# Transplantation of Human iPS Cell-Derived Neural Cells with an Artificial Nerve Conduit Leads to Cellular Retention in the Transplanted Area and Improves Motor Function in a Mouse Spinal Cord Injury Model

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**Abstract**

Spinal cord injury (SCI) causes motor dysfunction. Induced pluripotent cells (iPSCs) are becoming a new source for cells that can be used in transplantation therapy without concern for ethical issues or immune rejection, but an optimally effective clinical strategy for transplantation remains to be developed. Human iPSCs (hiPSCs) satisfy the requirement for grafted cells, and Nerbridge, a conduit made of polyglycolic acid (PGA) and collagen, has been used as a scaffold for grafted cells and employed clinically for regeneration of peripheral nerves. This scaffold may be applicable not only for peripheral nerve injury but also for central nervous system injury. We conducted an experimental study in which we combined hiPSCs with Nerbridge and transplanted the cells into a murine SCI model, which we created by complete transection at Th11. Cells were transplanted into the injury site. Transplantation of neural stem/progenitor cells (NSPCs) with the nerve conduit improved motor function more effectively than did transplantation of a single cell suspension of NSPCs or implantation of a nerve conduit without NSPCs. Histologic analyses revealed a high percentage of transplanted cells expressing human nuclear antigen and a high percentage of neurofilament M-positive axons at the site of injury. Our results suggest that the combined application of NSPCs and a nerve conduit has potential as treatment for SCI.

## Introduction

Spinal cord injury (SCI) leads to the loss of voluntary motor function and incomplete recovery[1,2]. Neural stem/progenitor cell (NSPC) replacement therapy has the potential to treat irreversible damage to and loss of spinal neurons. Cell replacement therapy was demonstrated to be beneficial for the recovery of patients with SCI using several embryonic stem (ES) cells or induced pluripotent stem cells (iPSCs) in the experimental trials, and autologous bone marrow stem cells and mesenchymal stem cells in the clinical trials[3–12].

It may be possible to transplant NSPCs to replace injured neurons, restore disrupted neural circuits, and re-myelinate axons in patients with SCI. iPSCs are becoming a new source for cells that can be used in transplantation therapy without concern for ethical issues or immune rejection; "patient-specific" iPSCs can be established from a patient's somatic cells. Like human ES cells, iPSCs have the ability to proliferate, self-renew, and differentiate[13].

We have succeeded in generating, from ES cells and human iPSCs (hiPSCs), NSPCs that can differen-

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tiate into cells with a spinal motoneuron phenotype[14–19]. Expression of neuron markers, neurofilament medium (NFM) and βIII tubulin, and of spinal motoneuron marker HB9 was increased by stimulation with retinoic acid (RA), noggin (NOG), and sonic hedgehog protein (SHH)[16,18,19]. Furthermore, transplantation of hiPSC-derived NSPCs into a murine SCI model improved motor function[16]. However, a severely damaged spinal cord may become an environment unsuitable for the survival of transplanted NSPCs. Low graft survival rates have been reported following direct therapeutic injection of neural cells[8,20].

Implantation of a nerve conduit has the potential to connect the completely transected spinal cord by surrounding and protecting transplanted NSPCs. Tissue engineering involving development of two- or three-dimensional scaffolds made up of stem cells or biomaterials has been applied in animal models of various types of tissue damage and also clinically[14,17,21–27]. Many types of artificial nerve conduits have been designed and used as alternatives to autologous nerve grafts for peripheral nerve injuries[23,28,29].

Nerbridge (Toyobo, Osaka, Japan) is a polyglycolic acid (PGA) conduit that is filled with collagen fibers and being used clinically in Japan for regeneration of peripheral nerves. PGA is said to have properties that allows it to act as a scaffold for neuronal process outgrowth[26,30–32]. This conduit is further reported to serve as a crosslinked material for nerve defects, to connect transected peripheral nerves, and to prevent scar tissue from infiltrating into the lumen of the neural conduit. It is bioabsorbable[32,33]. Due to these features, this nerve conduit may be applicable as a scaffold for central nervous system (CNS) regeneration.

In this study, we showed that transplantation of NSPCs with Nerbridge nerve conduit improved motor function in a murine SCI model more efficiently than did transplantation of NSPCs without the nerve conduit. A high graft survival rate appeared to lead to effective cellular connection and increased axon outgrowth. These results suggest that the combined application of NSPCs and a nerve conduit will be useful in the treatment of patients with SCI.

