Effect of Stem Cell Transplantation on Pain Behavior and Locomotor Function in Spinal Cord Contusion Model

The Journal Korean Society of Physical Therapy

Hea-Woon Park, MD, PhD; Su-Jeong Kim, PhD[1]; Yun-Woo Cho, MD, PhD[2]; Se-Jin Hwang, MD, PhD[3]; Won-Yub Lee, MD; Sang-Ho Ahn, MD, PhD[2]; Sung-Ho Jang, MD, PhD[2]

Department of Rehabilitation Medicine, School of Medicine, Catholic University of Deagu; [1]Institute of Medical Science, Yeungnam University; [2]Department of Rehabilitation Medicine, College of Medicine, Yeungnam University; [3]Department of Anatomy and Cell Biology, College of Medicine, Hanyang University

Purpose: Many trials for new therapeutic approaches such as stem cell-based transplantation have been conducted to improve the repair and regeneration of injured cord tissue and to restore functions following spinal cord injury (SCI) in animals and humans. Adipose tissue-derived stromal cells (ATSCs) have multi-lineage potential to differentiate into cells with neuron-like morphology. Most studies of stem cell transplantation therapy after SCI are focused on cellular regeneration and restoration of motor function, but not on unwanted effects after transplantation such as neuropathic pain. This study was focused on whether transplantation of ATSCs could facilitate or attenuate hindpaw pain responses to heat, cold and mechanical stimulation, as well as on improvement of locomotor function in a rat with SCI.

Methods: A spinal cord injury rat model was produced using an NYU impactor by dropping a 10 g rod from a height of 25 mm on to the T9 segment. Human ATSCs (hATSCs; approximately $5x10^5$ cells) or DMEM were injected into the perilesional area 9 days after the SCI. After transplantation, hindpaw withdrawal responses to heat, cold and mechanical allodynia were measured over 7 weeks. Motor recovery on the Basso, Beattie, and Bresnahan (BBB) locomotor rating scale and on the inclined plane test were also evaluated.

Results: The present study demonstrated that increased hindpaw withdrawal responses to cold allodynia was observed in both groups after transplantation, but the development of cold-induced allodynia in the hATSC transplantation group was significantly larger than in the control group. The difference between the two groups in locomotor functional improvement after SCI was also significant.

Conclusion: Careful consideration not only of optimal functional benefits but also of unintended side effects such as neuropathic pain is necessary before stem cell transplantation therapy after SCI.

Keywords: Adipose tissue-derived stromal cells, Spinal cord injury, Neuropathic pain, Locomotor function, Cold allodynia

Received: May 19, 2010
Revised: June 9, 2010
Accepted: June 15, 2010
Corresponding author: Se-Jin Hwang, hwangsj@hanyang.ac.kr; Sang-Ho Ahn, spineahn@ynu.ac.kr

I. Introduction

Spinal cord injury (SCI) results in not only permanent sensorimotor impairment and dysfunction of autonomic nervous system but also chronic neuropathic pain below level of injury. Many people with SCI are living with devastated their quality of life.[1] Various approaches for new therapeutic strategy have been studied to repair and regenerate of injured cord and restore functions following SCI.[2,3] It has been taken a growing interest in stem cells-based transplantation studies to treat SCI in animals[4-9] and humans,[10-12] although clinical trials for the treatments of SCI are quite unsatisfactory.

Various types of adult-derived stem or progenitor cells have been studied for autologous cell transplantation in a model of SCI because of ethical problems and immunological reaction. They are included as olfactory ensheathing cells (OECs),[10,12] adult neural precursor cells (NPCs)[9] and mesenchymal stem cells, including bone marrow-derived stem cells (BMSCs)[8,11] and

adipose tissue-derived stromal cells (ATSCs).[6] Among them, it has also been known that ATSCs have multi-lineage potentials to differentiate into neuron-like morphologies.[13,14] Kang et al has reported that ATSCs transplantation into the rats with cerebral ischemia and spinal cord injury resulted in functional improvement.[6,15]

Usually, most studies for stem cell transplantation therapy after SCI are focused on cellular regeneration and restoration of motor function but not interested in side effects like neuropathic pain after transplantation. Unfortunately, it has been reported that cell transplantation therapy may increase the possibility of development of neuropathic pain.[16-19] Previous studies about side effects like neuropathic pain development after stem cell transplantation therapy in a model of SCI have been relatively scarce. Therefore, the present study is focused on whether the stem cell transplantation therapy could facilitate or attenuate the hindpaw pain responses of thermal, cold and mechanical stimulation as well as improve locomotor function in a rat with SCI.

