Subarachnoid Space Transplantation of Schwann and/or Olfactory Ensheathing Cells Following Severe Spinal Cord Injury Fails to Improve Locomotor Recovery in Rats

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Abstract- Treatment of spinal cord injury by exogenous cells has brought both successful and unsuccessful results. Olfactory ensheathing cells and Schwann cells have been widely used for transplantation purposes. In this study, we investigated the effects of these cells on contused spinal cord by introducing cells into subarachnoid space. Fifty thousand Schwann cells or olfactory ensheathing cells or a mixture of both cell types were transplanted one week after a 3-second clip compression injury at T-9 spinal cord level in rats. Starting from the day one of spinal cord injury, animals were assessed for six months by BBB test and then were sacrificed for immunohistochemistry labeling of the spinal cord injury site. There was no locomotor recovery in any of the treatment groups including controls. Immunohistochemistry assessment indicated positive labeling of P75 and S100 markers in the cell-transplanted groups compared with control. Our data suggest that transplantation of Schwann cells and/or olfactory ensheathing cells into the subarachnoid space does not improve motor recovery in severely injured spinal cord, at least with the number of cells transplanted here. This, however, should not be regarded as an essentially negative outcome, and further studies which consider higher densities of cells are required.

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Introduction

Transplantation of Schwann cell and Olfactory Ensheathing Cell (OEC) has shown promising results in animal models of spinal cord injury (1-3). These cells have functions such as secretion of growth factors, axon guidance, myelination and structural support (4). Previous studies suggest that these two cell types behave differently in vitro or in vivo when transplanted into the demyelinated CNS (5). Transplantation of Schwann cell in injured spinal cord has yielded beneficial results in the functional and structural recovery of spinal cord lesions (6-10). Other studies provide evidence that transplantation of OECs improves motor function either in contusion (11,12) or transection models (13,14). OEC also improves functional properties of lesioned dorsal column in an animal model of spinal cord injury (15). However, these cells are not always beneficial for regeneration purposes (5,16-19). Despite contradicting results in the use of Schwann cell and OEC in experimental spinal cord injury models, they have been amongst best candidates for the purpose of cell therapy (20).

The idea of a combination of Schwann cell and OEC for spinal cord injury transplantation is pertinent to their specifications. For example, OECs have a better ability for migration; and when co-cultured with astrocytes in vitro, do not disintegrate from them, as is the case for Schwann cells (5). Schwann cells, on the other hand, have been shown superior in promoting axonal growth and myelination (4). There are a few studies which used

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OEC and Schwann cell in combination (8,21,22). It has been reported that OEC exerts beneficial effects on Schwann cells when transplanted in combination (23).

We have previously shown that transplantation of Schwann cell through subarachnoid route improves motor performance in moderate spinal cord contusion in rats (24). Yet our human study has not yielded such improvement in transplanted cases (25). In order to consider very severe injuries of spinal cord compared to our previous study (24), we investigated subarachnoid transplantation of OEC and Schwann cell, alone or in combination, in contused spinal cord of rat; and evaluated behavioral recovery in long-term.

Materials and Methods

Study design

A total number of sixty female inbred Wistar rats weighing 180-200 g were randomly divided into seven groups of Control (n=8), sham-operated (n=4), DMEM (n=8), OEC (n=10), Schwann (n=10), Mixed (OEC+Schwann, n=10) and fibroblast (n=10) based on injected suspension. All procedures were carried out according to the guidelines of ethics committee of Tehran University of Medical Sciences. Behavioral recovery of animals was assessed by BBB locomotor rating scale for six months (26). Animals were then sacrificed and prepared for histology and immunohistochemistry assessments.

Spinal cord injury

Aseptic surgical procedures were performed under anesthesia induced by a mixture of Ketamine (100 mg/kg, ip) and Xylazine (10 mg/kg, ip). Following anesthesia, assessed by corneal reflex, an incision was made through skin spanning T4-T12 thoracic vertebra. Laminectomy was performed at the T9-T10 level under loupe magnification. A curved aneurysm clip with closing force of 1.23 N was used to compress the cord for three seconds, with lower and upper blades of the clip compressing ventral and dorsal surfaces of the cord. Flanking muscles and skin were then closed with 4-0 chromic catgut and silk sutures respectively. Normal saline solution was injected after the surgery (5 ml, ip) and animals were transferred to recovery cages. Aspirin was dissolved in drinking water (100 mg/L) and supplied for three days as an analgesic. Special care included twice-a-day bladder expression and subcutaneous injection of Cefazolin (150 mg/kg) and Gentamicin (5 mg/kg) for up to two months in cases where animals were still incapable of urination or when urinary tract infection was observed.

