Original Article

Efficient transdifferentiation of human adipose-derived stem cells into Schwann-like cells: A promise for treatment of demyelinating diseases

Shanhaz Razavi, Nafiseh Ahmadi[1], Mohammad Kazemi, Mohammad Mardani, Ebrahim Esfandiari

Departments of Anatomical Sciences and Molecular Biology, Isfahan University of Medical Sciences, Isfahan, [1]Biology, Islamic Azad University, Science and Research Branch, Tehran, Iran

Abstract

Background: Schwann cells (SCs) can provide a suitable option for treatment not only diseases of peripheral nervous system (PNS), but also diseases of central nervous system (CNS). It is difficult to obtain sufficient large number of SCs for clinical purpose because of their restricted mitotic activity, and by sacrificing one or more functioning nerves with the consequence of loss of sensation. So, providing an alternative source for transplantation is desired. The aim of this study was isolation, characterization of human adipose derived stem cells (ADSCs), and transdifferentiation into Schwann-cells.

Materials and Methods: After isolation of ADSCs by mechanical and enzymatic digestion of adipose samples, characterization human ADSCs using flow cytometry was carried out. Human ADSCs were sequentially treated with various factors for neurosphere formation and terminal differentiation into Schwann-like cells. We used Schwann cell markers, GFAP and S100 to confirm the effectiveness of the differentiation of human ADSCs using Immunostaining and real time RT-PCR techniques.

Results: Flow cytometry analysis of ADSC showed isolated stem cells were positive for CD90 and CD44 markers of mesenchymal stem cells, but for CD45 and CD34 markers were negative. Dual immunofluorescence staining and real time RT-PCR analysis for GFAP and S100 markers were revealed that approximately 90% of differentiated cells expressed co-markers.

Conclusion: We indicated that human ADSCs have a suitable option to induce Schwann-like cells for autologous transplantation, offer promise for treatment in demyelinating diseases.

Key Words: Human ADSCs, Schwann-like cell, S100, transdifferentiation

Address for correspondence:
Dr. Shahnaz Razavi, Department of Anatomical Sciences and Molecular Biology, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: razavi@med.mui.ac.ir

Received: 25.12.2011, **Accepted:** 23.02.2012

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.96067

INTRODUCTION

Schwann cells (SCs) in the peripheral nervous system play a major role in developmental, neurodegenerative, and regenerative processes.[1-3] It is documented that Schwann cells can provide a suitable option for treatment not only diseases of the peripheral nervous system (PNS), but also disease of the central nervous system (CNS) such as multiple sclerosis and

How to cite this article: Razavi S, Ahmadi N, Kazemi M, Mardani M, Esfandiari E. Efficient transdifferentiation of human adipose-derived stem cells into Schwann-like cells: A promise for treatment of demyelinating diseases. Adv Biomed Res 2012;1:12.

spinal cord injury.[4-7] SCs can be obtained from nerve biopsies for autologous transplantation without the need for subsequent immunosuppression. Although transplanted cultured SCs can remyelinate and restore neural function of demyelinated axons of PNS and CNS, it is difficult to obtain sufficient large number of SCs for clinical purpose because of their restricted mitotic activity, and by sacrificing one or more functioning nerves with the consequence of loss of sensation, scarring and, possibly, neuroma formation. Therefore, the clinical application of SCs is limited. So, providing an exogenous source for transplantation is desired.

Recent studies demonstrated that bone marrow mesenchymal stem cells can transdifferntiate into Schwann-cell like *in vitro*[8,9] and *in vivo*.[10] However, bone marrow mesenchymal stem cells procurement procedures are invasive, painful, and the frequency of mesenchymal stem cells upon harvest from bone marrow is relatively low.[11] Therefore, an alternative cell source is preferred.