II. Materials and Methods

1. Animals and spinal cord injury

All experimental procedures were carried out according to the Institutional Animal Care and Use Committee guidelines at the Yeungnam University, South Korea. Adult female Sprague-Dawley rats (200~250 g, 6~8 weeks old) were used for this study. Animals were acclimatized to controlled laboratory environments (12 hr light/dark cycles) with free access to food and water. Animals were anesthetized prior to surgery by intraperitoneal injection of Zoletil (Virbac Laboratories, France, 50 mg/kg). Under anesthesia, laminectomy was performed and exposed the T9 segment of spinal cord. We used a weight-drop device developed at New York University and produced the spinal cord injured rat model.[20] Spinal cord contusion was induced by dropping a 10-g rod (2.5 mm in diameter) from a height of 25 mm. For sham surgery, animals were laminectomized and only vertebral clips of the impactor were placed without impact injury. After surgery, incised muscle and skin was sutured in layers and animalswere warmed with heating pad to restore the temperature. Throughout 7 days after surgery, animals were received antibiotics daily and bladder was emptied twice daily by bladder compression manually until the function of bladder was recovered.

2. ATSCs culture

The adipose tissue was obtained from abdominal omentum of patient operated by liposuction. Adipose tissue was washed at least three times in phosphate buffer saline (PBS, GibcoBRL) to remove blood. The tissue was cut off and digested with collagenase I (2 mg/ml, GibcoBRL) for 50 minutes (min) at 37℃ using shaking incubator. The cells were filtered through a 250 μm nylon mesh and centrifuged for 5 min at 412 g. The floating adipocytes were isolated from stromal vascular fraction. The pellet resuspended in red blood lysing buffer (8.3 g/L NH_4Cl, 0.01 M Tris-HCl, pH 7.5, Sigma) for 10 min. After centrifuged at 264 g for 5 min, preadipocytes in the stromal vascular fraction were plated in culture dishes at 4.0×10^3 cells/cm^2. The cells were cultured with Dulbecco's Minimal Essential Medium (DMEM, GicoBRL) containing 10% fetal bovine serum (FBS, GibcoBRL) and 1% antibiotics (GibcoBRL). The cells were incubated at 5% CO_2 and 37℃. After 24 h, non-adherent cells were removed. Thereafter, the culture media was changed every 3 days. When the cells reached 80% confluence, the cells were harvested with 0.25% trypsin-EDTA (GibcoBRL) for 3 min at 37 ℃, centrifuged for 5 min at 264 g and replated at a density of 4.0×10^3 cells/cm^2. The cells were expanded by serial passage and used for in vitro cell characterization and in vivo transplantation.

3. Flow cytometric analysis

Immunophenotypic characteristics of ATSCs were determined at 5 passages by fluorescence-activated cell sorting (FACS) analysis using primary antibodies conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE).

The cultured hATSCs were detached by 0.25% trypsin-EDTA and washed twice in PBS containing 1% FBS. The density of 2.5×10^5 cells were reacted with 5 μl of individual primary antibodies in 200 μl PBS containing 1% FBS for 20 min in the dark and at room temperature. The cells were washed twice in 500 μl PBS containing 1% FBS, centrifuged for 5 min at 100 g and finally diluted in 500 μl PBS containing 1% FBS. Samples were analyzed using a FACScan flow cytometer (FACScanlibur, BD). Analysis was performed using CellQuest software (BD).

4. Cell transplantation

Rats with BBB score 7 to 8 the day before transplantation (day 8 after injury) were randomly assigned to hATSCs (n=8) and DMEM (n=8) transplantation group.

Transplantation of hATSCs was performed at 9 days after SCI. The hATSCs were harvested just before injection into the injured spinal cord site. Animals were anesthetized as above, and the laminectomy site was re-exposed. A 10 µl Hamilton syringe (Hamilton, Reno, NV) with a 33 gage needle was lowered into the spinal cord using a stereotactic manipulator arm. Cell suspensions were injected along the midline of the spinal cord at a depth of 1.2 mm into two sites 1.5 mm cranial and caudal to the lesion epicenter, in a total volume of 5 µl DMEM without growth factors (approximately 5×10^5 cells) at a rate of 1µl/min. The injected needle was removed after 3 min to reduce the possibility of leakage of the injected suspension from the site. Control animals received DMEM vehicle only. After the surgery, animals were housed in pairs, and manually bladder evacuation was performed at least twice per day. The injured animals received prophylactic antibiotics during the first week after injection.

