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Transplantation effects of dental pulp-derived cells on peripheral nerve regeneration in crushed sciatic nerve injury

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Abstract: The effects of transplanted human dental pulp-derived cells (DPCs) on peripheral nerve regeneration were studied in a rat model of sciatic nerve crush injury. In one group, DPCs were transplanted into the compression site (cell transplantation group); the control group underwent no transplantation (crushed group). Sciatic nerve regeneration was determined based on the recovery of motor function and histological and immunohistochemical analyses. The cell transplantation group showed improved motor function compared with the crushed group using the CatWalk XT system, which corresponded to a higher ratio of tibialis to anterior muscle weight 14 days after surgery. Histological analysis revealed a smaller interspace area and few vacuoles in the sciatic nerve after cell transplantation compared with the crushed group. The myelin sheath was visualized with Luxol Fast Blue (LFB) staining and anti-myelin basic protein (anti-MBP) antibody labeling; the percentages of LFB- and MBP-positive areas were higher in the cell transplantation group than in the crushed group. Human mitochondria-positive cells were also identified in the sciatic nerve at the transplanta-

tion site 14 days after surgery. Taken together, the observed correlation between morphological findings and functional outcomes following DPC transplantation indicates that DPCs promote peripheral nerve regeneration in rats.

Keywords: dental pulp-derived cells; peripheral nerve crush injury; regeneration; sciatic nerve; transplantation.

Introduction

Peripheral nerve injury is a major cause of morbidity associated with surgical procedures. Although endogenous peripheral nerve regeneration is possible, it is often insufficient for complete functional recovery (1). The inferior alveolar, lingual, and hypoglossal nerves are typically affected in the maxillofacial region, and the incidence of nerve damage after the removal of mandibular third molar teeth ranges from 0.4% to 8.4% (2). Surgical repair, including nerve grafting, is usually required to treat transection nerve injuries, whereas drug therapy or physical rehabilitation is required to treat moderately severe nerve crush injuries (3).

Over the past decades, cell transplantation has been proposed as a promising strategy for peripheral nerve reconstruction, and a variety of cell types, including neural stem cells, bone marrow stromal cells, and umbilical cord-derived mesenchymal stromal cells, have been used to create an adequate environment for nerve regeneration through neurotrophic factor production and Schwann cell differentiation (4-6).

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Adult mesenchymal stem cells (MSCs) have been used in regenerative medicine due to their trilineage, osteogenic, chondrogenic, and adipogenic differentiation potential and ability to differentiate into neuron-like cells, astrocytes, and/or microglia (7,8). MSCs also have the ability to migrate to the site of injury, where they modulate immune and inflammatory responses and mobilize intrinsic cell reservoirs through paracrine mechanisms (9). MSCs can be isolated from different tissues such as bone marrow, adipose tissue, skeletal muscle, and dental pulp and are known to produce neurotrophic factors; thus, they are useful for cell-based therapy for peripheral nerve injury (9-14).

Dental pulp is a highly innervated and vascularized connective tissue encapsulated in a mineralized inextensible structure formed by enamel, dentin, and cementum. Dental pulp stem cells have been identified as an adult MSC population capable of regenerating tooth-related tissues (15-18). Importantly, dental pulp stem cells are derived from the ectomesenchymal neural crest, which exhibits the biological features of MSCs and neural crest stem cells. Human dental pulp stem cells have a higher rate of proliferation, stronger capacity for repair, lower immunogenicity, greater plasticity (15), and higher capacity for neural differentiation than those of other mesoderm-derived MSCs (14,19,20). Many *in vivo* and *in vitro* studies have shown that dental pulp stem cells can prevent and repair neuronal damage (21-23) by stimulating nerve regeneration (24-26), including that of the facial nerves (27,28). Unlike other mesoderm-derived MSCs, dental pulp stem cells have the advantage of being easy to isolate from extracted teeth without causing secondary damage or ethical controversy.

Two experimental models have been established to determine the regeneration potential of transplanted cells in peripheral nerve injury. The transection model uses the suture technique and materials that induce inflammation, which may obfuscate the evaluation of tissue repair (29). Spontaneous recovery of motor function has been reported in a sciatic nerve crush injury model several decades ago as well as more recently (30,31); however, nerve regeneration under these conditions is incomplete, and serious neurological deficits, determined by electrophysiological parameters, as well as inadequate nerve myelination and regeneration persist until 4 weeks (29,32). Therefore, for the sake of simplicity, in this study, we used the nerve crush injury model to investigate the peripheral nerve regeneration potential of transplanted human dental pulp-derived cells (DPCs).

