Int J Clin Exp Med 2016;9(9):17985-17994
www.ijcem.com /ISSN:1940-5901/IJCEM0031994

Original Article

Neural precursor cells generated from Induced pluripotent stem cells with gelatin sponge-electrospun PLGA/PEG nanofibers for spinal cord injury repair

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Received May 10, 2016; Accepted July 26, 2016; Epub September 15, 2016; Published September 30, 2016

Abstract: Objective: This study attempted to graft neural precursor cells (NPCs) differentiated from mouse induced pluripotent stem cells (iPSc) with gelatin sponge-electrospun Poly (lactic-co-glycolic acid)-Polyethylene glycol (PLGA/PEG) nanofibers into transected rat spinal cords and to investigate whether the tissue engineering scaffolds could promote motor functional recovery. Methods: iPSc were differentiated into NPCs and identified with immunofluorescence and patch clamp analysis. Transferring the NPCs on the gelatin sponge-electrospun PLGA/PEG or PLGA alone 3D scaffolds. Tissue engineering scaffolds were transplanted into transected rat spinal cords and motor functional recovery was observed. Results: iPSc-NPCs could survive, self-renew and differentiate into neurons within the scaffolds i*n vitro*. Electrospun PLGA/PEG nanofibers significantly promoted iPS-derived NPCs adhesion and proliferation as compared to PLGA. The iPS-derived NPCs have a better differentiation potential on the surface of the PLGA/PEG biomaterials. Furthermore, scaffolds combined with iPSc-NPCs treatment showed improvements in the functional recovery of the spinal cord after transection SCI. The PLGA/PEG group showed more significant promotions in motor functions than the PLGA alone and untreated control group when evaluated by the Basso-Beattie-Bresnahan (BBB) score. Conclusion: We demonstrated that neural precursor cells generated from induced pluripotent stem cells with gelatin sponge-electrospun PLGA/PEG nanofibers could promote functional recovery after spinal cord injury.

Keywords: Induced pluripotent stem cells, neural precursor cells, electrospun, PLGA/PEG, spinal cord injury

Introduction

Traumatic injuries to the nervous system, especially spinal cord injury (SCI), are considered to be among the common causes of disability and mortality worldwide [1]. During SCI, primary and secondary insults often contribute to neuronal death and axonal disconnection. Glial scars formation limits the recovery of the damaged neurons, resulting in the failure of severed axons regrowth [2, 3]. Tissue engineering scaffolds have recently emerged as a bridge for SCI repair, in particular, biodegradable nerve guidance conduits combining with stem cells can help to reconstruct spinal cord tissue and promote neural functional recovery [4, 5].

Recent study has indicated that neural precursor cells (NPCs) transplantation is a promising therapy for the treatment of SCI [6]. However, NPCs are usually derived from embryonic stem cells or extracted from nervous tissue, which causes ethical disputes and nervous system damage [7-9]. Furthermore, concerns regarding allogeneic immunological rejection after allotransplantation of NPCs have also been raised by scholars [10]. Induced pluripotent stem cells (iPSc), on the contrary, presenting minimal immunological rejection after auto transplantation and maximal neuronal differentiation potential without ethical issues, is regarded as an outstanding alternative to the above options and a significant breakthrough in regenerative medicine.

Poly lactic-co-glycolic acid (PLGA) has been broadly applied in nerve regeneration because of its biodegradability, biocompatibility and low

toxicity in the past decade [11, 12]. It is reported that employing PLGA scaffolds in the damaged site is an effective way to treat SCI [13]. Electrospun fibers made of natural polymers usually possess inferior mechanical properties. The unique pore features of the fibers such as the high bulk porosity, fully interconnected pore structures, and the wide-open surface promote nutrient and vapor transportation which are desirable for cell growth [14]. In addition, the high hydrophilic nature, low toxicity and immunogenicity, make the poly (ethylene glycol) (PEG) a promising tool for the biomedical applications when modified PLGA fiber [15].

This study was aimed to investigate the effects of iPS-derived NPCs with biofunctionalized gelatin sponge-electrospun PLGA/PEG nanofibers on the neural recovery after SCI.

Materials and methods

iPS cell culture and characterization

Feeder-dependent mouse iPS cells (purchased from the Guangzhou Institute of Biological Medicine and Health, Chinese Academy of Science) were routinely cultured at 37°C with 5% CO_2 in humidified atmosphere. They were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) supplemented with 15% fetal bovine serum (FBS; Gibco, USA), 1% L-glutamine (Gibco, USA), 1% nonessential amino acids (Invitrogen, USA), 1% β-mercaptoethanol (Gibco, USA), and 0.1% recombinant leukemia inhibitory factor (LIF; Millipore, Germany).

