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**Title:** Bone marrow stromal cell : mediated neuroprotection for spinal cord repair  
**Issue Date:** 2014-02-27
Chapter 1

Introduction
Introduction Part A

Stem Cells for Central Nervous System Repair and Rehabilitation

Gaby J. Ritfeld, Raymund A.C. Roos, Martin Oudega

Modified from PM&R 2011;6(3):S117-122
ABSTRACT

The central nervous system (CNS) has limited capacity for self-repair. Current treatments are often incapable of reversing the debilitating effects of CNS diseases that result in permanent and/or progressive physical and cognitive impairments. One promising repair strategy is transplantation of stem cells, which can potentially replace lost neurons and/or glia or promote repair through secretion of trophic factors. Various types of stem cells exist, each with their own advantages and disadvantages. Although no consensus exists regarding the optimal cell type to use, moderate functional improvements have been shown in animal models of CNS diseases using different types of stem cells. However, the precise mechanism of action behind their beneficial effects remains unknown. In addition, many barriers to clinical use still need to be resolved before transplantation of stem cells can be used as effective biologics. These barriers include—depending on the stem cell type—possible tumor formation, difficulty with harvest, limited in vivo differentiation and integration, and ethical issues regarding use.
INTRODUCTION

Central nervous system (CNS) diseases are often characterized by complex immune-mediated cytotoxic and apoptotic processes that result in the loss of function and permanent loss of neural cells\(^1\). Although many CNS diseases result from a loss of viable cells, a therapeutic approach must consider the type of cell lost to have a beneficial outcome. For example, Parkinson disease requires replacement of lost dopaminergic substantia nigra neurons, whereas multiple sclerosis requires reconstitution of functional oligodendrocytes. Stem cells have the potential to address this demand for specific cells for specific diseases because of their multipotency, and thus stem cell therapy is a promising biologic therapy to consider for persons with CNS diseases.

In the past decade an explosive amount of stem cell research has been conducted, resulting in an insightful scope of knowledge on stem cell biology. Continuing research will be essential before effective bedside treatments for CNS diseases may be developed. This review aims to provide a critical overview of stem cell use for repair of CNS diseases relevant for rehabilitation medicine.

DEFINITION, ORIGIN, AND VARIOUS TYPES OF STEM CELLS

By definition, a stem cell is capable of self-renewal and of differentiating into at least one other cell type. The zygote is referred to as a totipotent stem cell. The blastocyst contains an inner cell mass consisting of self-replicating cells that can become all but trophoblast cells (the outer layer blastocyst cells that later become the placenta); these cells are known as pluripotent stem cells (Figure 1). When these cells enter into 1 of the 3 primary germ layers—ectoderm, mesoderm, or endoderm—they are referred to as multipotent stem cells. These cells then can become precursor cells, which are unipotent cells that differentiate into the final cell types within differentiated tissues (Figure 1).

When stem cells are harvested from embryonic tissue, they are considered embryonic stem cells (ESC). When stem cells are taken from tissues from the adult body, they are
referred to as adult stem cells (ASC [note: adipose tissue-derived stem cells are sometimes also referred to as ASC]) or somatic stem cells. The existence of ASC was first demonstrated within the adult hematopoietic system, which throughout natural life gives rise to new blood cells\(^2\). After this discovery, ASC were demonstrated within numerous other adult tissues such as neural stem cells (NSC) in the brain, epidermal neural crest stem cells (EPI-NCSC) in hair follicles, muscle-derived (mesenchymal) stem cells in muscles, and bone marrow stromal cells (BMSC) in bone marrow. The functions of ASC are poorly understood, but one rational possibility would be that ASC support repair of the tissues in which they reside. At present, however, this theory has not been confirmed unequivocally, and it certainly does not appear to be the case in the CNS, where endogenous restoration is poor and disease or trauma typically elicits permanent damage.

![Hierarchy of stem cells. Totipotent cells can develop into all cell types of the body, pluripotent cells can become all but trophoblast cells, multipotent cells can give rise to all cells within 1 of the 3 germ layers, and precursor cells are unipotent cells that will become terminally differentiated cells of specialized tissue. Ecto = ectoderm germ layer; meso = mesoderm germ layer; endo = endoderm germ layer.](image)

Recently, a third type of stem cell has emerged—the induced pluripotent stem (iPS) cell, discovered by Takahashi and Yamanaka in 2006\(^3\). The iPS cell is generated from an adult somatic cell by introducing transcriptional factors whose ectopic expression reprograms the cell into a pluripotent cell. The groundbreaking discovery ‘that mature cells can be reprogrammed to become pluripotent’ has earned Yamanaka the Nobel Prize in
Physiology or Medicine 2012. The prize was shared with John B. Gurdon who in 1962 used an enucleated oocyte into which the nucleus of an adult cell was transferred to create a stem cell capable of forming a blastula and eventually a tadpole\(^4\). In June 2013 the somatic cell nuclear transfer method was for the first time successfully used for human embryonic stem cell generation\(^5\). The discovery of generated stem cells is opening exciting new avenues in the field of regenerative medicine (for review, see Bellin et al.\(^6\)).

