Evaluation of the Functional Recovery in Sciatic Nerve Injury following the Co-transplantation of Schwann and Bone Marrow Stromal Stem Cells in Rat

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ABSTRACT

Introduction: Transplantation of bone marrow stromal cells (BMSCs) or Schwann cells (SCs) can increase axonal regeneration in peripheral nerve injuries. Based on our previous investigations, the goal of the present work was to examine the individual and synergistic effects of the two different cell types in sciatic nerve injury . We pursued to evaluate the effects of BMSCs and SCs co-transplantation on the functional recovery after sciatic nerve injury in rat.

Methods: In this experimental research, adult male Wistar rats (n=32, 250-300g) were used, BMSCs and SCs were cultured, and the SCs were confirmed with anti S100 antibody. Rats were randomly divided into 4 groups (n=8 in each group): 1- control group: silicon tube filled with fibrin gel without cells; 2- BMSCs group: silicon tube filled with fibrin gel seeded with BMSCs; 3- SCs group: silicon tube filled with fibrin gel seeded with SCs and 4- co-transplantation group: silicone tube filled with fibrin gel seeded with BMSCs and SCs. The left sciatic nerve was exposed, a 10 mm segment removed, and a silicone tube interposed into this nerve gap. BMSCs and SCs were transplanted separately or in combination into the gap. BMSCs were labeled with anti-BrdU and SCs were labeled with DiI. After 12 weeks electromyographic and functional assessments were performed and analyzed by one-way analysis of variance (ANOVA).

Results: Electromyographic and functional assessments showed a significant difference between the experimental groups and controls. Electromyography measures were significantly more favourable in SCs transplantation group as compared to BMSCs transplantation and co-transplantation groups ($p<0.05$). Functional assessments showed no statistically significant difference among the BMSCs, SCs and co-transplantation groups ($p<0.05$).

Discussion: Transplantation of BMSCs and SCs separately or in combination have the potential to generate functional recovery after sciatic nerve injury in rat. The electromyography evaluation showed a greater improvement after SCs transplantation than BMSCs or the co-transplantation of BMSCs and SCs.

Key Words:
Bone Marrow Stromal Cells,
Schwann Cells,
Transplantation,
Peripheral Nerve Regeneration.

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1. Introduction

Peripheral nerve system (PNS) has the potential to regenerate nerve cells, and the peripheral nerve injury has been successfully recovered using various procedures such as nerve autograft or nerve guidance tubes (Belkas, Shoichet, & Midha, 2004). In peripheral nerve injury, one of the problems is suturing nerve ends when the resulting gap is too long (Millesi, 1984). The nerve ends can be connected with a nerve autograft to provide a guidance for the regenerating nerves. However, for more extensive nerve trauma, a longer graft is needed, and when the graft is thinner than the injured nerve, the transplantation of a bundle of nerve fibers becomes mandatory. Since the procedure requires a large graft from a healthy nerve, sensory and motor destruction may occur at the donor site (Ide, 1996; Ishikawa et al., 2009).

Axonal regeneration in a peripheral nerve injury which requires extrinsic factors to promote growth and supply guidance to the target. To overcome these problems, a variety of nerve guide tubes have been used to facilitate cell transplantation. The purposes of the cellular transplantation include: 1- bridging the gap; 2- providing a suitable environment to induce axonal regeneration and 3- to promote neovascularization. Different procedures have been used to improve regeneration of peripheral nerves. One of those is the seeding of the cells into the guide tubes (Belkas et al., 2004; Dezawa, 2005; Fan, Crawford, & Xiao, 2011; Ishikawa et al., 2009).

Bone marrow stromal cells (BMSCs) and Schwann cells (SCs) are cells with the capability to produce nerve growth factors such as nerve growth factor (NGF), brain-derived nerve growth factor (BDNF) and vascular endothelial growth factor (VEGF) . These factors play an important role in the survival and proliferation of axons. Thus BMSCs and SCs transplantation may possibly result in the recovery of peripheral nerves following injury (Braga-Silva et al., 2006; C. J. Chen et al., 2007; Lu et al., 2006; Schlosshauer, Muller, Schroder, Planck, & Muller, 2003).