NPCs differentiated from mouse iPS cells

Robustly proliferating iPS cells were dissociated with 0.125% trypsin-EDTA (Gibco, USA) and then transferred to non-adherent culture dishes. They were cultured in medium without LIF for 4 days to promote embryonic body (EB) formation. EBs were then treated with medium (without LIF) containing 1 μmol/L all-trans retinoic acid (RA; Sigma, USA) for another 4 days. After that, the EBs were transferred to culture plates coated with 0.1% poly-lysine and cultured in Insulin-Transferrin-Selenium (ITS) medium supplemented with a 1:1 mixture of neurobasal medium and DMEM/F12, 2% B27 (Invitrogen, USA), 1% N_2 (Invitrogen, USA) and 1% insulin, transferrin, selenium, sodium pyruvate solution (ITS-A, Invitrogen, USA). Neural differentiation medium was changed every 2 days. After about 7 days, differentiated cells were located around adherent EBs and incubated with accutase solution (Gibco, USA) for 20 min, and then mechanically disrupted. These products were transferred to non-adherent culture dishes and cultured in DMEM/F12 supplemented with 1% L-glutamine, 20 ng/ml basic fibroblast growth factor (bFGF; PeproTech, USA) and 20 ng/ml epidermal growth factor (EGF; PeproTech, USA) for 7 days. The culture medium was changed every 3 or 4 days to promote the formation of neurospheres that were aggregations of NPCs [16].

Neurons and astrocytes derived from NPCs

In order to confirm the existence of NPCs differentiating from iPS cells, their capability of generating three major lineages of the nervous system, including neurons, astrocytes and oligodendrocytes was tested. Neurospheres were plated onto 0.1% poly-lysine-coated adherent culture dishes in differentiation medium consisting of DMEM/F12 supplemented with 5% FBS and 1 μmol/L all-trans RA. The cells were then consecutively cultured for 12-15 days.

Identification of iPS cells and their differentiated progenies

Targeted cells were fixed with 4% formaldehyde (Amresco, USA), blocked with 3% bovine serum albumin (Biosharp, China), and permeabilized with 0.1% Triton X-100 (Invitrogen, USA) before incubated with Nestin (1:1000; Abcam, Britain), MAP-2 (1:1000, Millipore, Germany), or rabbit anti-mouse antibodies Oct4 (1:1000), Sox2 (1:1000), SSEA1 (1:1000), GFAP (1:1000) and MBP (1:1000), which all purchased from Santa Cruz, (USA) at 4°C overnight. Then, the cells were incubated with a goat anti-rabbit IgG-FITC antibody (1:50; ABGENT, USA) or donkey anti-rabbit IgG-FITC antibody (1:400; Invitrogen, USA) at room temperature (21~24°C) for 1 hour. Nuclei were counterstained with Hoechst 33342. Stained cells were observed under a fluorescence microscope (Leica, Germany). Alkaline phosphatase (AP) color development kit (Shanghai, Beyotime Biotech Co., Ltd., China) was used to confirm the pluripotency of iPS cell colonies according to the manufacturer's protocol.