Cell preparation

OEC and Schwann cell cultures were prepared from the olfactory bulb and sciatic nerve of neonatal rats inbred with recipient animals, respectively. Schwann cells were purified by a protocol described previously with some modifications (24,27). Briefly, sciatic nerves were extracted from 3- to 5-day-old rats; the epineurium was removed, and the nerve was split into several explants where subsequently placed on poly-L-lysine coated plates filled with culture media (DMEM+10% FCS). In order to reduce fibroblast contamination, explants were subcultured 3 to 4 times during four weeks. The cold jet technique was engaged to increase the purity of the Schwann cells. In this way, our cultures had a purity of about 95% determined by labeling with the anti-S100 antibody (28). Since fibroblast contamination always exists, we added a seventh group to evaluate the effect of pure fibroblast transplantation. Purification of OEC was according to the method of Nash *et al*., (29). Briefly olfactory bulbs from neonates were extracted, and the nerve fiber layer was dissected away and trypsinized. Cell suspension incubated for 18 hours, and then the supernatant was transferred to a second plate which incubated for 36 hours. The remaining supernatant was transferred into poly-L-lysine coated plates and maintained until cells reached a confluency of 70-80%.

Cell transplantation

One week after spinal cord injury, injury site was re-exposed for cell transplantation. Scar tissue was detached, and care was taken not to damage the exposed dura mater. The suspension for injection of either OEC or Schwann cells contained 5×10^4 cells of each in 5 μL of DMEM, and for the mixed suspension, a half concentration of each was prepared. Fibroblasts, with a similar concentration, were also prepared. Cell suspensions were injected into the subarachnoid space within 10 seconds using a Hamilton syringe with a fine 30-gauge needle bent in the middle to minimize unwanted tissue damage. The needle tip was inserted into the dura mater 3-4 mm caudal from the injury epicenter in order to compensate its bent portion distance. After injection, the needle was kept still for 10 seconds to minimize leakage of the suspension and then carefully withdrawn.

Behavioral assessment

Assessment of hindlimb function using Basso,

Beattie, Bresnahan (BBB) locomotor rating scale was performed every week for nine weeks and afterward, twice or once a month until 160 days post-injury, by two examiners blinded to the animal treatment (26,27). For this purpose, animals were placed in a round plastic container with a diameter and wall of 85 and 30 centimeters, respectively.

Immunohistochemistry

At the end of the study, all surviving animals were transcardially perfused with 4% paraformaldehyde. Two-centimeter segments of spinal cord containing the center of the lesion in the middle were then collected, embedded in paraffin and sectioned (3 μm). After rehydration, the sections underwent antigen retrieval: slides were placed in a humidified chamber and rinsed with a solution of hydrogen peroxide with methanol (1:9) for 20 minutes. Following washing with distilled water, slides were incubated in Tris solution (pH=6) at 120° C for 15 minutes. Blocking was performed by normal donkey serum (1:10) for 30 minutes. Anti-P75 (rabbit polyclonal, 1:500; Sigma) and anti-S100 (S2644; rabbit polyclonal, 1:500; Sigma) antibodies were used for 1 hour at room temperature. Sections were then washed in Tris/Tween 20 solution for 10 minutes. Then HRP-conjugated secondary antibody was used to incubate the sections for 30 minutes at room temperature. After another wash with Tris/Tween 20, diaminobenzidine tetrahydrochloride (DAB) was administered as the chromogen for up to 10 minutes. Finally, all specimens were counterstained with Hematoxylin. Slides were then dehydrated, mounted, and observed under light microscope. In a separate staining, every four sections were stained with Hematoxylin and Eosin (H and E) and observed by a pathologist for gliosis and inflammation.

Statistical analysis

Statistical comparisons of BBB scores between experimental groups were performed using two-way analysis of variances (ANOVA). A *P*.value less than 0.05 was considered significant in this study.