Materials and Methods

All experimental procedures involving animals were performed in accordance with Institutional Animal Care guidelines, and ethical approval was obtained from the Ethics Committee of Aichi-Gakuin University School of Dentistry (no. 406). Experiments were performed in accordance with the Helsinki Declaration.

Culture and isolation of DPCs

Normal human third molars were extracted from six patients (age, 18-29 years) at the Aichi-Gakuin University Dental Hospital after obtaining written, informed consent. The dental pulp of six teeth was gently removed, and DPCs were isolated as previously described (15), with slight modification. Briefly, the harvested dental pulp tissue was minced into 1-3-mm² pieces and enzymatically digested. A total of 3×10^4 cells were isolated based on their ability to form cell colonies and seeded onto 35-mm dishes (BD Biosciences, San Jose, CA, USA) after filtration through a 40- μ m cell strainer (BD Biosciences) and lysis of red blood cells with IOTest3 lysing solution (Beckman Coulter, Fullerton, CA, USA). Cells were detached and subcultured when they reached 70% confluence. At passage 6, DPCs had a flat, spread-out, and fibroblast-like morphology; these cells were used in subsequent experiments.

Surgical procedures

Animal protocols were approved by Aichi-Gakuin University Animal Research Committee (AGUD258) and were performed under the Regulations on Animal Experimentation of Aichi-Gakuin University.

A total of 18 adult male Fischer 344 rats (Japan SLC, Shizuoka, Japan) weighing 190-210 g were used in this study. The rats were initially anesthetized with 3.0% isoflurane (Intervet, Summit, NJ, USA) and maintained under anesthesia with 1.7% isoflurane. The left sciatic nerve was exposed under the microscope (Fig. 1a) and subjected to crush injury for 30 min (Fig. 1b, c). The crush position was marked by placing a 10-0 nylon suture in the epineurium. The animals were divided into three groups ($n = 6$ each): the crushed group, in which the left sciatic nerve was crushed with a vascular clip (0.59 N; BEAR Medic Corporation, Ibaraki, Japan); cell transplantation group, in which the left sciatic nerve was crushed with a vascular clip and then wrapped with an absorbable hemostat (SURGICEL; Johnson & Johnson, Tokyo, Japan) filled with human DPCs (3.0×10^5 cells) in 10 μ L of Hank's buffered saline solution containing 1% atelocollagen (Koken, Tokyo, Japan), 20 μ g/mL fibronectin, and 10 μ g/mL laminin (Fig. 1d); and sham operation group,

Fig. 1 Preparation of rat sciatic nerve crush injury model. (a) Left sciatic nerve. (b) The left sciatic nerve was crushed for 30 min using a microvascular clip. (c) Crushed sciatic nerve after clipping (arrowhead). (d) Dental pulp-derived cells (3.0×10^5 cells) in 10 μ L of mixed collagen solution were seeded in oxidized cellulose and the complex was wrapped around the crushed portion of the nerve in the cell transplantation group.

in which the sciatic nerve was exposed but not crushed. In all animals, the wound was then sutured and closed with 4-0 silk (Natsume Seisakusho, Tokyo, Japan). To avoid immune rejection of transplanted cells, 0.05 mg/kg tacrolimus (Prograf; Astellas Pharmaceutical, Tokyo, Japan) was intraperitoneally administered daily from preoperative day 1 to postoperative day 14.

Analysis of functional recovery

The walking track analysis with a CatWalk XT system (Noldus Information Technology, Wageningen, the Netherlands) was used to evaluate functional recovery. Gait characteristics were measured according to time and surface parameters of paw prints. When a rat walked voluntarily along the glass floor from one side of the apparatus to the other, a video camera under the walkway recorded its paw prints as it contacted the glass floor (33,34). Both injured and uninjured sides were assessed in terms of toe spread (TS), intermediate TS (ITS), and print length (PL) on postoperative days 7, 10, and 14. TS is the distance between the first and fifth toes, ITS is the distance between the second and fourth toes, and PL is the distance between the third toe and the heel of the hind limb. TS, ITS, and PL are referred to as experimental TS (ETS), experimental ITS (EITS), and experimental PL (EPL) on the injured site and normal TS (NTS), normal ITS (NITS), and normal PL (NPL) on the uninjured site, respectively. To compare the crushed and cell transplanta-

tion groups, we calculated TS factor (TSF) as NTS-ETS, intermediate TSF (ITSF) as NITS-EITS, and PL factor (PLF) as EPL-NPL. Similarly, we also calculated TSF, ITSF, and PLF in the sham operation group as a positive control.