Adipose-derived stem cells (ADSCs) can be obtained easily and expanded *in vitro* for autologous transplantation. So, ADSCs may be an ideal alternative cell source for SC. Recently, it is reported that ADSCs from rat can transdifferentiate into SC-like cells.[12-14]

The aim of this study transdifferentiation of human ADSCs into Schwann-like cell and evaluation markers of Schwann cells in differentiated cells using immunostaining and real time RT-PCR techniques.

MATERIALS AND METHODS

Isolation and culture of human ADSCs

All chemicals, except where specified otherwise, were purchased from Sigma-Aldrich, St. Louis, MO.

Human adipose tissue was obtained from six samples of abdominal fat from female donors (age range: 20–45 years) after receiving informed consent and cultured as described in a previous study.[15] Briefly, samples were washed extensively with sterile phosphate-buffered saline (PBS) to remove contaminating debris and red blood cells. Washed aspirates were treated with 0.075% collagenase type I in PBS for 30 min at 37°C with gentle agitation. The collagenase I was inactivated with an equal volume of DMEM: F12/10% fetal bovine serum (FBS) and the infranatant centrifuged for 10 min at 800 rpm. The cellular pellet was resuspended in DMEM: F12/10% FBS and plated at 20000 cells/cm^2 in T75 flasks in DMEM: F12 medium supplemented with 10% FBS and %1 Penicillin/streptomycin. After 24 h, it removed the nonadherent cells and expanded the adherent ADAS cells by serial passage. In this study, we used cells at passages 3–5 for all experiments and all experiments repeated at least in triplicate.

Characterization of human ADSCs

In order to determine "stemness" isolated cells, human ADSCs within 3–5 passages were harvested by trypsinization, and then the cells were fixed in neutralized 2% paraformaldehyde (PFA) solution for 30 min. The fixed cells were washed twice with PBS and incubated with antibodies against CD90, CD44, CD34, and CD45 (all from Chemicon,Temecula, CA, USA) for 30 min. Primary antibodies were directly conjugated with FITC and Phycoerythrin. For isotype control, nonspecific FITC-conjugated IgG was substituted for the primary antibodies. Flow cytometry was performed with a FACscan flow cytometer (Becton Dickinson, San Jose, CA).

Differentiation to a Schwann cell phenotype

Human ADSCs were induced into neurospheres. Briefly, we harvested human ADSCs (80–90% confluence) and then plated them in plastic dish a concentration of 1–2 × 10^5/cm^2 in DMEM: F12 supplemented with 20 ng/mL EGF (Peprotech, London, UK), 20 ng/mL basic fibroblast growth factor (bFGF) (Pepro Tech) and 2% B27 (1:50, Gibco) at 37°C in 5%CO2. We added fresh medium every 3 to 4 days. After 7 days, neurospheres were triturated using a fire-polished Pasteur pipette and re-plated in Laminin coated six-well chamber slides contain DMEM: F12 supplemented with 10% FBS, 14 μM forskolin (FSK; Alexis, Switzerland), 5 ng/mL platelet-derived growth factor-AA (PDGF; PeproTech, UK), 10 ng/mL bFGF(Pepro Tech) and 200 ng/mL recombinant human heregulin-beta1 (HRG; PeproTech) for terminal differentiation. The cells were incubated for 9 days under these conditions, and then harvested for investigation.

We used Schwann cell markers, S100 as calcium binding protein with GFAP as intermediate filament of glial cell to confirm the effectiveness of the differentiation of Human ADSCs with immunostaining and real time RT-PCR analysis.

MTT assay

To examine the survival of Schwann-like cells, MTT (3-[4,5-dimethythiaziazol-2yl]-2,5-diphenyl tetrazoliumbromide) was dissolved in PBS at 5 mg/mL. The stock solution was added to the culture medium at a dilution 1:10.Then plates were incubated at 37°C for 4 h. In live cells, the tetrazolium ring is cleaved into a visible purple formazan reaction product. The medium was aspirated and 200 μL of DMSO was added to extract the MTT formazan. Absorbance of each well was detected by microplate reader (Hiperion MPR 4+,

Germany) at the wavelength of 540 nm.