Our previous study showed that BMSCs and SCs can be effective on functional recovery of the sciatic nerve injury on their own (Zarbakhsh et al., 2012). To consolidate the earlier findings, here we pursued to compare the effects of the co-transplantation of these cells (BMSCs and SCs) with sole transplantation of these cells on the peripheral nerve recovery as this has not so far been evaluated under similarly controlled conditions.

2. Methods

In this experimental research, male Wistar rats (n=32, 250-300g) bred in Tehran Pasteur Institute were used. All animals had free access to laboratory chow, and tap water. Rats were randomly divided into 4 groups (n=8 in each group): 1- control group; 2- BMSCs transplantation group; 3- SCs transplantation group and 4- Co-transplantation group. All procedures in this study, including the use and care of animals, were approved by the Research Council of Tehran University of Medical Sciences (Tehran, Iran).

2.1. BMSCs Culture

Briefly, to obtain BMSCs, rats were killed and femurs and tibias were dissected out. The marrow was extruded with 10 ml of Dulbecco`s Modified Eagle Medium (DMEM) (Sigma, Aldrich) and cultured in DMEM (Azizi, Stokes, Augelli, Digirolamo, & Prockop, 1998). BMSCs were subcultured four times and were labeled with anti-BrdU antibody (Bromodeoxyuridin) (Sigma Aldrich) as the primary antibody and rhodamine (Sigma Aldrich) as the secondary antibody in the sciatic nerve. (Fig. 1, 2) (Li et al., 2006; Liao et al., 2001; Zurita & Vaquero, 2006; Zarbakhsh et al., 2012).

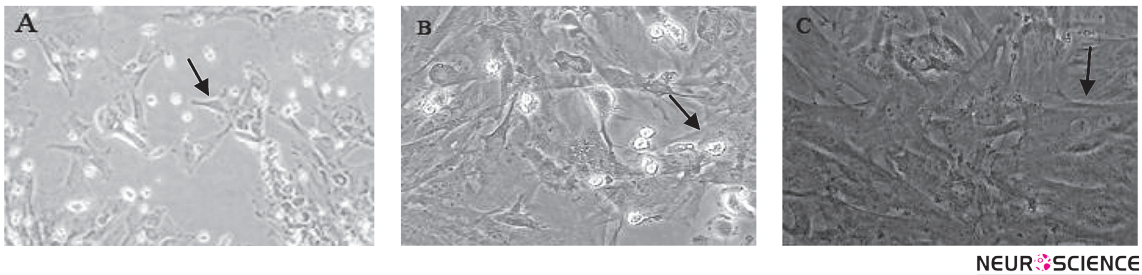

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Figure 1. Cultured bone marrow stromal cells. (A) In P0 stage adherent cells exhibited small round, spindle-shaped (arrow) (×200). (B) In P2 stage. Most cells grew, and exhibited fibroblast-like morphology. The small round cells adhered to the surface of these cell layers (arrow) (×200). (C) In P4 stage round cells disappeared and the fibroblast-like cells became morphologically homogeneous (arrow) (×200).

2.2. SCs Culture

Briefly, to obtain SCs, rats were killed and their sciatic nerves were dissected bilaterally. After removing epineurium and connective tissue, the sciatic nerves were cut into 2-3 mm fragments and cultured in DMEM (Zurita, Bonilla, Otero, Aguayo, & Vaquero, 2008). SCs were subcultured three times and were confirmed by anti S100 antibody. Dilution range of anti S100 antibody was ratio of 1 to 500 of rabbit anti S100 antibody (Sigma) in (PBS+0.3% Triton X+10% Normal Goat serum) (Fig. 3) (Rodriguez, Verdu, Ceballos, & Navarro, 2000). SCs were labeled with DiI (1,1`-dioctadecyl-3,3,3`,3`-tetramethylindocarbocyanine perchlorate) (Sigma Aldrich) (170 mg/ml in DMSO and diluted 1:10 in saline) in the sciatic nerve. Briefly SCs were suspended in DMEM and 5µl/ml DiI was added. After incubation for 20 min, the cells were centrifuged and washed twice with PBS. Then they were resuspended in PBS for transplantation. 4 weeks after the transplantation, prepared frozen sections and the labeled cells were detected using the fluorescent microscope (Olympus AX70) (Fig. 4) (Li et al., 2008; Pourheydar et al., 2012; Al et al., 2007; Bakhtyari et al., 2009; Haastert et al., 2006, Zarbakhsh et al., 2012).