**UTILITY OF NEURAL AND NON-NEURAL STEM CELLS**

NSC can contribute in different ways to repair of the brain and spinal cord. They can potentially differentiate into neurons and/or glial cells and replace those that were lost as a result of the disease or trauma. Alternatively, NSC can serve as vectors for growth factors that could support cell survival, cell proliferation, axon regeneration, and blood vessel formation, which can all positively influence CNS repair. It is also possible that stem cells serve as a substrate for regenerating axons and thus contribute to repair. Thus far, numerous studies have demonstrated the potential of stem cells for CNS repair. Interestingly, the mechanisms underlying their benefits remain elusive.

Embryonic NSC have a robust capacity to differentiate into neural cells and are therefore suitable for repair strategies based on cell replacement. However, their impressive differentiation capacity comes with uninhibited proliferation, which could result in tumor formation after transplantation. This factor, together with ethical concerns surrounding their harvest, has limited the application of ESC for CNS repair. Adult NSC also are capable, albeit less so than ESC, of differentiating into neural cells, and in contrast to ESC, they are not known for causing tumors after transplantation. Thus adult NSC are good candidate cells for neural replacement approaches. A disadvantage of adult NSC is that they are difficult to obtain because they need to be harvested from the adult brain or spinal cord.

Compared with embryonic and adult NSC, non-neural stem cells are more readily obtainable. For instance, BMSC reside in bone marrow, EPI-NCSC in hair follicles, and muscle-derived (mesenchymal) stem cells in muscles, and all these tissues are relatively
easy to harvest from adults. Some of these non-neural stem cells offer additional advantages such as the low expression of major histocompatibility complex I molecules by BMSC that would help evade immunologic rejection. Importantly, it was reported that several types of non-neural stem cells could (trans)differentiate into neural cells\textsuperscript{7}, which has opened new avenues for CNS repair. However, at present, this potential to become a neuron, astrocyte, or oligodendroglial cell has not been unambiguously proven and is in fact a subject of controversy. If this ability to transdifferentiate into neural cells is low or absent, their benefits in replacement strategies would be poor. On the other hand, non-neural stem cells may offer effective means to repair the CNS through their ability to secrete repair-supporting molecules such as growth factors. Moreover, in accordance with their decreased differentiation capacity, these cells are less inclined to unrestrained proliferation and are therefore less tumorigenic. Table 1 provides an overview of the relative advantages and disadvantages of different types of stem cells. The current controversies and challenges within the field of regenerative medicine are best illustrated with the following example. For a mesodermally derived BMSC to be suitable for CNS repair based on cell replacement, it will need to transdifferentiate into a neuron or neural glial cell. For this transdifferentiation to occur, the BMSC will first need to revert into a pluripotent cell, subsequently differentiate into an ectodermal precursor cell, and

<table>
<thead>
<tr>
<th></th>
<th>ESC</th>
<th>ASC</th>
<th>iPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differentiation/cell replacement potential</td>
<td>Very good</td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td>Harvest</td>
<td>Controversial</td>
<td>Difficult</td>
<td>Easy</td>
</tr>
<tr>
<td>Tumorigenicity</td>
<td>High</td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>

Abbreviations: ESC, embryonic stem cell; ASC, adult stem cell; NSC, neural stem cell; MSC, mesenchymal stem cell; iPS, induced pluripotent stem cell
then differentiate into a neuron, astrocyte, or oligodendrocyte. Several studies have shown that BMSC can be induced in vitro to express neuronal markers and even to have some electrical neuronal properties, but true transdifferentiation into a fully functioning neuron is strongly debated. Similarly, a few in vivo studies have shown expression of neuronal markers and/or anatomic integration after transplantation of BMSC, but neuronal functionality (i.e., synapse formation, firing of action potentials, and release of neurotransmitters) or glial functionality has not been shown unequivocally. For example, Kopen and colleagues (1999) reported the expression of glial fibrillary acidic protein, a marker for astrocytes, in BMSC after transplantation into mice brain ventricles and concluded that they had transdifferentiated into mature astrocytes. The expression of specific neural markers is an important first step toward applying BMSC for CNS cell replacement, but it appears to be a rare event, and it is not a demonstration that the cell has become a functional component of the nervous system.

