DPSCs also promoted transected axon regeneration by directly inhibiting a variety of AGI signals through a paracrine mechanism. Moreover, SHEDs exerted a protective effect against myelin degeneration, and Sakai reported that the total number of TUNEL-positive cells was significantly reduced after the transplantation of SHEDs, suggesting that SHEDs exerted a strong neuroprotective effect on all neuronal cell lineages, thereby minimizing the amplification of damage secondary to SCI [66].

In a rat model of complete spinal cord transection, Yang et al. reported that DPSCs, especially DFSCs, attenuated the inflammatory response by suppressing the expression of interleukin-1β, promoted neuronal regeneration by suppressing the expression of ras homologous gene family member A (RhoA), and attenuated progressive hemorrhagic necrosis by suppressing the expression of sulfonylurea receptor 1 (SUR1); additionally, some of the grafted cells survived and differentiated into mature neurons and oligodendrocytes rather than astrocytes, which promoted axonal outgrowth [64]. In addition, Nagashima et al. reported that surviving DPSCs increased the activity of neurotrophic factors at SCI lesion sites, thereby promoting axonal regeneration and motor function recovery [67]. Moreover, DPSCs can induce macrophage transformation from M1 to M2, promote the secretion of various neurotrophic factors through the PKA-CREB pathway, and ultimately promote axon regeneration [68]. In summary, we can conclude that DPSCs can promote nerve repair and axonal regeneration after nerve injury secondary to SCI through the above pathways(Fig. 4; Table 3).

### Reducing apoptosis

Approximately 90% of neurons at the site of injury is killed within the first 8 h after SCI, mainly as a result of apoptosis [69]. Traditionally, studies on neuronal and supporting cell death after SCI have focused on necrotic pathways resulting from actual mechanical tissue damage and passive inflammatory processes. However, programmed apoptosis, an actively mediated cell death process, may occur after acute SCI [70]. Glutamatergic

**Table 2** Anti-inflammatory mechanisms of DPSCs in the treatment of SCI

| Year | Team | Animal Model | Cells Concentration | Machine | Reference |
|---|---|---|---|---|---|
| 2015 | Matsubara et al. | Contusive SCI model | 2.5 × 10^5 DPSCs; intralesional injection | Increased anti-inflammatory effect of inducing M1 to M2 conversion after inhibition of DPSCs. | [60] |
| 2023 | Qiao et al. | Rat periodontitis model | \ | | [58] |
| 2016 | Bianco et al. | Contusive SCI model | \ | DPSCs reduce the inflammatory response by modulating immune cells to reduce the release of inflammatory factors. | [62] |
| 2020 | Albashari et al. | Contusive SCI model | $2 \times 10^6$ DPSCs; fibrin scaffold implantation | | [63] |
| 2023 | Qiao et al. | Rat periodontitis model | \ | DPSCs exosomes regulate inflammation by inhibiting the IL-6/JAK2/STAT3 signaling pathway. | [58] |

**Abbreviations**: DPSCs, Dental pulp stem cells; SCI, Spinal Cord Injury; M1, Macrophages1; M2, Macrophages2; IL-6, Interleukin-6; JAK2, Tyrosine protein kinase 2; STAT3, Signal transducer and activator of transcript 3

**Table 3** Axonal regeneration mechanisms of DPSCs in the treatment of SCI

| Year | Team | Animal Model | Cells Concentration | Machine | Reference |
|---|---|---|---|---|---|
| 2012 | Sakai et al. | Complete SCI model in rats | $1 \times 10^6$ DPSCs; intralesional injection | Direct inhibition of multiple AGIs signals through a paracrine mechanism promotes axonal regeneration. | [66] |
| 2017 | Yang et al. | Complete SCI model in rats | $1 \times 10^6$ DPSCs; local implantation | Suppression of RhoA expression and promotion of axonal regeneration through inhibition of IL-1β expression. | [64] |
| 2017 | Nagashima et al. | Complete SCI model in rats | $1 \times 10^6$ DPSCs; local implantation | Promotes the secretion of various neurotrophic factors through the PKA-CREB pathway, thereby promoting axonal regeneration. | [67] |
| 2020 | Zhang et al. | Complete SCI model in rats | $5 \times 10^5$ DPSCs; intravenous injection | | [68] |

**Abbreviations**: DPSCs, Dental pulp stem cells; SCI, Spinal Cord Injury; AGIs, Axon growth inhibitors; RhoA, Ras homologous gene family member A; IL-1β, Interleukin-1β; PKA, Protein kinase A; CREB, cAMP-Response element binding protein

excitotoxicity leads to calcium influx and subsequent calmodulin-mediated activation of neuronal nitric oxide