5. Outcome measures

All behavioral tests were performed independently by two investigators who were unaware of the experimental groupings and the protocols of each rat. Pain response for thermal hyperalgesia, cold allodynia and mechanical allodynia was assessed 2 days prior to injury, and weekly from 2 weeks after implantation until 7 weeks. Locomotor function was assessed on 2 days prior to injury and implantation, and weekly until 7 weeks for BBB locomotor rating scale, and on 2 days prior to injury, and weekly from 3 weeks after implantation until 7 weeks for inclined test.

Thermal hyperalgesia was determined by measuring the thermal withdrawal response of the hindpaw. Briefly, rats were placed in a Plexiglas chamber positioned on a glass platform. The plantar surface of the hindpaw was subjected to a radiant heat source (Model 336 combination unit, IITC/life Science Instruments, Woodland Hill, CA, USA) until the rat showed a quick withdrawal response. Each hindpaw was tested five times by alternating between the left and right with 30 seconds (s) interval. The mean latency of hindpaw withdrawal response was calculated.[21,22]

Cold allodynia was also determined by measuring the cold withdrawal response of the hindpaw to acetone application. Briefly, rats were placed in a clear plastic cage with a metal mesh floor and adapted for 15 min before measurements and then, acetone drop was touched to the plantar surface of the hindpaw. Each hindpaw was tested five times by alternating between the left and right with 5 min interval. The mean frequency of hindpaw withdrawal response was calculated.[23]

Mechanical allodynia was tested by measuring the mechanical withdrawal response of the hindpaw using von Frey filaments. Briefly, rats were placed in a clear plastic cage with a metal mesh floor and adapted for 15 min before measurements. Beginning with 0.1 g probe, filaments were applied to the plantar surface of the hindpaw for 6 to 8 seconds in a stepwise ascending or descending order following negative or positive withdrawal response. Fifty percent probability thresholds of mechanical paw withdrawal were calculated. In the absence of foot withdrawal in response to the application of a 26 g von Frey filament, 26 g was then assigned as the mechanical threshold.[24]

The locomotor function was assessed by open field test using BBB locomotor rating scale. Briefly, the test was performed after placing the rats individually for 4 min in an open field which was a molded-plastic circular enclosure with a smooth, a non slippery surface (100 cm in the diameter, height of 20 cm). The BBB locomotor rating scale was composed of 22-point scale, from 0 indicating no movement of the hind limb to 21 being normal rat.[25] The inclined plane test was also performed. Briefly, rat's ability to maintain body position on an inclined board was assessed by gradually raising the angles. The maximal angle that rat body axis was perpendicular to the axis of the inclined plane was measured.[26]

The collected data were encoded into SPSS/PC version 14.0 and analyzed. Data were expressed as mean±standard deviation (SD). Baseline measures were analyzed by Wilcoxon signed rank test and Mann Whitney U test for behavioral tests. Differences with $p<0.05$ were considered statistically significant.

III. Results

1. hATSCs characterization

After five passages of initial plating of the primary culture, hATSCs were determined the immunophenotype. The results show that hATSCs are highly positive for CD13, CD29, CD44, CD49e and CD90 with mean percent of positive cells of 97.89±

0.74, 96.30±1.56, 95.85±1.84, 92.54±5.90, and 95.66±2.77, respectively, but are negative for CD34, CD45 and CD31 with percent of negative cells of 1.76±1.75, 1.98±1.83, and 2.00±2.04, respectively.

2. Behavioral tests for pain responses with ATSCs

Behavioral responses for thermal hyperalgesia, cold allodynia and mechanical allodynia were measured as soon as the hind paws of the injured animals could support their weight. Behavioral test could be applied from 2 weeks after implantation in this study.

1) Thermal hyperalgesia

The hindpaw withdrawal latency for heat stimuli following SCI was decreased in both hATSC and DMEM implantation throughout the experimental period compared to basal values. Although the paw withdrawal latency was more decreased in group of hATSC transplantation from 5 weeks, there was no significant difference between hATSC and DMEM implantation (Figure 1). The transplantation of hATSC could not induce further development of thermal hyperalgesia compared to DMEM implantation.