ANATOMIC AND FUNCTIONAL REPAIR AFTER STEM CELL TRANSPLANTATION IN ANIMAL MODELS OF CNS DISEASE

In the past decade a number of studies showed that transplantation of NSC can result in histologic and/or functional improvements in rodent models of various CNS diseases. Cummings and colleagues demonstrated remyelination of axons and functional improvements after transplantation of NSC into a mouse spinal cord injury model. Improved motor function was observed in hemiplegic mice after implantation of monkey ESC. Table 2 provides a selected overview of studies that have transplanted neural and non-neural stem cells in different CNS disease models and reported repair. Typically, in ESC transplantation paradigms, some degree of differentiation into neurons and glia is shown. From the results it appears that NSC preferentially differentiate into astrocytes. Some may also differentiate into oligodendrocytes, but very few differentiate into neurons. Whether these newly generated neural cells then integrate within the host CNS tissue is not always clearly demonstrated. Despite the alleged in vitro ability, it is not often reported that non-neural stem cells become neural cells after transplantation into the CNS. Nevertheless, anatomic and/or functional repair has been demonstrated. Sieber-
Blum\textsuperscript{12} showed improvements in sensory connectivity and in touch perception after transplantation of EPI-N CSC in a mouse spinal cord injury model. In this study it was proposed that the neural crest cell–derived EPI-N CSC have the advantages of ESC and ASC because they are able to differentiate into oligodendrocytes and neuroblasts without being tumorigenic and are easily obtained from the bulge of hair follicles.

Table 2. Selected overview of studies that have implanted stem cells in rodent models of spinal cord injury, stroke or Parkinson’s disease

<table>
<thead>
<tr>
<th>Type of stem cell</th>
<th>Disease Model</th>
<th>Differentiation</th>
<th>Functional Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESC</td>
<td>SCI, rat</td>
<td>Astrocytes, oligo’s, neurons</td>
<td>↑BBB</td>
<td>McDonald et al.\textsuperscript{36}</td>
</tr>
<tr>
<td>Fetal NPC</td>
<td>SCI, rat</td>
<td>Neurons</td>
<td>↑Pellet retrieval</td>
<td>Ogawa et al.\textsuperscript{37}</td>
</tr>
<tr>
<td>BMSC</td>
<td>SCI, rat</td>
<td>-</td>
<td>↑BBB</td>
<td>Hofstetter et al. 2002\textsuperscript{21}</td>
</tr>
<tr>
<td>NSC</td>
<td>SCI, mouse</td>
<td>Neurons, oligo’s</td>
<td>↑BBB</td>
<td>Cummings et al.\textsuperscript{10}</td>
</tr>
<tr>
<td>EPI-N CSC</td>
<td>SCI, mouse</td>
<td>Neurons, oligo’s</td>
<td>↑Touch perception</td>
<td>Sieber-Blum\textsuperscript{12}</td>
</tr>
<tr>
<td>iPS</td>
<td>SCI, mouse</td>
<td>Neurons, oligo’s, astrocytes</td>
<td>↑BMS</td>
<td>Tsuji et al.\textsuperscript{13}</td>
</tr>
<tr>
<td>ESC</td>
<td>Stroke, mouse</td>
<td>Neurons</td>
<td>↑Beam walking, rotarod</td>
<td>Ikeda et al.\textsuperscript{11}</td>
</tr>
<tr>
<td>BMSC</td>
<td>Stroke, rat</td>
<td>Oligo’s, astrocytes</td>
<td>↑body swing test, ↑footprint analysis</td>
<td>Chen et al.\textsuperscript{9}</td>
</tr>
<tr>
<td>NSC</td>
<td>Stroke, rat</td>
<td>Neurons, astrocytes</td>
<td>-</td>
<td>Kelly et al.\textsuperscript{38}</td>
</tr>
<tr>
<td>BMSC</td>
<td>Parkinson, rat</td>
<td>-</td>
<td>↑Rotational behavior</td>
<td>Wu et al.\textsuperscript{15}</td>
</tr>
</tbody>
</table>

Abbreviations: ESC, embryonic stem cell; SCI, spinal cord injury; oligo’s, oligodendrocytes; BBB, Basso, Beattie, and Brasnahan-scale; NPC, neural progenitor cell; BMSC, bone marrow stromal cell; NSC, neural stem cell; EPI-N CSC, epidermal neural crest stem cells; iPS, induced pluripotent stem cell.
Another cell type that has putative neural differentiation capacity without being tumorigenic is the iPS cell. The therapeutic potential of iPS cells was nicely demonstrated in a mouse spinal cord injury model, which revealed that transplanted iPS cell–derived neurospheres differentiated into all 3 neural cell types, participated in remyelination, promoted axonal outgrowth, and improved locomotor function\(^\text{13}\). Additionally, this study circumvented tumor formation by pre-evaluation and selection of the neurospheres for tumorigenicity\(^\text{13}\). This study was partially confirmed by Nutt and colleagues, who showed successful transplantation and integration of iPS cell-derive NPCs into an early chronic spinal cord injury model, however, without evidence of functional improvement.\(^\text{14}\)