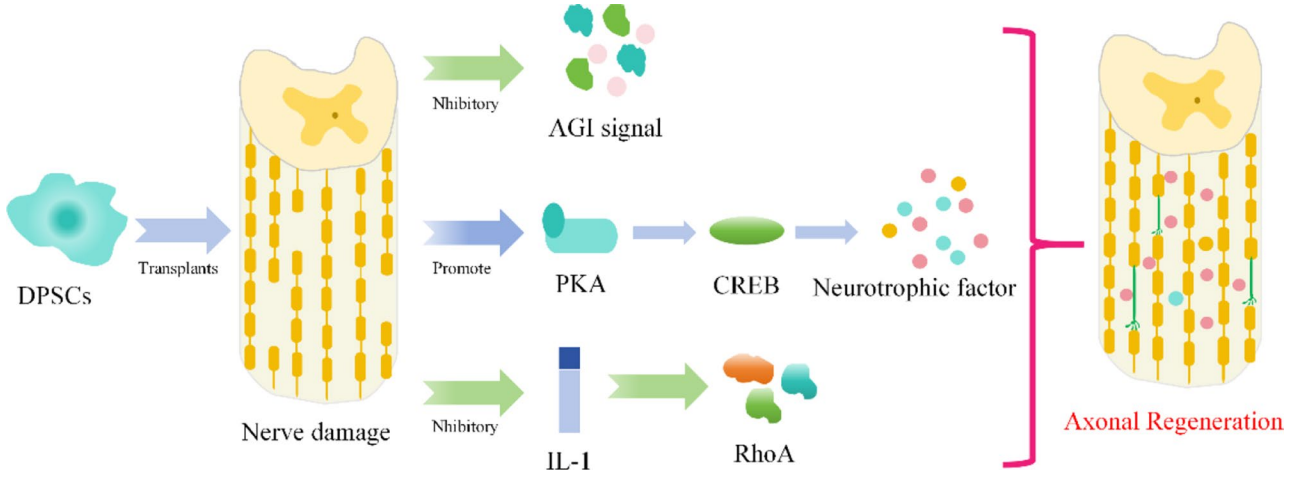


**Fig. 4** DPSCs transplanted into the injured spinal cord can reduce nerve injury and axonal regeneration by inhibiting AGE signaling, promoting the release of neurotrophic factors through the PKA/CREB pathway, and inhibiting the increase in IL-1/RhoA. Abbreviations: DPSCs, dental pulp stem cells; SCI, spinal cord injury; AGIs, axon growth inhibitors; RhoA, Ras homologous gene family member A; IL-1β, interleukin-1β; PKA, protein kinase A; CREB, cAMP-response element binding protein

synthase (nNOS); activation of nNOS may be involved in the process of mitochondrial membrane damage, which in turn activates mechanisms associated with apoptosis [71]. All these effects lead to abnormal apoptosis of cells after SCI.

To verify whether cell therapy using DPSCs has an anti-apoptotic effect on SCI, Nicola et al. randomly divided the rats into four groups, the control group (naive) and surgical control group were both given SHEDs; sham group(no SCI after laminectomy); SCI group(SCI after laminectomy, medication administered); and SHED group(SCI treated with intravertebral transplantation of $3 \times 10^5$ SHEDs at 1 h post-SCI). Functional assessments and morphological analyses were performed to confirm the effects of SCI and the benefits of SHED transplantation on behavior, tissue protection, and motor neuron survival. Six hours after injury, flow cytometry was performed on double-labeled samples containing MAP-2 antibody and cleaved cysteine 3 antibody to quantify apoptotic neurons. SHED transplantation was found to reduce early neuronal apoptosis in rats with SCI compared to that in controls. At 72 h, the TNF-α-treated group exhibited a significantly lower expression than the control group. This enabled transplanted SHEDs to diminish the rise in TNF-α levels after SCI and decrease the release of inflammatory factors, thus diminishing programmed cell death activation, augmenting anti-apoptotic factor expression, and averting early neuronal apoptosis in the spinal cord following contusion injury [50]. To verify whether cell therapy using DPSCs has an anti-apoptotic effect in SCI, Nicola et al. found that at 6 h after injury, the levels of excitatory amino acid transporter 3 (EAAT) were increased in both SCI and SHED groups. EAAT3 expression was significantly lower in the SHED group compared with the SCI group, as was nNOS. EAAT and nNOS expression levels were positively correlated. This suggests that SHED transplantation can reduce the activation of apoptotic mechanisms associated with nNOS, thereby reducing apoptosis. Of note, flow cytometric analysis of double-labeled samples with MAP-2 and cleaved caspase-3 (CC3) antibodies was performed at 6 h after injury. Although SHED transplantation was associated with a decrease in CC3-positive neurons compared with the control group, it is important to recognize that caspase-3 activation in CNS injury may not fully reflect apoptosis [72, 73].