Results

Eight animals died during the study, and their records were not included in the analysis of data. Prior to the injury, all animals were scored 21 according to the BBB scale. One day after injury BBB was evaluated, and seven animals which scored 1 or more in the scale were excluded from the study to assure that all of the animals sustain an intensive injury. Other animals, fulfilling injury criteria, were assigned into experimental groups. Assessment of BBB score started at one week post-injury and carried out for 21 weeks, revealed no significant difference between the treatment and control groups during this time (Figure 1).

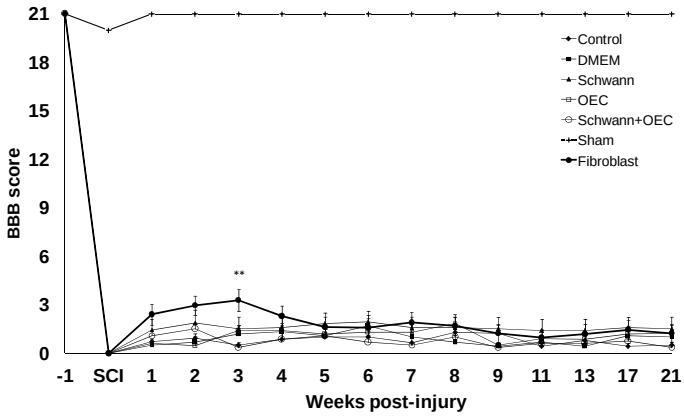

Figure 1. Mean of BBB scores (±SEM) of the all experimental groups. Animals were assessed one week prior to the injury, and all scored 21, which is considered normal locomotion. Except the higher score of fibroblast group at the third week (**P*<0.05), there is no significant difference between the experimental groups during the study.

The only exception was the fibroblast group, which showed a slight difference at the third week compared to control, DMEM, OEC, and Schwann cell/OEC groups, but not Schwann cell group. However, it's BBB score declined at the fourth week and in following evaluations had no difference with any of the groups. Sham-operated

animals showed no functional impairment one week after injury and thereafter. When analyzed for the time factor in each single group, BBB score showed literally no improvement even 21 weeks after the injury.

In H and E stained sections, fibrosis and gliosis were evident at the injury site in all groups; macrophages were rarely detected in some sections (data not shown). Labeling of the spinal cord with antibodies against P75 or S100 reveals positive staining in white matter areas of injury site in OEC, Schwann, and mixed groups, but not in the control and DMEM groups (Figure 2).

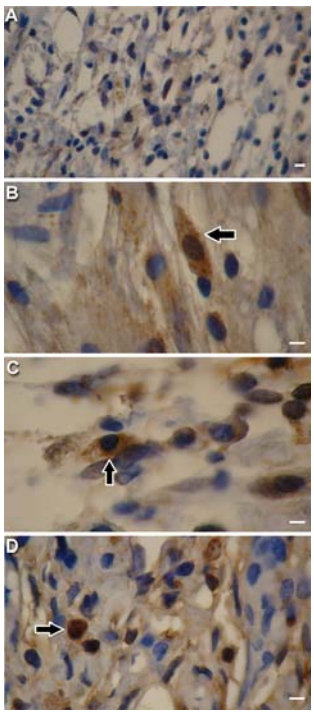

Figure 2. Injury site of spinal cord showing a portion of the white matter. Immunochemistry against P75 and S100 was visualized by the enzymatic HRP-tagged secondary antibody. Arrows show cells which stained positively for either P75 (B and C) or S100 (D). A: Anti-P75 was used in an animal from the control group. No positive staining is evident. B: Anti-P75 positively stains a cell in an animal which received OEC. C and D: Positive staining of both anti-P75 (C) and anti-S100 (D) in an animal which received Schwann cell. Scale bar: 10 μm.

Discussion

In this study, we found that transplantation of Schwann cell and/or OEC through the subarachnoid space did not improve motor function in rats with severe spinal cord injury. The only improvement in motor performance was seen in the fibroblast group at the third-week post-injury, which followed by an unexpected decline. Further studies are needed to clearly address the role of exogenous fibroblasts transplanted into the injured cord. Although motor recovery is the ideal goal of most transplantation studies, sensory improvement is also important which was not assessed in this study.