**IMPROVING THE OUTCOME AFTER STEM CELL TRANSPLANTATION INTO THE DAMAGED CNS**

Notwithstanding recent reports that transplanted stem cells can become neural cells, the key mechanism for functional improvements observed after ASC transplantation in the CNS is thought to be neuroprotection, that is, limiting the loss of tissue. Neuroprotection can be accomplished through the secretion of growth factors, such as brain-derived neurotrophic factor, glial cell line–derived neurotrophic factor, and nerve growth factor. A cell transplant provides long-term delivery of growth factors, which is an important advantage over direct injection, because growth factors dilute rapidly and typically have short half-lives. For this reason, recent studies have genetically engineered stem cells to overexpress such growth factors, with the aim of enhancing their neuroprotective capacity and, as a result, their repair-supporting potential. Wu and colleagues\(^\text{15}\) showed a neuroprotective effect of glial cell line–derived neurotrophic factor-overexpressing BMSC in a Parkinson model. Axonal regeneration and enhanced functional recovery was found after transplantation of brain-derived neurotrophic factor-overexpressing BMSC in a spinal cord injury model\(^\text{16}\).

The beneficial effects of stem cells can also be increased by pre-differentiating the cells in vitro prior to transplantation. This pre-differentiation can be achieved by growing the cells according to a particular induction protocol that pushes the cells into a desired lineage.
Davies and colleagues\textsuperscript{17} showed improved axon regeneration and locomotor function in rats with spinal cord injuries after transplantation of astrocytes differentiated from embryonic glial-restricted precursors, but not undifferentiated glial-restricted precursors. Hofstetter and co-workers\textsuperscript{18} transduced NSC with neurogenin-2 to suppress astrocytic differentiation prior to transplantation into rats with spinal cord injuries and demonstrated prevention of graft-induced sprouting, decreased allodynia, and improved functional recovery. Although these 2 studies achieved some degree of restoration with use of opposite differentiation protocols, it is clear that both demonstrated that the repair-supporting abilities of stem cells can be positively influenced prior to transplantation.

Another way to improve the outcome is by combining stem cell transplantation with putative additive or synergistic treatments. Even though combinatorial strategies are thought to be essential to achieve biologically significant repair, exploration of these strategies has been sparse. Combining BMSC transplantation with an inhibitor of Rho-kinase\textsuperscript{19}, a molecule known to prevent neurite outgrowth, or with olfactory ensheathing cells\textsuperscript{20}, another adult cell type that has been shown to benefit anatomic and functional CNS repair, did not improve functional outcomes in rats with spinal cord injuries more than BMSC transplantation alone. In a similar model, the combination of BMSC with physical exercise did not improve function compared with control subjects\textsuperscript{21}. On the other hand, in both a spinal cord injury\textsuperscript{22} and a stroke\textsuperscript{23} model, the outcome after transplantation of brain-derived adult NSC with olfactory ensheathing cells was improved compared with transplantation of each of the cell types alone. In a rat model of cerebral ischemia, the combination of BMSC with erythropoietin showed a synergistic effect on neurogenesis and memory performance but not on locomotor function\textsuperscript{24}. Because of our limited current knowledge about mechanisms underlying single treatments, it is difficult to select the appropriate combinations in which the single interventions would exert additive or synergistic effects. Future elucidation of mechanisms will allow more rationally targeted combinatorial repair strategies.
It is clear that, despite some promising results, stem cell–based repair of the damaged CNS still has major challenges to overcome before it can be successfully applied in a clinical setting. These challenges vary between tumorigenic and ethical concerns with ESC, differentiation issues with ASC, and survival of transplanted stem cells in general. Poor survival of stem cells after injection into damaged CNS tissue can be due to poor vascularization of the transplantation site or a result of inflammation with accompanying secretion of cytotoxic molecules and rejection by activated immune cells. To optimally benefit from transplanted stem cells, it will be necessary to develop strategies to improve survival of the cells after transplantation. Although concomitant treatment with immunosuppressive drugs, improving the timing of cell transplantation, and transplantation of cells within a scaffold all have been shown to benefit cell survival, the majority of cells still die within weeks of transplantation. Clearly, further studies that focus on optimizing stem cell survival after transplantation into the damaged CNS are imperative.

**CLINICAL TRIALS**

Despite our incomplete knowledge, several clinical trials are currently being conducted in which stem cells are being transplanted in patients with Parkinson disease, stroke and other neurological disorders, with variable results thus far. After transplantation of human fetal mesencephalic tissue in patients with Parkinson disease, grafted neurons have been reported to survive and integrate, with improvements in several outcome parameters. These improved parameters included a 37% reduction in Levodopa dose, a 40% improvement in 18F-Flurodopa uptake (a measurement of dopaminergic activity in the putamen), a 44% improvement in the Unified Parkinson Disease Rating Scale Motor Score (while being off medication), a 39% decrease in “off medication” time, and a 49% decrease in “on medication” time with dyskinesia, according to a meta-analysis.

