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**Potential of dental pulp stem cells and their products in promoting peripheral nerve regeneration and future applications**

Xing WB *et al.* Application of DPSC in PNI

## **Abstract**

Peripheral nerve injury (PNI) seriously affects people's quality of life. Stem cell therapy is considered a promising new option for the clinical treatment of PNI. Dental stem cells, particularly dental pulp stem cells (DPSCs), are adult pluripotent stem cells derived from neuroectoderm. DPSCs have significant potential in the field of neural tissue engineering due to their numerous advantages, such as easy isolation, multidifferentiation potential, low immunogenicity, and low transplant rejection rate. DPSCs are extensively used in tissue engineering and regenerative medicine, including for the treatment of sciatic nerve injury, facial nerve injury, spinal cord injury, and other neurodegenerative diseases. This article reviewed research related to DPSCs and their advantages in treating PNI, aiming to summarize the therapeutic potential of DPSCs for PNI and the underlying mechanisms and providing valuable guidance and a foundation for future research.

**Key Words:** Dental pulp stem cells; Peripheral nerve injury; Regenerative medicine; Neural regeneration; Schwann cells; Regenerative medicine

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**Core Tip:** This article reviews the potential applications of dental pulp stem cells (DPSCs) and their derivatives in the field of nerve regeneration. First, this paper describes the current status of stem cell therapies for peripheral nerve injury (PNI) and discusses the advantages of DPSCs in this field. Then, the status of research on the neuroregenerative ability of DPSCs and their derivatives is reviewed. Finally, the potential of DPSCs in treating PNI and

the underlying mechanism are summarized, providing valuable guidance and a basis for future research.

## INTRODUCTION

It has been reported that approximately 2.8% of trauma patients suffer from peripheral nerve injury (PNI), resulting in permanent disabilities<sup>[1]</sup>. PNI can lead to muscle function loss, sensory impairment, and painful neuropathy<sup>[2]</sup>. While autologous nerve transplantation is a standard treatment option, its drawbacks, such as the difficulty in obtaining donor nerves, the need for surgical procedures to acquire donor nerves, secondary deformities at the donor site, and potential mismatch issues, limit its widespread use<sup>[3,4]</sup>. However, with advancements in biomaterials and tissue regeneration, significant progress has been made in nerve regrowth techniques<sup>[5]</sup>. The restoration of function after injury is of paramount importance, and experiments on stem cells have shown that they can accelerate nerve regeneration<sup>[6]</sup>. Compared to other types of stem cells, dental pulp stem cells (DPSCs) are more advantageous because they can be obtained *via* noninvasive operation, can be preserved at low temperature long-term, are simple to use, are associated with few ethical problems, and have low immunogenicity, so they are ideal materials for tissue engineering<sup>[7]</sup>. Human DPSCs (hDPSCs) exhibit remarkable self-renewal, multilineage differentiation, and cloning capabilities<sup>[8]</sup>. Schwann cells play a critical role in nerve regeneration, and stem cells with the same embryonic origin as Schwann cells are suitable tools for PNI treatment. DPSCs originate from neural spinal cells<sup>[9]</sup> and share homology with Schwann cells<sup>[10]</sup>. Therefore, due to DPSCs' unique neural differentiation and nerve regeneration abilities, DPSC-derived Schwann cells are viable tools for achieving nerve regeneration after PNI *in vitro*<sup>[11]</sup>. To keep abreast of the latest developments in this field, articles published in PubMed

between 2010 and 2023 were screened using the following search terms: “peripheral nerve”, “tooth-derived stem cells”, “exosomes”, and “dental stem cells”. The primary aim of this review is to provide information on the recent applications of DPSCs in PNI treatment, with a specific focus on both cellular therapies and noncellular therapies involving DPSCs. Additionally, the review discusses the therapeutic effects of DPSCs and their potential future applications in treating PNI.

## **PNI**

PNI is a prevalent clinical condition often resulting in long-term functional impairments. The effectiveness of surgical treatments is frequently unsatisfactory<sup>[12]</sup>, and the restoration of nerve function is often not optimal<sup>[13]</sup>. Peripheral nerve regeneration is a complex process involving Wallerian degeneration, axon sprouting, and myelin regeneration<sup>[14]</sup>. While nerves may regenerate over relatively short distances after mild nerve injury, the outcomes of nerve regeneration are often unsatisfactory. In cases of peripheral nerve amputation, a series of molecular and cellular changes occur, known as Waller’s degeneration<sup>[15,16]</sup>. Subsequently, monocytes and macrophages migrate to the nerve stump to clear axon fragments and myelin sheaths at the damaged end, while the proliferation of Schwann cells results in the formation of longitudinal cell columns, known as Bungner bands<sup>[17,18]</sup>. According to the literature, various factors, such as scaffolds for axonal migration, Sertoli cells (including Schwann cells and macrophages), growth factors, and the extracellular matrix, play crucial roles in regeneration after PNI<sup>[2]</sup>.

