

ORIGINAL COMMUNICATION

Treatment of Spinal Cord Injury: A Review of Engineering Using Neural and Mesenchymal Stem Cells

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Over time, various treatment modalities for spinal cord injury have been trialed, including pharmacological and nonpharmacological methods. Among these, replacement of the injured neural and paraneural tissues via cellular transplantation of neural and mesenchymal stem cells has been the most attractive. Extensive experimental studies have been done to identify the safety and effectiveness of this transplantation in animal and human models. Herein, we review the literature for studies conducted, with a focus on the human-related studies, recruitment, isolation, and transplantation, of these multipotent stem cells, and associated outcomes. Clin. Anat. 00:000–000, 2014. © 2014 Wiley Periodicals, Inc.

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INTRODUCTION

Stem cells are nondifferentiating cells that have high proliferation, differentiation, and self-renewal potentials. The proliferation and self-renewal potential is achieved via the asymmetric division, in which one of the daughter cells becomes further differentiated, whereas the other maintains the characteristics of the cell of origin. In regard to their differentiation capacity, the stem cells can be divided into totipotent, pluripotent, multi- or oligo-potent, and unipotent stem cells. An example of totipotent stem cells is the fertilized egg, and they are capable of generating embryonal and extra-embryonal cells. With further specialization, pluripotent stem cells are formed, and they are capable of differentiation into any cell of the three germ line (ectoderm, mesoderm, and endoderm). Good examples of these cells are the embryonic stem cells (ESC) and the induced pluripotent stem cells (iPSC). Multipotent stem cells are able to differentiate into different types of cells within each germ line. An example of these is the neural progenitor/stem cell. It is evidenced that these cells are able to transdifferentiate

into other germ lines' cells. However, induced transdifferentiation may result in induced carcinogenesis. Unipotent stem cells can differentiate into only one type of cells, and include neuroblast and glioblast, which give rise to neurons or glial cells, respectively (Nandoe Tewarie et al., 2009; Sahni and Kessler, 2010; Cherian et al., 2011). Both pluripotent and multipotent cells may be used as a source of transplanted cells. For the purpose of cellular replacement, the more differentiated is the transplanted cell, the less risk of consequent teratoma. Thus, differentiation of the applied stem cells is required before transplantation (Sahni and Kessler, 2010).

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NEURAL PROGENITORS/STEM CELLS

Neural stem cells (NSC) were first identified and isolated from the striatum of an adult mouse brain by Reynolds and Weiss (1992; Tsuji et al., 2011). Now, two decades after their discovery, along with extensive and prolonged experimental studies, the characteristics, origin, and differentiation of these cells are widely uncovered. The NSC are one type of the multipotent stem cells that have the capability of differentiation into multiple unipotent cells, including neural and non-neural tissues (e.g., oligodendrocytes and astrocytes). This ability to replace the major lost elements of degenerated nervous system gave NSC a central role in the regenerative attempts and trials.

The source of the NSC can be divided according to the developmental level of isolation into embryonic and adult sources. The embryonic source of the NSC includes the ESC and the developing central nervous system (CNS), whereas the adult sources include the adult neural tissue and iPSC.

hNSC DERIVED FROM FETAL NEURAL TISSUES

Isolation, culture, and expansion of the NSC have been widely carried out on embryonic rodent brains (Davis and Temple, 1994; Johe et al., 1996; Reynolds and Weiss, 1996; Svendsen et al., 1996; Qian et al., 1997) and spinal cords (Ray and Gage, 1994; Kalyani et al., 1997). For enhancing the growth and expansion of these, different mitogenic factors have also been used, including the epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF).