## Materials and Methods

### Induction of neural differentiation of hiPSCs

We transplanted NSPCs derived from hiPSCs into a murine SCI model. The model itself is described below, and timelines showing both NSPC devel-opment and transplantation into the model are shown in **Figure 1**. We developed NSPCs from hiPSCs, as reported previously[14,16]. The hiPSC line 253G1 was obtained from RIKEN BRC (Ibaraki, Japan, RRID: CVCL_B518, http://cellbank.brc.riken.jp/cell_bank/CellInfo/?cellNo=HPS0002) and maintained according to the standard protocol[34]. In brief, embryoid body (EB) formation began on Day −8. Free-floating EB aggregates developed from undifferentiated hiPSCs in 4 days. The cells were then cultured in fibronectin (FN, BD Biosciences, San Diego, CA, USA)-coated dishes for 4 days. We introduced 1 μM retinoic acid (RA, Sigma-Aldrich, St. Louis, MO, USA), 10 nM noggin (NOG)-Fc (R&D Systems, Minneapolis, MN, USA), and 10 nM Sonic hedgehog protein (SHH, R&D Systems) into the culture twice (on Days −3 and −1)[14,16,35–38]. In preparation, the cells were disaggregated into single-cell suspensions and transplanted on Day 0. The suspensions were confirmed to contain less than 0.01% feeder cells.

To evaluate the influence of the conduit on neural cell differentiation, we ran a separate experiment in which we injected NSPCs ($5 \times 10^5$ cells) into Nerbridge conduit (2.0 mm in diameter, 3.0 mm in length, and kindly provided by the manufacturer). We used it as a scaffold for the NSPCs. Cell suspensions (100,000 cells) were seeded into the conduit and cultured in FN-coated wells (12 mm in diameter) of 48-well culture plates for 7 days. The medium was DMEM/F12 with N2 supplement (ThermoFisher Scientific, Waltham, MA, USA) and 0.001% FN without mitogens or growth factors. In an *in vitro* cell culture with the nerve conduit, cultured NSPCs were washed in phosphate-buffered saline (PBS), then fixed in 4% paraformaldehyde (Merck, Darmstadt, Germany) for 15 minutes. Cells and cryostat sections were blocked for 2 hours in PBSTG (PBS containing 0.2% Tween 20 [Kanto Chemical, Tokyo, Japan] and 5% goat serum [ThermoFisher Scientific]). Immunofluorescence staining with antibody to βIII tubulin (G712A, Promega, Madison, WI, USA) was then performed, as previously reported[14–17]. Cells in the conduit were examined morphologically, and immunofluorescence was examined with a stereomicroscope and confocal microscope (LSM-510, Zeiss, Oberkochen, Germany).

### Creation of the murine SCI model

For generation of the murine SCI model, we used 8- to 10-week-old female wild-type mice bred on a C57BL/6J background. The mice were obtained from Japan SLC, Shizuoka, Japan. Female mice were
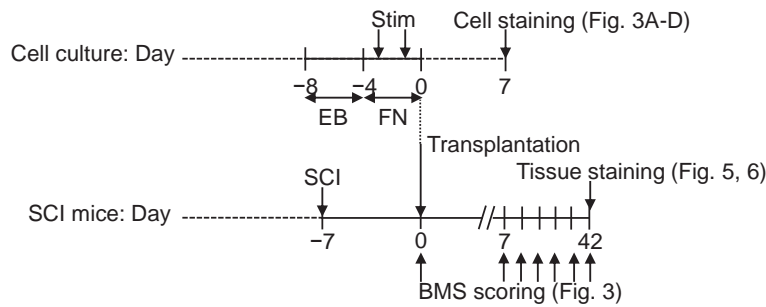
**Figure 1.** Timelines showing induction of neural differentiation of human in-
duced pluripotent Stem cells (hiPSCs) and their transplantation into a
murine spinal cord injury (SCI) model.

Undifferentiated hiPSCs were maintained in growth medium on a
feeder layer consisting of mouse embryonic fibroblasts. On Day −8,
undifferentiated hiPSCs dissociated from dishes for embryoid body
(EB) formation in the 4-day floating culture. Cells were then cultured
in fibronectin (FN)-coated dishes for 4 days. EBs were stimulated
(Stim) with retinoic acid (RA), noggin (NOG), and sonic hedgehog
protein (SHH) on Days −3 and −1. Transplantation of NSPCs into the
SCI model was performed on Day 0. Spinal laminectomy was per-
formed at Th11 on Day −7. Seven days later, the SCI mice were trans-
planted with NSPCs with the artificial nerve conduit (the conduit + cell
group), neural cells only (the cell group), and artificial nerve conduit
only (the conduit group). A group of SCI mice was injected with PBS
(the PBS group). Motor function was evaluated by means of the open-
field Basso Mouse Scale (BMS) on Day 1 after SCI and once a week
thereafter. Seven weeks after SCI, spinal cord tissues were obtained
from the mice for histologic analysis.