Schwann cells originate from neural crest cells<sup>[19]</sup> and play a crucial role in the regeneration of peripheral nerves, influenced by neurotrophic factors (NTFs) such as Krox-20, Oct-6, and Sox-10<sup>[18]</sup>. These cells play an instrumental

role in nerve regeneration by selectively promoting axonal regrowth of both motor and sensory nerves<sup>[20-22]</sup>. After nerve injury, Schwann cells may undergo dedifferentiation, a process mainly regulated by the negative regulatory factor c-Jun. This dedifferentiation is vital because it can help nerve survival and facilitate axonal regeneration<sup>[23]</sup>. Researchers have explored the effect of transplanting Schwann cells isolated from peripheral nerves into rat sciatic nerve injury models and found that these cells promote the regeneration of nerve axons, proving the potential of autologous stem cell transplantation in treating PNI<sup>[24]</sup>. However, such regenerative strategies are associated with challenges, as collecting Schwann cells is difficult, and their survival rate after transplantation is low<sup>[25]</sup>. In recent studies, DPSC-derived conditioned medium (DPSC-CM) was shown to promote the proliferation of Schwann cells and increase the production of myelin-associated proteins<sup>[26]</sup>. This may provide insight for the development of a new method for the treatment of peripheral nerve diseases.

#### **STEM CELL THERAPY FOR PNI**

Earlier studies have revealed that allogeneic stem cell transplantation has a favorable effect on nerve regeneration and thus warrants further investigation<sup>[27]</sup>. Acellular nerve allografts (ANAs) promote axon regeneration through Schwann cell proliferation<sup>[28]</sup>. However, the therapeutic efficacy of ANAs diminishes over time after nerve injury, likely due to the limitations of Schwann cell function in the host nerve. Therefore, there is an urgent need to explore alternative or more effective therapies for Schwann cell replacement.

An increasing number of studies have demonstrated the remarkable potential of stem cells in promoting neural regeneration. Adipose-derived stem cells have been shown to differentiate into Schwann-like cells and

effectively promote nerve regeneration<sup>[29-31]</sup>. Additionally, Shimizu *et al*<sup>[32]</sup> reported that human bone marrow stromal cells (MSCs) can serve as Schwann cell substitutes, making them a viable option for nerve regeneration applications. Furthermore, muscle-derived stem/progenitor cells have shown the ability to differentiate into myelinated Schwann cells *in vivo*, thereby promoting axonal regeneration<sup>[33]</sup>. Al-Zer and Kalbounieh<sup>[11]</sup> successfully induced the differentiation of DPSCs into Schwann cells by utilizing retinoic acid, mercaptoethanol, and neuromodulin  $\beta$ 1. Among stem cell sources, dental stem cells have garnered significant attention due to their excellent nerve regeneration capabilities and ease of availability.

Recent studies have revealed that during tooth development, glial cells related to the peripheral nervous system produce a significant number of <sup>3</sup> mesenchymal stem cells, including dental pulp cells and odontoblasts<sup>[34]</sup>. DPSCs, stem cells from human exfoliated deciduous teeth (SHEDs), periodontal ligament stem cells (PDLSCs), stem cells from the apical papilla, and dental follicle progenitor cells all fall under the category of dental stem cells<sup>[35]</sup>. These dental tissue-derived MSCs exhibit the ability of multidirectional differentiation and hold great potential in immunomodulation and tissue regeneration<sup>[36]</sup>. Moreover, MSCs derived from hair follicles, the dental pulp, and nipples show similar biological characteristics as those from the teeth of the same donor and display osteogenic, adipogenic, and chondrogenic differentiation capabilities<sup>[37]</sup>.

Apical pulp-derived cells and coronal pulp cells have demonstrated the ability to differentiate into nerve cells *in vitro* and express markers associated with neural crest cells (p75, Snail, and Slug) as well as neural stem cell markers (Nestin and Musashi1)<sup>[38]</sup>. SHEDs can differentiate into many types of cells, including nerve cells<sup>[39]</sup>. Pereira *et al*<sup>[40]</sup> utilized polyglycolic acid tubes to induce SHEDs to differentiate into Schwann cells. Human PDLSCs have

exhibited the ability to promote axonal regeneration after optic nerve injury, potentially through the secretion of brain-derived neurotrophic factors (BDNFs)<sup>[41]</sup>. The ERK1/2 signaling pathway plays a role in the differentiation of PDLSCs into Schwann cells<sup>[42]</sup>, thus making them a viable alternative source for autologous Schwann cells<sup>[43]</sup>. Ng *et al*<sup>[44]</sup> successfully induced adult PDLSCs to differentiate into retina-like cells with the biological characteristics of nerve cells. Dental embryonic stem cells have the potential to achieve nerve tissue regeneration due to their common origin with the nervous system<sup>[5,14]</sup>, making them a promising source of stem cells for nerve tissue regeneration<sup>[45]</sup>. The neurological potential of DPSCs makes them a viable candidate cell type for the treatment of peripheral nerve diseases<sup>[46]</sup>. DPSCs can differentiate into neuron-like cells, and after 5 d of neuron differentiation, Tub3 is activated, accompanied by increased Nestin expression<sup>[47]</sup>. Insulin-like growth factor binding protein 5 promotes the formation of neurospheres by DPSCs. Angiogenic markers such as vascular endothelial-derived growth factor (VEGF), platelet derived growth factor subunit A, and angiopoietin-1 and neurogenic markers such as neural cell adhesion molecule, Nestin,  $\beta$ III-tubulin and tyrosine hydroxylase are upregulated in DPSCs, reflecting the vascular and neurogenic differentiation potential of DPSCs<sup>[48]</sup>. Additionally, DPSCs can secrete NTFs that support nerve cell function<sup>[49]</sup>.