Determination of tibialis anterior muscle weight ratio

Functional recovery was assessed based on the ratio of the tibialis muscle to anterior muscle 14 days after the surgery. Under deep anesthesia, the tibialis anterior muscles on the injured and contralateral uninjured sides of rats were harvested and immediately weighed to determine the wet weight ratio; this was defined as the ratio of muscle wet weight on the injured side in relation to the contralateral uninjured side, which is a more reliable measure than muscle wet weight only. All measurements were performed by two blinded observers.

Histological analysis

To histologically confirm the enhancement of axonal regeneration in DPC-transplanted animals, sciatic nerve segments 1.5 cm below the repair site were harvested under a light microscope. Specimens were harvested from the crushed group on postoperative days 1, 3, 7, 14, and 30 and from the cell transplantation group on postoperative day 14. The specimens were fixed with 4% (w/v) paraformaldehyde in phosphate-buffered saline for 24 h at 4°C, embedded in paraffin, and longitudinally cut into 5 μ m-thick sections that were stained with hematoxylin and eosin (H-E) and Luxol Fast Blue (LFB) to visualize tissue morphology and myelin sheath structure, respectively.

In H-E-stained sections on postoperative day 14, the interspace area between myelin sheaths was measured with ImageJ software (National Institutes of Health, Bethesda, MD, USA) in a standardized field (0.42×2.27 mm), and values obtained for the crushed and cell transplantation groups ($n = 3$ each) were compared. The number of vacuoles in the field of view (0.21×0.37 mm) at high magnification (40 $\times$) was also counted and compared between the two groups. A total of 10 sites (five sites each on the proximal and distal sides) were randomly selected in each group ($n = 3$ per group). The number of vacuoles and density of LFB-positive and myelin basic protein (MBP)-positive cells were determined using previously described methods (35). In LFB-stained sections on postoperative day 14, the LFB-positive areas in the two groups ($n = 3$ each) were measured with ImageJ software (National Institutes of Health) in the standardized field (0.54×3.22 mm) and compared.

Fig. 2 Macroscopic observations of hind limb and sciatic nerve on postoperative day 14. (a, b) Hind limb on the injured side in the crushed (a, black arrowhead) and cell transplantation (b, white arrowhead) groups. (c, d) Macroscopic view of the sciatic nerve in the crushed (c) and cell transplantation (d) groups.

Immunohistochemical analysis

Regenerated sciatic nerves were examined with immunohistochemistry. Paraffin-embedded sections (5- μ m thick) were prepared from regenerated sciatic nerve specimens on postoperative day 14. Rabbit polyclonal anti-MBP antibody (ab40390, 1:1,000; Abcam, Cambridge, UK) was used to label myelin sheaths and mouse monoclonal anti-human mitochondria antibody (ab92824, 1:50; Abcam) was used to determine whether transplanted human DPCs were incorporated into the regenerated sciatic nerve. The antibodies were applied overnight at 4°C after endogenous peroxidase activity blocking with 3% hydrogen peroxide in methanol for 20 min at room temperature and antigen retrieval in HistoVT One (Nacalai Tesque, Kyoto, Japan) for 20 min at 90°C.

As a negative control, 2.5% normal horse serum was applied instead of primary antibodies. The ImmPRESS horseradish peroxidase anti-rabbit or anti-mouse IgG (peroxidase) polymer detection kit (Vector Laboratories, Burlingame, CA, USA) was then applied for 30 min at room temperature. The sections were incubated with diaminobenzidine substrate (Vector Laboratories) to visualize the immunoreactivity. The MBP-positive areas in the crushed and cell transplantation groups ($n = 3$ each) were measured with ImageJ software (National Institutes of Health) in a standardized field (0.47×3.12 mm) and compared on postoperative day 14.

Statistical analysis

Data are shown as the mean $\pm$ standard deviation. All statistical analyses were performed with Excel 2013 for Windows (Microsoft, Redmond, WA, USA). Statistical

Fig. 3 Paw prints on postoperative day 14. Walking track analysis was performed using a CatWalk XT system.

differences were evaluated with the Student's *t*-test, and $P < 0.05$ was considered statistically significant.