2.3. Transplantation Procedure

Rats were anesthetized and after skin incision, the sciatic nerve was exposed using a muscle splitting incision. Under an operating microscope the left sciatic nerve was exposed at the mid-thigh, and a 10 mm segment of the nerve was removed. A 12 mm silicone tube (1 mm inner diameter, 2 mm outer diameter) was interposed into this nerve gap (Y. S. Chen et al., 2000). Both proximal and distal ends of the nerve were anchored into the conduit with 10-0 nylon suture (Fig.5). The silicone tube in the BMSCs group was filled with fibrin gel seeded with about 500,000 BMSCs, in the SCs group was filled with fibrin gel seeded with about 500,000 SCs, in the co-transplantation group was filled with fibrin gel seeded with about 250,000 BMSCs and 250,000 SCs; and the control group with fibrin gel without any cell. Finally the skin was sutured with 5-0 silk.

2.4. Electromyography (EMG) Study

Twelve weeks after the transplantation, rats were anesthetized and the sciatic nerves were exposed. Electric stimulation was utilized to the proximal site of the injured nerve. The compound muscle action potential was recorded in the gastrocnemius with a needle electrode and a reference cap electrode inserted at the knee joint. The stainless steel needle used as the ground electrode was inserted into the tail skin (Chen et al., 2007; Mimura, Dezawa, Kanno, Sawada, & Yamamoto, 2004; Zarbakhsh et al., 2012).

2.5. Functional Assessment

The functional assessment of the sciatic nerve regeneration was expressed by the sciatic function index (SFI). Briefly, twelve weeks after the transplantation, rats hind feet were dipped in ink and the rats were allowed to walk through a plastic tunnel so that the footprints could be recorded on paper loaded onto the bottom of the tunnel. The distance among the fingers, toes

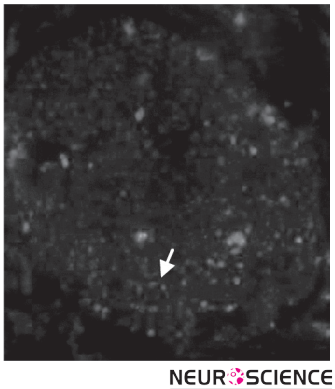

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Figure 2. Cross-section of the sciatic nerve, 4 weeks after the transplantation of BMSCs labeled with anti BrdU antibody shown as red spots in the sciatic nerve (arrow) (×100).

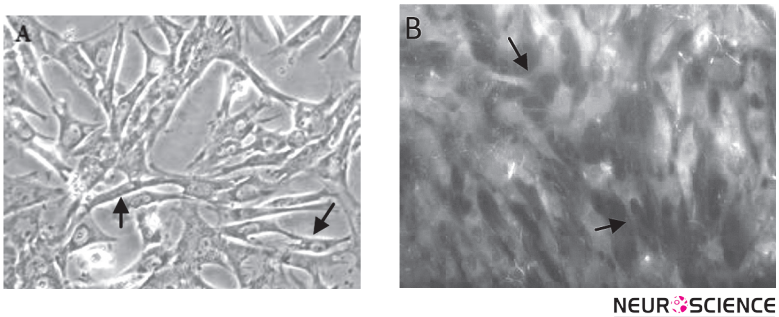

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Figure 3. (A) Cultured Schwann cells were small, elongated, and spindle shaped in P2 stage (arrows) (×200). (B) Schwann cells were labeled with anti S100 antibody, and showed a tendency to line up side by side or end to end and to form interconnected networks (arrows) (×200).

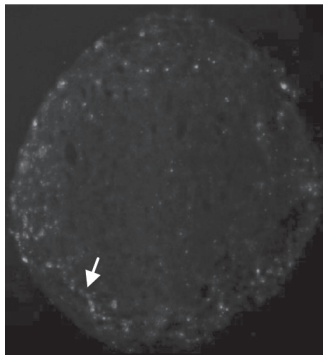

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Figure 4. Cross-sections of the sciatic nerve 4 weeks after the transplantation of Schwann cells labeled with DiI shown as red spots in the sciatic nerve (arrow) (×100).