Compared with other stem cells, DPSCs have stronger multidifferentiation potential, self-renewal ability and colony formation ability<sup>[8]</sup>. The interaction of Eph/eaffin indicates that unlike that of other stem cells, the formation of DPSCs involves neural crest<sup>[50,51]</sup>. Because of their spinal origin, DPSCs have the ability to differentiate into other spinal cord-related cells<sup>[52]</sup>. Unlike bone marrow mesenchymal stem cells (BM-MSCs), DPSCs are heterogeneous, and they also express markers of endothelial cells (vascular cell adhesion molecule 1 and MUC-18), smooth muscle ( $\alpha$ -smooth muscle actin), bone (alkaline



phosphatase, type I collagen, osteonectin, osteopontin and osteocalcin) and fibroblasts (type III collagen and fibroblast growth factor 2)<sup>[39,53]</sup>. DPSCs express the neural precursor and glial cell markers nestin and glial fibrillary acidic protein (GFAP), which indicates that DPSCs are similar to BM-MSCs and have the potential to differentiate into neural cells<sup>[8]</sup>. Compared with BM-MSCs, DPSCs can more strongly inhibit the proliferation of PHA-stimulated T cells and exert immunosuppressive effects<sup>[54]</sup>. Compared with umbilical cord stem cells, the secretion of vascular endothelial growth factor-A and follistatin in DPSCs was more obvious, while umbilical cord stem cells tended to secrete vascular endothelial growth factor-C, which does not produce angiogenic effects. The levels of vascular endothelial growth factor-A and vascular endothelial growth factor-D secreted by DPSCs were higher than those secreted by BM-MSCs<sup>[55]</sup>. DPSCs can survive for a long time under extreme stress conditions<sup>[56]</sup>. DPSCs have the same immunomodulatory effect as BM-MSCs in the treatment of nervous system diseases. DPSCs are easier to obtain than other stem cells, have high multidifferentiation potential and a strong proliferation ability, and are not carcinogenic. They are a good substitute for stem cells in the treatment of PNI<sup>[5]</sup>.

Moreover, compared with dental follicle- and papilla-derived stem cells, DPSCs have a stronger Na<sup>+</sup> current, indicating that they have a higher potential for neural differentiation<sup>[57]</sup>. Both DPSCs and BM-MSCs implanted in the vitreous body secrete nerve growth factor (NGF), BDNF and neurotrophin 3 (NT-3), and the amount of NGF and BDNF secreted by DPSCs is significantly higher than that secreted by BMSCs, which support nerve survival and axonal regeneration<sup>[58]</sup>. Adipose tissue-derived stem cells also have the potential for nerve regeneration but mainly play an active role in stimulating endogenous stem cells by releasing NTFs<sup>[59]</sup>. Wang *et al*<sup>[60]</sup> successfully induced vascular endothelial cells and BM-MSCs to differentiate

into neurons with tricyclodecane-9-yl xanthate. Cryopreserved DPSCs were also shown to be able to differentiate into cholinergic neurons by tricyclodecane-9-yl xanthate<sup>[61]</sup>. By comparing stem cells from different sources, Isobe *et al*<sup>[62]</sup> found that BM-MSCs and synovial fluid-derived stem cells showed significant osteogenic effects. An increase in alkaline phosphatase and osteocalcin levels suggested that synovial fluid-derived stem cells had the highest cartilage formation ability. Reverse transcription-polymerase chain reaction showed increased expression of class III  $\beta$ -tubulin and microtubule-associated protein 2, suggesting that DPSCs and human deciduous tooth stem cells have the potential for neural regeneration. In addition, studies by Isobe *et al*<sup>[62]</sup>, showed that DPSCs have stronger neural differentiation potential than pluripotent stem cells isolated from bone marrow and synovial fluid.

In light of these findings, DPSCs have emerged as excellent candidate stem cells for treating PNI, with stronger tissue regeneration potential than other types of stem cells (Figure 1). Consequently, the focus of this research will be on research progress related to the effectiveness of DPSCs in treating PNI.

#### **EFFECT OF DPSCS IN THE TREATMENT OF PNI**

Teeth originate from the cranial neural crest<sup>[63]</sup>. Chai *et al*<sup>[64]</sup> were the first to confirm the involvement of cranial neural crest cells in the formation of dental pulp cells. In recent years, there has been a growing interest in research on utilizing DPSCs for nerve repair<sup>[65]</sup>. Janebodin *et al*<sup>[52]</sup> demonstrated that DPSCs originate from spinal nerves and possess the potential to differentiate into tissues derived from other neural crest-derived structures. Gronthos *et al*<sup>[53]</sup> provided evidence that the dental pulp contains cells with the ability to form clones, proliferate, and regenerate tissue, classifying them as stem cells.