Isolation and differentiation was also induced in human fetal neural tissue-derived NSC. The major issue was in the proper culture and expansion of these cells before transplantation. For this purpose, different protocols were applied, including the use of recombinant adenoviruses by Sabate et al. (1995). The most significant breakthrough was made by Svendsen et al. (1997). The isolated human NSC (hNSC) were expanded as neurospheres in a growth culture using EGF and bFGF. For the first time, these stem cells were capable of migration and differentiation into the three neural phenotypes (neurons, oligodendrocytes, and astrocytes) after transplantation into rat brains. Nevertheless, cellular markers detected only very few cellular survivals 20 weeks after transplantation. One year later, Svendsen et al. (1998) were able to further expand the neurosphere culture and maintain a rapid cellular growth to 1.5 million-folds over a period of 200 days using a different method of sphere separation during NSC passage. In this protocol, instead of mechanical dissociation of the neurospheres, they were divided into quarters using a microscopic technique. This method maintained the cell-cell contacts and permitted the rapid and continual growth of each individual quarter over a period of 14 days. Differentiation of these cells was limited to neurons and astrocytes.

Vescovi et al. (1999) isolated the NSC from a human embryonic brain and maintained their self-

renewal and prolonged proliferation in a neurosphere culture using EGF and bFGF. In contrast to murine NSC, the use of either of these growth factors would suppress the hNSC proliferation. Transplantation of these cells into rat brains was associated with migration and differentiation, and survival up to 1 year post-transplantation. In the same year, Quinn et al. (1999) derived NSC from a human fetal spinal cord, and expanded them using combination of EGF and bFGF in a neurosphere forming culture. At later passages, the only cell lines that could be maintained were those with the potential differentiation to astrocytes. According to the authors, this might indicate that spinal cord neural precursors were restricted to the astrocytic lineage under these conditions.

In the same year, Carpenter et al. (1999) added the leukemia inhibitory factor (LIF), EGF, and bFGF to the NSC culture derived from human embryonic brain. When compared with a control group where only EGF and bFGF were added, the culture exhibited a faster and larger expansion, which was not evident until 50–60 days in-culture. The mechanism for this delayed effect might be related to the maintenance of the cell survival beyond the typical NSC lifespan (50–60 days), or it might be related to inhibited differentiation of the NSC and consequent maintained proliferation. The bFGF also proved to be essential in enhancing the expansion of the hNSC in a concentration-dependant fashion. In contrast, the absence of EGF did not affect the cellular growth and neuron formation, but no data has shown the long-term effect of its absence. This protocol facilitated hNSC growth, renewal, and expansion to as much as 10^7 -fold, and maintained the differentiation potential for over 1 year, with unknown ultimate capacity.

Thus, this approach became favorable in obtaining the hNSC for experimental transplantation in later studies (Kelly et al., 2004; Iwanami et al., 2005; Li et al., 2005, 2008; Kim et al., 2006; Tarasenko et al., 2007; Yamamoto et al., 2009). Zhang et al. (2011) used a different method by applying a combination of EGF and brain-derived growth factor. The addition of the latter was associated with enhanced proliferation and migration of the hNSC through a PI3K/Akt kinase pathway.

In a study by Ostenfeld et al. (2000), they concluded that the hNSC have shorter telomeres than both their rodent counterparts and the ESC. However, its exact effect on the cellular replication has not been determined, despite the shortening associated with each replication in some cellular populations, and their absence at certain stages with related slowed growth. Moreover, with the transplantation of 200 thousand, 1 million, or 2 million cells per animal, they found that the smaller the transplant, the more likely they are to differentiate and extend neuronal fibers, and the less likely they are to provoke immune rejection. The authors suggested the existence of an upper limit to the optimal number of transplanted cells. This is in contrast to previous belief that high density transplants are more suitable for regeneration.