Cellular pyroptosis is a mode of programmed cell death. Liu et al. established a rat model of SCI based on shock injury with weight-drop injury model, and then injected conditioned medium from human DPSCs were injected intraperitoneally into the rats. SCI rats' recovery of sensory and motor functions was greatly aided by conditioned medium (CM), which also decreased the expression of NLRP3, GSDMD, caspase-1, and interleukin-1β microglial focal death markers; stimulated axon regeneration and myelin sheath regeneration; and inhibited neuroglial scarring. Furthermore, in a lipopolysaccharide-induced BV2 microglial model, CM from human DPSCs protected cells from scorched death by inhibiting the NLRP3/caspase-1/interleukin-1β pathway. Conditioned medium from human DPSCs similarly reduces microglial cell pyroptosis by inhibiting the NLRP3/caspase-1/interleukin-1β pathway, which promotes the recovery of neurological function after SCI. All of the above experiments demonstrated that DPSCs can affect the apoptotic pathway after SCI in many of the ways described above, reducing the death of a variety of cells, including SCI cells and secondary neuronal cells [74] (Fig. 5; Table 4).

#### Differentiation and regeneration

Cell-based transplantation strategies have great potential for injured spinal cord repair. Due to their simple isolation process, ability to differentiate into various cell types, minimal risk of immune reaction, and low likelihood of rejection post-transplantation, DPSCs are commonly employed as primary cells in tissue engineering and regenerative medicine [75]. Transplantation of DPSCs promotes regeneration of neurons and vasculature [76, 77]. As early as 2010, Wang et al. discovered that SHEDs could differentiate into cell populations of neurons when cultured under neural-inducing conditions [78]. Zhu et al. transplanted DPSCs into a rat model of SCI, and control experiments revealed that DPSCs also differentiated into CD13 + pericytes. Rats were necropsied at 14 and 30 days after the transplantation of DPSCs for Western blot, histology, and immunofluorescence staining. N-calmodulin expression was significantly greater in basic fibroblast growth factor (bFGF) cells in the bFGF-DPSC group, and N-calmodulin and bFGF cells were located in the perivascular area. All the analyses suggested that N-calmodulin perivascular cells differentiated from grafted DPSCs may be critical for alleviating hypoxia at the SCI site. Immunofluorescence staining revealed that cells expressing N-calmodulin partially colocalized with CD13 pericytes but not with CD31 endothelial cells, suggesting that N-calmodulin is secreted by CD13 pericytes. DPSCs can differentiate into CD13 + pericytes to compensate for the massive death of CD13 + pericytes after SCI. NeuN and GAP43 were used to label regenerating neurons and growing axons. The results showed that the number of neurons and axons in the bFGF-DPSCs group was significantly higher than that in the SCI group and the CON-DPSCs group, suggesting that AAV-5HRE-bFGF-DPSCs promoted neuronal survival and axon regeneration, indicating that bFGF-DPSCs not only differentiated into pericytes but also into neurons. It can be inferred, based on the above experiments, that DPSCs can differentiate