2) Cold allodynia

Before SCI and naïve states, rats rarely responded to the application of acetone on the hindpaw. From 2 weeks after implantation, hindpaw withdrawal frequency for cold stimuli was observed and gradually increased in both groups until 7 weeks similarly. In addition, in hATSC transplantation the development of cold allodynia was larger than in DMEM implantation only. The difference of the development of cold allodynia was significant at 3, 6 weeks after implantation (Figure 2)($p<0.05$). It seems that the hATSC transplantation might lead to induce cold allodynia much more than DMEM implantation only.

3) Mechanical allodynia.

Before SCI and naïve states, rats were rarely respond to the mechanical hindpaw stimulation with a 26 g von Frey filaments. After SCI, in both ATSCs and DMEM transplantation group, hind paws of rat did not respond to application of the subsequent series of von Frey filaments (0.1～26 g) throughout the experimental period (Data not shown). Mechanical allodynia at hindpaw did not develop after SCI in this study and the effect of transplantation of hATSC on the production of mechanical allodynia could not check.

3. Motor assessment for functional recovery with ATSCs

We compared the difference of locomotor functional recovery between ATSCs and DMEM transplantation group with BBB

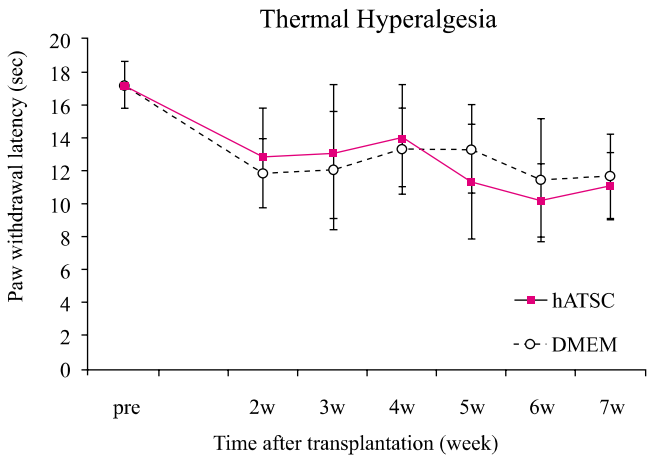

Figure 1. The effects of transplantation of hATSC and DMEM on thermal hyperalgesia after SCI. The hindpaw withdrawal latency to heat stimuli was gradually decreased in both groups. Although the latency was more decreased in hATSCs transplantation group from 5 weeks, there was no significant difference between two groups.

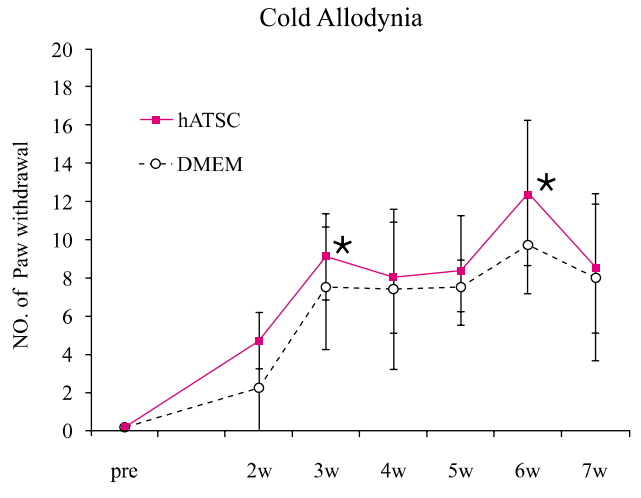

Figure 2. The effects of transplantation of hATSC and DMEM on cold allodynia after SCI. The hindpaw withdrawal frequency to cold stimuli was gradually increased in both groups. The hindpaw withdrawal frequency was more distinct in hATSCs transplantation group at 3, 6 weeks significantly (*$p<0.05$).