To study the regional or temporal characteristics with regard to growth and differentiation, Kim et al. (2006) isolated NSC from almost all parts of the CNS,

including telencephalon, diencephalon, midbrain, cerebellum, pons, and medulla, and spinal cord. Based on their study, they found that, compared with the midbrain and hindbrain, the forebrain-derived NSC grew faster and ultimately gave rise to significantly more neurons. There was also a reduction in neuronal emergence from the respective neurospheres over time in culture, except in those derived from cerebellum, which were significantly increased. Moreover, distinctive molecular markers of regional identity were expressed by neurospheres of different compartments and were maintained during long-term passaging in vitro. However, these region-specific markers are not irreversible and may change in response to local inductive cues.

TRANSPLANTATION OF NSC DERIVED FROM FETAL NEURAL TISSUES

Cummings et al. (2005) reported on transplantation of fetal brain-derived NSC into mice with spinal cord injury (SCI) at T9. Immunocytochemistry revealed extensive human cell survival and engraftment within the injured mouse spinal cord, along with migration of the neural cells, starting at 24 hr after transplantation. The engrafted cells showed less differentiation into astrocytes than neurons and oligodendrocytes. These neurons and oligodendrocytes showed signs of active synaptic function restoration and effective myelination, respectively. The Basso, Beattie, and Bresnahan (BBB) score assessment at 16 weeks post-transplantation showed significant locomotor improvement compared with control group.

In the same year, Iwanami et al. (2005) reported on fetal spinal cord-derived NSC transplantation into C5 injured spinal cords of common marmosets at 9 days after injury. Histological study 8 weeks post-transplantation showed cellular survival and migration outside the grafted site. Differentiation into neuronal and oligodendrocyte lineage was also seen. Functional recovery assessment using bar grip tests revealed locomotor improvement starting 2 weeks post-transplantation. The authors also concluded that concomitant use of scaffolds, the blockage of axonal regeneration inhibitors, and the administration of neurotrophic factors can be effectively use with NSC transplantation.

In the experiment of Tarasenko et al. (2007), NSC were isolated from fetal brain and allowed to expand by the addition of EGF, bFGF, and LIF. These cells were then transplanted into T10 injured spinal cords of rats 9 days after injury. Differentiation into functioning neurons and oligodendrocytes, which have survived for at least 3 months, has also been seen. The BBB evaluation showed significant locomotor improvement starting 2 weeks after grafting that reached a plateau 40 days post-transplantation. According to the authors, these timing represent an important window for transplantation.

Emgard et al. isolated and transplanted fetal brain-derived NSC into injured spinal cords of rats. These cells showed remarkable proliferation and migration, starting 6 hr post-transplantation and thereafter over

the next 3 weeks. However, they did not assess the locomotor outcome of this transplantation (Emgard et al., 2009).

hNSC DERIVED FROM ADULT NEURAL TISSUES

Since Reynolds and Weiss (1992), many studies have attempted to isolate NSC from different areas of adult CNS, including the subependymal zone (Reynolds and Weiss, 1992; Morshead et al., 1994; Johe et al., 1996; Johansson et al., 1999a), the subventricular zone (SVZ; Lois and Alvarez-Buylla, 1993; Doetsch et al., 1999; Sanai et al., 2004), and the hippocampus (Gage et al., 1995; Palmer et al., 1997). Other areas may include the spinal cord (Kalyani et al., 1997; Mujtaba et al., 1998; Rao et al., 1998), striatum, and neocortex (Palmer et al., 1995, 1999). As in the case of fetal-derived NSC, proliferation of the adult-derived NSC needs the presence of EGF and FGF mitogens. Withdrawal of these growth factors will induce differentiation to neurons, astrocytes, and oligodendrocytes in vitro (Akiyama et al., 2001). The same method is applied to obtain NSC from human CNS (Eriksson et al., 1998; Johansson et al., 1999b; Roy et al., 2000; Akiyama et al., 2001; Nunes et al., 2003; Sanai et al., 2004).