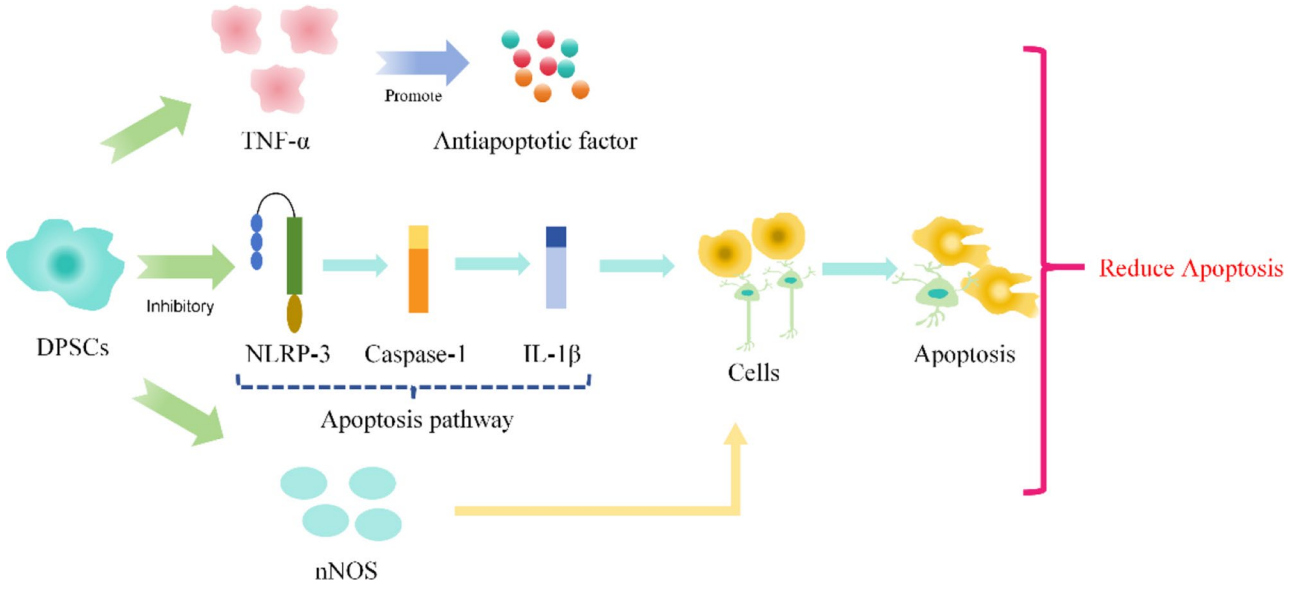


**Fig. 5** DPSCs can increase the level of antiapoptotic factors by inhibiting the release of TNF-α, reduce the activation of nNOS-related apoptotic mechanisms, thereby reducing apoptosis, and protect cells from apoptosis by inhibiting the NLRP3/caspase-1/IL-1β apoptotic pathway. Abbreviations: DPSCs, dental pulp stem cells; SCI, spinal cord injury; TNF-α, tumor necrosis factor; nNOS, neuronal nitric oxide synthase; NLRP3, inflammatory vesicle sensor protein; IL-1β, interleukin-1β

**Table 4** Reduce apoptosis mechanisms of DPSCs in the treatment of SCI

| Year | Team | Animal Model | Cells Concentration | Machine | Reference |
|---|---|---|---|---|---|
| 2017 | Nicola et al. | Contusive SCI model | $5\times10^5$ DPSCs; intravenous injection | Inhibits the release of TNF-α and increases the level of anti-apoptotic factors. | [73] |
| 2021 | Zhu et al. | Contusive SCI model | $1\times10^6$ DPSCs; intravenous injection | | [50] |
| 2017 | Nicola et al. | Contusive SCI model | $2\times10^6$ DPSCs; intra-arterial injection | Reduced activation of apoptotic mechanisms associated with nNOS, thereby reducing apoptosis. | [73] |
| 2024 | Liu et al. | Rat SCI model of impact injury | $1\times10^6$ DPSCs; intralesional injection + exosomes (20 μg/day) | Protects cells from apoptosis by inhibiting the NLRP3/Caspase-1/IL-1β apoptotic pathway. | [74] |

**Abbreviations**: DPSCs, Dental pulp stem cells; SCI, Spinal Cord Injury; TNF-α, Tumor necrosis factor; nNOS, Neuronal nitric oxide synthase; NLRP3, Inflammatory vesicle sensor protein; IL-1β, Interleukin-1β

into CD13 pericytes, secrete bFGF, augment N-calmodulin expression, facilitate adhesion of CD13 pericytes to vascular endothelial cells, and foster vascular restoration and construction - thus enhancing the hypoxic microenvironment caused by SCI. Additionally, DPSCs can also differentiate into neurons, which promotes the overall recovery after SCI [50](Fig. 6; Table 5).

## Preclinical studies of DPSCs in the treatment of SCI

There is a very large amount of DPSC research involving many diseases, including SCI, Alzheimer's disease, dental repair and regeneration, radiation-induced esophageal injury, retinal nerve repair, and stress urinary incontinence [79–84]. The following preclinical studies of DPSCs in the treatment of SCI in recent years were retrieved from PubMed (Table 6).

DPSCs have the potential for neural differentiation and play an immunomodulatory role in the microenvironment, making them an ideal cell source for the repair of CNS injury, including SCI. As early as 2012, Sakai et al. created a SCI model using rats, then implanted human dental pulp stem cells (DPSCs) into the model and contrasted it with a model implanted with human bone marrow stromal cells or skin-derived fibroblasts, discovering that the improvement in motor function was significantly lower in the latter group compared to the former. Moreover, they found that human DPSCs promoted nerve regeneration after SCI injury mainly through three pathways. Initially, they prevented the apoptosis of neurons, astrocytes, and oligodendrocytes following SCI, leading to enhanced maintenance of neuronal filaments and myelin. Subsequently, they blocked various axon growth inhibitory factors like chondroitin sulfate proteoglycans and myelin-associated glycoproteins through paracrine signaling, which facilitated the regrowth of severed axons. Under the most intense SCI conditions, dental-derived stem cells have been found to be capable of replacing lost cells by differentiating into mature