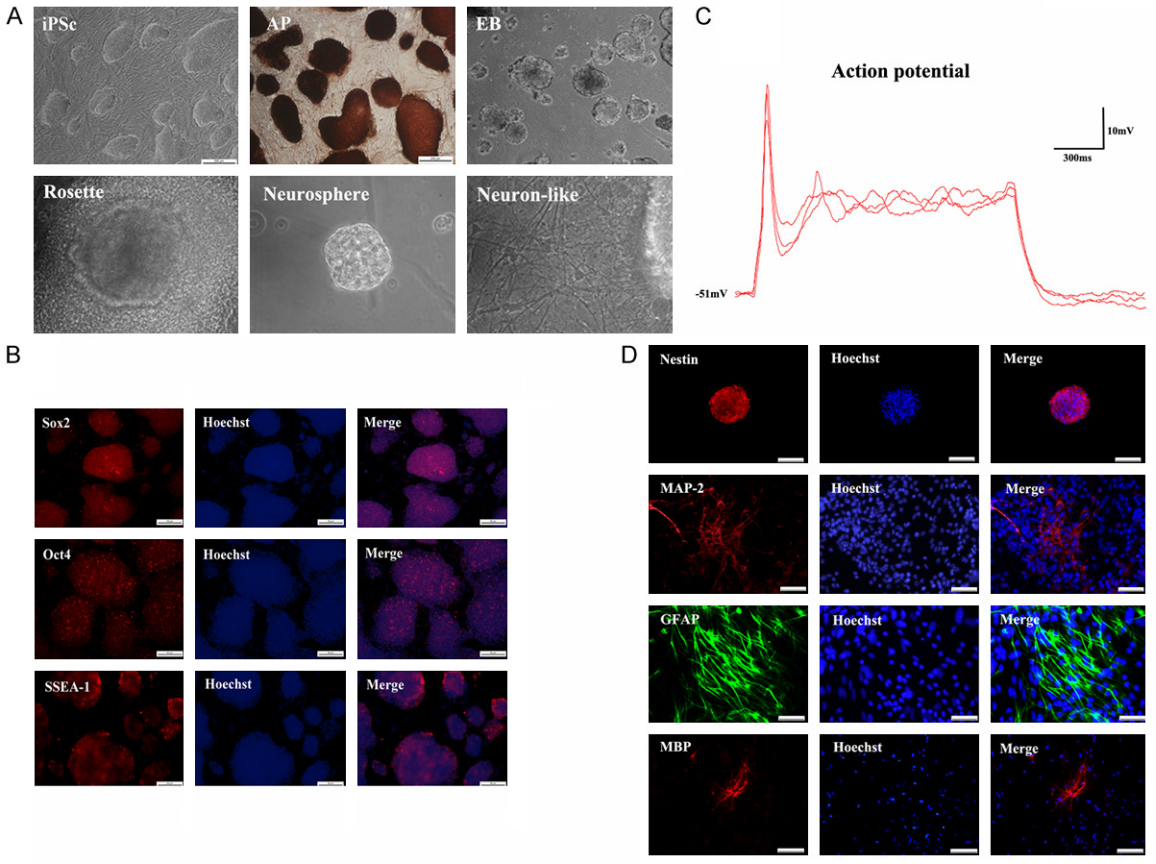

Figure 1. Identification of iPS cells and neural differentiation. Photomicrographs of mouse iPS cells and alkaline phosphatase staining. Pluripotent markers were assessed by immunofluorescence, including Sox2, Oct4, SSEA1. Embryonic body (EB), rosette structures, neurospheres and neuron-like cells were observed. Whole-cell patch-clamp recordings exhibited functional membrane properties and action potentials. Neurospheres, neurons, astrocytes and oligodendrocytes were assessed by immunofluorescence for Nestin, MAP2, GFAP and MBP. Scale bars =200 μm (iPSc, EB, Nestin, MBP & B) and 100 μm (AP, Neurosphere, MAP2) and 50 μm (Rosette, Neuro-like, GFAP).

Patch clamp analysis

After 3 weeks of differentiation, iPS-NPC-derived neurons were put on a microscopic workbench for patch clamp analysis. Throughout the whole process, neurons were immersed in the extracellular fluid filled with 95% O_2 and 5% CO_2. Cells were visualized using patch clamp microscope (BX51WI, Olympus). Medium-sized neurons with a bright cell margin and smooth surface were selected to perform patch clamp. Current clamp was inserted into cells and stimulated with electric current, during which patch clamp amplifier system and Igor Pro (WaveMetrics, Lake Oswego, OR, USA) were used to record the active potential.

Preparation of gelatin sponge-electrospun PLGA/PEG 3D scaffolds

In order to improve the hydrophilicity of PLGA (75:25) fibers (Polyscience, USA), 1.5% PEG (molecular weight 4000, w/w to PLGA, Polyscience, USA) was added to the electrospinning solution. Briefly electrospinning solution in 1, 1, 1, 3, 3, 3-Hexafluoro-2-propanol (Amresco, USA) was fed into a 2.0 mL plastic syringe fitted with a needle in the diameter of 0.4 mm. The fibers were fabricated at an applied voltage of 15 kV with a voltage regulated DC power supply (DW-P203-1ACFD, Tianjin Dongwen High Voltage Power Supply Plant, China) at a feeding rate of 1.0 mL h^{-1}. The distance between the syringe needle tip and the cylindrical tube (diameter of 2 mm) collector was 12 cm. As a control, PLGA fibers were electrospun with a diameter similar to that of PLGA/PEG under the same conditions without incorporating PEG.