used because we have previously found survival of
male mice to be poor due to severe bladder and rectal
disturbance resulting from the SCI. The mice were
anesthetized by intraperitoneal injection of 4.0 mg/kg
midazolam (Sandoz, Yamagata, Japan), 0.3 mg/kg
medetomidine hydrochloride (Meiji, Tokyo, Japan),
and 5.0 mg/kg butorphanol tartrate (Meiji). Spinal
laminectomy at Th11 was then performed (**Figure 2**).
We first slit the dura mater and exposed the spinal
cord and then transected the spinal cord completely
using No. 11 microscissors. We probed the vertebral
cavity several times with a fine spatula to ensure
complete spinal cord transection. After transection,
the muscle layers over the laminectomy site and the
skin on the back were sutured[14,16]. Daily rehabilita-
tion and lower abdominal massage were performed to
encourage urination and defecation because complete
transection of the spinal cord generally results in
bladder and rectal dysfunction.

All experimental procedures were performed in
accordance with the Guide for the Care and Use of
Laboratory Animals, 8th edition (National Research
Council) and were approved by the local Animal
Care Committee (Animal Care and Use Committee,
St. Marianna University School of Medicine).

### Division of mice into groups and various treat-
### ments applied

After transection, mice were randomly assigned
to one of four treatment groups. Treatments were as
follows: transplantation of NSPCs with Nerbridge
conduit (the cell + conduit group, n = 19); transplan-
tation of NSPCs without conduit (the cell group, n =
34); transplantation of conduit without NSPCs (the
conduit group, n = 18); and injection of phosphate-
buffered saline (PBS) only (the PBS group, n = 23).
Treatment was performed 7 days after SCI (Day
0)[24,25]. Before Nerbridge implantation, the conduit
was immersed in PBS for 3 minutes and then injected
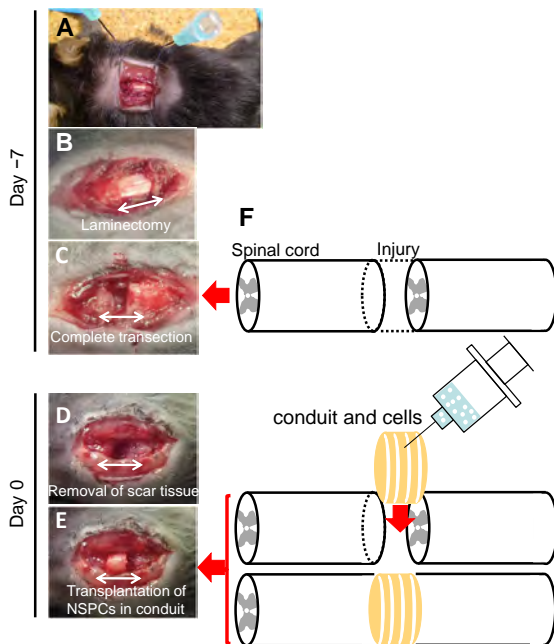with the NSPC suspension ($5 \times 10^5$ cells/2 μL PBS

**Figure 2.** Representative photographs obtained intraoperatively and schematic representation of NSPC transplantation with the nerve conduit in the murine SCI model.

(**A, B**) The spinal cord was exposed and laminectomy was performed at Th11. (**C**) The spinal cord was then completely transected. (**D**) For placement of the conduit, a cavity was made in the spinal cord by reinjuring the transected site. (**E**) NSPCs were transplanted with an artificial nerve conduit on Day 0.

(**F**) Diagram illustrates the site of transection and transplantation of cells with conduit.

(for the cell + conduit group) or with 2 µl PBS (for the conduit group).

The conduits were placed without sutures at the site of complete transection, as previously reported[39,40]. We transplanted NSPCs ($5 \times 10^5$ cells/2 µl PBS) and injected 2 µL PBS at the sites of complete transection in the cell group and the PBS group, respectively. We then covered the sites with a thermoresponsive gelation polymer (TGP, Mebiol, Tokyo, Japan). After treatment, the muscle layer and epidermis on the backs of all mice were sutured. The spinal cord itself was not sutured, but we confirmed that the transplanted cells had been retained. To prevent rejection, 10 mg/kg of cyclosporine (Novartis, Basel, Swiss/Novartis Pharmaceuticals Tokyo, Japan) and 0.2 mg/kg of dexamethasone (Sigma-Aldrich) were administered to the mice 1 hour before transplantation. Mice in each of the four groups were given 10 mg/kg of cyclosporine once daily, beginning on the day after transplantation and continuing until the time of sacrifice.

Also included in the study was a group of female C57BL/6J mice in which SCI was not produced (the intact normal mice group, n = 7).

### Analysis of motor function

Motor function of mice in each of the groups was evaluated according to the open-field Basso Mouse Scale (BMS)[14,16,41], which was originally developed on the basis of frequently observed mouse locomotor activity. BMS scores for hindlimb locomotion range from 0 (no movement) to 9 (normal movement). We determined BMS scores on the day preceding SCI, on the day after SCI, and once a week thereafter up to Day 42 (7 weeks after the injury).