Immunocytochemistry

After fixation with 4% PFA/PBS, cells were treated with blocking solution (PBS containing 4% goat serum and 0.1% Triton X-100) for 45 min at RT. The cells were incubated in primary antibodies in PBS/0.1% Triton X-100 and 1% goat serum overnight at 4°C. The following antibodies were used: anti-Nestin (1:300; Abcam, UK), anti-Glial Fibrillary Acidic Protein (GFAP, 1:300; Abcam, UK) and anti-S100 (1:500; Abcam, UK). After washing with PBS, slides were treated with secondary antibody, rabbit anti-mouse FITC(1:500; Abcam, UK) and rabbit anti-mouse PE (1:200; Abcam, UK)-conjugated secondary antibodies incubated at RT for 1 h. Then, nuclei were stained with DAPI for cell counting. For negative controls, primary antibody was omitted from the reaction series in each experiment. Cells were observed using fluorescence microscope (Olympus, BX51, Japan). To perform quantitative analysis, the number of positive cells was counted on each acquired image by ImageJ1.42 (NIH), and the ratio to the number of nuclei was analyzed for each antigen whereas the number of immuno-positive cells counted in a minimum total of 200 cells per slide.

Real time RT-PCR

Real-time (SYBR Green) PCR was performed using a Thermal Cycler Rotor-Gene 6000 (Step one Plus AB), with 12.5 µL Rotor-Gene SYBR Green PCR Master Mix (Qiagen), 5 pM of each primer, and 1.5–2 µL cDNA for each reaction in final volume of 20 µL. Cycle conditions were carried out according to the protocol (Qiagen). Relative gene expression was analyzed using the comparative Ct method ($2^{-\Delta\Delta Ct}$). All measurements were done in triplicates. Real-time specific primer pairs were indicated [Table 1].

Statistical analysis

To analyze data, SPSS (PASW) version 18 was applied. The experiments were replicated at least three times. Data were presented as mean ± SEM. Kruskal–Wallis one way ANOVA with Dunn's comparison test was used to determine the statistical significance between data, A value of $P \leq 0.0\,5$ was considered statistically significant.

RESULTS

Characterization of stem cell cultures

Human ADSCs within 3–5 passages after initial plating

Table 1: Primers used for real-time PCR

Gene	Primers
GFAP	For: 5′-CCGACAGCAGGTCCATGTG-3′ Rev: 5′-GTTGCTGGACGCCATTGC-3′
S 100	For:5′-GGAGACGGCGAATGTGACTT-3′ Rev: 5′-GAACTCGTGGCAGGCAGTAGTAA-3′
Nestin	For: 5′-AGCCCTGACCACTCCAGTTTAG-3′ Rev: 5′-CCCTCTATGGCTGTTTCTTTCTCT-3′
GAPDH	For:5′-GAAATCCCATCACCATCTTCCAGG-3′ Rev: 5′-GAGCCCCAGCCTTCTCCATG-3′