Impressively, up to sixteen years after transplantation, dopaminergic innervations in basal ganglia could be restored to normal levels and was associated with relief of motor symptoms. Variability across studies, however, is high. The differences between the observations in different clinical trials are likely due to variances in recipient
characteristics (e.g., younger patients seem to show better recovery after neural grafting),
the use of different surgical techniques, and/or (lack of) immunosuppressive drug
administration, resulting in decreased graft survival.

The first clinical trial using human ESC for spinal cord injury was approved in 2009 by the
United States Food and Drug Administration (FDA). In this Phase I trial, oligodendrocyte
progenitor cells derived from human ESCs were safely transplanted into five severe spinal
cord injury patients. In November 2011 however, the trial was discontinued for financial
reasons.\textsuperscript{31} Another Phase I/II trial by StemCells Inc using human CNS stem cells for spinal
cord injury is currently underway in Switzerland and Canada and has recently (October
2013) been approved by the FDA\textsuperscript{32}. Small clinical trials in other neurologic diseases,
including stroke\textsuperscript{33} and Huntington disease\textsuperscript{34}, seem to support the potential use of stem
cells, because moderate functional improvements are being achieved in some patients.
For example, after transplantation of neuronal cells in 12 patients who have sustained a
basal ganglia stroke, 6 patients showed improvements on the European stroke scale (a
gain of 3 to 10 points) 12 to 18 months after transplantation\textsuperscript{33}. Transplantation of fetal
neural tissue in 5 patients with Huntington disease resulted in cognitive improvements in
3 patients the first 2 years after surgery, which then faded after 4-6 years, as measured by
the Unified Huntington Disease Rating Scale and neuropsychological tests. Safety was
shown up to ten years postoperatively\textsuperscript{35}. Several other clinical trials are in or have
completed phase I/II of safety, but large trials of efficacy of stem cells for neurological
disorders are still lacking.

CONCLUSION

Transplantation of stem cells can potentially be used for treatment of various CNS
diseases. Progress is being made in the laboratory, and in various animal models of CNS
disease/disorders, moderate functional improvements are being reported. The underlying
mechanisms are still mostly unclear. In addition, determination of which stem cell type
would be best for a particular CNS disease/disorder is still largely unresolved. Clearly many
issues need to be elucidated before safe and effective stem cell-based therapies can be
designed for bedside treatments of neurologic disorders. All these issues warrant further investigations before stem cells can live up to their potential as effective biologic treatments for CNS disease.

REFERENCES


Introduction Part B

Bone Marrow Stromal Cells for Repair of the Injured Spinal Cord

Rishi D.S. Nandoe, Martin Oudega, Gaby J. Ritfeld

Modified from New Advances in Stem Cell Transplantation (2012).
INTRODUCTION

In 1927 Harvey Cushing described the outcome for soldiers with spinal cord injury (SCI) sustained during World War I: "Fully 80 percent died in the first few weeks in consequence of infection from bedsores and catheterization. Only those cases survived in which the spinal cord lesion was a partial one" \(^1\). Nowadays, this has been reversed. In well-organized systems of care for trauma and SCI and due to improved critical-care medicine most patients survive the initial hospitalization. At present, there is no treatment available that effectively re-establishes disrupted axonal circuitries that are necessary to restore injury-induced functional deficits. Due to the lack of a cure and the improved health care, the number of wheelchair bound people increases steadily each year. Currently, in the United States there are an estimated 400,000 people with SCI, with an annual incidence of 11,000 (The National Spinal Cord Injury Statistical Center, Birmingham, AL). In Western European countries similar leading causes of SCI are obtained as in the United States, with vehicular crashes and falls as leading causes of SCI and predominantly young males affected\(^2\)\(^3\). In contrast to the developed countries, in the less developed countries a shift of etiology can be observed towards falls\(^4\) and violence\(^5\)\(^-\)\(^7\).

Following the first medical care in a hospital, continuing medical care is necessary to maintain the SCI patient’s health and quality of life. This does not lead to functional repair. Repair-promoting pharmaceutical and/or surgical interventions will be necessary to significantly change the functional outcome after SCI. Transplantation of repair-supporting cells is considered a candidate repair approach. A bone marrow stromal cell (BMSC) transplant has shown great promise for spinal cord repair. This chapter will give an overview of the pathophysiology, clinical consequences, assessments, and treatments of SCI and will then focus on BMSC as a possible therapy for SCI. In addition, the SCI model system used in subsequent chapters will be explained.