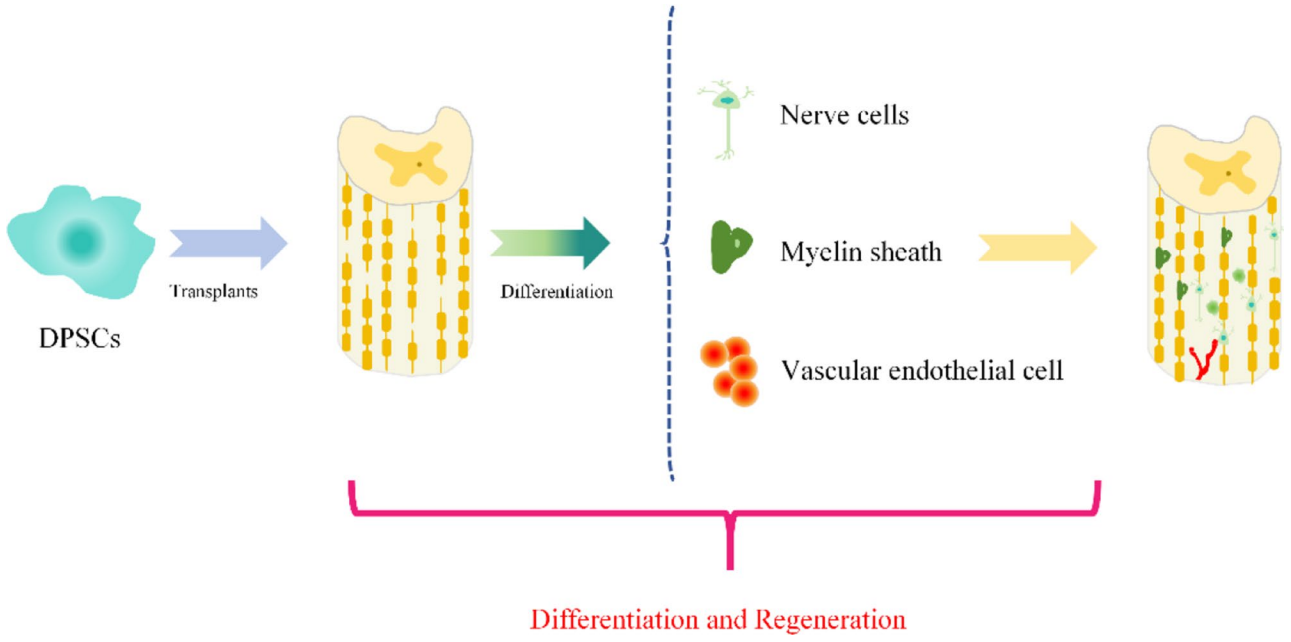


**Fig. 6** DPSCs differentiate into neurogenic, myelinating, and vasculogenic cells and proliferate to compensate for the loss of cells. Abbreviations: DPSCs, dental pulp stem cells; SCI, spinal cord injury

**Table 5** Differentiation and regeneration mechanisms of DPSCs in the treatment of SCI

| Year | Team | Animal Model | Cells Concentration | Machine | Reference |
|---|---|---|---|---|---|
| 2010 | Wang et al. | Parkinsonian rat model | $2\times10^6$ DPSCs; intrathecal injection | DPSCs differentiate towards neurogenic, myelinating, and vasculogenic cells, and proliferate to compensate for the loss of cells. | [78] |
| 2016 | Majumdar et al. | \ | \ | | [76] |
| 2019 | Zhu et al. | SCID mice model | $5\times10^5$ DPSCs; direct implantation | | [77] |
| 2021 | Zhu et al. | Contusive SCI model | $1\times10^6$ DPSCs + bioactive scaffold | | [50] |

Abbreviations: DPSCs, Dental pulp stem cells; SCI, Spinal Cord Injury; SCID, severe combined immunodeficient

oligodendrocytes. This suggests that these cells may offer a beneficial treatment for SCI through their capacity to autonomously and paracrinely regenerate neurons [66]. In 2015, Matsubara et al. reported that the ability of SHED-CM to induce recovery was associated with the induction of immunomodulatory activity in anti-inflammatory M2-like macrophages. Secretome analysis of SHED-CM revealed a set of previously unidentified anti-inflammatory M2-like macrophage-inducing factors, monocyte chemotactic protein-1 (MCP-1), and isoforms of secreted sialic acid-binding Ig-like lectin-9 (ED-Siglec-9). Their data suggested that the unique combination of MCP-1 and ED-Siglec-9 can repair the injured spinal cord by inducing the production of anti-inflammatory M2-like macrophages [60]. In 2022, it was documented that exosomes derived from ]DPSCs can alleviate macrophage M1 polarization through the ROS-MAPK-NF-κB p65 signaling pathway in order to address SCI [85]. In 2019, Nicola et al. transplanted human dental pulp stem cells into rats and randomized them into a control group (did not receive any manipulation), an SCI group (underwent laminectomy, then underwent SCI and was treated with a vector), and a SHED group (SCI rats were treated with intraspinal SHED transplantation, 1 h post-SCI). After measurement by the Basso, Beattie, and Bresnahan scales and ELISA and Western blot analyses, SHED was shown to act as a neuroprotectant after transplantation, possibly through paracrine signaling to reduce glial scar formation and induce tissue plasticity and functional recovery [86].