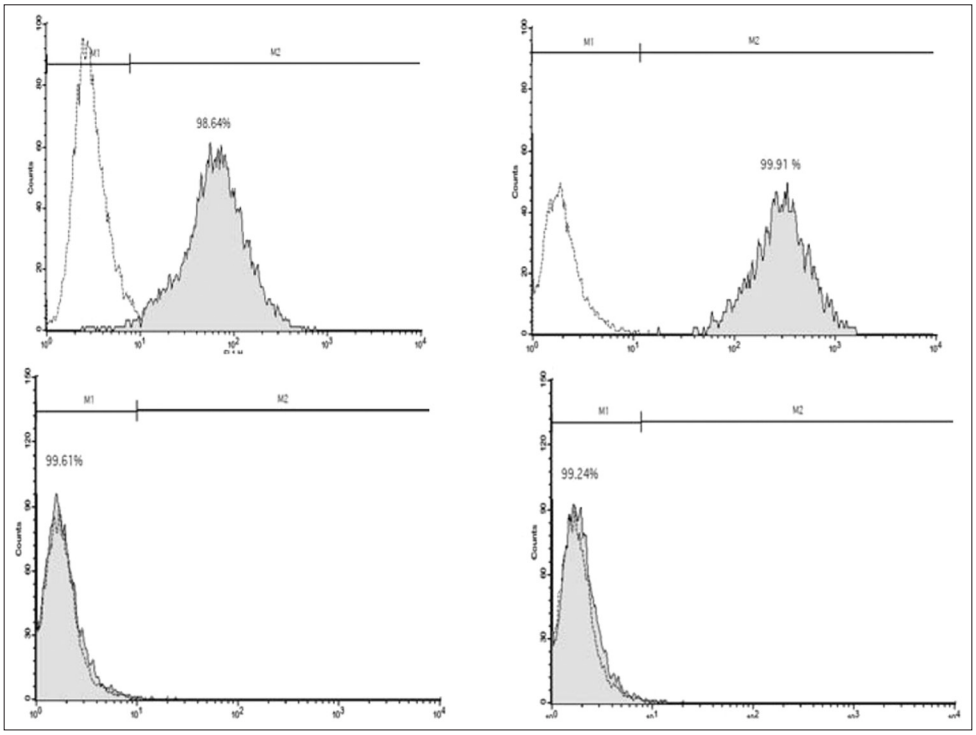

Figure 1: Flow cytometric analysis of human ADSCs (3–5 passages) using specific FITC and PE coupled antibodies against surface markers. An isotype control is included in each test (Gray lines). Flow cytometric analysis shows that human ADSCs express CD90 and CD44 markers but don't express CD34 and CD45 markers

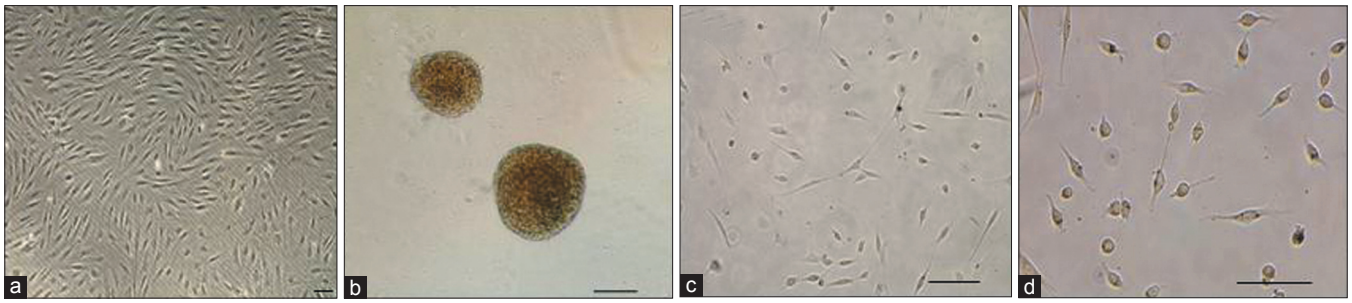

Figure 2: Phase contrast image of transdifferentiation of Schwann – like cells from human ADSCs. (a) Phase contrast image of human ADSCs showed flattened fibroblast like morphology. (b) Neurosphere extracted from human ADSCs after 7 days post induction. (c and d) human ADSCs differentiated Schwann cells phenotype shows bi or tri – polar and spindle-like shape upon treatment using specific growth factors with two magnifications

of the primary culture appeared to be a monolayer of large and flat cells. As the cells approached confluency, they assumed a more spindle shaped fibroblastic morphology. To further characterize these cells, cell surface markers were examined by flow cytometry. Flow cytometry analysis of human ADSCs showed that ADSCs were positive for CD90 and CD44, but negative for CD45and CD34 [Figures 1 and 2a].

Differentiation to a Schwann cell phenotype

Human ADSCs were re-plated in DMEM: F12 medium supplemented with EGF and bFGF. A lot of spheres of floating cells appeared after 7 days in conversion culture [Figure 2b]. Then, the neurospheres triturated and re-plated in differentiation medium. ADSCs changed from a mono-layer of large and flat morphology to a bi – or tri-polar, spindle-like shape [Figures 2c and d].