In several studies of Schwann cell or OEC transplantation, behavioral recovery is reported in treatment groups (8,12,31-34). On the other hand, it has been reported that transplanted Schwann cells fail to migrate and myelinate areas of demyelinated axons in CNS (16-19,35). Similarly, failure of OEC for improvement of hindlimb function and axon regeneration (29,36-39), and even unwanted hyperalgesic effects have been reported (40). A recent human study has reported improvement in three patients, which suggests that autologous OEC transplantation can be beneficial (41). In most transplantation studies which report improvement in motor function, the weight drop model of spinal cord injury were used, whereas we used severe clip compression which could be considered complete contusion (42). We have previously shown beneficial results in transplantation of Schwann cell in moderate spinal cord contusion by clip compression in rats (24).

The most important difference between our previous study and the current is the exact contusion model of the spinal cord. Here, the cord was compressed with a curved aneurysm clip, with the blades totally spanning and pressing dorsal and ventral surfaces, while in our previous study the clip was straight, and the blades compressed lateral surfaces. Hence, in our previous study, there was a possibility that the most ventral parts of the spinal cord remained intact; a possibility that was controlled in the current study. Another point of dissimilarity is the 0.07-N higher closing force than the previously-used straight clip. These two factors, position and the closing force of the clip, seems to be responsible for the greater injury seen in this study. Indeed, in the current study, the BBB scores of the control group are less than half of those of our previous study. There are factors that potentially affect the outcome of transplantation which vary from cell preparation and engraftment to routes of transplantation. The source of OECs was the olfactory bulb in this study, which is suggested not to be different from those of olfactory mucosa (43). Survival of transplanted cells is another determining factor. For example in the case of Schwann cell, it has been reported that most cell death occurs in the first 24 hours; but even seven days after transplantation, necrosis and apoptosis is observed (44). It has been documented that density of cell suspension is an

important factor in the outcome of transplantation in that high concentration of cells- 50,000 to 100,000 /µl- yields better results (45). Thus, a cell population of 50,000 in 5 µL in our study is relatively a sparse transplantation; however, it was effective in our previous study. Bladder expression and its timing before behavioral assessment have been implicated as a potential source of varied results (38). In neither the previous nor the current study, we performed bladder expression prior to BBB scoring.

Part of the recovery from spinal cord injuries is because of the plasticity in both spinal cord and higher motor centers (46). It has been suggested that a greater injury leaves less spared tissue. When less than 15 percent of the fibers are left, plasticity cannot take place in higher centers rostral to the site of injury (47). Compared to our current intensive injury, the milder injury in our previous study would, therefore, leave more spared tissue, raising the possibility for greater plasticity with the resultant tangible recovery.

Immunohistochemical labeling against P75 and S100 reveals positive labeling in animals received Schwann cell, OEC or both. Although Schwann cell and OEC express S100 and P75, these markers are not specific for them and thus positively labeled cells cannot be exclusively attributed to transplanted Schwann cells and/or OECs. It has been shown that P75 expression increases in oligodendrocytes following spinal cord injury (48); and on the other hand, gliosis is the well-known proliferation of astrocytes which express S100 (49). Nonetheless, as shown in figure 1, sparse positive staining of P75 and S100 in the white matter of injury center in cell-treated groups, suggests integration of transplanted cells into the injury site. Nevertheless, even this sparse labeling is absent in control group. Current methods do not specifically purify OECs from its mixture with Schwann cells (8,22,50,51). This is especially important when OECs are extracted from lamina propria or olfactory nerve layer, which may raise Schwann contamination (51,52). We purified OEC from the olfactory bulb to minimize Schwann contamination.

The timing of cell transplantation is also an important issue and acute transplantation usually gives better results (53,54). In the present study, transplantation was sub-acute and was done one week after injury. The first few hours following injury are extremely critical and after this time the spinal cord undergoes secondary injury cascades (55) and is difficult to repair by exogenous cell transplantation. Co-transplantation studies in acute phase have shown promising results (34,56).

This study suggests that after severe compression of the spinal cord, subarachnoid transplantation of Schwann cell and/or OEC does not improve motor performance. In other words, when the injury is severe, transplanted cells fail to exert functional motor recovery. However, it should not be concluded that OEC or Schwann cell are incapable of supporting regeneration and recovery under other models of spinal cord injury and transplantation, or higher concentration of cells. The injured spinal cord is one of the most hostile milieus for transplanted cells to survive. Regarding the low density of cells in injection bolus, higher densities should be applied especially because of the potential dispersion of cells in subarachnoid space. Due to the paucity of cell therapy studies through this route of transplantation, a definite conclusion cannot be made based on studies to date and further research is suggested in this field.