The cylindrical tube collector was submerged in 75% ethanol for 2 minutes to get PLGA cylindrical tube and was cut transversely into smaller rings of 2 mm in length. Sterilized gelatin

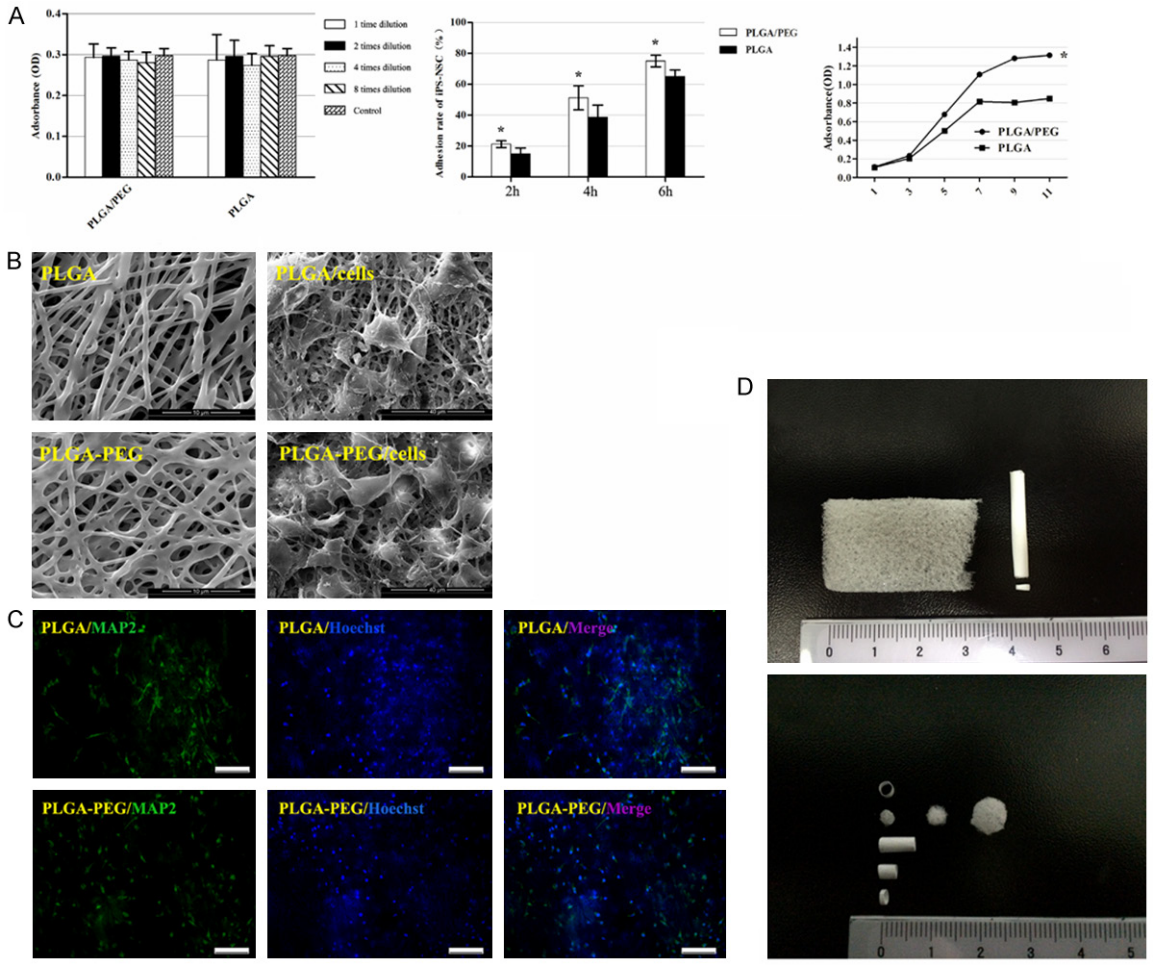

Figure 2. iPSc-derived NPC survival, adhesion and proliferation on electrospun nanofibers (A). Immunofluorescent staining and SEM morphological examination (B, C). Gelatin sponge (GS) electrospun scaffolds in different diameters and heights (D). Scale bars =10 μm (PLGA & PLGA-PEG), 40 μm (PLGA/cells & PLGA-PEG/cells), 100 μm (C).

sponge (GS) (Nanjing, Jinling Co., Ltd., China) was shaped into short tubes of 3 mm in diameter and 2 mm in length (**Figure 2D**). Before using, the PLGA rings were sterilized by ultraviolet irradiation for 30 minutes and a total of 1×10^6 cells in 10 μl culture medium were seeded to each GS scaffold.