PATHOPHYSIOLOGY AND CLINICAL CONSEQUENCES

A direct force to the vertebral column can cause damage to bony and soft tissue structures. Torn ligaments or fractures can cause instability of the vertebral column with
potential risk of additional damage. Fracture dislocation and hematomas can directly compress the spinal cord and cause immediate neural cell death, axon damage and demyelination, resulting in instant loss of motor and sensory function. After the first destructive events, a sequence of molecular and cellular pathophysiological events, including an aggressive inflammatory response within the damaged tissue, leads to additional tissue loss at the injury epicenter and at distant sites (secondary injury). The functional consequences of SCI are highly variable and depend on the degree of tissue damage, which in turn depends on the impact severity. In patients with SCI with a relatively small amount of tissue damage, some endogenous recovery of function can be observed, which is most likely resulting from plasticity of the spinal nervous tissue. In people with SCI with extensive tissue damage the neurological deficits are generally major and permanent. There are very few reports of people with a large injury that regain motor function to a degree that independence can be achieved.

Over 95% of SCI patients survive their initial hospitalization. The relatively young age when SCI occurs, improved medical care, and lack of effective therapies are responsible for the continually increasing number of paralyzed people with SCI. This puts a high financial burden on the patient, his/her family, and society. The psychological consequences of SCI should not be underestimated and appropriate guidance of patient and family should have an important place in the management of SCI. Patients need time to accept their deficits. One can expect an initial period of denial and/or inability to fully comprehend the consequences of the paralysis caused by the injury. After the patient realizes his/her fate to the fullest extent, a period of acceptance will have to run its course. After that, the patient needs to learn to live with his/her disabilities, and this may be accompanied by bouts of depression. The mental state of the patient can have its effect on his/her medical treatments.

SCI is the second most expensive condition to treat in the United States after respiratory distress syndrome in infants and is ranked third in medical conditions requiring the longest stay in hospitals. The costs of lifetime care for a SCI patient varies between 1 and 3
million dollars. The Center for Disease Control in the United States estimated that about 10 billion dollars are spent yearly on SCI treatment excluding the management of pressure ulcers, a common adverse effect of SCI, which adds another billion dollars per year\textsuperscript{17}.

**TREATMENT**

An acute and a chronic phase can be distinguished after SCI. Since SCI is often a consequence of severe accidents, initial treatment is generally focused on stabilization of the patient. There is insufficient evidence that would support standards of care during the acute phase of SCI. It is advised to maintain patients in an intensive care unit for close monitoring of respiratory and hemodynamic complications. For adequate spinal perfusion, which can be at risk due to injury-induced edema, a mean arterial pressure of 85-90 mmHg should be maintained\textsuperscript{18}. Depending on the type of injury, surgical interventions should be considered to decompress the spinal cord and or stabilize the spinal column\textsuperscript{19,20}. Decompression surgeries may accelerate functional improvements and result in shorter hospitalization and rehabilitation periods\textsuperscript{17,21}. However, it does not result in an improved functional outcome\textsuperscript{22}. A lack of consensus of care during the acute phase of SCI is in part due to the large variability among injuries and makes its early management complicated. If bone fragments continue to compress the spinal cord, early surgery may be vital to prevent exacerbation of spinal cord tissue destruction. However, in cases without a clear sign of such urgency there is no consensus on whether and what type of early surgical/clinical interventions must be implemented. The type of surgical intervention should be considered on a case-to-case basis, which makes it complicated to study the efficacy of intervention in the acute phase after SCI in randomized and controlled clinical trials.

Besides surgical interventions, pharmacological treatments to limit the secondary injury after SCI are often considered. The best-known treatment is a high dose of the glucocorticosteroid, methylprednisolone sodium succinate (MPSS) within 8 hours after the injury\textsuperscript{23-25}. Experimentally it was demonstrated that a high dose of MPSS reduces the inflammatory response and limit tissue loss after damage to the spinal cord. The effects of
MPSS in patients with SCI were investigated in 3 consecutive National Acute Spinal Cord Injury Studies (NASCIS)\textsuperscript{23-25}. The results demonstrated that MPSS treatment in the acute phase of SCI resulted in neurological improvements up to 6 months after injury. After a thorough review of the results from the NASCIS studies and a more comprehensive assessment of the benefits and risks involved in high dose MPSS treatment, the therapeutic benefits are now disputed\textsuperscript{26-28}. Especially in patients with complete SCI high dose steroid treatment can lead to adverse effects such as myopathy and wound infection that may negatively influence functional outcome and in some cases may be life-threatening\textsuperscript{28,29}. Currently, many SCI clinics worldwide have discontinued the ‘standard’ acute administration of MPSS after SCI.