### Immunohistochemical staining

The mice were killed on Day 42 (7 weeks after SCI), then perfused with 4% paraformaldehyde (PFA)/PBS. Fixed spinal cords were cut into 30-µm-thick slices with a cryostat (CM 1850, Leica Biosystems, Nussloch, Germany). The slices were stained with hematoxylin and eosin (Wako, Osaka, Japan) so that we could determine the location and morphologic features of the cells. Immunohistochemical analysis was performed as previously reported[14–17].

Slices were mounted on glass slides and incubated with antibodies to NFM (AB1987, Millipore, Billerica, MA, USA) and human nuclear antigen (hNuc, Anti-Nuclei Antibody, MAB1281, Sigma-Aldrich) at 4°C overnight. For control, the adjacent slices were incubated without primary antibodies. All slices were then stained with an Alexa 488- or Alexa 594-conjugated secondary antibody. Slices were examined under a confocal microscope. Regardless of the type of treatment performed, we counted at least 200 cells to determine the number (and thus the percentage) of cells expressing NFM and hNuc. This was done at least three times, and results are expressed as the mean and standard error of the mean (SEM).

### Flexion injury evaluation

Photographs of the spinal cord of each mouse were taken during the operative procedure, and sagittal spine curvature was measured on photographs obtained just after dissection. ImageJ was used for these

measurments[42].

### *Statistical analyses*

Data are shown as mean ± SEM or median values. Between-group differences were analyzed by Steel-Dwass test. All statistical analyses were performed with JMP 8.0.2 (SAS, Cary, NC, USA), and p < 0.05 was considered significant.

## Results

### *Differentiation of neural precursor cells into βIII tubulin-positive neural cells in conduit*

Conduits harvested after the 7-day culture *in vitro* are shown in **Figure 3**. On stereomicroscopic examination, NSPCs were observed in but not outside the conduit (**Figure 3A**). On examination of conduits with NSPCs, however, the NSPCs were found to express βIII tubulin (**Figure 3C, D**).

### *Improved motor function of SCI mice by transplantation of NSPCs with a conduit*

In comparing BMS scores obtained at 6 weeks, we found scores to be significantly higher in the cell + conduit group than in the other three groups (2.79 ± 0.20 in the cell + conduit group vs, 1.68 ± 0.14 in the cell group, 1.11 ± 0.14 in the conduit group, and 0.7 ± 0.10 in the PBS group; p < 0.0001 for each comparison). The score was significantly higher in the cell group than in the PBS group (p < 0.0001). The other combinations with no significant difference

were the cell group vs the conduit group (p = 0.11) and the conduit group vs the PBS group (p = 0.15) (**Figure 4**).

### *Engraftment of NSPCs and nerve fiber density*

Immunohistochemical analysis performed on Day 42 (7 weeks after SCI) revealed the following. The spinal cord tissues were found to express NFM in a high percentage of cells in the intact mice group (84.7 ± 3.9%) and in the cell + conduit group (51.9 ± 6.3%) (**Figure 5A, E, F**). In comparison to the percentage of NFM-expressing cells in the intact mice group, percentages of such cells in the cell group (26.4± 3.7%), the conduit group (2.83 ± 0.66%), and the PBS group (0.60 ± 0.40%) were significantly lower (p = 0.020, p = 0.041, and p = 0.046, respectively) (**Figure 5B–D, F**. In comparison to the percentage of such cells in the injured area in the cell + conduit group, percentages in the cell group, the conduit group, and the PBS group were significantly lower (p = 0.037, p = 0.028, and p = 0.048, respectively), and in comparison to the percentage in the cell group, percentages were significantly lower in the conduit and the PBS group (p = 0.020 and p = 0.033, respectively). The other combinations with no significant difference were the cell + conduit group vs the intact normal mice group (p = 0.063) and the conduit group vs the PBS group (p = 0.24) (**Figure 5F**).

In evaluating hNuc expression at the site of in-
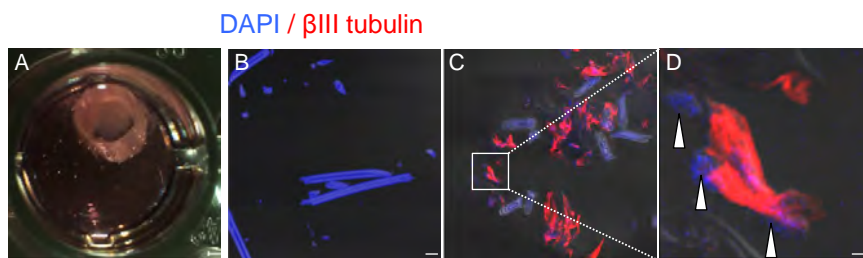
DAPI / βIII tubulin