The embryonic origin of DPSCs endows them with the potential to serve as stem cells for neural tissue engineering.

DPSCs have been shown to effectively promote axonal regeneration both *in vivo* and *in vitro*<sup>[66]</sup> (Tables 1 and 2). Typically, DPSCs exhibit several characteristics of pluripotent stem cells and display high proliferation rates, expressing CD44+, CD90+, and CD166<sup>[65]</sup>. Moreover, DPSCs <sup>4</sup> express embryonic stem cell markers such as Oct-4, Nanog, SSEA-3, SSEA-4, TRA-1-60, and TRA-1-81, along with several other mesenchymal stem cell markers<sup>[67]</sup>. DPSCs have the remarkable capability to differentiate into several stromal-derived cell types (Figure 2). These cell types include osteoblasts<sup>[68-72]</sup>, neurons<sup>[73]</sup>, adipogenic cells<sup>[69]</sup>, chondrogenic cells<sup>[69,70]</sup>, smooth and skeletal muscle cells<sup>[74]</sup>, dental pulp cells<sup>[75]</sup>, Schwann cells<sup>[76]</sup>, pancreatic cell lineage cells<sup>[70,77]</sup>, blood vessel cells<sup>[78]</sup>, and hepatocytes<sup>[79,80]</sup>. These findings strongly support the potential of DPSCs to be applied for tissue engineering<sup>[7]</sup>.

Subsequent studies have revealed that a large number of dental mesenchymal stem cells originate from glial cells associated with peripheral nerves<sup>[34]</sup>. Long-term cryopreserved DPSCs retain their potential to differentiate into cholinergic nerves, indicating their suitability for prolonged preservation<sup>[61]</sup>, and they have demonstrated beneficial anti-inflammatory effects in nerve injury<sup>[81]</sup>. Paes *et al*<sup>[82]</sup> conducted a pioneering comparison between monolayer and spheroid cultures of hDPSCs, demonstrating that both exhibit promising nerve regeneration potential through different mechanisms. Furthermore, hDPSCs were found to be effective in treating diabetic polyneuropathy<sup>[83]</sup>. In a groundbreaking study, Omi *et al*<sup>[84]</sup> treated neuropathy in diabetic rats through DPSC transplantation for the first time and observed nerve function recovery that was potentially attributed to basic fibroblast growth factor (bFGF), VEGF, NGF, and NT-3 secreted by DPSCs. As an effective therapeutic tool for treating PNI, DPSCs are anticipated to be

widely used in clinical settings in the future. With the deepening of research (Figure 3), it has been confirmed that DPSCs can be used to treat PNI *via* many mechanisms.

#### **CELL THERAPEUTIC ALTERNATIVE FOR DPSCS**

DPSCs can differentiate into nerve-like cells, making them of great significance in the field of nerve regeneration. Dental pulp mesenchymal stem cells exhibit strong expression of nerve and glial cell markers and can be induced to adopt a nerve-like morphology<sup>[5]</sup>. DPSCs can be induced to differentiate into cells that exhibit a neuronal morphology and express neuronal-specific markers, such as the immature neuron markers Nestin and PSA-NCAM, by a neural induction regimen *in vivo* and *in vitro*<sup>[85]</sup>. Moreover, DPSC transplantation has been shown to promote macrophage polarization toward the anti-inflammatory M2 phenotype<sup>[86]</sup>. Saez *et al*<sup>[87]</sup> demonstrated that DPSCs can effectively promote peripheral nerve regeneration when used to treat unilateral nerve crush injury in rats.

The peripheral nerve transection model is widely utilized in the study of peripheral PNI, and DPSCs have demonstrated good nerve regeneration ability in this model<sup>[88]</sup>. Stocchero *et al*<sup>[89]</sup> transplanted dental pulp cells into Wistar rats with sciatic nerve defect and observed significant promotion of nerve regeneration during the first two weeks. Combining dental pulp cells with biodegradable materials is a promising approach to avoid secondary surgery<sup>[90]</sup>. Additionally, Sasaki *et al*<sup>[91]</sup> embedded dental pulp cells in silicone tubes to repair facial nerve defects in rats, and the results indicated the formation of blood vessels and myelin sheath tissue by dental pulp cells. DPSCs have exhibited a robust capacity to promote nerve regeneration in peripheral nerve transection models, and optimizing material combination

schemes to fully unleash DPSCs' potential in nerve regeneration remains a prominent research focus in this field.