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References

1. Czepiel M, Boddeke E, Copray S. Human oligodendrocytes in remyelination research. Glia 2015;63:513-30.
2. Fortuna J, Hill CE, Bunge MB. Combinatorial strategies with Schwann cell transplantation to improve repair of the injured spinal cord. Neurosci Lett 2009;456:124-32.
3. Barnett SC, Riddell JS. Olfactory ensheathing cells (OECs) and the treatment of CNS injury: advantages and possible caveats. J Anat 2004;204:57-67.
4. Oudega M. Schwann cell and olfactory ensheathing cell implantation for repair of the contused spinal cord. Acta Physiologica (Oxford) 2007;189:181-9.
5. Franklin RJ. Remyelination of the demyelinated CNS: The case for and against transplantation of central, peripheral and olfactory glia. Brain Res Bull 2002;57:827-32.
6. Ban DX, Kong XH, Feng SQ, Ning GZ, Chen JT, Guo SF. Intraspinal cord graft of autologous activated Schwann cells efficiently promotes axonal regeneration

and functional recovery after rat's spinal cord injury. Brain Res 2009;1256:149-61.
7. Lavdas AA, Papastefanaki F, Thomaidou D, Matsas R. Schwann cell transplantation for CNS repair. Curr Med Chem 2008;15:151-60.
8. Pearse DD, Sanchez AR, Pereira FC, Andrade CM, Puzis R, Pressman Y, et al. Transplantation of Schwann cells and/or olfactory ensheathing glia into the contused spinal cord: Survival, migration, axon association, and functional recovery. Glia 2007;55:976-1000.
9. Pearse DD, Pereira FC, Marcillo AE, Bates ML, Berrocal YA, Filbin MT, et al. cAMP and Schwann cells promote axonal growth and functional recovery after spinal cord injury. Nat Med 2004;10:610-6.
10. Yang L, Ge Y, Tang J, Yuan J, Ge D, Chen H, et al. Schwann Cells Transplantation Improves Locomotor Recovery in Rat Models with Spinal Cord Injury: a Systematic Review and Meta-Analysis. Cell Physiol Biochem 2015;37:2171-82.
11. Raisman G. Repair of spinal cord injury by transplantation of olfactory ensheathing cells. C R Biol 2007;330:557-60.
12. Plant GW, Christensen CL, Oudega M, Bunge MB. Delayed transplantation of olfactory ensheathing glia promotes sparing/regeneration of supraspinal axons in the contused adult rat spinal cord. J Neurotrauma 2003;20:1-16.
13. Lopez-Vales R, Fores J, Navarro X, Verdu E. Chronic transplantation of olfactory ensheathing cells promotes partial recovery after complete spinal cord transection in the rat. Glia 2007;55:303-11.
14. Lu J, Feron F, Mackay-Sim A, Waite PM. Olfactory ensheathing cells promote locomotor recovery after delayed transplantation into transected spinal cord. Brain 2002;125:14-21.
15. Keyvan-Fouladi N, Raisman G, Li Y. Functional repair of the corticospinal tract by delayed transplantation of olfactory ensheathing cells in adult rats. J Neurosci 2003;23:9428-34.