Treatment paradigms in the chronic stage after SCI are multidisciplinary and intensive. Different complications may occur that each demands specific interventions. For instance, SCI can lead to pain\textsuperscript{14,15}, decreased fertility\textsuperscript{30}, and autonomic dysreflexia with loss of bladder and bowel control\textsuperscript{31}. It has to be taken into consideration that many SCI patients get accustomed to the specific injury-related pain they experience and as a result reveal their distress to their physician often at a late stage\textsuperscript{32,33}. For some SCI-related conditions, such as decreased fertility, it is the patient’s personal desire that should guide the physician’s actions. Other common problems that arise after SCI are septicemia, respiratory insufficiency, and pneumonia due to muscle atrophy. These complications may cause clinical deterioration and could eventually result in death. They often occur without typical symptoms. It is imperative that SCI patients receive annual screenings and long-term follow-ups to prevent these secondary complications. It is advised to treat patients on a regular basis with pneumococcal and influenza vaccine to prevent opportunistic infections. Monitoring the skin and urinary tract and implementing aggressive treatments against pressure ulcers and urinary tract infections is needed to reduce the risk of septicemia. Appropriate nutrition and exercise should also be incorporated in the (new) lifestyle. Rehabilitation programs should be implemented to reduce the risk of cardiovascular disease\textsuperscript{34}. 
BONE MARROW STROMAL CELL THERAPY

Mesenchymal stem cells from bone marrow (here referred to as bone marrow stromal cells (BMSCs)) have therapeutic potential for the injured spinal cord\textsuperscript{35}. BMSC were shown to differentiate into bone, fat, tendon and cartilage cells\textsuperscript{36}. Although still debated, it has been reported that BMSC can transdifferentiate in vitro into liver cells\textsuperscript{37}, skeletal cells\textsuperscript{38}, cardiac muscle cells\textsuperscript{39}, and neural cells\textsuperscript{37,40}. Besides this ability, BMSC are also known to produce different types of growth factors that could potentially influence nervous tissue repair positively. Together, these abilities make BMSC interesting for repair strategies for the injured spinal cord.

Several other aspects make BMSC interesting candidates for cell-based approaches for central nervous system repair. Firstly, BMSC are relatively easy to obtain from a fairly routine bone marrow extraction followed by a quick centrifuge and culture procedure to remove the hematopoietic cells. Secondly, BMSC are easy to culture as they do not need complicated growth media or special culture circumstances. Basic cell culture equipment is sufficient to successfully culture millions of BMSC. Thirdly, BMSC are easy to transduce with viral vectors which, if necessary, may be helpful to boost the overall reparative abilities of the cells. The use of viral vectors to genetically modify cells prior to transplantation has not yet become mainstream as there are some biological and ethical issues that need to be resolved. Finally, BMSC do not have the ethical concerns that embryonic or fetal stem cells have, and therefore circumvent public rejection as a possible treatment for neural and non-neural trauma and disorders.

At this time, there is no irrefutable evidence that BMSC transplanted into the damaged nervous tissue differentiate into neural cells that successfully replace lost cells. Also, there is no convincing evidence that neural cells derived from grafted BMSC contributed to functional improvements after transplantation. As long as the potential of BMSC for differentiation into neural cells is in debate, the ability to produce and secrete different types of growth-promoting molecules, which include several neurotrophins and cytokines, is the more interesting and more likely characteristic of BMSC that makes these cells...
important candidates for spinal cord repair approaches. By releasing these molecules, BMSC can positively influence the consequences of spinal cord injury and support anatomical and functional repair (Figure 1).

Fig. 1. BMSC secrete various growth factors, including BDNF, VEGF, NGF and NT-3. These factors are thought to limit the loss of tissue in the injured spinal cord, contributing to the increased functional outcomes after BMSC transplantation.

RAT MODEL SYSTEM

Promising therapies for spinal cord injury are typically tested in rodent models, and mostly in rats. Similar as in humans, a SCI in the rat results in progressive loss of the grey and white matter creating large fluid filled cysts. Proliferation and activation of astrocytes result in formation of scar tissue, which acts as a barrier for axonal regeneration. Importantly, as in humans, there is no spontaneous regeneration in the injured spinal cord in rodents. The histological similarity between human and rat spinal cord injury has made the rat an extensively studied model for experimental therapeutic strategies, including BMSC transplantation.

The most widely used model of spinal cord injury involves a spinal cord contusion inflicted by an impactor device. A contusion is clinically the most frequently occurring type of spinal
cord injury; approximately 75% of all human injuries are contusions. The consequences of a contusive injury in rats are similar as the known consequences in the contused human spinal cord. Figure 1 shows the rat model system for spinal cord contusive injury.

An alternative model for a contusion-like spinal cord injury is the clip compression model. The main difference between the impactor-inflicted contusion and the clip-inflicted compression is time. With an impactor the spinal cord is compressed for a brief moment of time while with a clip the spinal cord is compressed for a longer, regulatable, time. The clip model is clinically more relevant as most spinal cord injuries are inflicted by a lasting compression rather than a brief one.