As previously reported, DPSCs, being derived from the neural ridge, possess a strong ability to differentiate into neuronal lineages. Numerous studies have explored various methods to induce the neural differentiation of DPSCs, and these cells have been applied to treat PNI<sup>[92]</sup>. After neural differentiation induction, DPSCs generate neural progenitor cells, expressing the neural markers Nestin, TuJ-1, and GFAP<sup>[93]</sup>. Additionally, inner ear neurotrophins, such as BDNF and NT-3, along with glial cell-derived neurotrophic factor (GDNF), have been found to promote the differentiation of DPSCs into spiral ganglion neuron-like cells<sup>[94]</sup>. Notably, when Schwann cells differentiated from human DPSCs were employed to treat a rat sciatic nerve defect model, significant regeneration of blood vessels and nerve processes was observed, highlighting the crucial role of revascularization in supporting nerve regeneration and survival<sup>[95]</sup>. Moreover, Zheng *et al*<sup>[96]</sup> demonstrated that coculture of chitosan scaffold with bFGF can enhance the neural differentiation of DPSCs. The ERK signaling pathway, a classical MAPK pathway, plays a pivotal role in this process. As a downstream effector of bFGF, DPSCs are actively engaged in neural differentiation. Numerous neural induction protocols exist for DPSCs; however, an optimal scheme that is simple, efficient, and quick is currently lacking.

Although significant progress has been made in the tissue field, finding a nerve conduit that can match the effectiveness of autologous transplantation remains a challenge in the treatment of PNI. Das and Bellare<sup>[97]</sup> developed a uniform bead-free nanofibrous scaffold primarily composed of polycaprolactone and gelatin A, which has been shown to support DPSC regeneration and neural differentiation. In our previous studies, we confirmed that combining DPSCs with a third-generation nerve regeneration

conduit can be used to effectively repair 15 mm long defects of the sciatic nerve in rats. Remarkably, the effect of this approach is comparable to that of autotransplantation. Furthermore, we observed that nerve tissue at the repair site mainly originated from differentiating DPSCs. This promising finding offers a potential tissue engineering strategy for the treatment of PNI<sup>[98]</sup>.

Ullah *et al*<sup>[99]</sup> made an intriguing discovery that there was no significant difference in nerve regeneration ability between hDPSCs and differentiated neuronal cells derived from hDPSCs. More research on this topic is needed to clarify the underlying mechanisms and find a more effective treatment strategy.

#### ***Differentiation into Schwann cells***

Previous studies have demonstrated that autologous adult Schwann cells have a remarkable capacity to support extensive peripheral nerve regeneration and can evade rejection<sup>[100]</sup>. Currently, Schwann cells remain a focal point of animal studies on PNI. Substitutive cell therapies bring renewed hope for nerve regeneration<sup>[20]</sup>. Schwann cells play a pivotal role in Wallerian degeneration, myelin regeneration, and supporting axon growth.

Martens *et al*<sup>[76]</sup> were the first to demonstrate that Schwann cells differentiated from hDPSCs can promote nerve regeneration. These differentiated Schwann cells express neural markers such as laminin, p75, GFAP, and CD104 and exhibit an increase in the expression of NTFs such as BDNF, b-NGF, NT-3, and GDNF. Medium conditioned by these differentiated Schwann cells was found to increase the survival of dorsal root ganglion cells and stimulate axon elongation. Subsequently, Al-Zer *et al*<sup>[101]</sup> induced the differentiation of DPSCs into Schwann cells by using forskolin, bFGF, platelet-derived growth factor, and recombinant human neuregulin- $\beta$ 1. The results revealed that the differentiated neural stem cells strongly expressed the

Schwann cell marker Sox10. Additionally, Sanen *et al*<sup>[102]</sup> demonstrated that hDPSCs, after differentiating into Schwann-like cells, have the potential to stimulate endothelial cell migration and tubule formation. Neuronally differentiated DPSCs secrete higher levels of the angiogenic factor VEGF-A, suggesting that DPSCs retain their angiogenic ability even after differentiating into Schwann cells. However, the underlying mechanism by which DPSC-derived Schwann cells promote endothelial cell proliferation requires further investigation. These findings collectively indicate the ability of DPSCs to differentiate into Schwann cells, making them potential candidates for tissue engineering-based approaches for treating nerve injuries. Lambrichts *et al*<sup>[103]</sup> showed that hDPSC-derived Schwann cells could form myelin sheaths and dorsal root ganglia. Similarly, Carnevale *et al*<sup>[12]</sup> discovered that STRO-1+/c-Kit+/CD34+ hDPSCs, which originate from the neural ridge, promote axonal regeneration in an animal model of PNI and express S100b, a typical marker of Schwann cells. The differentiation of DPSCs into Schwann cells thus is potential strategy for nerve regeneration, and the refinement of differentiation protocols and materials remains an important area of investigation.

#### ***Differentiation into oligodendrocytes***

The primary function of oligodendrocytes is to form myelin, creating intricate connections between neurons in the nervous system. The transcription factor OLIG-2 and proteoglycan NG2 are markers of oligodendrocyte progenitor cells (OPCs)<sup>[104]</sup>. In a previous study, Sakai *et al*<sup>[105]</sup> transplanted SHED into rats with spinal cord transection and found that these cells exerted promising effects in promoting axon regeneration. This effect was achieved through the inhibition of various axon growth inhibitor signals and the differentiation of the transplanted cells into oligodendrocytes to replace damaged cells.