Results

Macroscopic observation

No wound dehiscence or infection was observed in any groups. Gross examination revealed that the functioning of the left hind leg in rats transplanted with DPCs was nearly normal on postoperative day 14 (Fig. 2a, b); moreover, there was no difference in the appearance of the injured sciatic nerve between the crushed and cell transplantation groups on postoperative day 14 (Fig. 2c, d).

Recovery of motor function after DPC transplantation

There was a significant improvement in motor function in the cell transplantation group compared with the crushed group (Fig. 3). TSF in the crushed and cell transplantation groups was 1.16 ± 0.21 and 0.86 ± 0.21 cm on postoperative day 7; 1.09 ± 0.25 and 0.83 ± 0.18 cm on postoperative day 10; and 0.72 ± 0.34 and 0.38 ± 0.25 cm on postoperative day 14, respectively. ITSF in the crushed and cell transplantation groups was 0.58 ± 0.16 and 0.37 ± 0.16 cm on postoperative day 7; 0.50 ± 0.19 and 0.36 ± 0.13 cm on postoperative day 10; and 0.41 ± 0.17 and 0.12 ± 0.13 cm on postoperative day 14, respectively. Thus, at all the three time points, TSF and ITSF differed significantly between the two groups ($P < 0.05$). PLF in the crushed and cell transplantation groups was 1.43 ± 0.04 and 1.18 ± 0.20 cm on postoperative day 7; 1.42 ± 0.13 and 1.04 ± 0.09 cm on postoperative day

Fig. 4 Analysis of motor function in the rat sciatic nerve crush injury model. Toe spread (TS), intermediate TS (ITS), and print length (PL) were measured on the injured and uninjured sides on postoperative days 7, 10, and 14. TS factor (TSF) and intermediate TSF (ITSF) significantly differed between the crushed and cell transplantation groups on postoperative days 7, 10, and 14 ($P < 0.05$). PL factor (PLF) also significantly differed between the two groups on postoperative days 7 and 10 ($P < 0.05$).

10; and 0.89 ± 0.18 and 0.84 ± 0.21 cm on postoperative day 14, respectively. Thus, PLF differed significantly between the two groups on postoperative days 7 and 10 ($P < 0.05$). These results indicated that animals transplanted with DPCs were better able to support their body weight on the injured limb than those without treatment, starting from postoperative day 7 (Fig. 4).

The results in the sham operation group were as follows: TSF was 0.01 ± 0.06 , 0.01 ± 0.04 , and 0.05 ± 0.06 cm; ITSF was 0.01 ± 0.06 , 0.02 ± 0.09 , and 0.01 ± 0.03 cm; and PLF was 0.03 ± 0.05 , 0.00 ± 0.04 , and 0.04 ± 0.05 cm on postoperative days 7, 10, and 14, respectively.

Recovery of target muscle after DPC transplantation
Tibialis anterior muscle mass was decreased in the crushed and cell transplantation groups on postoperative day 14. We measured the wet weight ratio (%) of the crushed and normal sides and found that the average wet weight of the crushed side was 259.50 ± 36.74 mg in the crushed group and 312.17 ± 44.19 mg in the cell transplantation group, whereas the average wet weight of the normal side was 391.5 ± 46.37 mg in the both groups (Fig. 5 and Table 1). The ratio of muscle wet weight of the crushed side relative to that of the normal side was

Fig. 5 Macroscopic view of the tibialis anterior muscle on postoperative day 14.

Table 1 Tibialis anterior muscle wet weight and wet weight ratio

Group	Wet weight (mg)	Wet weight ratio (%)
Crushed	259.50 ± 36.74	67.21 ± 4.44
Cell transplantation	312.17 ± 44.19	78.66 ± 6.89

* $P < 0.05$

$67.21\% \pm 4.44\%$ in the crushed group and $78.66\% \pm 6.89\%$ in the cell transplantation group. Sciatic nerve paralysis in both the groups induced the contraction of the tibialis anterior muscle, but the muscle weight ratio was higher in the cell transplantation group than in the crushed group ($P < 0.05$; Table 1).

Morphological analysis of nerve regeneration at the injury site of rat sciatic nerve

Axonal degeneration was observed on postoperative day 1 and continued until day 14. Inflammatory cells accumulated at the site of injury. Complete myelination and axonal regeneration were observed on postoperative day 30.