16. Vroemen M, Caioni M, Bogdahn U, Weidner N. Failure of Schwann cells as supporting cells for adult neural progenitor cell grafts in the acutely injured spinal cord. Cell Tissue Res 2007;327:1-13.
17. Iwashita Y, Blakemore WF. Areas of demyelination do not attract significant numbers of Schwann cells transplanted into normal white matter. Glia 2000;31:232-40.
18. Iwashita Y, Fawcett JW, Crang AJ, Franklin RJM, Blakemore WF. Schwann cells transplanted into normal and X-irradiated adult white matter do not migrate extensively and show poor long-term survival. Exp Neurol 2000;164:292-302.
19. Miyagoe-Suzuki Y, Nakagawa M, Takeda S. Merosin and Congenital Muscular Dystrophy. Microsc Res Tech 2000;48:181-91.
20. Zhang X, Yin F, Guo L, Zhao D, Gong G, Gao L, et al. Transplantation of neural stem cells, Schwann cells and olfactory ensheathing cells for spinal cord injury : A Web of Science-based: lterature analysis. Neural Regen Res 2012;7:2818-25.
21. Sun T, Ye C, Zhang Z, Wu J, Huang H. Cotransplantation of olfactory ensheathing cells and Schwann cells combined with treadmill training promotes functional recovery in rats with contused spinal cords. Cell Transplant 2013;22:S27-38.
22. Pearse DD, Marcillo AE, Oudega M, Lynch MP, Wood PM, Bunge MB. Transplantation of Schwann cells and olfactory ensheathing glia after spinal cord injury: does pretreatment with methylprednisolone and interleukin-10 enhance recovery? J Neurotrauma 2004;21:1223-39.
23. Cao L, Zhu YL, Su Z, Lv B, Huang Z, Mu L, et al. Olfactory ensheathing cells promote migration of Schwann cells by secreted nerve growth factor. Glia 2007;55:897-904.
24. Firouzi M, Moshayedi P, Saberi H, Mobasheri H, Abolhassani F, Jahanzad I, et al. Transplantation of Schwann cells to subarachnoid space induces repair in contused rat spinal cord. Neurosci Lett 2006;402:66-70.
25. Saberi H, Moshayedi P, Aghayan HR, Arjmand B, Hosseini SK, Emami-Razavi SH, et al. Treatment of chronic thoracic spinal cord injury patients with autologous Schwann cell transplantation: an interim report on safety considerations and possible outcomes. Neurosci Lett 2008;443:46-50.
26. Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma 1995;12:1-21.
27. Rahimi-Movaghar V, Jazayeri SB. When we do start Basso, Beattie, and Bresnahan assessment after experimental spinal cord injury? Acta Med Iran 2013;51:5183.
28. Firouzi M, Sabouni F, Deezagi A, Pirbasti ZH, Poorrajab F, Rahimi-Movaghar V. Schwann cell apoptosis and p75(NTR) siRNA. Iran J Allergy Asthma Immunol 2011;10:53-9.
29. 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.
30. Nash HH, Borke RC, Anders JJ. Ensheathing cells and methylprednisolone promote axonal regeneration and functional recovery in the lesioned adult rat spinal cord. J Neurosci 2002;22:7111-20.