Bagheri-Hosseinabadi *et al*<sup>[106]</sup> successfully induced DPSCs to differentiate into oligodendrocytes using cerebrospinal fluid and retinoic acid and obtained cells with a fibroblastic morphology and high adherence potential. Moreover, Askari *et al*<sup>[107]</sup> successfully induced DPSCs to differentiate into OPCs by transfecting them with a virus carrying the human Orig2 gene. The differentiated oligodendrocytes displayed a typical morphology and expressed neural markers such as GFAP, oligodendrocyte lineage transcription factor 2, and MBP. Subsequently, they applied transplanted oligodendrocytes into sciatic nerve demyelination model mice and demonstrated their effectiveness in nerve repair<sup>[108]</sup>. The nerve regeneration potential of DPSCs has been substantiated through various studies, offering hope for the treatment of peripheral nerve demyelination.

#### *Differentiation into endothelial cells*

Adequate blood supply is crucial for the survival of stem cells and nerve regeneration following injury. When mesenchymal stem cells differentiate into Schwann cells, they increase the secretion of angiogenic factors, including angiopoietin-1 and VEGF-A<sup>[109]</sup>. DPSCs can increase the migration of endothelial cells and promote angiogenesis *in vitro* and *in vivo*<sup>[110]</sup>. Sanen, K and others also confirmed this phenomenon<sup>[102]</sup>. Moreover, research has demonstrated that stem cells can directly differentiate into endothelial cells. In a rat facial nerve defect model, it was observed that DPSCs can directly differentiate into RECA1-positive endothelial cells, promoting nerve regeneration by increasing blood supply<sup>[91]</sup>. DPSCs were found lining the blood vessel wall of newly formed braided bone, indicating that angiogenesis occurred *in vitro*. Osteoblasts and endothelial cells were found after transplantation, and ultimately, bone-containing blood vessels were produced. flk-1 is very important for the coupling of osteogenesis and



angiogenesis. d'Aquino *et al*<sup>[69]</sup> proved that DPSCs can differentiate into flk-1<sup>+</sup>/STRO-1<sup>+</sup>/CD44<sup>+</sup>/CD54<sup>+</sup> endothelial progenitor cells. Sasaki *et al*<sup>[91]</sup> found that regenerated nerves contained S100-positive Schwann cells and RECA1-positive endothelial cells derived from dental pulp 14 d after DPSC transplantation into rats with facial nerve defect. Newborn blood vessels are composed of endothelial cells from both recipient and donor sources. It has been suggested that DPSCs can differentiate into nerve cells and endothelial cells at the same time to promote nerve and vascular regeneration to treat PNI<sup>[91]</sup>. Subsequently, Maraldi *et al*<sup>[71]</sup> also proved that transplanted DPSCs can differentiate into endothelial cells *in vivo*. The ability of DPSCs to differentiate into endothelial cells further increases their potential for nerve regeneration.

#### *Paracrine action*

PNI triggers the dedifferentiation of Schwann cells and induces the formation and secretion of protogranules, which play a crucial role in nerve repair and the promotion of axonal growth<sup>[111]</sup>. Yamamoto *et al*<sup>[112]</sup> discovered that mobilized DPSCs (MDPSCs) treated with a granulocyte-colony stimulating factor (G-CSF) gradient express a variety of NTFs. These NTFs not only stimulate Schwann cells but also regulate their apoptosis and proliferation. Additionally, the differentiation of hDPSCs into Schwann cells increases the expression of glial markers and the secretion of NTFs, including BDNF, GDNF, NGF, NTF3, ANGPT1, and VEGFA<sup>[45,76]</sup>. Furthermore, linearly arranged dental pulp cell slices have been shown to guide and support axonal regeneration, and the abundant NTFs produced by the cells were found to make a significant contribution to this phenomenon<sup>[113]</sup>. Implantation of DPSCs into the vitreous can effectively treat retinal ganglion cell injuries in adult rats, with the secretion of NTFs being a critical factor. The

neuroprotective effect of DPSCs is weakened when K252a and Trk are blocked, indicating the importance of the NTFs NGF, BDNF, and NT-3 in this process<sup>[58]</sup>. In the repair of sciatic nerve crush injury in rat models, transplanted dental pulp cells may secrete NTFs<sup>[114]</sup>. In a rat model of sciatic nerve injury with a 10 mm defect, DPSCs were found to exert their effects on Schwann cells through paracrine signaling, leading to significant promotion of axonal regeneration<sup>[45]</sup>. The strong paracrine effect of DPSCs, coupled with their potential for nerve regeneration, makes them promising candidates for nerve tissue engineering.