**Figure 3.**    Representative photomicrographs of differentiation of NSPCs into neural cells in the nerve conduit.
Human iPS cells were differentiated into NSPCs, and $1×10^5$ of these cells were cultured in the artificial nerve conduit for 1 week. (**A**) Stereomicroscopic view of a nerve conduit in which NSPCs were cultured in one well (diameter of 12 mm) of a 48-well culture plate. Nerve conduits with/without NSPCs were stained with anti-βIII tubulin (red) and DAPI (blue) and observed under a confocal microscope. (**B**) Nerve conduit without NSPCs showed a strong blue autofluorescence signal due to their materials, but not red autofluorescence signal. Scale bar = 20 μm. (**C, D**) Conduit with NSPCs expressed βIII tubulin. Panel **D** is a magnified view of the inset of panel **C**. White arrowheads indicate DAPI-positive nuclei. Scale bar = 5 μm
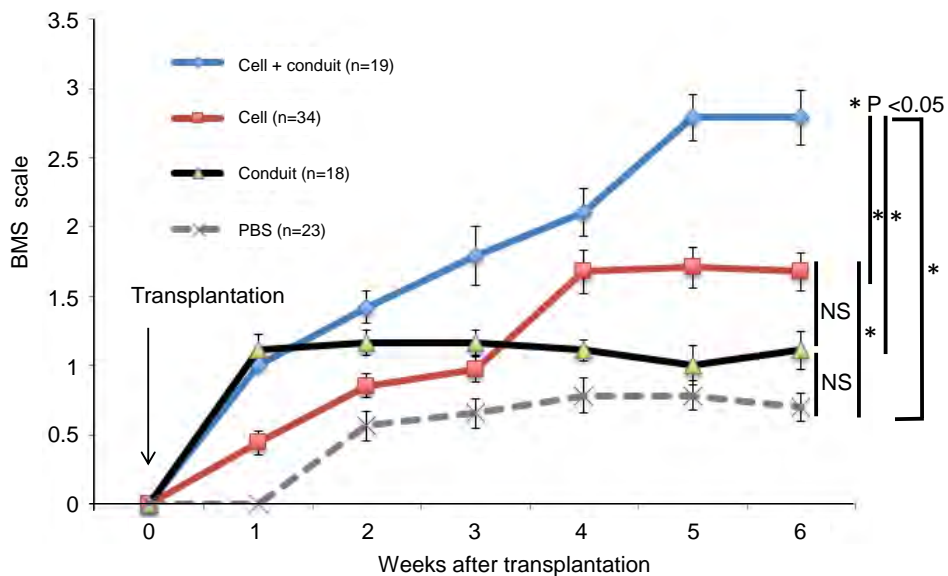
**Figure 4.**   Time course of BMS scores in the SCI mice after NSPC transplantation.
Mean ± SEM scores are shown for each group.

jury, we found hNuc expression in 55.8 ± 7.4% of cells in the cell + conduit group, in 29.3± 3.3% of cells in the cell group, in 1.23 ± 0.50% in the conduit group, and in 0.40 ± 0.24% in the PBS group; hNuc was expressed in 0.11 ± 0.1% of cells at the corresponding site in the intact mice group (**Figure 5A–E**). Percentages differed significantly between the cell + conduit group and the cell group, the conduit group, the PBS group, and the intact mice group (at p = 0.037, p = 0.028, p = 0.043, and p = 0.027, respectively). Percentages differed significantly between the cell group and the conduit group, the PBS group, and the intact mice group (at p = 0.020, p = 0.033, and p = 0.019, respectively). Percentages did not differ significantly between the conduit group and the PBS group (p = 0. 63), the conduit group and the intact mice group (p = 0.090), or the PBS group and the intact mice group (p = 0.99) (**Figure 5G**).

Hematoxylin and eosin staining of spinal cord tissues revealed that cell density at the site of transection was greater and that scar tissue was reduced in the cell + conduit group (**Figure 6D**) in comparison to those in the cell group and in the PBS group (**Figure 6B, C**).

The flexion angle was significantly smaller in the PBS group (median = 93.2°), the cell group (120°) and conduit group (112°) than that in the intact normal mice group (141°) (p = 0.006, p = 0.03 and p = 0.028, respectively) (**Figure 6E1, F1, G**).

Transplantation ameliorated flexion injury significantly more in the cell + conduit group (126°) and the cell group than in the PBS group (p = 0.002 and p = 0.037, respectively) (**Figure 6E2, F2, G**). No difference in flexion angle was found between the cell + conduit group and the cell group (p = 0.48). The other combinations with no significant difference were the cell + conduit group vs the intact normal mice group (p = 0.57), the cell + conduit group vs conduit group (p = 0.16), the cell group vs the conduit group (p = 0.97), and the conduit group vs the PBS group (p = 0.08).

