

Spinal cord injury currently has no clinical treatment. One promising therapy is the transplantation of oligodendrocyte progenitor cells (OPCs) which can potentially remyelinate axons (1,2). We previously devised a protocol for directed differentiation of OPCs from mouse fibroblasts, via an induced pluripotent stem (iPS) cell intermediary. Here we show that such derived OPCs survive after transplantation in the injured rat spinal cord, and thus represent a new strategy for potential treatment of spinal cord injury.

IPS cells were generated from mouse fibroblasts expressing eGFP, using the Yamanaka method (3). These cells were subsequently cultured according to our protocol, established for generating OPCs from mouse iPS cells, as outlined in Figure 1. OPCs were characterized during the differentiation protocol via immunocytochemistry, RT-PCR marker analysis, and transplantation into dysmyelinated mouse brains, where their myelinating capacity was demonstrated (shown previously). Moderate spinal cord injury was induced in adult rats, using the NYU spinal cord impactor, and the OPCs were transplanted 9 days after the injury. The rats were sacrificed at two and three weeks following transplantation. Immunohistochemical studies were performed on the spinal cord tissue to look for the continued presence of transplanted cells.

Stage 1 IPS cells	A	DAY 0 Undifferentiated mouse IPS cells, with transmembranous GFP IPS cell media with LIF
Stage 2 Neuroglial Patterning	B	Embryoid body formation DAY 1 – 3 KSR media DAY 4 KSR media + RA DAY 5 KSR media + RA + Pur DAY 6 – 8 N2 media + RA + Pur
Stage 3 OPC Expansion	C	DAY 9 – 25 OPC expansion OPC media with PDGF-AA, SHH, FGF

OPCs were cultured to day 25 of the protocol, and then characterized by immunocytochemistry and RT-PCR, prior to being transplanted. Immunocytochemistry confirmed expression of OPC markers, such as Olig 1, Olig 2, PDGFR α , NG 2, Nestin, and A2B5, Figure 2. RT-PCR analysis demonstrated expression of genes characteristic of OPCs (Fig. 3B), absence of genes expressed in stem cells (Fig 3A), mature oligodendrocytes, and neurons (Fig. 3C). The percentage of cells in culture expressing OPC markers was greater than 80%. Such generated OPCs were injected into spinal cord of rats, 9 days after moderate spinal cord injury. Fluorescence microscopy demonstrated presence of eGFP positive cells in the injured rat spinal cord at two and three weeks following transplantation, Figure 4 - green:eGFP, red:Tuj 1. The eGFP signal was found to align with cells of the spinal cord. The transplanted cells migrated away from the injection sites, and developed elongated processes.

Figure 1 displays six panels of fluorescence microscopy images showing the expression of Olig2-GFP and various markers in the adult SVZ. The panels are arranged in a 3x2 grid. The left column shows Olig2-GFP (green) and the right column shows various markers (red) with DAPI (blue) nuclear staining. The markers are A2B5, NG2, PDGFR α , Nestin, and Olig1. The panels are labeled as follows: Top-left: Olig2 GFP; Top-right: Olig 2 A2B5; Middle-left: NG2 DAPI; Middle-right: PDGFR α DAPI; Bottom-left: Nestin DAPI; Bottom-right: Olig1 DAPI.

A)

Oct 4

Stage	Relative Expression
D0	0.38
D4	0.32
D8	0.02
D15	0.05
D20	0.08
D25	0.02
E14	0.02
3wk	0.02

Nanog

Stage	Relative Expression
D0	0.05
D4	0.02
D8	0.55
D15	0.05
D20	0.02
D25	0.02
E14	0.02
3wk	0.02

Nestin

Stage	Relative Expression
D0	0.01
D4	0.01
D8	0.01
D15	0.08
D20	0.15
D25	0.12
E14	0.28
3wk	0.02

Pax 6

Stage	Relative Expression
D0	0.002
D4	0.005
D8	0.008
D15	0.012
D20	0.015
D25	0.018
E14	0.018
3wk	0.005

B)

Olig 2

Stage	Relative Expression
D0	0.005
D4	0.005
D8	0.005
D15	0.015
D20	0.025
D25	0.035
E14	0.015
3wk	0.05

Olig 1

Stage	Relative Expression
D0	0.005
D4	0.005
D8	0.015
D15	0.09
D20	0.09
D25	0.05
E14	0.05
3wk	0.12

PDGFR α

Stage	Relative Expression
D0	0.01
D4	0.01
D8	0.01
D15	0.35
D20	0.55
D25	0.5
E14	0.15
3wk	0.1

C)

Tuj 1

Stage	Relative Expression
D0	0.05
D4	0.05
D8	0.05
D15	0.05
D20	0.05
D25	2.5
E14	3.5
3wk	3.8

Syn 1

Stage	Relative Expression
D0	0.05
D4	0.05
D8	0.05
D15	0.05
D20	0.05
D25	0.35
E14	0.95
3wk	0.95

CNP

Stage	Relative Expression
D0	0.01
D4	0.01
D8	0.01
D15	0.01
D20	0.02
D25	0.05
E14	0.05
3wk	0.4

MBP

Stage	Relative Expression
D0	0.005
D4	0.005
D8	0.005
D15	0.005
D20	0.005
D25	0.005
E14	0.005
3wk	0.05

This fluorescence micrograph displays a dense network of fibers within a tissue section. The fibers are stained with two different dyes, resulting in a complex pattern of green and red fluorescence against a dark background. The green staining highlights a broad, interconnected web of fibers, while the red staining appears as more distinct, elongated structures, possibly representing specific cell types or extracellular matrix components. The overall texture is highly fibrous and intricate.

OPCs derived from mouse iPS cells survive transplantation into the injured rat spinal cord. They demonstrate ability to migrate away from the injection sites, and develop processes that align with cells of the spinal cord, suggestive of their differentiation into oligodendrocytes. iPS cells represent a new source of OPCs that can be utilized as a model to study OPC effects on the environment of spinal cord injury. Future studies include further characterization of the transplanted cells via electron microscopy, and long term functional analysis of injured rats after OPC transplantation.