#### **CELL-FREE THERAPEUTIC ALTERNATIVES INVOLVING DPSCS**

An increasing number of studies have demonstrated that odontogenic stem cells treat nerve injury through paracrine mechanisms<sup>[105]</sup>. NTFs, such as NGF, BDNF, and GDNF, can stimulate axonal growth, making DPSCs potential candidates for acellular therapy<sup>[115,116]</sup>. Kumar *et al*<sup>[117]</sup> showed that DPSCs secrete high levels of cytokines such as G-CSF, interferon gamma, and transforming growth factor (TGF)  $\beta$ , which promote nerve differentiation and axonal growth. Unlike some other stem cell types, DPSCs demonstrate strong potential for nerve regeneration, supporting the use of their secreted factors for exocrine therapy for PNI. Kanada *et al*<sup>[118]</sup> found that both DPSCs and secreted factors from DPSCs (DPSC-SFs) exerted therapeutic effects in a rat model of diabetic polyneuropathy. The effects included increases in sciatic nerve motor/sensory nerve conduction velocity and sciatic nerve blood flow. DPSC-SFs were found to include angiogenic, neurotrophic, and immunomodulatory proteins. However, DPSC transplantation may provide benefits over a longer duration of time<sup>[118]</sup>.

With the continuous development of research on DPSC-derived exosomes (DPSC-Exos), DPSC-Exos have been demonstrated to exert potential

therapeutic effects in various diseases. They have shown promise for the treatment of periodontitis<sup>[119]</sup>, Parkinson's disease<sup>[120]</sup>, SCI<sup>[121]</sup>, degenerative diseases<sup>[122]</sup>, and cerebral ischemia<sup>[123]</sup> and in achieving pulp regeneration <sup>[124]</sup>. DPSC-Exos have garnered significant attention due to their immunomodulatory properties<sup>[125]</sup>, ability to promote angiogenesis<sup>[126]</sup>, inhibitory effect on inflammation<sup>[127]</sup>, and marked neuroregenerative and neuroprotective effects<sup>[128]</sup>. Studies have revealed that DPSC-Exos can increase the formation of SH-SY5Y cell axons, leading to improved neuronal ultrastructure and increased expression of neural markers<sup>[129]</sup>. Research on acellular therapies involving DPSCs is still relatively limited, and the therapeutic potential of DPSC-Exos and DPSC lysates requires further experimentation and validation. Progress has been made in the techniques used to extract exosomes and analyze their composition, but there is still much work to be done before their clinical application can be realized.

CM derived from D-MSCs has been shown to promote axonal growth<sup>[45]</sup>. Sultan *et al*<sup>[130]</sup> conducted a study on the neuroprotective effect of DPSCs and DPSC-CM on isolated TGNCs for the first time. The results demonstrated that DPSC-CM can increase neuronal survival and promote axonal growth through the action of various NTFs, including GDNF, BDNF, NT-3, and CNTF<sup>[130]</sup>. CM from human dental pulp cells was found to contain bone morphogenetic protein 7, FGF7, insulin-like growth factor (IGF)-1, FGF4, growth hormone, and VEGF-D, all of which are related to nerve regeneration and protection, vascular regeneration, and osteogenesis. Furthermore, the addition of B-27 to CM was observed to enhance the promotion of axon growth<sup>[131]</sup>. DPSC-CM has shown the ability to alleviate polyneuropathy in diabetic rats and reduce the number of macrophages in diseased peripheral nerves<sup>[132]</sup>. DPSC-CM holds significant promise in the treatment of peripheral

nerve injuries. However, further research is needed to explore the underlying mechanisms of nerve regeneration.

Neurotrophin, BDNF, GDNF, IGF, NGF and VEGF were detected near transplanted DPSCs 14 d after transplantation. The secretion of vascular endothelial growth factors supports angiogenesis, thus promoting nerve regeneration<sup>[112]</sup>. BM-MSCs can also secrete NTFs such as BDNF, b-FGF and CNTF to promote peripheral nerve regeneration and thus treat peripheral nerve defects<sup>[133]</sup>. G-CSF-MDPSCs express higher levels of granulocyte macrophage CSF, matrix metalloproteinase 3, VEGF, and NGF than BM-MSCs. The effects of G-CSF-MDPSCs in angiogenesis, neurite extension, and migration and their anti-apoptotic effects were found to be stronger than those of BM-MSCs in the same environment<sup>[134]</sup>. Compared with medium conditioned by CD31<sup>-</sup> cells derived from bone marrow and fat, medium conditioned by CD31<sup>-</sup> cells from pulp results in higher levels of angiogenesis/NTFs and exerts stronger angiogenic and neurogenic effects<sup>[135]</sup>. Kumar *et al*<sup>[117]</sup> also proved that DPSCs and their secreted factor may be exert beneficial effects in treating neurological disorders and injuries. Exosomes derived from DPSCs can inhibit the differentiation of CD4<sup>+</sup> T cells into helper T cells 17 (Th17), reduce the secretion of the proinflammatory factors interleukin (IL)-17 and tumor necrosis factor-alpha (TNF- $\alpha$ ), promote the polarization of CD4<sup>+</sup> T cells into regulatory T cells, and increase the release of the anti-inflammatory factors IL-10 and TGF- $\beta$ . Compared with BM-derived exosomes, exosomes derived from DPSCs have a stronger immunoregulatory effect. In CD4<sup>+</sup> T cells stimulated by exosomes derived from DPSCs, the expression levels of IL-10 and TGF- $\beta$  mRNA were found to be the highest, while the transcription levels of IL-17 and TNF- $\alpha$  were found to be the lowest. Exosomes derived from DPSCs have stronger anti-inflammatory effects than exosomes derived from BM-MSCs<sup>[125]</sup>. Exosomes derived from DPSCs/BM-