Cell viability, adhesion and proliferation

To determine iPSc-derived NPC survival on PLGA fibers and PLGA PEG-modified fibrous scaffolds, Cell Counting Kit-8 (Nanjing, KeyGEN Biotech Co., Ltd., China) was applied according to the manufacturer's procedure. Briefly, targeted cells were cultured in 96-well plates for 2 days before subjected to CCK-8 solution for 2 h at 37°C, and the absorption value was measured under 450 nm with microplate reader (Elx800, Biotek, USA).

iPSc-derived NPC (3×10^4 per well) were seeded onto PLGA fibers and PLGA-PEG modified fibrous scaffolds in a 96-well plate. After incubating for 2, 4 and 6 hours, samples were washed twice with PBS, during which cells that did not adhere to the samples were removed. The remaining cells were dissociated with 0.25% trypsin-EDTA and cell amount was counted under inverted optical microscope (NIKON TS100, Japan).

iPSc-derived NPC (1×10^3 per well) were seeded onto PLGA fibers and PLGA/PEG-modified fibrous scaffolds in a 96-well plate. After incubating for 1, 3, 5, 7, 9 or 11 day(s), cultured cells were submitted to CCK-8 solution for 2 h at 37°C and the absorption value was measured under 450 nm with microplate reader (Elx800, Biotek, USA). The proliferation curve of iPSc-derived NPC on fibers was depicted.

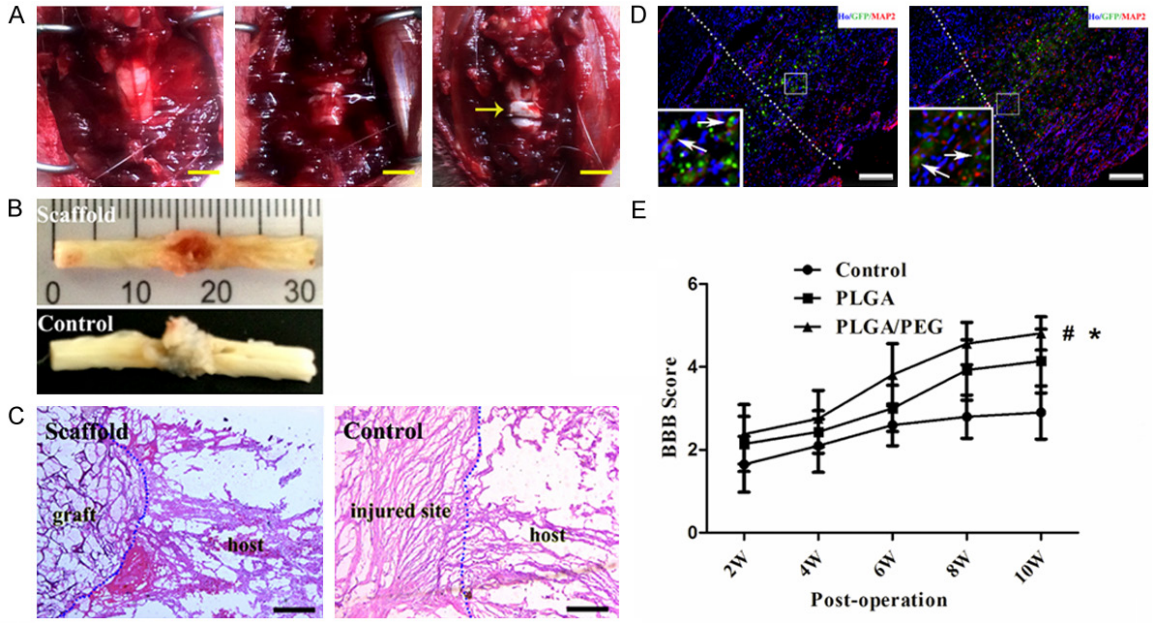

Figure 3. Surgical procedures of transected SCI model at T10 and was filled with the iPS-derived NPCs-GS-electrospun scaffolds (A). Gross morphology and hematoxylin & eosin (H&E) staining (B, C). MAP-2-positive neurons were observed in graft site of PLGA/PEG and PLGA group respectively (D). The functional recovery was improved in both of PLGA and PLGA/PEG group (*, P<0.05), the PLGA/PEG group was better than the PLGA group (#, P<0.05) (E). Scale bars =3 mm (A), 200 μm (C), 400 μm (D).

Scanning electron microscopy (SEM) morphological examination, immunofluorescent images of neurons cultured on different nanofibers

Samples were rinsed with PBS solution three times, fixed with 2.5% glutaraldehyde (Amresco, USA) at 4°C overnight, and then washed three times with PBS (5 min each time) before dehydrated in a series of 30%, 50%, 70% and 100% ethanol (7 min each, Amresco, USA). After air-drying, samples were covered with gold using sputter coating (IB5 ion coater, EIKO, Japan). The cell morphologies were observed with an SEM (Quanta 200, FEI, USA). The diameters of the nanofibers were measured in the SEM photographs using Image J software. For the morphological studies, cell-biomaterial compounds were fixed with formaldehyde after 7-day culture, blocked with bovine serum albumin, and permeabilized with 0.1% Triton X-100 (Invitrogen, USA). The compounds were then incubated with rabbit anti-mouse MAP-2 (1:1000, Millipore, Germany) antibodies at 4°C overnight. After that, the cells were incubated with a donkey anti-rabbit IgG-FITC antibody (1:400; Invitrogen, USA) at room temperature (21~24°C) for 1 hour. Nuclei were counterstained with Hoechst 33342 (10 μg/ml, Invitrogen, USA) for another 3 min. Stained cells were finally observed under a fluorescence microscope (Leica, Germany).