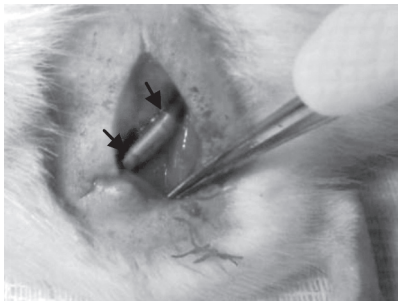

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Figure 5. Nerve graft. The left sciatic nerve was exposed at the mid-thigh and a 10 mm segment of the nerve was removed. A 12 mm silicone tube was interposed into this nerve gap. Arrows show two heads of the nerve in the silicone tube.

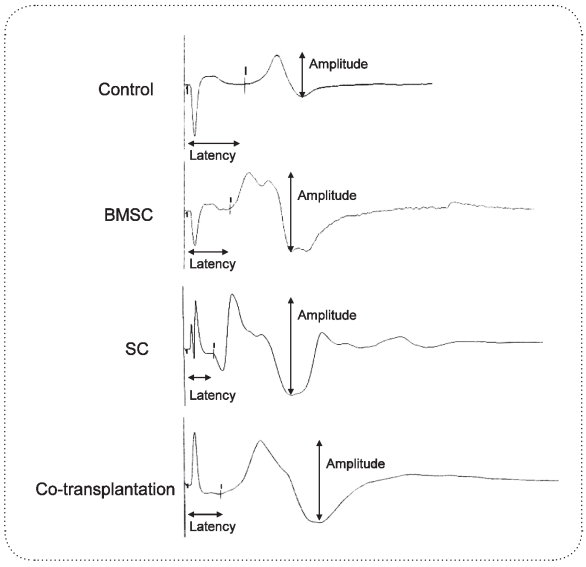

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Figure 6. Electromyographic waves for control, BMSCs, SCs and co-transplantation groups 12 weeks after surgery. Amplitude and latency are shown for each group. The time calibration bar was 2 ms and the amplitude calibration bar was 10 mV. The stimulation intensity was 2.3 mA and the duration was 0.1 ms.

and heels was measured. The SFI was calculated as follow: SFI= -38.3×(EPL-NPL)/NPL+109.5×(ETS-NTS)/NTS+13.3×(EITS-NITS)/NITS-8.8. In general, the SFI oscillates around 0 for normal nerve function, whereas around -100, the SFI indicates a total dysfunction (Chen et al., 2007; Mimura et al., 2004; Zarbakhsh et al., 2012).

2.6. Statistical Analyses

All data were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey test. Obtained data were presented as means ± standard error and a level of $p<0.05$ was considered statistically significant.

3. Results

3.1. BMSCs Culture

BMSCs obtained from the femurs and tibias of adult rats comprised heterogeneous groups of cells after seeding and growing in culture plates. After initial plating, the adherent cells exhibited a small rounded-shape, a spindle-shape or a large flattened morphology (Fig.1A). Most cells grew and exhibited a fibroblast-like morphology on reaching confluence. The small rounded cells adhered to the surface of these cell layers (Fig.1B). These rounded cells disappeared after repeated passages, whereas the fibroblast-like cells became enriched. Upon the 4th passage, the fibroblast-like cells became morphologically homogeneous (Fig.1C).

3.2. Labeled BMSCs in the Sciatic Nerve Tissue

Immunohistochemistry technique showed the BMSCs labeled with the anti-BrdU antibody were red spots in the cross section of the sciatic nerve (Fig.2). Presence of the red color was due to the use of rhodamine as the secondary antibody. Our results confirmed not only the the presence but the viability of the transplanted cells in the silicone tube bridging the gap 4 weeks after the transplantation.