## Discussion

Development of novel cell-based and scaffold-based strategies for treatment of SCI is ongoing. Such approaches have been reported to have therapeutic potential for severe SCI and use of nerve conduits has been reported as a therapeutic alternative to use of autologous nerve grafts for various peripheral nerve injuries[32,33]. Previous studies have also shown transplantation of nerve conduits to be as effective as autologous nerve transplantation for repair of injured peripheral nerves in mice, and combined use of nerve conduits and iPSCs has been shown to enhance axonal regeneration and myelination without teratoma formation[26,30–32]. Although embedding conduit has yielded satisfactory results in patients with digital nerve injury, the optimum means of transplanting
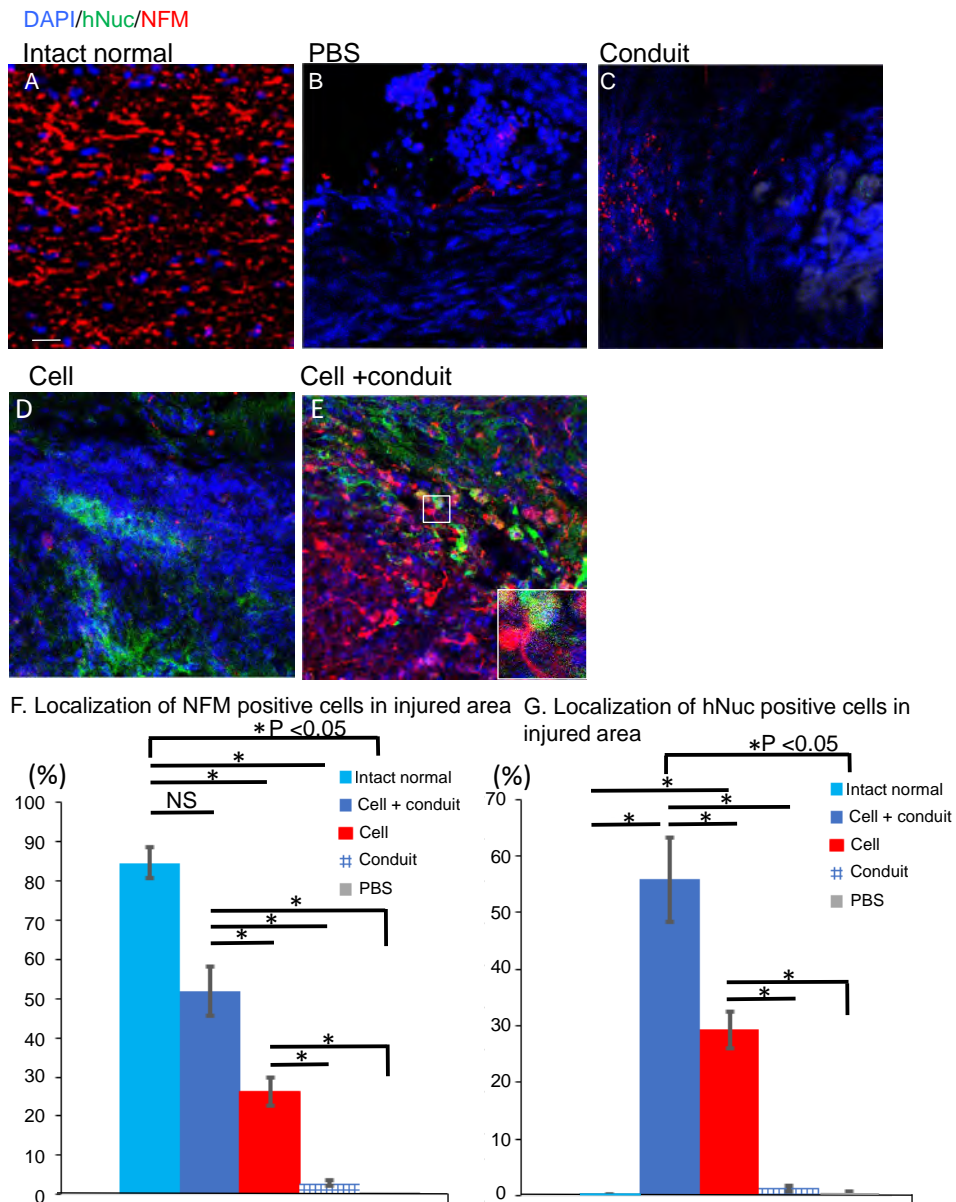
**Figure 5.**  NSPCs transplanted with the nerve conduit located in the injured area.