Spinal cord transection and transplantation

NPCs were transduced with GFP retrovirus for the following use in the animal study. All animal procedures were approved by the Animal Care and Use Committee of Sun Yat-sen University (Approval Number: SYXK2012-0083) and were consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. SD rats (200-250 g, Slac Laboratory Animal, Shanghai, China) were randomly divided into three groups: PLGA group (n=10), PLGA/PEG group (n=10), and SCI group (n=10) and were housed in ventilated, humidity and temperature controlled (23-25°C) rooms. Standard food pellets and water were supplied. Following intraperitoneal anesthesia with 10% chloral hydrate (0.3 ml/100 g, Amresco, USA), the spinal cord was transected after laminectomy at T10-11 level. A 2 mm spinal cord segment was completely removed at T10 spinal cord level. Right after the SCI, the scaffolds (2-mm-long, 3-mm-diameter) with or without the NPCs (GFP^+) were implanted into transected gap (**Figure 3A**). For postoperative care, the bladder was emptied manually twice a day for 2 months.

And besides, all rats received intramuscular injection of penicillin (50,000 U/kg/day, Sigma, USA) for 3 days after surgery and cyclosporine (10 mg/kg/day, Sigma, USA) was used for the suppression of immune rejection.

Tissue histology and immunofluorescence microscopy

Eight weeks after transplantation, animals were anesthetized with 10% chloral hydrate (Amresco, USA) before perfused with 4% paraformaldehyde (Amresco, USA) in PBS. Longitudinal repairing spinal cord sections were cut and stained with hematoxylin and eosin (H&E, Amresco, USA). For tissue immunofluorescence, spinal cords were dissected and postfixed overnight at 4°C for 24 hours, and placed in 30% phosphate buffered sucrose at 4°C for 48 h. 1.5-cm-long horizontal sections of spinal cords encompassing the lesion/graft site were dissected on a cryostat set at 30 μm thickness. The slides were fixed in acetone for 10 min at 4°C, and then incubated in PBS containing 5% BSA (Biosharp, China) for 1 h at room temperature. The samples were incubated with primary antibody anti-MAP-2 (1:1000; Millipore, Germany) overnight at 4°C, and followed by secondary antibody Alexa555-labeled (1:400; Life technology, USA) for 1 h at room temperature. Hoechst 33342 (Invitrogen, USA) was used for nuclear staining.

Hindlimb locomotor functional analysis

Locomotion recovery after SCI was scored according to Basso, Beattie, and Bresnahan (BBB) locomotor scale ranging from 0 (complete paralysis) to 21 (normal locomotion) by three observers blinded to the operation procedures. The test was performed one day postoperatively after transplantation and twice weekly up to the tenth week after operation.

Statistical analysis

Descriptive data were presented as means ± SDs. All statistical analyses were performed using the statistical software SPSS17.0. The data were analyzed with Student t-test when two groups of data were compared, while one-way analysis of variance (ANOVA) was applied when three groups of data were compared. The significant level was set at 0.05.

Results

iPS cells identification and NPCs differentiated from iPS cells

iPS cell colonies prominently expressed embryonic stem (ES)-cell-like pluripotent markers, including AP, Sox2, Oct4 and SSEA1 (**Figure 1A**, **1B**). When iPS cells were allowed in suspension culture, embryoid bodies (EBs) grew as round spheres with a high marginal sharpness that could be seen on the second day. After adding 1 μmol/L RA into the differentiation medium, EBs showed no obvious morphological change under microscopic observation. When these EBs were incubated on poly-lysine-coated culture plates in ITS medium for 2-3 days, differentiated cells with different morphological characteristics (smaller cell body and nucleus) were revealed at the edge of EBs and presented rosette structures. The number of differentiated cells increased gradually to a plateau on the seventh day after EB adherence. Fragment cell colonies were obtained by accutase. After 1-2 days of culture, these suspension colonies proliferated or aggregated together to form neurospheres. The neurospheres were round granular spheres with a bright margin, which were quite different from EBs and grew larger gradually under microscopic observation (**Figure 1A**).