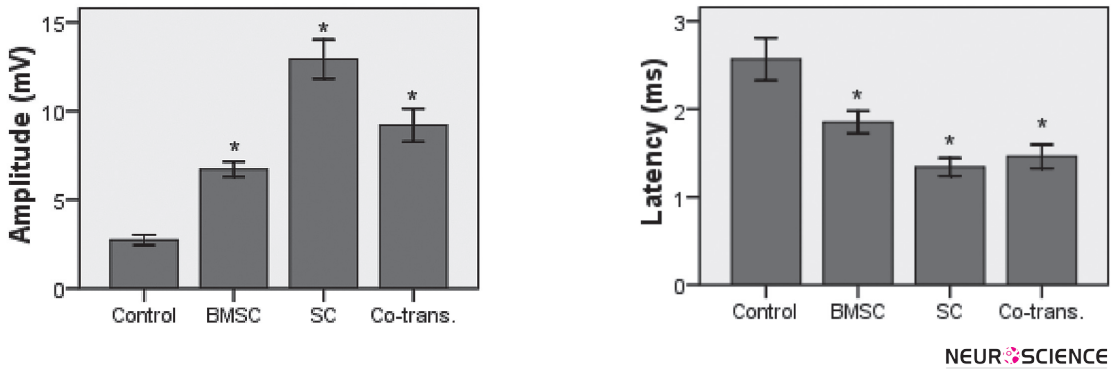

Figure 7. The results of electromyography tests of amplitude and latency showed there were statistically significant differences between the control and experimental groups (BMSCs, SCs and co-transplantation of BMSCs and SCs)* and SCs transplantationresulted in a more favorable results compared to the other groups (*p<0.05).

3.3. SCs Cultured

The spindle-shaped cellular morphology of the SCs seen on the culture plate was viable. Most of the cells were small, elongated, and spindle shaped (Fig.3A). Fluorescence microscopy showed the SCs were S100-positive cells. In the culture dishes, the SCs had a tendency to line up side by side or end to end and to form interconnected networks (Fig.3B).

3.4. Labeled SCs in the Sciatic Nerve Tissue

Histochemistry technique showed the SCs labeled with DiI as red-positive spots in the cross section of sciatic nerve (Fig.4). The results confirmed the presence and viability of the transplanted cells in the silicone tube bridging the gap 4 weeks after the transplantation.

3.5. Electromyography (EMG)

The results of the EMG tests comprised both amplitude and latency measures. The time calibration bar was 2 milliseconds (ms) and the amplitude calibration bar was 10 millivolts (mV). The stimulation intensity was 2.3 milliampere (mA) and the duration was 0.1 ms (Fig.6).

The results showed a statistically significant difference between control and experimental groups (BMSCs, SCs and co-transplantation). Moreover, results from the SCs-transplant group were significantly more favorable compared to the other groups (p<0.05) (Fig.7).

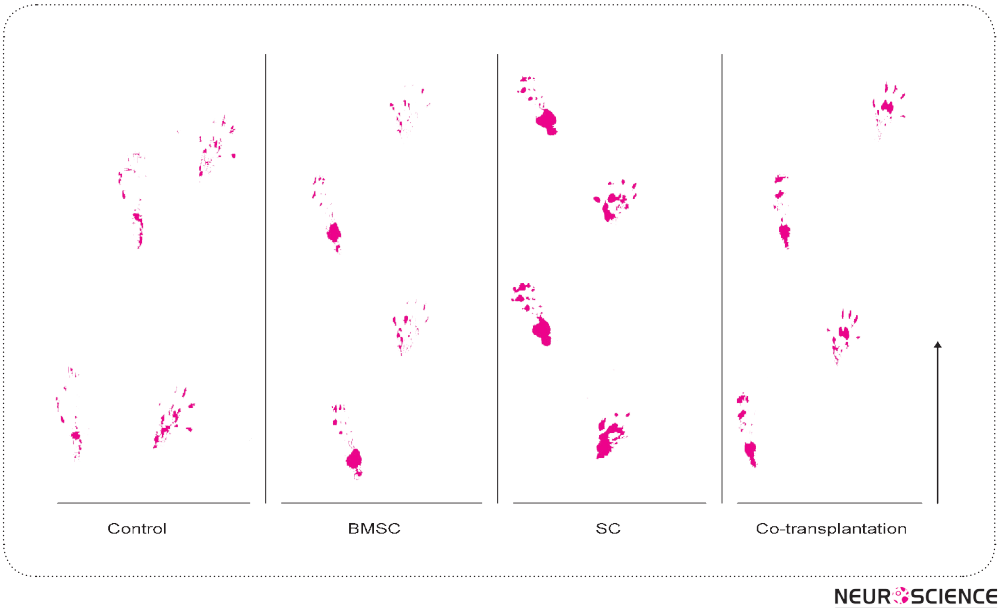

Figure 8. Foot print of control, BMSCs, SCs and co-transplantation groups 12 weeks after surgery. Arrow shows walking direction.