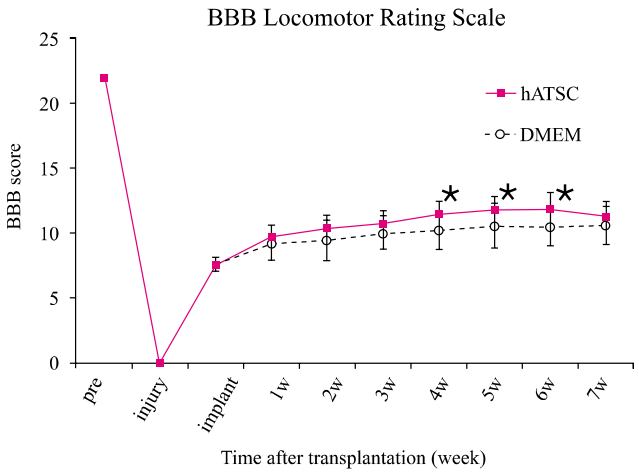

Figure 3. Locomotor test with BBB rating scale after transplantation of ATSCs and DMEM. The BBB socre was improved gradually in both groups. The improvement of BBB score was more significant in hATSCs group at 4, 5, and 6 weeks after transplantation (*p<0.05).

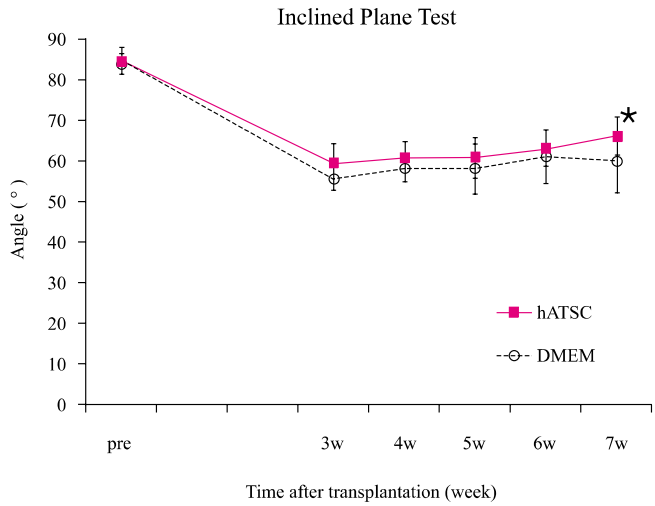

Figure 4. Locomotor test with inclined test after transplantation of ATSCs and DMEM. The maximum angles were gradually increased in both groups. The improvement of maximum angle was more significant in hATSCs group at 7 weeks after transplantation (*p<0.05).

score and the maximum angles of inclined board. In BBB test, after transplantation of ATSCs or DMEM, the hindpaw function was improved gradually in both groups, respectively. After 7 weeks of transplantation, motor function of both groups were improved to the average of BBB score 11.4±0.9 and 10.6±0.7. There were significant differences between 2 groups at 4, 5, and 6 weeks after transplantation (Figure 3)($p<0.05$). In inclined test, the maximum angles were also improved gradually in both groups. After 7 weeks of transplantation, the mean maximum angles of both groups were 65.9±4.6° and 59.6±7.5°, respectively. The maximum angles were significantly larger in ATSCs transplantation group at 7 weeks after transplantation (Figure 4)($p<0.05$). The transplantation of hATSCs could improve the locomotor function after SCI compared with DMEM transplantation.

IV. Discussion

The present study demonstrated that the development of cold allodynia was more distinct as compared with transplantation of DMEM only after transplantation of ATSCs in SCI rat model. In addition, more locomotor functional improvement was also observed in hATSCs transplantation.

It has been reported that ATSCs isolated from adipose tissue had the ability to differentiate into multiple lineages, including neurogenic, adipogenic, osteogenic and chondrogenic differentiation and more than 75% of subpopulation of primate ATSCs had neurogenic potential.[27,28] Transplantationof ATSCs after cerebral ischemia and spinal cord injury in rats has shown significant functional improvement.[6,15] Furthermore, injection of cytoplamic extracts from ATSCs after SCI has reduced apoptotic cell death, astrogliosis and hypomyelination.[7] Consistent with previous study, this study also showed that significant functional recovery after SCI was observed in hATSCs transplantation compared with control. These findings support hATSCs likewise other types of stem cells also have potential to restore spinal motor function after SCI.

Unfortunately, stem cell transplantation therapy seems to influence unwanted sensory function as well as motor function of nervous system.[16-19] Transplantation of neural stem cells in the injured spinal cord has been shown forelimb thermal and mechanical allodynia and increased sprouting of nociceptive afferents without improvement of locomotor function.[19] Differentiation into astrocytes, are known as the sources of neurotrophic factors, might result in painful response to stimuli. Hofstetter et al[17] also has reported that grafting of adult neural stem cells into a rat with SCI causes allodynia-like hypersensitivity and axonal sprouting, which is prevented by suppression of astrocytic differentiation by transduction of neural stem cells with neurogenin-2. Furthermore, the human trials using auto-

logous bone marrow cell transplantation also showed 20% of patients developed neuropathic pain.[11] Interestingly, in consistent with previous study, the present study demonstrated the development of cold allodynia on hindpaw was exacerbated after transplantation of hATSCs in rats with SCI. Although we also supposed that differentiation into astrocytes may affect the development of pain response, we could not evaluate the histologic findings. However, the other study of our laboratory showed increased astrocytes in below and above area of spinal cord lesion (Unpublished data).