Identification of iPS cell-derived neurospheres and their progenies

Differentiated cells with slender neurites and a small cell body were able to migrate from adherent neurospheres as shown by the increase of neurites under microscopic observation (**Figure 1A**). Neurons exhibited functional membrane properties. Whole-cell patch-clamp recordings exhibited functional membrane properties and action potentials (**Figure 1C**). In addition, immunofluorescence staining demonstrated the presence of iPS cell-derived NPCs, neurons, astrocytes and oligodendrocyte that exhibited positive staining for Nestin, MAP-2, GFAP and MBP respectively (**Figure 1D**).

iPSc-derived NPC survival, adhesion and process extension on electrospun nanofibers

iPSc-derived NPC viability was not significant difference from the control. Cell adhesion rates on PLGA-PEG fibers were significantly greater than those on PLGA at each time point

($^{*}P<0.05$). The number of the cells was on rise from the first day to the ninth day of the incubation before reached a plateau. The number of iPSc-derived NPC in PLGA-PEG group was significantly higher than that in PLGA group ($^{*}P<0.05$) (**Figure 2A**).

Immunofluorescent and SEM images of iPSc-derived NPC cultured on different fibrous scaffolds and the structure of GS scaffolds

The diameters of PLGA/PEG and PLGA fibrous scaffold were 883.33±80.15 nm and 674.35± 74.70 nm, respectively. PEG was uniformly mixed into the PLGA, resulting in a larger-diameter fiber. Neurite-like structures were observed under microscopy after neurospheres adherence (**Figure 2B**). Some neurons showed spindleshaped bodies with two elongated neuritis, but there were also neurons that had polygonal morphology with multiple neurites. At the same time, neurite-like branching was also observed, which occasionally extended into the territory of another cluster of neurons. Immunofluorescence staining demonstrated the presence of iPS cell-derived NPCs that differentiated into neurons in both scaffolds with positive staining for MAP-2 (**Figure 2C**). GS scaffolds in different diameters and heights (**Figure 2D**).

Surgical procedures of SCI, gross morphology and tissue staining study

Spinal cord was transected after laminectomy at T10 level. A 2 mm spinal cord segment completely removed at T10 spinal cord level. The 3D scaffolds of 2 mm in length with NPCs were transplanted to the gap (**Figure 3A**). Both the nanofibers and control groups contributed to tissue structural integrity, whereas more scars in the lesion site were found in the control group (**Figure 3B**). Hematoxylin & eosin (H&E) staining was performed to investigate morphological change of the spinal cord and scaffolds 8 weeks post-surgery. In both PLGA/PEG and control groups, there were cavity areas (**Figure 3C**). We performed tissue immunofluorescent staining to the spinal cord lesion to assess the survival and differentiation of iPSc-derived NPCs *in vivo*. Eight weeks post-implantation, immunostaining of MAP2/GFP indicated that implanted iPSc-derived NPCs survived and differentiated into neurons in the surroundings of the lesion site (**Figure 3D**).

Functional recovery post-SCI

In the first two weeks, BBB score was low, which indicated successful model construction. SCI animals showed a gradual functional recovery in the following 8 weeks. In addition, the hindlimb function in PLGA/PEG group recovered faster and better than that in other groups. At the end of the tenth week, the BBB scores in PLGA/PEG (4.812±0.403, n=8) and PLGA (4.143±0.77, n=7) groups were significantly higher than that in the control group (2.900± 0.641, n=10). The results also showed that functional recovery was improved best in the PLGA/PEG group (**Figure 3E**).

Discussion

Stem cells with tissue engineering biomaterials have been served as a common strategy in spinal tissue repair [17, 18]. The ideal cell source for transplantation should first meet the safety criteria for the recipient and then offer the highest therapeutic potential [10]. Well-controlled NPCs with high safety have revealed promising therapeutic benefits in neurological disorders without causing serious side effects after transplantation [19, 20]. iPS cells and their derived NPCs show several advantages over embryonic stem cells or NPCs originating from neural tissues for the treatment of SCI. Principally, iPS cells are generated by reprogramming somatic cells that are acquired easily compared with NPCs dissection from embryonic neural tissue [21]. In addition, iPS cells bypass the ethical problems associated with destroying embryos and working with fetal tissue. Finally, reduced immunological rejection following autotransplantation of iPS cell-derived NPCs makes this strategy a perfect surrogate for allograft rejection of NPCs from different individuals [22].