At low magnification, DPC-transplanted nerves had a more organized arrangement of axons than did nerves in the crushed group on postoperative day 14 (Fig. 6A). Notably, regenerated axons in the proximal area of the injured sciatic nerve transplanted with DPCs appeared similar to the axons in the intact nerve (Fig. 6A, B). The space between myelin sheaths (interspace) and vacuoles was clearly visible in the regenerated nerve area. The total interspace area in low-magnification images and number of vacuoles in high-magnification images were compared between the crushed and cell transplantation groups (Fig.

Fig. 6 Histological analysis of the excised sciatic nerve. (A) Low-magnification view. (a-g) Hematoxylin and eosin (H-E) staining in the crushed group on postoperative days 1, 3, 7, 14, and 30 (a-e); in the cell transplantation group (f) and sham operation group (g) on postoperative day 14. (B) High-magnification view. (h-q) Images of distal (h, j, l, n, p) and proximal (i, k, m, o, q) portions of the crush injury in the crushed group shown in Fig. 6A (a-e). (r, s) Image of distal (r) and proximal (s) portions of the crush injury in the cell transplantation group shown in Fig. 6A (f). (t) Image of the sham operation group shown in Fig. 6A (g).

6B). The proportion of interspace to standardized area was smaller in the cell transplantation group ($7.04\% \pm 3.34\%$) than in the crushed group ($17.99\% \pm 1.65\%$; $P < 0.05$; Fig. 7a), whereas the mean number of vacuoles was lower in the cell transplantation group (177.0 ± 13.06) than in the crushed group (319.33 ± 20.24 ; $P < 0.05$; Fig. 7b).

H-E staining revealed that the total myelin area and myelin area per axon were greater in DPC-transplanted nerves than in non-transplanted nerves. We therefore used LFB staining to compare remyelination in the crushed and cell transplantation groups (Fig. 8a-h). The LFB-positive area was larger in the cell transplantation

group ($42.29\% \pm 5.48\%$) than in the crushed group ($0.82\% \pm 1.11\%$) on postoperative day 14 ($P < 0.05$; Fig. 8i). In addition, the proximal and distal areas differed in the cell transplantation group (Fig. 8e, f). An increase in myelination and decrease in the number of vacuoles were positively correlated with the integrity of the nerve tissue, which reflects the extent of nerve regeneration (36).

Immunohistochemical analysis

We speculated that DPC transplantation increased the area of LFB staining as a result of axon guidance promoted by Schwann cells. Because MBP is a major constituent of the myelin sheath produced by Schwann

Fig. 7 Interspace area and number of vacuoles in the rat model of sciatic nerve crush injury. (a, b) The interspace area relative to the standardized field was smaller (a) and the number of vacuoles was lower (b) in the cell transplantation group than in the crushed group ($P < 0.05$).

Fig. 8 Luxol Fast Blue (LFB) staining of the excised sciatic nerve on postoperative day 14. (a-c) Sciatic nerve in the crushed group. (b, c) Distal (b) and proximal (c) portions of the crushed nerve in the crushed group. (d-f) Sciatic nerve in the cell transplantation group. (e, f) Distal (e) and proximal (f) portions of the crushed nerve in the cell transplantation group. (g, h) Low- (g) and high-magnification (h) views of the sciatic nerve in the sham operation group. (i) The LFB-positive area relative to the standardized field was larger in the cell transplantation group than in the crushed group ($P < 0.05$).

cells in the peripheral nervous system, we examined MBP expression by immunohistochemistry (Fig. 9a-h). MBP immunoreactivity was increased in DPC-transplanted nerves ($37.68\% \pm 1.47\%$) compared with non-transplanted nerves ($15.73\% \pm 2.08\%$) on postoperative day 14, as reflected by the larger anti-MBP-positive area ($P < 0.05$; Fig. 9i). The observed difference between the proximal and distal areas was consistent with the results obtained by LFB staining (Fig. 9e, f). Immunohistochemical detection of human mitochondria 14 days after transplantation revealed that human DPCs were present around or in the sciatic nerve in the cell transplantation group (Fig. 10a-d).

Fig. 9 Myelin basic protein (MBP) expression in the excised sciatic nerve on postoperative day 14. (a-h) Sciatic nerve in the crushed (a-c), cell transplantation (d-f), and sham operation groups (g, h). (b, c, e, f) Distal (b, e) and proximal (c, f) portions of the sciatic nerve in the crushed (b, c) and cell transplantation (e, f) groups. (g, h) Low- (g) and high-magnification (h) views of the sciatic nerve in the sham operation group. (i) The MBP-positive area relative to the standardized field was larger in the cell transplantation group than in the crushed group ($P < 0.05$).