Contradictorily, opposite studies was also reported in stem cell based therapy. The transplantation of fibroblasts, genetically modified to express BDNF and NT-3 into perilesional spinal cord (T8/9) of rat with spinal contusion injury had not exacerbated the development of forepaw thermal hypersensitivity.[29] Furthermore, in T13 hemisection SCI model, intrathecal transplantation of RN46A-B14 serotonergic precursor cells, which secrete serotonin and BDNF had reduced the hindlimb mechanical allodynia and thermal hyperalgesia.[30] Predifferentiated embryonic stem cells also had reduced mechanical allodynia and cold allodynia of mice pain model after unilateral intraspinal injection of quisqualic acid.[31] Perilesional transplantation of human mesenchymal stem cell into rat spinal cord had shown not only the functional improvement but also attenuation of mechanical allodynia in a rat with spinal contusional injury.[8] Variable conditions including the type of implanted cells, spinal cord injury model and animals may influence the difference of pain response after cell transplantation following SCI. To our knowledge, this is the first study about the effects of transplantation with ATSCs after SCI on the pain response related with stem cell therapy. In the present study, perilesional transplantation of ATSCs after spinal contusional injury may affect not only improve the hindpaw motor function but also the exacerbation of hindpaw pain rather than attenuation of hindpaw pain.

The limitation of present study is that we could not add histologic or physiologic evidence of causal relationship of hATSCs transplantation for motor improvement and increased pain response. Further studies are needed toverify the pathomechanism of various positive or negative effects of ATSCs transplantation and minimize the adverse effects for spinal cord repair.

V. Conclusion

The present study demonstrated that after transplantation of ATSCsin rats with SCI, the development of cold allodynia as well as locomotor functional improvement were more distinct as compared with transplantation of DMEM. For safe stem cell-based therapeutic strategies for human trial, careful considerations about not only optimal functional benefits but also accompanying unintended side effects like neuropathic pain should be necessary before adipose tissue-derived stromal cell transplantation therapy after SCI.

Author Contributions

Research design: Park HW
Acquisition of data: Lee WY, Kim SJ, Cho YW
Analysis and interpretation of data: Park HW, Ahn SH, Hwang SJ
Drafting of the manuscript: Park HW, Jang SH
Administrative, technical, and material support: Kim SJ, Cho YW
Research supervision: Ahn SH, Park HW, Hwang SJ

Acknowledgements

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2007-331-E00174).

References

1. Siddall PJ, McClelland JM, Rutkowski SB et al. A longitudinal study of the prevalence and characteristics of pain in the first 5 years following spinal cord injury. Pain. 2003; 103(3):249-57.
2. Okano H, Kaneko S, Okada S et al. Regeneration-based therapies for spinal cord injuries. Neurochem Int. 2007;51 (2-4):68-73.
3. Rossignol S, Schwab M, Schwartz M et al. Spinal cord injury: time to move? J Neurosci. 2007;27(44):11782-92.
4. Bambakidis NC, Butler J, Horn EM et al. Stem cell biology and its therapeutic applications in the setting of spinal cord injury. Neurosurg Focus. 2008;24(3-4):E20.
5. Eftekharpour E, Karimi-Abdolrezaee S, Fehlings MG. Current status of experimental cell replacement approaches to spinal cord injury. Neurosurg Focus. 2008;24(3-4):E19.
6. Kang SK, Shin MJ, Jung JS et al. Autologous adipose tissue-derived stromal cells for treatment of spinal cord injury. Stem