In this study, we differentiated mouse iPSc into NPCs that presented a similar morphology to neurosphere and were able to differentiate into neurons and glial cells *in vitro*. In addition, after the transplantation of scaffolds with iPS-derived NPCs into rats, cells were observed to survive and differentiate into neurons. Even though promising results have been shown above, there are still some problems needed to be resolved before translating iPS cells and their differentiated cells from bench to bedside. These issues include improving the low reprogramming efficiency of iPS cells and

decreasing the potential tumorigenicity of iPS cells and their derivatives after transplantation [23]. Genetic aberrations of iPS cells during culture *in vitro* also have to be evaluated before they can be safely applied in clinical treatments [24].

Numerous biomaterials have been applied for neural tissue engineering, including synthetic polymers, such as poly (L-lactic acid) (PLLA), Poly (D,L-lactide-co-glycolic acid) (PLGA), poly (-caprolactone) (PCL), and natural polymers, such as chitosan (CS), gelatin (Gel), as well as their composites such as PCL-gelatin (PCL-Gel) and PCL-collagen [25]. It was reported that PLLA nanofiber substrates could support serum free growth of primary motor and sensory neurons. However, a significant limitation of the use of PLLA is lacking biological recognition sites that can interact with the cells [26]. Chitosan (CS) and gelatin (Gel) are also two widely used natural biomaterials for tissue engineering applications because of their good biocompatibility. By comparing PCL/gelatin electrospun scaffolds at 100:0, 70:30 and 50:50 weight ratios, the presence of gelatin improved the hydrophilicity of PCL scaffolds. While results demonstrated that higher hydrophilicity, also possessed loose and weak fibrous structures and enhanced degradation rate that may not be favorable for nerve regeneration [27]. PLGA degradability and mechanical property make it a good candidate that easily fills an injury cavity without further damaging the surrounding tissues [28, 29]. But the PLGA nanofiber hydrophilicity is insufficient, the cell adhesion and proliferation is deficient. PEG, composed of repetitive oxygen vinyl, is commonly used as scaffold due to the hydrophilic components [15]. In addition, PEG could protect key axonal cytoskeleton protein to promote the repair of spinal cord injury [30].

In this study, we synthesized an electrospun PLGA/PEG biomaterial and tested its ability to promote adhesion, proliferation and neurites outgrowth of iPSc-derived NPCs *in vitro*. The results indicated that PLGA/PEG nanofiber have a larger diameter and was better for the attachment and proliferation of differentiated cells compared with PLGA alone. This finding could possibly due to a large crosslinking space, mesh size and a higher expansion ratio created by PEG, which facilitate cell adhesion and proliferation. Furthermore, the two scaffolds showed no obvious cell toxicity, suggesting the safe application of both techniques in clinical practice. SEM and immunofluorescence results indicated that iPSc-derived NPCs differentiated into neural cells and neurites grew well on the both surface of the fibers.

To restore the continued morphology of spinal cord and provide a good support structure, we stuffed electrospun PLGA/PEG or PLGA nanofiber with GS to construct a 2 mm-long cylinder with 3 mm diameter. After transplanted into a rat spinal cord transection injury model for 2 months, HE analysis was performed on longitudinal sections of the injured spinal cords. We observed disordered structure in both PLGA/PEG and PLGA groups. Immunofluorescence staining revealed that iPSc-derived NPCs were evenly distributed within the tissues and could survive and differentiate into neurons in the surroundings of the lesion site. Furthermore, the BBB score revealed that functional recovery was improved best in the PLGA/PEG group compared with the PLGA and control groups.

Spinal cord injury treatment with cell transplanting and tissue engineering is an exciting strategy. However, several vital issues are still needed to be overcome in our study. The yields iPCs-derived NPCs should be sorted to a high purity and new approaches should be developed to convert one somatic cell type to another without full reprogramming to avoid viral integration of the genes encoding reprogramming factors. In summary, we have demonstrated that neural precursor cells generated from induced pluripotent stem cells with gelatin sponge-electrospun PLGA/PEG nanofibers could promote functional recovery after spinal cord injury. It is conceivable that this engineered scaffold provides a platform in which non-viral induced neural precursor cells can be applied to improve the recovery after SCI.

Acknowledgements

This study was supported by the Natural Science Foundation of China (31170947, 31470949, 81472122), the Guangdong Natural Sciences Foundation of China (S20130100-16413), the Guangdong Medical Scientific Research Foundation of China (A2015128) and the China Postdoctoral Science Foundation (2014M552272). We thank Pro. Liumin He from

the Institute of Biomedical Engineering, College of Life Science and Technology, Jinan University, China for providing us with the electrospun PLGA/PEG nanofibers. We also thank Pro. Bin Jiang from the Neuroscience Research Center, Department of Physiology, Zhongshan School of Medicine, Sun Yat-Sen University in China for patch clamp analysis.

Disclosure of conflict of interest

None.

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