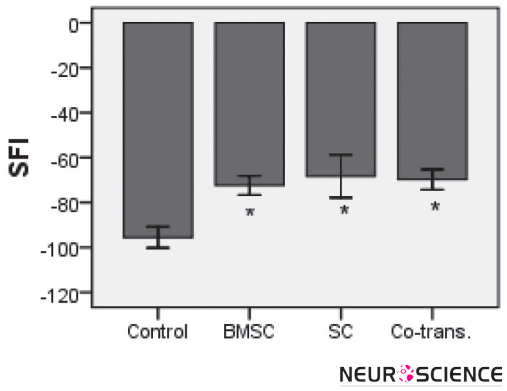

Figure 9. The results of the sciatic function index (SFI) test showed there were statistically significant differences between control and experimental groups (BMSCs, SCs and co-transplantation of BMSCs and SCs)* while showing no statistically significant difference among the experimental groups (*p<0.05).

3.6. Functional Analysis

The results of SFI showed a statistically significant difference between the control and experimental groups (BMSCs, SCs and co-transplantation), however, no statistically significant difference was observed amongst the experimental groups (p<0.05) (Fig.8, 9).

4. Discussion

Peripheral nerve injury is considered as one of the most challenging microsurgical problems. These damages may lead to a considerable disability due to the loss of both motor and sensory functions. Since the auto graft procedure involves multiple surgeries, loss of function, and loss of sensation at the donor site, development of a high quality replacement for auto grafts is required (Belkas et al., 2004; Dezawa, 2005; Ishikawa et al., 2009).

In the previous study we compared the effects of transplantation of BMSCs and SCs on the recovery of rats' sciatic nerve injury. We also showed that BMSCs and SCs can be effective by their own and SCs were significantly more effective than the BMSCs (Zarbakhsh et al., 2012). In this study, we compared the effects of the sole transplantation of BMSCs, SCs and the co-transplantation of these cells on the recovery of rats' sciatic nerve injury under similar condition. Several investigators have shown that BMSCs and SCs can repair the sciatic nerve injuries (Braga-Silva et al., 2006; C. J. Chen et al., 2007; Lu et al., 2006; Schlosshauer et al., 2003), however, BMSCs and SCs have not been used in combination for the same purpose. Comparing the effects these cells' co-transplantation (BMSCs and SCs) with the effect of their sole transplantation on the recovery of the injured peripheral nerve under similar conditions may introduce a novel clinical approache in utilizing these cells to replace peripheral nerve recovery auto grafts.

Due to the benefits of stem cells and Schwann cells in producing nerve growth factors such as NGF, BDNF and VEGF, as well as substantiating extracellular matrix proteins such as collagen IV and laminin (Braga-Silva et al., 2006; C. J. Chen et al., 2007; Fan et al., 2011; Feng, Zhou, Rush, & Ferguson, 2008; Ide, 1996), there is good evidence to support the hypothesis that transplantation of BMSCs and SCs may repair peripheral nerve injuries. Moreover, using the co-transplant of these cells may possibly be an important step in the selection of a repair procedure based on the peripheral nerve recovery measures.

In this study, we showed that the sole- and co-transplantation of BMSCs and SCs may lead to functional recovery of the injured sciatic nerve. This was documented by the EMG test in the gastrocnemius muscle as well as the walking behavior measured by the foot print analysis. The results of the EMG tests revealed that there were statistically significant differences between the control and experimental groups (BMSCs, SCs and co-transplantation of these cells). Some other reported data support our findings (C. J. Chen et al., 2007; Murakami et al., 2003; Rodriguez et al., 2000; Wang et al., 2008). The greater recovery in the SCs group as compared to the BMSCs group could probably be due to the direct and essential role of SCs in regeneration and recreation of axonal bridges. Similarly, The greater recovery in the SCs group as compared to the co-transplantation group was probably due to the number of SCs in SCs group than the co-transplant group. Furthermore, in the co-transplant group these cells might have overlapped resulting in a less repaire rate. The results of the SFI tests

showed there were statistically significant differences between the control and experimental groups. These results are in agreement with findings of other investigations (C. J. Chen et al., 2007; Hou, Zhang, Quan, Liu, & Zhu, 2006; Kim, Lee, & Lee, 2007; Nie et al., 2007), while the lack of any statistically significant difference amongst the experimental groups is a new finding and has not been previously addressed under similar conditions. Presumably after 12 weeks, due to the growing axons, a significance difference was observed in the results of EMG amongst the experimental groups, while myelin formation was not completed. Accordingly, there was no statistically significant difference amongst the experimental groups with regard to the SFI results. Nevertheless, we might possibly have observed a significance difference in the results of SFI amongst the experimental groups if waited longer.