(**A–E**) Immunohistochemistry of the spinal cord at the transplantation site 6 weeks after the injury. Transplanted NSPCs were stained with anti- hNuc antigen (green), DAPI (blue), and anti-NFM antibody (red). Representative images obtained from the (**A**) intact spinal cord group, (**B**) the PBS group, (**C**) the conduit group, (**D**) the cell group, and (**E**) the cell + conduit group. Spinal cord tissues in the cell + conduit group expressed NFM to a similar extent as the intact normal mice group. (**F**) Percentages (mean ± SEM, n = 6) of NFM-expressing cells to total nucleated cells (DAPI-positive cells) in the injured area, per group. (**G**) Percentages (mean ± SEM, n = 6) of hNuc-expressing cells to total nucleated cells in the injured area, per group.
We counted cells in 0.2-mm$^2$ fields, which is equivalent to the area of panels **A–E**. Scale bar indicates 20 μm in **A** and 5 μm in the inset of **E**.
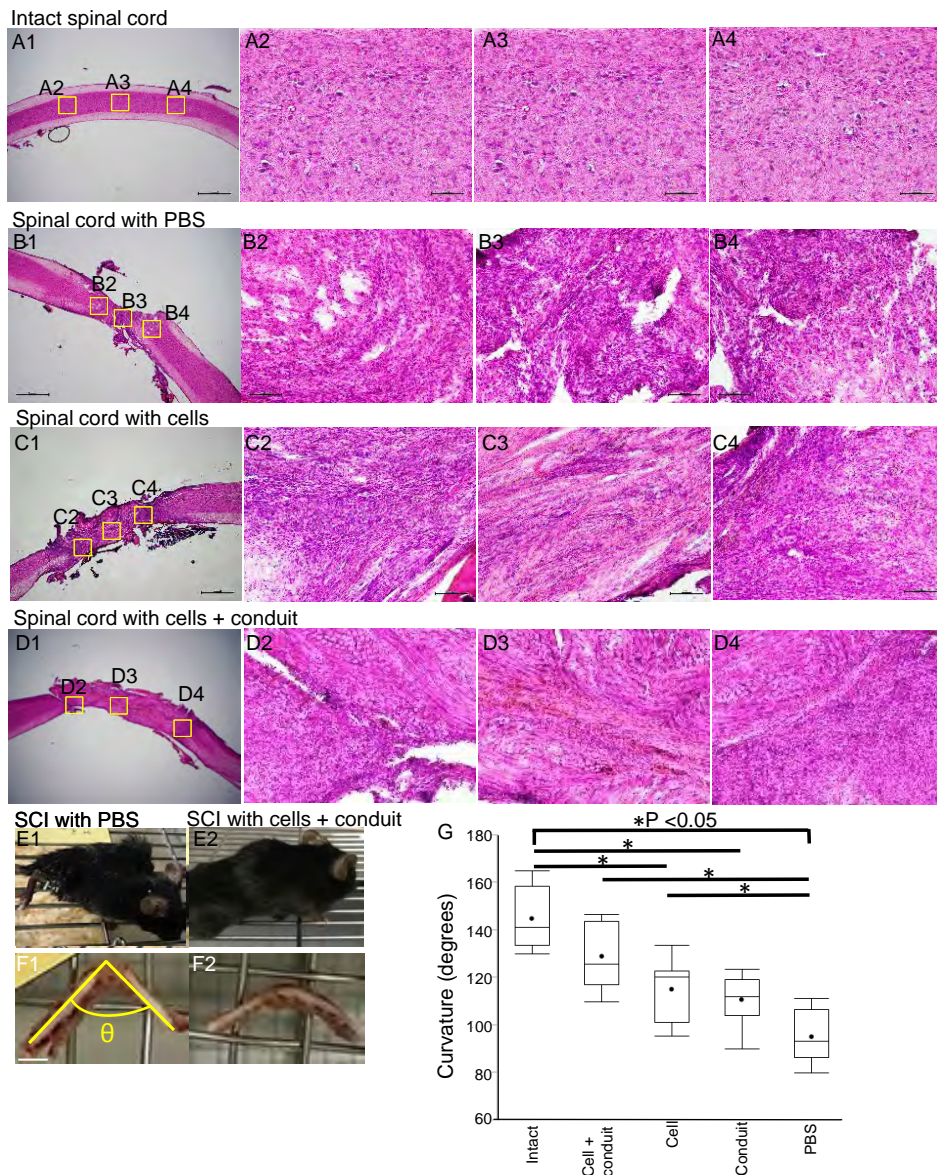
**Figure 6.**  NSPC transplantation with the nerve conduit reduced SCI lesions.