TRANSPLANTATION OF NSC DERIVED FROM ADULT NEURAL TISSUES

In an experimental study by Akiyama et al. (2001), NSC were identified and isolated from adult brain subependymal and SVZs. These cells were allowed to expand by applying the mitogens bFGF and EGF. Hence, they were transplanted into dorsal columns of the lumbar region of injured spinal cords of rats. Observation of the site of transplant showed NSC differentiation into myelinating Schwann cells and, to a lesser extent, oligodendrocytes for 6–8 weeks. This directed differentiation is different from fetal neural tissue-derived NSC, and could be related to intrinsic capacity of the adult progenitor cells and the cellular and extracellular milieu of the transplant zone, including cytokines released from microglia or macrophages.

Using the same protocol, Karimi-Abdolrezaee et al. (2006) isolated NSC from the SVZ of rats. These cells were then expanded using EGF and bFGF, and transplanted into T6–T7 region at 2 and 6 weeks after SCI. The addition of EGF, bFGF, and platelet-derived growth factor (PDGF-AA) into the spinal subarachnoid space promoted their survival and proliferation. After 8 weeks of transplantation, the cells were able to proliferate and migrate along the site of injury, and differentiate into myelinating oligodendrocytes and, to a lesser extent, astrocytes. Functional assessment, using the BBB locomotor rating scale, footprint analysis, and grid walk assessment, showed significant locomotor recovery. These findings, however, were only detected in the early (subacute) transplant and not in the late (chronic) transplant.

Some scientists prefer the use of NSC over ESC for clinical application, as they are supposed to have a lower risk of tumorigenesis. However, some challenges are present in using these cells, including the need for pure populations of differentiated cells, inefficient tracking systems, and moderate cell survival after transplantation. Nowadays, the iPSC still present the promising source of progenitor cells (Ronaghi et al., 2010).

hNSC DERIVED FROM hESC AND hiPSC

ESC are derived from the inner cell mass of the blastocyst, an early-stage embryo. These cells were studied extensively over a period of almost two decades before they were isolated from human embryos by Thomson et al. (1998).

It has always been thought that late during fetal development, the ESC start to differentiate and gradually acquire a specific cell type in a unidirectional fashion. However, this was opposed in 2006, when Takahashi and Yamanaka were able to reverse the cellular cycle by reprogramming the somatic fibroblast cells into pluripotent cells using defined pluripotency-related transcription factors (i.e., Oct3/4, Sox2, c-Myc, and Klf4). These cells were then called iPSC. The major advantages of these cells include solving the ethical problem related to ESC derivation, and eliminating the need for immunosuppressive factors as they represent an autologous transplant. The major disadvantages include the genetic instability and high teratogenic potential associated with the process of reprogramming and culture. For neural cell replacement within the SCI, the pluripotent stem cells are used as a source of the neural precursors/stem cells, motor neurons, and oligodendrocytes.

TRANSPLANTATION OF NSC DERIVED FROM hESC AND hiPSC

In 2009, Hatami et al. induced NSC formation from hESC cultured in DMEM/F12 medium supplemented with N2 and exposed to bFGF, retinoic acid (RA), Noggin, sonic hedgehog, and LIF at different stages. Their protocol resulted in around 50% NSC formation. They were then transplanted along with collagen scaffold into the T10 spinal cord hemisection of rats. The transplanted cells migrated and incorporated into the damaged sites of the spinal cord and differentiated into neurons and glial cells, as evident by immunocytochemistry. When compared with the control group, significant hind limb locomotor function recovery, assessed by the BBB scoring system, was noted 5 weeks post-transplant. They also noted improved sensory response in the study group. No complications were observed on long-term observation.

The use of the biodegradable collagen scaffold by Hatami et al. (2009) in spinal cord transplants, and laminin and fibronectin scaffolds by Tate et al. (2009) in brain transplants, showed advantages compared with control groups. The synthetic biodegradable scaffold

provide adhesive support and may also induce the release of some growth factors such as neurotrophin-3 (NT-3) and PDGF (Ronaghi et al., 2010).