Our results suggested that the sole- or co-transplantation of BMSCs and SCs have the potential to generate functional recovery of rats' injured sciatic nerve. EMG evaluation revealed that the SCs transplantation results in a greater functional improvement in the injured nerve as compared to BMSCs transplantation or co-transplantation of BMSCs and SCs. Our findings support use of SCs transplant as opposed to BMSCs or combination of BMSCs and SCs transplant for the clinical repair of the injured peripheral nerves.

References

Al, J., Epstein, P. N., Gozal, D., Yang, B., Wurster, R., & Cheng, Z., J. (2007). Morphology and topography of nucleus ambiguus projections to cardiac ganglia in rats and mice. Neuroscience, 149, 845-860.

Azizi, S. A., Stokes, D., Augelli, B. J., Digirolamo, C., & Prockop, D. J. (1998). Engraftment, and migration of human bone marrow stromal cells implanted in the brains of albino rats similarities to astrocytes grafts. Proc Natl Acad Sci USA, 95(7), 3908-3913.

Bakhtyari, M., Abootaleb, H., & Mansouri, K. (2009). Electrophysiological study of sciatic nerve regeneration through tubes seeded with schwann cells. Iranian Journal of Neuroscience, 1(3), 49-56.

Belkas, J. S., Shoichet, M. S., & Midha, R. (2004). Peripheral nerve regeneration through tubes. Neural Res, 26(2), 151-160.

Braga-Silva, J., Gehlen, D., Roman, J. A., Menta, C., Atkinson, E. A., Machado, D. C., et al. (2006). Bone marrow stem cells and platelet-rich plasma effects on nervous regeneration and functional recovery in an acute defect model of rats` peripheral nerve. Acta Ortop Bras, 14(5), 273-275.

Chen, C. J., Ou, Y. C., Liao, S. L., Chen, W. Y., Chen, S. Y., Wu, C. W., et al. (2007). Transplantation of bone marrow stromal cells for peripheral nerve repair. Exp. Neurology, 204(1), 443-453.

Chen, Y. S., Hsieh, C. L., Tasi, C. C., Chen, T. H., Cheng, W. C., Hu, C. L., et al. (2000). Peripheral nerve regeneration using silicone rubber chambers filled with collagen, laminin and fibronectin. Biomaterials, 21(15), 1541-1547.

Dezawa, M. (2005). Future views and challengges to the peripheral nerve regeneration by cell based therapy. Rinsho Shinkeigaku (Japanes), 45(11), 877-879.

Fan, W., Crawford, R., & Xiao, Y. (2011). The ratio of VEGF/PEDF expression in bone marrow mesenchymal stem cells regulates neovascularization. Differentiation, 81(3), 181-191.

Feng, S. Q., Zhou, X. F., Rush, R. A., & Ferguson, I. A. (2008). Graft of pre-injured sural nerve promotes regeneration of corticospinal tract, and functional recovery in rats with chronic spinal cord injury. Brain Res, 1209, 40-48.

Haastert, K., Lipokatic, E., Fischer, M., Timmer, M., & Grothe, C. (2006). Differentially promoted peripheral nerve regeneration by grafted Schwann cells over-expressing different FGF-2 isoforms. Neurobiology of Disease, 21(1), 138-153.

Hou, S. Y., Zhang, H. Y., Quan, D. P., Liu, X. L., & Zhu, J. K. (2006). Tissue engineered peripheral nerve grafting by differentiated bone marrow stromal cells. Neuroscience, 140(1), 101-110.

Ide, C. (1996). Peripheral nerve regeneration. Neurosci. Res., 25(2), 101-121.

Ishikawa, N., Suzuki, Y., Dezawa, M., Kataoka, K., Ohta, M., Cho, H., et al. (2009). Peripheral nerve regeneration by transplantation of BMSC-derived schwann cells as chitosan gel sponge scaffolds. Willey Periodicals, Inc. J Biomed Mater Res., 89(4), 1118-1124.