(**A–D**) The transplanted site of the spinal cord was examined by hematoxylin and eosin staining in mice having the (**A1–4**) intact spinal cord and in those belonging to (**B1–4**), the PBS group, (**C1–4**), cell group, and (**D1–4**), cell + conduit group.

(**E–F**) Macroscopic views of (**E**) SCI model mice and (**F**) spinal cords from the model. (**E1, F1**), the PBS group mouse. (**E2, F2**), the cell + conduit group mouse. We evaluated flexion injury in the 4 groups and intact normal mice group. Spinal cords just after dissection were subjected to measurements of the degree of spine curvature θ, and (**G**) collected data were summarized in the graph. Data for each group were compiled to draw a box plot showing the minimum, first quartile, median, third quartile, and maximum values, which correspond to the lower whisker, lower side of box, middle bar, upper side of the box, and upper whisker, respectively. The dot in each box indicates the mean value.

Scale bar in **A1–D1** and **F1** indicates 5 mm for panels of the first column; scale bar in **A2–D4** indicates 250 μm for the second through fourth columns.

stem cells, especially hiPS-derived NSPCs, for treatment of CNS injury needs to be established. Reported experimental transplantation of NSPCs derived from neural tissue (such as rat spinal cord or human fetal brain tissue) with PGA conduit for such CNS injury as SCI led to neural regeneration and remedied the SCI[39,40]. We consider the study described herein as a next-step study in which we applied the Nerbridge conduit, a clinically safe polymer scaffold, in combination with hiPSC-derived NSPCs, believing hiPSCs to be a safe and stable source of cells to be transplanted for treatment of SCI.

We have reported transplantation of mouse ES cell-derived neurons and hNSPCs, the latter being induced with RA, NOG-Fc, and SHH protein[14–16], and recovery from SCI was achieved[1,14–17]. The NSPCs differentiated into mature HB9-, Islet1-, LIM1-, and NFM-positive neurons[16]. In terms of motor function in the study reported herein, significantly higher BMS scores were observed in the cell + conduit group than in the other transplantation groups. Furthermore, our histopathological analyses showed neural cell density at the site of spinal cord transection to be greater in the cell + conduit group, than in the cell and the PBS groups; scar tissue formation was also reduced in this group. We found the high number of NFM-expressing cells in the injured area in the cell + conduit group as in the intact normal mice group.

In our previous *in vitro* experiments, hiPSCs differentiated into neural progenitor cells expressing mainly nestin[16,43] on the day of transplantation, and further incubation in conduits promoted differentiation into neurons, most of which expressed βIII tubulin, an early neuronal marker (**Figure 3C**). In our preliminary *in vitro* experiments, a low number of glial cells expressing glial fibrillary acidic protein (GFAP) or galactocerebroside (GalC) was found at a similar stage of the cell culture after βIII tubulin expression. It is conceivable that the transplanted nestin-positive cells should mainly differentiate into neurons.

In the transplantation experiment, the artificial conduit harbored many hNuc-positive cells, most of which expressed NFM. NFM is a marker of differentiated post-mitotic neuronal cells. Thus, transplanted NSPCs appeared to differentiate further into neuronal cells (**Figure 5**). The remaining cells in the injured area consisted of hNuc (+) / NFM (−) and hNuc (−) / NFM (−) cells, suggesting that they were undifferentiated transplanted cells or migrated host cells[44]. In our preliminary study, in the transplanted area, a few GFAP-expressing cells (astrocytes) and GalC-ex-

pressing cells (oligodendrocytes) were found. This differed from what was seen in the area of injury alone in mice belonging to the PBS group[16]. Glial scar formation is reported to be an obstacle to successful axonal regeneration. The glial reactivity can vary, and some types of glial cells are thought capable of conferring either beneficial or detrimental effects on axonal regeneration, depending on the injury condition[44]. In the experiment reported herein, we analyzed transplanted NSPCs that compensated for the neural connection lost by complete transection of the spinal cord. Further studies are needed to determine whether glial cells contribute to the anatomical and functional recovery conferred by transplantation.

The SCI that we inflicted on the mice caused flexion injury. The degree of flexion in the PBS group was significantly greater than that in the intact mice group, and spine curvature in the cell + conduit group was moderate, but significantly restored, in comparison to that in the PBS group (**Figure 6G**). In other words, transplantation of NSPCs seeded in Nerbridge ameliorated flexion injury (**Figure 6F, 6G**), which suggests that the combination of PGA and collagen fibers is applicable as a scaffold for NSPC transplantation in the treatment of SCI. Although we confirmed that the scaffold dissolved and was absorbed inside the lesion after approximately 3 months and that the transplanted cells remained there, long-term observation is needed to clarify whether the beneficial effect of such transplantation persists.

In summary, transplantation of hiPSC-derived NSPCs with the conduit, Nerbridge restored motor function and ameliorated flexion injury in our murine SCI model. Histological examination indicated that transplanted NSPCs should mainly differentiate into neuronal cells and survived better compared to NSPC transplantation without conduit. Further exploration is needed for a more precise understanding of the mechanisms explaining the neuronal differentiation process. The results of our study suggest that the combined application of NSPC and a nerve conduit will be useful in the treatment of patients with SCI.

## Conflicts of Interest

The authors have nothing to disclose.

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