Nori et al. (2011) reported on iPSC reprogramming from adult human dermal fibroblasts, using Oct3/4, Sox2, Klf4, and c-Myc. These cells were then induced to form embryoid body, using RA and NSC using bFGF, which were transplanted into T10 SCI sites of mice. Electrophysiological function and functional recovery was monitored using the Motor-Evoked Potential (MEP) and Basso mouse scale (BMS) score, which showed progressive improvement of motor function 12 days after transplant and thereafter, followed by a plateau. After 47 days of transplant, histological study showed survival, migration, and differentiation into neurons and glial cells. They also promoted angiogenesis, axonal regeneration, and local-circuitry reconstruction. No complications, including tumor formation, were observed.

Using the same protocol, Fujimoto et al. (2012) induced iPSC reprogramming. After expansion of iPSC-derived NSC cells using bFGF and EGF, they were transplanted into T10 injured spinal cords of mice. The results were compared with human fetal spinal cord-derived NSC. Hind limb motor function revealed no difference between the two types of cells as compared with the control group using the BMS for at least 8 weeks. At 12 weeks, the MEP amplitudes were significantly higher than those of control group. As evident by immunohistochemistry, transplanted cells survived and migrated to both rostral and caudal directions around the lesion site. Differentiation into neurons and glial cells, along with synaptic formation and enhanced survival of endogenous neurons, were also observed. Moreover, injection of these cells into the motor cortex of the hind limb area showed signs of corticospinal tract (CST) reconstruction, but without CST axonal re-extension. To test the theory that lentiviral infection affects the differentiation potential, induced differentiation of infected and uninfected cells were observed, and no differences could be detected.

MESENCHYMAL STEM CELLS

As in the case of NSC, the mesenchymal stem cells (MSC) are a type of multipotent stem cells that have the ability to differentiate into any cells within the mesenchymal lineage, including osteoblasts, chondrocyte, adipocytes, and stroma (Sandner et al., 2012). They are also able to transdifferentiate into cells of endodermal and ectodermal lineages, such as hepatocytes and neurons, respectively (King et al., 2012). However, this does not occur in a quantitatively relevant fashion (Sandner et al., 2012). MSC can be isolated from adult and neural tissues, with the major sources being bone marrow in adults and umbilical cord blood in fetuses and neonates. The fact that the MSC can be used as autologous cell grafts that easily can expand from relatively small amounts of bone marrow aspirates, along with their inflammatory and immune-modulating function, makes them an attractive source for cellular transplantation (King et al.,

2012; Sandner et al., 2012). Moreover, despite the fact that they can rapidly and extensively be expanded in cell cultures, there is no evidence of tumor production *in vivo*, and they have a strong capacity to increase tissue preservation, decrease cyst and injury size, and increase recovery of function (Bhanot et al., 2011). They are also capable of producing a number of growth factors *in vivo*, including NGF, brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor, hepatocyte growth factor, and NT-3, which significantly promotes neuronal survival and functional recovery, with the BDNF being the most important and potent factor. The human MSC (hMSC) can also be genetically modified for further secretion and consequent enhanced functional improvement (Lee et al., 2007; Sasaki et al., 2009; Hu et al., 2010; Shang et al., 2011).

Transplantation of human umbilical cord blood-derived MSC (hUCB-MSC) and human bone marrow-derived MSC (hBM-MSC) has been applied on animal models via direct (Lee et al., 2007; Sasaki et al., 2009; Hu et al., 2010; Shang et al., 2011; Zhilai et al., 2012) or intravenous injection (Osaka et al., 2010; Zhilai et al., 2012). These studies were mostly applied on acute SCI and were associated with significant locomotor and somatosensory improvement assessed by the BBB scoring system, somatosensory-evoked potential (SSEP), and MEP. It was also reported that hUCB-MSC have greater *ex vivo* expansion capabilities, faster proliferation, and lower immunogenicity than hBM-MSC (Troyer and Weiss, 2008; Weiss et al., 2008). The combined treatment with intrathecal Taxol, an anticancer drug, and hUCB-MSC may lead to further decrease in astrocytic activation, increased axonal preservation, and consequent enhanced functional recovery (Zhilai et al., 2012).