DPSCs undergo multidirectional differentiation and can promote neural repair in SCI, and many preclinical studies have identified a number of factors that enhance the differentiation of DPSCs into neuronal cells in SCI [32]. Zhang et al. transplanted DPSCs with chitosan scaffolds into a rat model of SCI and found that the chitosan scaffolds were noncytotoxic and provided a favorable microenvironment for the survival and neural differentiation of DPSCs [87]. It can promote the neural differentiation of DPSCs in SCI. Similarly, Zheng et al. reported that chitosan scaffolds in combination with bFGF better promoted the neural differentiation of DPSCs in SCI [75]. The utilization of scaffolds, cells, and growth factors in tissue engineering approaches shows potential for being

**Table 6** Preclinical studies of DPSCs in the treatment of SCI

| Year | Team | Animal Model | Cells Concentration | Content of the study | Reference |
|---|---|---|---|---|---|
| 2012 | Sakai et al. | Complete SCI model in rats | $2\times10^6$ DPSCs; intralesional injection | DPSCs promote motor recovery after spinal cord transection in rats through multiple neuroregenerative mechanisms. | [66] |
| 2015 | Matsubara et al. | Contusive SCI model | SHED-CM (MCP-1/ED-Siglec-9); intravenous injection | A novel set of M2 inducers, MCP-1 and ED-Siglec-9, were identified from SHED-CM. induced M2-like cells activated multifaceted endogenous neural repair mechanisms to promote motor function in SCI rats. | [60] |
| 2016 | Zhang et al. | Rat laminectomy model | $1\times10^5$ DPSCs; 3D chitosan scaffold culture | Potential role of chitosan scaffolds for inducing neural differentiation of human DPSCs in the treatment of SCI. | [87] |
| 2017 | Nicola et al. | Contusive SCI model | $1\times10^6$ SHED; intrathecal injection | Transplantation of SHED after SCI inhibits early neuronal apoptosis thereby protecting the nerves. | [73] |
| 2017 | Nagashima et al. | Complete SCI model in rats | $5\times10^5$ DPSCs + FGF2 (10 ng/mL); intralesional injection | Stimulation of DPSCs with FGF2 initiation promotes axonal regeneration and motor function recovery after SCI. | [67] |
| 2018 | Luo et al. | Rat laminectomy model | $1\times10^6$ DPSCs + bFGF (20 ng/mL); intralesional | Transplanted HP hydrogels containing DPSCs and bFGF had a significant effect on spinal cord repair and regeneration and may offer a promising strategy for neuronal repair, functional recovery and tissue regeneration after SCI. | [88] |
| 2019 | Nicola et al. | SCI model after laminectomy | $2\times10^6$ SHED; intravenous injection | SHED acts as a neuroprotective agent after transplantation, possibly through paracrine signalling to reduce glial scarring and induce tissue plasticity and functional recovery. | [86] |
| 2019 | Sugiyama | Rat laminectomy model | $1\times10^6$ DPSCs; intralesional injection | Using FGF2 to explore genes associated with their efficacy in DPSCs in a SCI model. | [89] |
| 2020 | Guo et al. | complete spinal cord transection model | Pre-vascularized DPSC constructs; implantation | The neuroregenerative and angiogenic potential of bioengineered pre-vascularised DPSC-embedded constructs was demonstrated in a rat spinal cord complete transection model. | [91] |
| 2021 | Zheng et al. | Complete SCI model in rats | $1\times10^5$ DPSCs + bFGF (15 ng/mL); direct implantation | Chitosan scaffolds were not cytotoxic to the survival of DPSCs, and the combination of chitosan scaffolds and bFGF promoted neural differentiation of DPSCs. | [73] |
| 2022 | Liu et al. | Contusive SCI model | DPSC exosomes (100 μg/day); intravenous injection | DPSCs-derived exosomes reduce macrophage M1 polarisation, thereby attenuating the inflammatory response reducing nerve damage. | [85] |
| 2022 | Hu et al. | Allen Methods Acute SCI Rat Model | $1\times10^6$ DPSCs + PRP (0.5 mL); intralesional injection | Dual treatment with DPSCs and PRP resulted in a significant increase in neuronal apoptosis inhibition and improved recovery of spinal motor function. | [90] |
| 2023 | Qian et al. | Complete SCI model in rats | GelMA microspheres + $5\times10^5$ DPSCs; implantation | DLP-printed DPSC-loaded GelMA microspheres aid spinal cord repair. | [32] |
| 2023 | Zhou et al. | Complete SCI model in rats | ZIF-8 + $1\times10^6$ DPSCs; intralesional injection | ZIF-8 is a material that promotes neural differentiation and angiogenesis in DPSC by activating the MAPK signaling pathway. | [80] |
| 2023 | Tao et al. | Rat laminectomy model | DPSCs co-expressing OPN/IGF-1/CNTF; intravenous injection | Co-expression of OPN, IGF-1 and CNTF enhances the therapeutic effect of DPSCs in SCI. | [92] |
| 2023 | Ying et al. | Rat laminectomy model | ROS-scavenging hydrogel + $1\times10^6$ DPSCs; implantation | Shear-thinning, ROS-scavenging hydrogel combined with DPSCs promotes spinal cord repair. | [93] |
| 2024 | Liu et al. | Rat SCI model of impact injury | $2\times10^6$ DPSCs; intravenous injection | DPSCs can treat SCI by inhibiting microglia focal death. | [74] |