Fig. 10 Detection of human mitochondria in the excised sciatic nerve 14 days after DPC transplantation. (a, c) Human mitochondria-positive cells were present in the sciatic nerve (arrowheads). (b, d) Negative control section incubated with 2.5% normal horse serum instead of anti-human mitochondria antibody.

Discussion

Following crush injury, axons in the distal segment of the peripheral nerve undergo Wallerian degeneration. Functional recovery is possible but limited; cell-based therapy can enhance nerve regeneration. Under normal conditions, nerve fiber healing can take a few weeks or months (37-41); accordingly, we found that although healing was incomplete on day 14, complete nerve regeneration occurred by 30 days after the injury without cell transplantation.

Gait measured using TS is the most important index of sciatic nerve function (42). We observed a decrease in TSF, ITSF, and PLF following DPC transplantation,

demonstrating that motor nerve regeneration led to reinnervation of the target muscles. This was confirmed by the increase in the wet weight of the target muscle in the cell transplantation group, because the number of nerve fibers is positively correlated with muscle weight (43).

Transplanted MSCs produce trophic factors that promote remyelination (44-46). We found that DPCs transplanted into crushed peripheral nerves enhanced myelin formation in the regenerating nerve. In addition, the total interspace area and total number of vacuoles in the standardized space were lesser in the cell transplantation group than in the crushed group, indicating greater nerve regeneration in the crushed group. Interestingly, in the cell transplantation group, the difference in the interspace area was clearly observed in the distal but not in the proximal portion of the injured nerve because the nerve structure remained relatively intact in the proximal region. In peripheral nerve regeneration, axon extension is initiated from the proximal stump after remyelination, with regenerating axons penetrating through the myelin column formed by Schwann cells. Thus, peripheral nerve remyelination requires a longer time in the regeneration process. In fact, previous studies have shown that remyelination begins only 3 weeks after peripheral nerve crush injury (10). We speculate that the distal portion of the crushed peripheral nerve will be completely regenerated in the cell transplantation group after a period of >2 weeks.

Remyelination is critical in peripheral nerve regeneration because crushed peripheral nerves represent an axonotmesis-type injury in which the basal membrane of Schwann cells is preserved (47). We examined the effects of DPC transplantation on remyelination with LFB staining and immunohistochemical detection of MBP. LFB- and MBP-positive areas were larger in the cell transplantation group than in the crushed group, suggesting that in the crushed nerve, DPCs promoted peripheral nerve regeneration by stimulating Schwann cell differentiation. Indeed, Schwann cell transplantation is the preferred method for enhancing peripheral nerve regeneration after crush injury (48,49), although its use in the clinical practice remains controversial because of the difficulty of isolating Schwann cells (50,51). Our results demonstrated that DPCs are an alternative source of Schwann cells.

DPCs promote peripheral nerve regeneration through multiple mechanisms, including direct differentiation into Schwann cells and secretion of trophic factors (52). In fact, it has been reported that DPCs have neuronal differentiation potential (7-15,53). However, the differentiation of transplanted DPCs into Schwann cells has

not been detected in transected sciatic nerve models (54). On the other hand, DPCs have been shown to exert paracrine neurotrophic effects in injured peripheral nerves (7-14,44-46,55). Brain-derived neurotrophic factor, nerve growth factor, and glial cell line-derived neurotrophic factor play important roles in neuronal survival and axon regeneration and are necessary for inducing Schwann cell differentiation (56-61). However, further study is needed to identify the factors that mediate the nerve regeneration effects of DPCs.

To date, only one report has demonstrated the therapeutic potential of human DPCs in an *in vivo* model of sciatic nerve crush injury. In our study, we investigated whether transplanted DPCs could be incorporated into regenerated nerve tissue. Two weeks after transplantation, cells positive for human mitochondria were found at the transplanted sites. Thus, transplanted DPCs may have secreted neurotrophic factors to promote axonal growth into the distal stump within this time frame.

Overall, our results proved that DPCs can enhance peripheral nerve regeneration based on the correlation observed between morphological findings and functional outcomes following DPC transplantation. However, cell-based therapies for peripheral nerve repair have yet to be applied in the clinical setting, which is associated with other unique challenges.

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Conflict of interest

The authors declare no conflict of interest.

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