Nine days after differentiation, morphology of the cells was analyzed and percentage of Nestin$^+$ cells, SC marker GFAP$^+$/S100$^+$ and GFAP$^+$ cells were detected.

Immunocytochemistry analysis showed that approximately less than 5% of differentiated ADSCs only were positive for GFAP while, about 9o% of induced human ADSCs were positive for GFAP/S100 co-markers [Figure 3].

Cell viability

We examined viability and cell proliferation of differentiated cells by MTT assay. Comparison mean absorbance value of differentiated cell with human ADSCs show that mean of absorbance value of differentiated cells was more than human ADSCs (0.57 ± 0.13 *vs.* 0.49 ± 0.15). Therefore, the additive factors for Schwann cell differentiation not only toxic effect on human ADSCs cells but also insignificantly promoted proliferation of them ($P>0.05$).

Real time RT-PCR

In order to determine effectiveness transdifferentiation

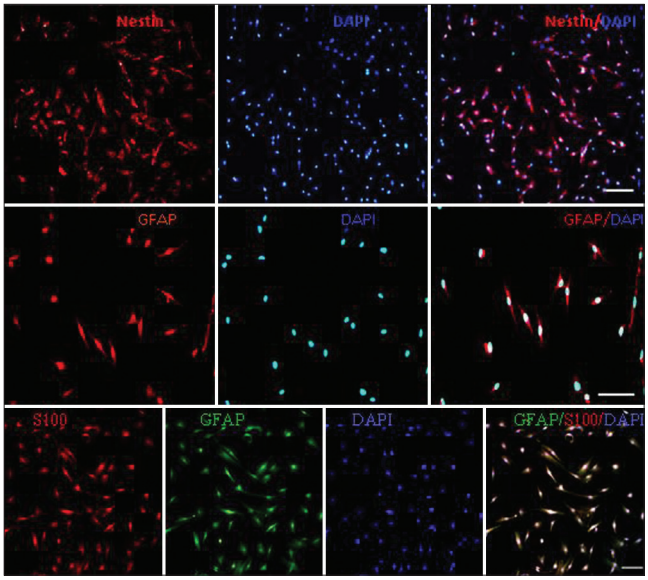

Figure 3a: Immunocytochemistry of Nestin, GFAP and GFAP/S100. The yellow color is an overlap of green and red showing double positively stained cells for both markers and cell nuclei are labeled with DAPI (blue) Bar: 20 μm (Nestin and GFAP/S100), 50 μm (GFAP)

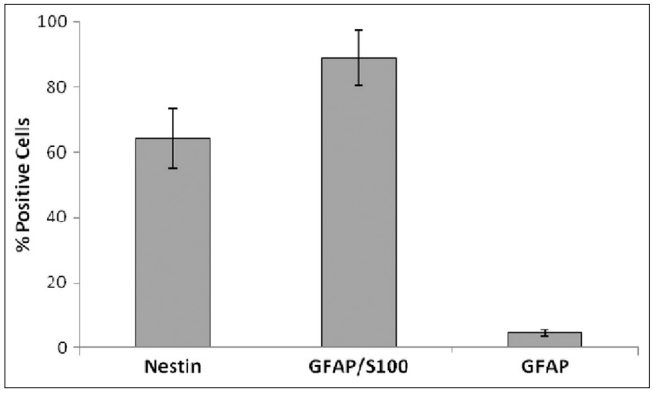

Figure 3b: Comparative analysis of Schwann cells markers (GFAP and GFAP/S100) and Nestin for differentiated cells

potential of Schwann-like cells, we examined expression of Nestin as neural progenitor cell marker, S100 and GFAP as Schwann cell markers. The results of Real time RT-PCR confirmed the potential of human