31. Ghosh M, Tuesta LM, Puentes R, Patel S, Melendez K, El Maarouf A, et al. Extensive cell migration, axon regeneration, and improved function with polysialic acid-modified Schwann cells after spinal cord injury. Glia 2012;60:979-92.
32. Silva NA, Sousa RA, Pires AO, Sousa N, Salgado AJ, Reis RL. Interactions between Schwann and olfactory ensheathing cells with a starch/polycaprolactone scaffold aimed at spinal cord injury repair. J Biomed Mater Res A 2011;100:470-6.
33. Wang H, Liu C, Ma X. Alginic acid sodium hydrogel co-transplantation with Schwann cells for rat spinal cord repair. Arch Med Sci 2012;8:563-8.
34. Wang JM, Zeng YS, Wu JL, Li Y, Teng YD. Cograft of neural stem cells and schwann cells overexpressing TrkC and neurotrophin-3 respectively after rat spinal cord transection. Biomaterials 2011;32:7454-68.
35. Franklin RJM, Blakemore WF. Requirements for Schwann cell migration within CNS environments: A viewpoint. Int J Dev Neurosci 1993;11:641-9.
36. Deumens R, Koopmans GC, Honig WM, Maquet V, Jerome R, Steinbusch HW, et al. Chronically injured corticospinal axons do not cross large spinal lesion gaps after a multifactorial transplantation strategy using olfactory ensheathing cell/olfactory nerve fibroblast-biomatrix bridges. J Neurosci Res 2006;83:811-20.
37. Lu P, Yang H, Culbertson M, Graham L, Roskams AJ, Tuszynski MH. Olfactory ensheathing cells do not exhibit unique migratory or axonal growth-promoting properties after spinal cord injury. J Neurosci 2006;26:11120-30.
38. Steward O, Sharp K, Selvan G, Hadden A, Hofstadter M, Au E, et al. A re-assessment of the consequences of delayed transplantation of olfactory lamina propria following complete spinal cord transection in rats. Exp Neurol 2006;198:483-99.
39. Deumens R, Van Gorp SF, Bozkurt A, Beckmann C, Fuhrmann T, Montzka K, et al. Motor outcome and allodynia are largely unaffected by novel olfactory ensheathing cell grafts to repair low-thoracic lesion gaps in the adult rat spinal cord. Behav Brain Res 2013;237:185-9.
40. Lang BC, Zhang Z, Lv LY, Liu J, Wang TY, Yang LH, et al. OECs transplantation results in neuropathic pain associated with BDNF regulating ERK activity in rats following cord hemisection. BMC Neurosci 2013;14:80.
41. Tabakow P, Jarmundowicz W, Czapiga B, Fortuna W, Miedzybrodzki R, Czyz M, et al. Transplantation of Autologous Olfactory Ensheathing Cells in Complete Human Spinal Cord Injury. Cell Transplant 2013;22:1591-612.
42. Rahimi-Movaghar V, Yazdi A, Karimi M, Mohammadi M, Firouzi M, Zanjani LO, et al. Effect of decompression on complete spinal cord injury in rats. Int J Neurosci 2008;118:1359-73.
43. Mayeur A, Duclos C, Honore A, Gauberti M, Drouot L, do Rego JC, et al. Potential of olfactory ensheathing cells from different sources for spinal cord repair. PLoS One 2013;8:e62860.
44. Hill CE, Hurtado A, Blits B, Bahr BA, Wood PM, Bunge MB, et al. Early necrosis and apoptosis of Schwann cells transplanted into the injured rat spinal cord. Eur J Neurosci 2007;26:1433-45.
45. Toft A, Scott DT, Barnett SC, Riddell JS. Electrophysiological evidence that olfactory cell transplants improve function after spinal cord injury. Brain 2007;130:970-84.
46. Fouad K, Krajacic A, Tetzlaff W. Spinal cord injury and plasticity: Opportunities and challenges. Brain Res Bull 2011;84:237-42.
47. Schucht P, Raineteau O, Schwab ME, Fouad K. Anatomical correlates of loco-motor recovery following dorsal and ventral lesions of the rat spinal cord. Exp Neurol 2002;176:143-53.
48. Casha S, Yu WR, Fehlings MG. Oligodendroglial apoptosis occurs along degenerating axons and is associated with FAS and p75 expression following spinal cord injury in the rat. Neurosci 2001;103:203-18.
49. Ohtani R, Tomimoto H, Wakita H, Kitaguchi H, Nakaji K, Takahashi R. Expression of S100 protein and protective effect of arundic acid on the rat brain in chronic cerebral hypoperfusion. Brain Res 2007;1135:195-200.
50. Ramo´n-Cueto A, Nieto-Sampedro M. Regeneration into the spinal cord of transected dorsal root axons is promoted by ensheathing glia transplants. Exp Neurol 1994;127:232-44.
51. Vincent AJ, Taylor JM, Choi-Lundberg DL, West AK, Chuah MI. Genetic Expression Profile of Olfactory Ensheathing Cells Is Distinct From That of Schwann Cells and Astrocytes. Glia 2005;51:132-47.
52. Lindsay SL, Riddell JS, S.C. B. Olfactory mucosa for transplant-mediated repair: a complex tissue for a complex injury? Glia 2010;58:125-34.
53. Tetzlaff W, Okon EB, Karimi-Abdolrezaee S, Hill CE, Sparling JS, Plemel JR, et al. A Systematic Review of Cellular Transplantation Therapies for Spinal Cord Injury. J Neurotrauma 2011;28:1611-82.
54. Centenaro LA, da Cunha Jaeger M, Ilha J, de Souza MA, Balbinot LF, do Nascimento PS, et al. Implications of olfactory lamina propria transplantation on hyperreflexia and myelinated fiber regeneration in rats with complete spinal cord transection. Neurochem Res 2013;38:371-81.

55. Barnett SC, Riddell JS. Olfactory ensheathing cell transplantation as a strategy for spinal cord repair--what can it achieve? Nat Clin Pract Neurol 2007;3:152-61.
56. Ban DX, Ning GZ, Feng SQ, Wang Y, Zhou XH, Liu Y, et al. Combination of activated Schwann cells with bone mesenchymal stem cells: the best cell strategy for repair after spinal cord injury in rats. Regen Med 2011;6:707-20.