EXPERIMENTAL TRIALS ON HUMANS

An experimental study by Pal et al. (2009) included lumbar intrathecal injection of hBM-MSC into 30 patients with complete SCI. Lumbar injection is considered safe, feasible, and beneficial, as the injected cells migrate eventually to the site of injury. The patients were divided into two groups, depending on the timing of injury: less than 6 months or more than 6 months. Over 1 year follow-up, the patients within the first group (less than 6 months) showed noticeable, yet variable, improvement of daily activities and quality of life, starting with recovery of bladder and bowel sensation and control, followed by sensory and then motor function. However, these sensory and motor recoveries were not sufficient to elicit a positive electrophysiological response on the MEP, SSEP, and nerve conduction velocity (NCV). Also, no MRI-changes could be noticed. On the other hand, patients within the second group (more than 6 months) failed to show any improvements. No major adverse reaction could be noticed in these patients, and only two patients reported neuropathic pain after the transplant.

Kishk et al. (2010) reported on the hBM-MSC transplantation in 43 patients with various degrees of SCI in the cervical and thoracic areas for a period of 6

months (subacute and chronic phases). These patients received lumbar intrathecal injection monthly for 6 months, along with rehabilitation therapies three times weekly. The functional recovery on the American Spinal Injury Association (ASIA) Impairment Scale, ASIA grading of completeness of injury, Ashworth Spasticity Scale, Functional Ambulation Classification, and bladder and bowel control questionnaire were very minimal as compared with the control group and were noticed almost always in patients with incomplete SCI. Adverse effects included spasticity, neuropathic pain in 24 patients, and encephalomyelitis in one patient with a history of postinfectious myelitis.

In the study of Bhanot et al. (2011), 13 patients with chronic (more than 8 weeks) cervical or thoracic complete SCI were chosen. Each patient received one intramedullary hBM-MSC injection at the site of injury and two lumbar intrathecal injections 1 and 2 weeks after the first injection, respectively. These patients were followed every 3 months with complete neurological evaluation and ASIA scale, and every 6 months with the electrophysiological studies, including SSEP, MEP, and NCV. In over 1 year of follow-up, only one patient showed a slight motor recovery, two patients had patchy improvement in pin prick sensation below the level of injury, and one patient subjectively developed the sensation of fullness of bladder. None of these patients had serious or persistent complications, but some of them experienced transient manifestations, including spasticity, fever, malaise, vomiting, and tingling or burning girdle sensation.

Park et al. (2012) reported on long-term follow-up of three patients out of the original 10 patients with incomplete or complete SCI. Their experiment included intramedullary injection of hBM-MSC into the site of injury more than one month (subacute and chronic) following trauma. Seven out of 10 patients showed no motor, electrophysiological, or MRI improvement. The other three patients, who had residual neurological function, showed motor improvement within the first 6 months of follow-up, and were further evaluated for more than 30 months. Over this period, they had remarkable motor and functional recovery. Electrophysiological study, assessed by SSEP and MEP, and MRI scan also, for the first time, showed significant improvement. This improvement may be related to several factors, including: (1) Direct delivery of the transplant into the site of injury is a more effective method for SCI recovery compared with intrathecal injection, especially in subacute and chronic phases. The latter, although proven safer, is associated with more limited migration and time window of recovery. At the same time, their study was the first to apply multiple intramedullary injections—two above the cavity and three into the cavity—and to use the fibrin glue to seal the site of injection. (2) The fact that the three patients who showed locomotor recovery already had residual neurological function following injury may indicate that this treatment could be more effective for patients with incomplete injuries rather than complete injuries. No serious complications were reported in any of these patients.