**Abbreviations**: DPSCs, Dental pulp stem cells; SCI, Spinal Cord Injury; M2, Macrophages2; MCP-1,Monocyte chemoattractant protein-1; ED-Siglec-9, Sialic acid-binding Ig-Like Lectin-9; SHED-CM, Human exfoliated deciduous teeth- culture medium; FGF2, Fibroblast growth factor; bFGF, Basic fibroblast growth factor; M1, Macrophages1; PRP, Platelet-rich plasma; DLP, Digital Light Procession; GelMA, Gelatin Methacryloyl; ZIF-8, Zeolite imidazolate framework 8; MAPK, Mitogen-activated protein kinase; OPN, Bone growth factor; IGF-1, Insulin-like growth factor 1; CNTF, Calcium-derived neurotrophic factor

an effective therapeutic method in the treatment of SCI. Luo et al. prepared heat-sensitive heparin-polyoxyethylene ether (HP) hydrogels containing DPSCs and bFGF and evaluated the effect of HP-bFGF-DPSCs on neuronal recovery after SCI. The results showed that transplanted HP hydrogels containing DPSCs and bFGF had a significant effect on spinal cord repair and regeneration and may constitute a promising strategy for neuronal repair, functional recovery, and tissue regeneration after SCI [88]. In 2019, Sugiyama et al. reported that pretreatment of human HDPCs with fibroblast growth factor (FGF2) upregulated GABRB1, MMP1, and DRD2, suggesting that FGF2 contributes to neurological recovery after SCI [89]. In 2017, Nagashima et al. used DPSCs cultured with FGF2 5–6 consecutive times immediately after complete transection of the spinal cord of rats transplanted

to the injury site. The FGF2 primer was found to protect DPSCs from the posttraumatic microenvironment and thus promote axonal regeneration and motor function recovery. FGF2 has an enhancing effect on DPSCs in SCI [67]. Zhou et al. explored the effects of zeolite imidazolate framework 8 (ZIF-8) on the neural differentiation of DPSCs as well as the effects of combination therapy on SCI and found that in the presence of ZIF-8, the number of axons and axon length of DPSC-differentiated neuronal-like cells were significantly increased, and at the same time, the grafted DPSCs were protected from apoptosis in the damaged microenvironment and promoted the neural differentiation of DPSCs and angiogenesis by activating the mitogen-activated protein kinase (MAPK) signaling pathway [80]. In addition, the transplantation of platelet-rich plasma (PRP) combined with dental pulp stem cells in a rat SCI model significantly inhibited neural yard apoptosis and promoted the transformation of HDPCs into neural cells [90]. Moreover, highly vascularized scaffolds embedded with human DPSCs, which have the potential for paracrine-mediated angiogenesis and neuroregeneration, can enhance and modulate injured spinal cord repair [91]. Tao et al. constructed an adenovirus (Ad-OIC) carrying bone growth factor (OPN), insulin-like growth factor 1 (IGF-1), and ciliary neurotrophic factor (CNTF). After the modification of Ad-OIC with DPSC supernatant, HT-22 cells were cocultured and their effect on DPSC-OIC was evaluated using a cell counting kit-8 (CCK-8) assay, PCR real-time analysis, laser confocal microscopy, and fluorescence-activated cell sorting (FACS) analysis. Subsequently, the injection of DPSC-OIC into the lesion area of the injured spinal cord allowed for the assessment of the transplanted cells' survival time through bioluminescence imaging. Recovery of the injured spinal cord was evaluated through behavioral scoring, radiographic imaging, and immunohistochemical analysis. The results showed that DPSC-OIC could enhance the proliferation and axonal growth of HT-22 cells and protect HT-22 cells from H2O2-induced apoptosis. This finding suggested that the co-overexpression of OPN, IGF-1, and CNTF can enhance the therapeutic effect of DPSCs on SCI [92].