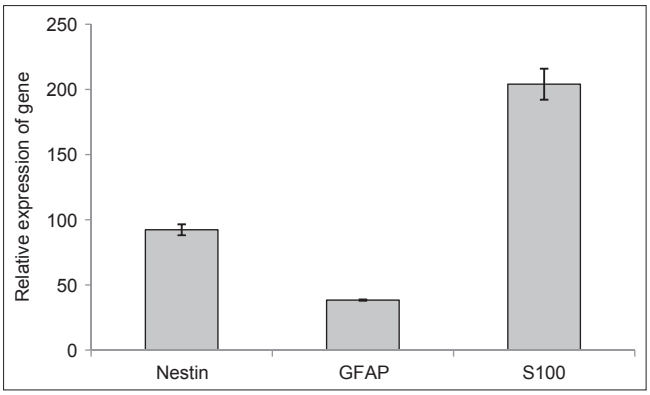

Figure 4: Comparative analysis of Schwann cells markers examined by real time RT-PCR. The mean of expression of Nestin, GFAP and S100 markers in differentiated cells

ADSCs differentiation to Schwann cells [Figure 4].

DISCUSSION

Previous studies have shown that human adipose-derived stem cells can be induced into adipocytes, osteoblasts, chondrocytes, myocytes, hepatocytes, endothelial, and neuron-like cells.[16-21] Here, we can induce differentiation of human ADSCs into Schwann-like cells with high efficiency. Transdifferentiation of human stem cells into Schwann-like cells has been reported in limited studies[22] and mainly of transdifferentiation into Schwann-like cells from rat ADSCs were studied.[12,23-28]

Caddick *et al.* have exposed bone marrow stem cells to a differentiation media including b-mercaptoethanol, all-transretinoic acid, platelet derived growth factor (PDGF), bFGF, glial growth factor (GGF), and forskolin. They have been established to induce glial-like differentiation of to induce Schwann cells. This produced a morphological change in the majority of the cells, toward an elongating spindle phenotype, characteristic of Schwann cells. Many of these cells also express the glial markers, GFAP, S100, and p75.[9]

Hermmen *et al.* believe that mesenchymal stem cell-derived neurospheres are similar to neural stem cell derived from fetal or adult CNS in phenotype and differentiation capacity.[29]

In this study, we also used a standard neuroprogenitor medium that contains bFGF and EGF to establish free floating. Then, neurosphere-singled cells induced with DMEM: F12 supplemented with 10% FBS, forskolin, PDGF, bFGF, and HRGβ1 for terminal differentiation. We can induce human ADSCs to cells that were spindle, bi or tri polar like Schwann cell morphology and immunopositive for Schwann markers GFAP/S100, like to native Schwann markers.

In addition, Xu *et al.* found that rat ADSC could be induced into cells that were spindle like in shape, could proliferate *in vitro,* and were immunopositive for SC marker GFAP and S100, similar to native SC and express myelin protein P0 when co-cultured with rat pheochromocytoma cell line (PC12 cells), SC-like cells could induce the differentiation of PC12 cells rapidly and form myelin structures with PC12 cells *in vitro.*[23]

Kingham *et al.* shown that rat ADSC treated with a mixture of glial growth factors expressed GFAP, S100 proteins and enhanced neurite outgrowth *in vitro,* suggesting transition to a Schwann cell phenotype. They indicated that 81% of the cells have a spindle-like morphology of which 44.5% expressed GFAP and approximate all of these GFAP-positive cells also stained for S100 protein (42.9 ± 3.3%) positive (24). Whereas Jiang *et al.* reported using double immunofluorescence staining most differentiated rat adipose-derived stem cells were S100 and GFAP positive. The ratio of positive cells reached to 72.4% and 69.8% for S100, GFAP after 5 days of differentiation. Although, the ratios of positively stained cells for S100 and GFAP on day 8 were lower than on day 5.[27]

In this study, less than 5% of differentiated human ADSCs were only positive for GFAP while near 90% of induced human ADSCs were positive for Co-markers GFAP and S100.