From previous studies, we can conclude that the use of autologous hBM-MSC represents a safe,

feasible, and reliable method of cellular transplantation for SCI treatment. That is the reason why they are the main source of transplant applied to humans. However, the use of these cells in subacute and chronic phase of injury showed only minimal promising results, and further studies are needed.

TRANSPLANTATION OF BONE MARROW STEM CELLS (BMSC) IN PATIENTS WITH SCI

BMSC are a mixed cell population including hematopoietic stem cells, MSC, endothelial progenitor cells, macrophages, lymphocytes, and marrow stromal cells. The use of the entire BMSC can combine the established neuroprotective effect of its components, including the hematopoietic cells and their secretory factors (Mehler et al., 1993; Chong et al., 2002), the stromal cells (Chen et al., 2002, 2005), and the MSC.

In 2005 and 2007, Park et al. (2005) and Yoon et al. (2007) reported on direct intramedullary transplantation of BMSC along with subcutaneous injection of granulocyte macrophage-colony stimulating factor (GM-CSF). The latter has been found to induce the growth of different hematopoietic cell lineages and prevent apoptotic cell death of the hematological and neuronal cells. In these studies, the authors also concluded that the GM-CSF had a direct effect on the transplanted BMC by enhancing their survival in the spinal cord and activating them to excrete neurotrophic cytokines. These factors were also found to stimulate microglial cells to produce neurotrophic cytokines such as BDNF. The experiment of Park et al. (2005) involved six patients with complete cervical SCI within 14 days (acute phase), only five received intramedullary transplant and one received only GM-CSF injection. Four of the six patients (including the one with GM-CSF alone) experienced significant motor and sensory recovery noted 3–7 months postoperatively. No permanent or serious complications were noted, although the patients experienced transient fever and myalgia related to GM-CSF injection. In the other experiment of Yoon et al. (2007), 35 patients with complete cervical SCI were divided into three groups based on the timing of injury into acute (less than 2 weeks), subacute (2–8 weeks), and chronic (more than 8 weeks). Over 10 months of follow-up, noticeable locomotor improvement was noted in the acute and subacute patients to variable degrees, but none in the chronic patients. No permanent or serious complications were noted. Neuropathic pain was only reported in seven patients.

The significant locomotor improvement noted in the study of Park et al. (2005) may be related to several factors, including: (1) Their study was the only one to apply stem cell therapy in acute SCI, and, based on animal studies, early cellular transplantation is always associated with enhanced recovery. This also can be noticed when their results are compared with those of Yoon et al. (2007) who used the same protocol but in patients with different phases of injury. (2) The intramedullary route of transplantation is pro-

ven to be superior to other routes, including intrathecal injection (see above). (3) The use of the entire BMSC. (4) The concomitant subcutaneous injection of GM-CSF. However, further studies are still needed to confirm the role of these factors.

In a study by Sykova et al. (2006), 20 patients with complete SCI were divided into two groups who received BMSC via intravenous and intra-arterial routes, respectively. Each group contained patients with acute and chronic phase injury. Over a 3-month follow-up period, very minimal motor and sensory recoveries were noticed in five of seven acute patients and in one of thirteen chronic patients. These patients were mainly from the intra-arterial group.

The study of Geffner et al. (2008) included eight people with acute and chronic SCI who received infusions of BMSC via multiple routes: directly into the spinal cord, directly into the spinal canal, and intravenous. Over 2 years of follow-up, almost all patients experienced variable degrees of functional improvements assessed by ASIA scores, the Barthel Index, Ashworth scores, and bladder function. In none of these patients was serious or permanent complications, including neuropathic pain and tumor formation, reported.

We have reviewed current literature regarding the efficacy of the experimented mesenchymal and stem cells used for the treatment of SCI. Mesenchymal and stem cells offer promising results following SCI and as our combined experience grows, so to does our understanding of how the spinal cord responds to injury.

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