Cells Dev. 2006;15(4):583-94.
7. Kang SK, Yeo JE, Kang KS et al. Cytoplasmic extracts from adipose tissue stromal cells alleviates secondary damage by modulating apoptosis and promotes functional recovery following spinal cord injury. Brain Pathol. 2007;17(3):263-75.
8. Lee KH, Suh-Kim H, Choi JS et al. Human mesenchymal stem cell transplantation promotes functional recovery following acute spinal cord injury in rats. Acta Neurobiol Exp (Wars). 2007;67(1):13-22.
9. Wrathall JR, Lytle JM. Stem cells in spinal cord injury. Dis Markers. 2008;24(4-5):239-50.
10. Mackay-Sim A, Feron F, Cochrane J et al. Autologous olfactory ensheathing cell transplantation in human paraplegia: a 3-year clinical trial. Brain. 2008;131(Pt 9):2376-86.
11. Yoon SH, Shim YS, Park YH et al. Complete spinal cord injury treatment using autologous bone marrow cell transplantation and bone marrow stimulation with granulocyte macrophage-colony stimulating factor: Phase I/II clinical trial. Stem Cells. 2007;25(8):2066-73.
12. Lima C, Pratas-Vital J, Escada P et al. Olfactory mucosa autografts in human spinal cord injury: a pilot clinical study. J Spinal Cord Med. 2006;29(3):191-203.
13. Ashjian PH, Elbarbary AS, Edmonds B et al. In vitro differentiation of human processed lipoaspirate cells into early neural progenitors. Plast Reconstr Surg. 2003;111(6):1922-31.
14. Safford KM, Hicok KC, Safford SD et al. Neurogenic differentiation of murine and human adipose-derived stromal cells. Biochem Biophys Res Commun. 2002;294(2):371-9.
15. Kang SK, Lee DH, Bae YC et al. Improvement of neurological deficits by intracerebral transplantation of human adipose tissue-derived stromal cells after cerebral ischemia in rats. Exp Neurol. 2003;183(2):355-66.
16. Davies JE, Proschel C, Zhang N et al. Transplanted astrocytes derived from BMP- or CNTF-treated glial-restricted precursors have opposite effects on recovery and allodynia after spinal cord injury. J Biol. 2008;7(7):24.
17. Hofstetter CP, Holmstrom NA, Lilja JA et al. Allodynia limits the usefulness of intraspinal neural stem cell grafts; directed differentiation improves outcome. Nat Neurosci. 2005;8(3):346-53.
18. Klein S, Svendsen CN. Stem cells in the injured spinal cord: reducing the pain and increasing the gain. Nat Neurosci. 2005;8(3):259-60.
19. Macias MY, Syring MB, Pizzi MA et al. Pain with no gain: allodynia following neural stem cell transplantation in spinal cord injury. Exp Neurol. 2006;201(2):335-48.
20. Gruner JA. A monitored contusion model of spinal cord injury in the rat. J Neurotrauma. 1992;9(2):123-6.
21. Dirig DM, Salami A, Rathbun ML et al. Characterization of variables defining hindpaw withdrawal latency evoked by radiant thermal stimuli. J Neurosci Methods. 1997;76(2):183-91.
22. Hargreaves K, Dubner R, Brown F et al. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain. 1988;32(1):77-88.
23. Choi Y, Yoon YW, Na HS et al. Behavioral signs of ongoing pain and cold allodynia in a rat model of neuropathic pain. Pain. 1994;59(3):369-76.
24. Chaplan SR, Bach FW, Pogrel JW et al. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods. 1994;53(1):55-63.
25. Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma. 1995;12(1):1-21.
26. Rivlin AS, Tator CH. Objective clinical assessment of motor function after experimental spinal cord injury in the rat. J Neurosurg. 1977;47(4):577-81.
27. Kang SK, Putnam LA, Ylostalo J et al. Neurogenesis of Rhesus adipose stromal cells. J Cell Sci. 2004;117(Pt 18):4289-99.
28. Zuk PA, Zhu M, Mizuno H et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng. 2001;7(2):211-28.
29. Mitsui T, Fischer I, Shumsky JS et al. Transplants of fibroblasts expressing BDNF and NT-3 promote recovery of bladder and hindlimb function following spinal contusion injury in rats. Exp Neurol. 2005;194(2):410-31.
30. Hains BC, Johnson KM, Eaton MJ etal. Serotonergic neural precursor cell grafts attenuate bilateral hyperexcitability of dorsal horn neurons after spinal hemisection in rat. Neuroscience. 2003;116(4):1097-110.
31. Hendricks WA, Pak ES, Owensby JP et al. Predifferentiated embryonic stem cells prevent chronic pain behaviors and restore sensory function following spinal cord injury in mice. Mol Med. 2006;12(1-3):34-46.