Moreover, Ziegler *et al.* were obtained approximately 60% of $GFAP^+/S100^+$ from differentiated human embryonic stem cell.[28] Therefore, we succeed to obtain SC-like cells with high efficiency than other studies. This variety of outcome could be due to different in kind of stem cells, differentiation medium, and length of differentiation process.

Morphological and phenotypic characteristics do not prove that differentiated adipose-derived stem cells possess the function of Schwann cells. Therefore, we using Immunofluorescence staining combined real time RT-PCR to confirm that differentiated adipose-derived stem cells produced protein indicators characteristic for Schwann cells, including S100 and GFAP.

The result of real time RT-PCR show that effectively up regulation Schwann cell markers in differentiated human ADSCs, but Nestin expression was down regulated in Schwann –like cells.

Previous researchers using RT-PCR or western blot to confirm Schwann cell markers, including S100 and GFAP proteins.[24,27,28]

CONCLUSION

We succeeded to induce the differentiation of human ADSCs into Schwann-like cells. Schwann-like cells could be applying as a model for understanding the myeliniztion process change-related neuropathology diseases. Moreover, we suggests that the Schwann-like cells induced from human ADSCs may be substitute for functionally native Schwann cells autologous transplantation, offer promise for treatment of PNS and CNS demyelinating diseases.

ACKNOWLEDGMENT

The authors are grateful to Iranian Council of Stem Cell Technology, Isfahan University of Medical Sciences for financial support (Grant no. 188055) and Dr. Freshteh Haghighat for providing human adipose tissue.