Reducing cell death is one way in which DPSCs are used to treat SCI. Many preclinical studies have revealed the specific mechanism involved. In 2017, Nicola et al. reported that SHED transplantation 1 h after SCI disturbed the balance between pro- and antiapoptotic factors and reduced early neuronal apoptosis [72]. In 2022, hydrogels that are too hard cannot be used to treat SCI due to the deformability and brittleness of the spinal cord. Ying et al. developed a hydrogel with shear-thinning ability that exhibits good deformation, allowing it to match the physical properties of the spinal cord. By combining DPSCs with this hydrogel and introducing it to the site of SCI, it was found that this combination could promote spinal cord repair by inhibiting iron death and could reduce muscle warming to enhance SCI recovery [93]. Recently, Liu et al. conditioned the medium of human DPSCs by intraperitoneal injection into SCI rats. The conditioned medium of human DPSCs was found to enhance SCI recovery by inhibiting microglial cell pyroptosis through inhibition of the NLRP3/caspase-1/interleukin-1β pathway [74].

## Discussion and conclusion

Through the above discussion, the advantages and mechanisms of DPSCs for the treatment of spinal cord injuries are understood. However, the therapeutic efficacy of DPSCs in SCI models is influenced by multiple interrelated factors. Cell survival and engraftment are critical determinants, as DPSCs' ability to secrete extracellular matrix proteins (e.g., laminin, fibronectin) facilitates their adhesion to lesion sites, while hypoxic preconditioning can further enhance their survival under ischemic conditions [94]. Paracrine signaling plays a dominant role: DPSC-derived exosomes enriched with miR-21-5p and miR-124-3p promote axonal regeneration by modulating PTEN/mTOR pathways, whereas VEGF and FGF2 secretion induces angiogenesis to improve the local microenvironment [95]. Studies have shown that in a rat contusion model, early intervention (within 7 days after injury) by intrathecal injection of $1 \times 10^6$ cells can achieve the greatest functional recovery, which is associated with reduced glial scar formation. At the same time, the acute SCI stage (24–72 h after injury) is characterized by severe inflammation, oxidative stress, and apoptosis, which gradually abate within 1–2 weeks. Dental pulp stem cells (DPSCs) exert anti-inflammatory effects by paracrine IL-10 and TGF-β, while their anti-apoptotic properties are mediated by BDNF and VEGF. However, these pathological events are transient, suggest that early intervention within 1–2 weeks may maximize the DPSC-mediated neuroprotective effect by targeting active inflammatory cascades [96, 97]. However, the route of administration impacts safety profiles. Intravenous delivery risks pulmonary entrapment, whereas direct intralesional injection minimizes off-target distribution but necessitates precise imaging guidance [98]. Immunogenicity concerns are mitigated by DPSCs' low MHC-II expression and capacity to induce regulatory T cells (regulatory T cells (Tregs)), though allogeneic transplantation in primates may trigger transient inflammatory responses [99]. Long-term safety evaluations (≥ 12 months) in canine SCI models reveal no tumorigenicity or ectopic tissue formation, attributable to DPSCs' restricted differentiation plasticity compared to pluripotent stem cells [62]. When considering the use of DPSCs for the treatment of SCI, it is important to take all of these factors into account.

Regenerative medicine is a branch of translational research that aims to rebuild irreparably damaged tissues and organs by implanting stem cells of specialized cell types to stimulate the body's own repair mechanisms. As a special cell type, DPSCs have attracted much attention among many cell types due to their advantages such as easy acquisition, multidirectional differentiation, low immune response, and few ethical issues. Transplantation of DPSCs to treat SCI has achieved good results in multiple animal models. The combination of DPSCs and biomaterials further amplifies the advantages of DPSCs-based treatment of SCI, provides an additional approach to SCI treatment, and can also be used for other diseases. For example, DPSCs showed efficacy in a cerebral ischemia model, with intracerebral transplantation reducing infarct volume by 38.2% and improving neurological scores via glial scar remodeling ($p < 0.01$). These findings are consistent with our observations of enhanced neurotrophic activity of SHED [100]. DPSCs have several advantages over MSCs; Nevertheless, certain aspects of these stem cells still need to be fully studied, such as their potential to differentiate into target cells, their ability to produce and secrete neurotrophic factors, their homing properties, and their immune response modulation ability. In addition, is there a biomarker that indicates that an individual is more responsive to DPSC-mediated therapeutic effects? Can it be applied to treat all types of SCI, or should it be differentiated according to different pathogenesis? This is an innovative and promising research direction that will provide great help for the clinical application of DPSCs in the treatment of SCI. With further research on this, we believe that DPSCs will be more widely recognized and used in the field of stem cell therapy for SCI.

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**Author contributions**
The authors' responsibilities were as follows: KZW and ZHL: designed the study; KZW, XYL: literature search and screening; KZW, XKJ, and SC: data extraction; ZBW, QWW, and HW: quality assessment; KZW: wrote the initial draft, which was modified after feedback from all coauthors; ZHL: had primary responsibility for content; and all authors: read and approved the final manuscript.

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**Data availability**
Not applicable.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Artificial intelligence**
The authors declare that they have not use AI-generated work in this manuscript and all work are owned by the authors.

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