MSCs significantly decrease the activity of caspase3/7 and exert a significant antiapoptotic effect to play a neuroprotective role by upregulating endogenous expression of neuronal survival factors. Specifically, the cell survival-related PI3K-Bcl-2 pathway protects hippocampal neurons from excitotoxicity. Exosomes derived from DPSCs have stronger antiapoptotic and anti-visceral necrosis effects than exosomes derived from bone marrow stem cells<sup>[122]</sup>. Interestingly, the factors secreted by DPSCs are different in different environments<sup>[136]</sup>. There is no doubt that the neuroprotective effect of DPSC-Exos is stronger than that of BM-MSC-derived exosomes. However, for clinical application, it is necessary to better determine the optimal composition, dosage and culture conditions of exosomes<sup>[121]</sup>.

## **CONCLUSION**

PNI is a prevalent clinical issue that often leads to long-term pain in patients. Recent research has demonstrated the beneficial effects of Schwann cells on axonal regeneration and functional recovery after injury<sup>[137]</sup>. However, isolating and cultivating Schwann cells are challenging due to their limited availability and low proliferation rate<sup>[138]</sup>. Tissue engineering strategies involving nerve grafts consisting of physical scaffolds combined with Sertoli cells and/or growth factors or other biomolecules are promising approaches for treating PNI<sup>[139]</sup>.

DPSCs may occupy the injury site and exert immunomodulatory effects, participate in paracrine signaling and directly differentiate into relevant cell types, thus promoting the repair and regeneration of diseased and injured tissues. To promote the application of DPSCs and their products in nerve regeneration in the future, clinical strategies and various administration methods are being studied on a large scale<sup>[140]</sup>. A study revealed that inducing DPSCs to differentiate into oligoprogenitor cells is a potential treatment

strategy for neurodegenerative diseases<sup>[141]</sup>. DPSCs have the multilineage differentiation potential and can differentiate into neurotrophoblasts. DPSC-CM and DPSC-Exos contain rich NTFs, and DPSC-Exos can also cross the blood-brain barrier. In addition, DPSCs have the ability to reduce inflammation, promote axonal growth and resist apoptosis. They have great potential in the treatment of PNI<sup>[142]</sup>.

Previous *in vivo* studies have focused on the sciatic nerve and facial nerve of rats and analyzed the behavioral and pathological manifestations of rats with sciatic and facial nerve injury, but electrophysiological studies are lacking. Moreover, it is also necessary to compare therapeutic effects among different models to identify the optimal application of DPSCs. However, there are still some questions to be answered. Do DPSCs have the same effect on nerve crush injury and amputation injury? Do different treatment methods have different effects on different models. Which is better, cell therapy or acellular therapy? What are the key factors in the neuroprotective and regenerative abilities of acellular therapies? In addition, researchers should pay more attention to the function of differentiated nerve cells *in vitro*. Furthermore, organoids may become a hot research topic in the future. A DPSC cell bank is also urgently needed<sup>[143]</sup>. It is believed that DPSCs will bring hope to patients with PNI soon.

The unique biological characteristics of DPSCs make them significant cell sources for the treatment of PNI. DPSCs exhibit neurogenic potential, are easily accessible, exhibit pluripotency, and can be preserved for extended periods, making them excellent candidates for tissue engineering applications. DPSCs (Figure 4) can be utilized directly or in combination with nerve conduits or hydrogels or used after differentiation into nerve-like cells to treat PNI. Moreover, DPSC lysates, DPSC-Exos, and DPSC-CM all hold promise in the treatment of PNI. However, overcoming safety and ethical

problems and avoiding tumor formation are still the keys for translating DPSCs for clinical use<sup>[144]</sup>.

This review focused on the therapeutic potential of DPSCs in treating PNI, elaborating on the neuroprotective and regenerative capabilities of DPSCs through both cell and acellular approaches. DPSCs hold significant promise for the treatment of PNI, and it is anticipated that they will play a crucial role in the clinical treatment of PNI in the future. As research on the effect of DPSCs on PNI continues to progress, more efficient and expedient treatment protocols are expected to be developed. However, the safety of cell therapies and the efficacy of acellular therapies in treating long-gap injuries require thorough evaluation. The search for convenient and effective treatment strategies involving DPSCs and their products for PNI remains ongoing. Furthermore, the underlying mechanisms and key components of DPSCs in peripheral nerve treatment warrant further investigation. As acellular therapies are novel stem cell therapies, there is a need to refine the purification process of cell materials and establish a standardized and effective cell production plan. Comparative studies assessing the safety, efficacy, and production costs of various therapies are also essential.

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