Kim, S. M., Lee, S. K., & Lee, J. H. (2007). Peripheral nerve regeneration using a three dimensionally cultured Schwann cell conduit. The Journal of Craniofacial Surgery, 18(3), 475-488.

Li, P., Akimoto, T., Zhang, M., Williams, R. S., & Yan, Z. (2006). Resident stem cells are not required for exercise-induced fiber-type switching and angiogenesis but are necessary for activity-dependent muscle growth. Am J Physiol Cell Physiol, 290(6), 1461-8.

Li, N., Yang, H., Lu, L., Duan, C., Zhao, C., & Zhao, H. (2008). Comparison of the labeling efficiency of BrdU, DiI and FISH labeling techniques in bone marrow stromal cells. Brain Research, 1215, 11-19.

Liao, G., Wu, F. Y., & Hayward, S. D. (2001). Interaction with the Epstein-Barr virus helicase target Zta to DNA replication compartments. J Virol, 75(18), 8792-802.

Lu, M. C., Chang, Y. H., Chiang, L. C., Wang, H. T., Cheng, C. Y., Yao, C. H., et al. (2006). Peripheral nerve regeneration through nerve guides filled with bilobalide and schwann cells. Biomed Eng Appl Basis Comn, 18(1), 8-12.

Millesi, H. (1984). Nerve grafting. Clin Plast Surg 11(1), 105-113.

Mimura, T., Dezawa, M., Kanno, H., Sawada, H., & Yamamoto, I. (2004). Peripheral nerve regeneration by transplantation of bone marrow stromal cell-derived Schwann cells in adult rats. J. Neurosurg, 101(5), 806-812.

Murakami, T., Fujimoto, Y., Yasunaga, Y., Ishida, O., Tanaka, N., Ikuta, Y., et al. (2003). Transplanted neuronal progenitor cells in a peripheral nerve gap promote nerve repair. Brain Res, 974(1-2), 17-24.

Nie, X., Zhang, Y. J., Tian, W. D., Jiang, M., Dong, R., Chen, J. W., et al. (2007). Improvement of peripheral nerve regeneration by a tissue-engineered nerve filled with ectomesenchymal stem cells. Int. J. Oral Maxillofac. Surg., 36(1), 32-38.

Pourheydar, B., Joghataei, M. T., Bakhtiari, M., Mehdizadeh, M., Yekta, Z., & Najafzadeh, N. (2012). Co-transplantation of bone marrow stromal cells with schwann cells evokes mechanical allodynia in the contusion model of spinal cord injury in rat. Cell Journal (yakhteh), 13(4), 213-222.

Rodriguez, F. J., Verdu, E., Ceballos, D., & Navarro, X. (2000). Nerve guides seeded with autologous Schwann cells improve nerve regeneration. Exp. Neurology, 161(2), 571-584.

Schlosshauer, B., Muller, E., Schroder, B., Planck, H., & Muller, H. (2003). Rat schwann cells in bioresorbable nerve guides to promote, and accelerate axonal regeneration. Brain Res, 963(1-2), 321-326.

Wang, D., Liu, X. L., Zhu, J. K., Jiang, L., Hu, J., Zhang, Y. J., et al. (2008). Bridging small-gap peripheral nerve defects using acellular nerve allograft implanted with autologous bone marrow stromal cells in primates. Brain Res, 1188, 44-53.

Zarbakhsh, S., Bakhtiari, M., Faghihi, A., Joghataie, M. T., Mehdizadeh, M., Khoei, S., et al. (2012). The effects of Schwann and bone marrow stromal stem cells on sciatic nerve injury in rat: A comparison of functional recovery. Cell Journal(Yakhteh) 14(1), 39-46.

Zurita, M., Bonilla, C., Otero, L., Aguayo, C., & Vaquero, J. (2008). Neural transdifferentiation of bone marrow stromal cells obtained by chemical agents is a short-time reversible phenomenon. Neuroscience Research, 60(3), 275-280.

Zurita, M., & Vaquero, J. (2006). Bone marrow stromal cells can achieve cure of chronic paraplegic rats: functional and morphological outcome one year after transplantation. Neurosci. Lett, 402(1-2), 51-56.