REFERENCES

1. Bunge RP. The role of the Schwann cell in trophic support and regeneration. J Neurol 1994;242:19-21.
2. Jessen KR, Mirsky R. Schwann cells and their precursors emerge as major regulators of nerve development. Trends Neurosci 1999;22:402-10.
3. Gulati AK, Rai DR, Ali AM. The influence of cultured Schwann cells on regeneration through acellular basal lamina grafts. Brain Res 1995;705:118-24.
4. Hadlock T, Sundback C, Hunter D, Cheney M, Vacanti JP. A polymer foam conduit seeded with Schwann cells promotes guided peripheral nerve regeneration. Tissue Eng 2000;6:119-27.
5. Mosahebi A, Woodward B, Wiberg M, Martin R, Terenghi G. Retroviral labeling of Schwann cells: *In vitro* characterization and *in vivo* transplantation to improve peripheral nerve regeneration. Glia 2001;34:8-17.
6. Dubois-Dalcq M, Ffrench-Constant C, Franklin RJ. Enhancing central nervous system remyelination in multiple sclerosis. Neuron 2005;48:9-12.
7. Takami T, Oudega M, Bates ML, Wood PM, Kleitman N, Bunge MB. Schwann cell but not olfactory ensheathing glia transplants improve hindlimb locomotor performance in the moderately contused adult rat thoracic spinal cord. J. Neurosci 2002;22:6670-81.
8. Dezawa M, Takahashi I, Esaki M, Takano M, Sawada H. Sciatic nerve regeneration in rats induced by transplantation of *in vitro* differentiated bone-marrow stromal cells. Eur J Neurosci 2001;14:1771-6.
9. Caddick J, Kingham PJ, Gardiner NJ, Wiberg M, Terenghi G. Phenotypic and functional characteristics of mesenchymal stem cells differentiated along a Schwann cell lineage. Glia 2006;54:840-9.
10. Akiyama Y, Radtke C, Kocsis JD. Remyelination of the rat spinal cord by transplantation of identified bone marrow stromal cells. J Neurosci 2002;22:6623-30.
11. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, *et al*. Multilineage cells from human adipose tissue: Implications for cell-based therapies. Tissue Eng 2001;7:211-8.
12. Chi GF, Kim MR, Kim DW, Jiang MH, Son Y. Schwann cells differentiated from spheroid-forming cells of rat subcutaneous fat tissue myelinate axons in the spinal cord injury. Exp Neurol 2010;222:304-17.
13. Wei Y, Gong K, Zheng Z, Liu L, Wang A, Zhang L, *et al*. Schwann-like cell differentiation of rat adipose-derived stem cells by indirect co-culture with Schwann cells *in vitro*. Cell Prolif 2010;43:606-16.
14. Kaewkhaw R, Scutt AM, Haycock JW. Anatomical site influences the differentiation of adipose-derived stem cells for Schwann-cell phenotype and function. Glia 2011;59:734-49.
15. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, *et al*. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002;13:4279-95.
16. Liqing Y, Jia G, Jiqing C, Ran G, Fei C, Jie K, *et al*. Directed differentiation of motor neuron cell-like cells from human adipose-derived stem cells *in vitro*. Neuroreport 2011;22:370-3.
17. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, *et al*. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002;13:4279-95.
18. Safford KM, Hicok KC, Safford SD, Halvorsen YD, Wilkison WO, Gimble JM, *et al*. Neurogenic differentiation of murine and human adipose-derived stromal cells. Biochem Biophys Res Commun 2002;294:371-9.
19. Strem BM, Hicok KC, Zhu M, Wulur I, Alfonso Z, Schreiber RE, *et al*. Multipotential differentiation of adipose tissue-derived stem cells. Keio J Med 2005;54:132-41.
20. Planat-Benard V, Silvestre JS, Cousin B, André M, Nibbelink M, Tamarat R, *et al*. Plasticity of human adipose lineage cells toward endothelial cells: Physiological and therapeutic perspectives. Circulation 2004;109:656-63.
21. Seo MJ, Suh SY, Bae YC, Jung JS. Differentiation of human adipose stromal cells into hepatic lineage *in vitro* and *in vivo*. Biochem Biophys Res Commun 2005;328:258-64.
22. Radtke C, Schmitz B, Spies M, Kocsis JD, Vogt PM. Peripheral glial cell differentiation from neurospheres derived from adipose mesenchymal stem cells. Int J Dev Neurosci 2009;27:817-23.
23. Xu Y, Liu Z, Liu L, Zhao C, Xiong F, Zhou C, *et al*. Neurospheres from rat adipose-derived stem cells could be induced into functional Schwann cell-like cells *in vitro*. Brain Res 2008 6;1239:49-55.
24. Kingham PJ, Kalbermatten DF, Mahay D, Armstrong SJ, Wiberg M, Terenghi G. Adipose-derived stem cells differentiate into a Schwann cell phenotype and promote neurite outgrowth *in vitro*. Exp Neurol 2007;207:267-74.
25. Xu Y, Liu Z, Liu L, Zhao C, Xiong F, Zhou C, *et al*. Neurospheres from rat adipose-derived stem cells could be induced into functional Schwann cell-like cells *in vitro*. BMC Neurosci 2008;9:21.
26. Keilhoff G, Goihl A, Langnäse K, Fansa H, Wolf G, Keilhoff G, *et al*. Transdifferentiation of mesenchymal stem cells into Schwann cell-like myelinating cells. Eur J Cell Biol 2006;85:11-24.
27. Jiang L, Zhu JK, Liu XL, Xiang P, Hu J, Yu WH. Differentiation of rat adipose tissue-derived stem cells into Schwann-like cells *in vitro*. Neuroreport 2008;19:1015-9.
28. Ziegler L, Grigoryan S, Yang IH, Thakor NV, Goldstein RS. Efficient generation of schwann cells from human embryonic stem cell-derived neurospheres. Stem Cell Rev 2011;7:394-403.
29. Hermann A, Gastl R, Liebau S, Popa MO, Fiedler J, Boehm BO, *et al*. Efficient generation of neural stem cell-like cells from adult human bone marrow stromal cells. J Cell Sci 2004;117:4411-22.

Source of Support: Iranian Council of Stem Cell Technology, Isfahan University of Medical Sciences for financial support (Grant no. 188055), **Conflict of Interest:** None declared.