There are a number of other, non-contusive, spinal cord injury models employed in laboratories around the world to test treatment paradigms. These are valuable in their own right to investigate the underlying mechanisms and/or validity of certain approaches. Partial transections of specific regions in the spinal cord are used especially to study the effects of treatments that aim to promote axonal regeneration; specific descending or ascending pathways can be damaged with relatively small local knife cuts and the regeneration response quantified at later time points. The involvement of specific axonal pathways in locomotor function can also be investigated using partial transections. The main disadvantages of partial transections are the low clinical relevance and the possible misinterpretation of results due to compensatory sprouting, i.e., other previously non-involved axonal pathways become involved in particular functions. Another model that has been used is the complete transection of the spinal cord. Although this is not often seen in the clinic, complete transections are particularly advantageous to study cell types for their ability to promote regeneration of damaged axons without contaminating sprouting of undamaged pathways and to serve as bridging material between spinal cord stumps. This model is also suitable to study the efficacy of synthetic or natural biomaterials for their efficacy to serve as carrier of cells or drugs. A disadvantage besides the low clinical relevance is that rats with a completely transected spinal cord are more laborious to maintain.
Fig. 2. Rat spinal cord contusion model. A. A laminectomy is performed exposing the underlying spinal cord. B. Enlarged view of the exposed spinal cord segment. C. A computerized impactor is used to contuse the spinal cord. The piston is attached to a sensor to record velocity, force and displacement to ensure consistency. D. A moderate contusion results in loss of function at and below the level of injury and loss of bladder function.

BMSC INJECTION

It is difficult to provide standard guidelines for cell preparation because every cell type requires special conditions and circumstances for optimal isolation and culturing. Cell injection procedures may vary but are essentially similar. The standard procedures to harvest, culture and genetically modify BMSC with lentiviral vectors encoding for green fluorescent protein (GFP) to enable easy identification in vivo, as well as to inject BMSC as used in our laboratory are depicted in Figure 5. The length of the culture (preparation) time for BMSC depends on how many cells are needed to fill the damaged area. Thus, the number of BMSC necessary depends on the overall loss of tissue which, in turn, depends on the severity of the initial insult and on the time between insult and transplantation. Imaging techniques may provide the necessary information to guide the decisions on damaged tissue volumes and number of cells.

There are a number of studies that have explored injection paradigms other than straight acute injections into the injury site. BMSC have been infused systemically or into the 4th ventricle, or transplanted acutely into the cervical or thoracic spinal cord or into the chronically injured cord.
Fig. 3. Transplantation of BMSC. A. BMSC are isolated from femurs of rats by cutting off the epiphyses and flushing out the bone marrow. Cells are plated onto plastic culture dishes. Non-adherent hematopoietic stem cells are removed and the plastic-adherent BMSC are infected with LV-GFP. B. Cells are injected into the spinal cord contusion epicenter using a Hamilton syringe with a pulled glass needle attached, held within a micromanipulator. C. Appearance of transplanted BMSC (green) in the contused rat spinal cord seven days post transplantation (20 μm thick section at 2.5 x magnification). The red color represents immunohistochemically stained glial fibrillary acidic protein (GFAP), a commonly used marker for astrocytes.

TIMING OF TRANSPLANTATION

In an experiment by Nandoe Tewarie and colleagues, BMSC were transplanted into a moderately contused adult rat spinal cord at 15 min, and at 3, 7, and 21 day post-injury and BMSC survival was closely assessed both during the transplantation procedure and up to four weeks after transplantation. In addition, the effect of the timing of BMSC transplantation on tissue sparing was determined. BMSC were collected from culture dishes, kept on ice, and passed through a glass pulled needle for injection into the contusion site. This procedure resulted in a majority (67 %) of the BMSC intended to be transplanted being present in the contusion at 15 min after transplantation. Thereafter, BMSC numbers rapidly decreased. The rate at which cell death occurs is different when transplanting acutely or delayed. In an acute transplantation paradigm (15 min post-contusion) and sub-acute transplantation paradigm (3 days post-injury) BMSC survival is better than in a delayed transplantation paradigm (7 days or 21 days post-injury). The percentages of BMSC in the contusion at seven days after transplantation are 32% and 52% for acute and sub-acute transplantation, respectively, and 9% for delayed transplantation. Four weeks after transplantation, almost no BMSC can be found in either paradigm (see figure 4). Interestingly, the presence of BMSC for this short period of time is sufficient to elicit tissue sparing. Acute and subacute transplantation, but not delayed
transplantation results in neuroprotection, and tissue volumes in these paradigms are strongly correlated with the number of BMSC present\textsuperscript{46}. These results indicate that timing of BMSC transplantation is important for optimal survival and neuroprotective effect, with acute and subacute transplantation being superior to delayed transplantation. However, because of the clinical relevance of delayed treatment, it seems imperative to find strategies to improve BMSC survival in delayed paradigms.

![Fig. 4](image)

**Fig. 4.** A. BMSC numbers within a moderate contusion in the adult rat thoracic spinal cord decrease during 28 days post-injection. The rate at which cell death occurs is higher when BMSC are transplanted 7 or 21 days post-contusion, compared to BMSC transplantation 15 min or 3 days after contusion. B. The decreasing transplant is shown at 15 min (A–C), 7 days (D–F), and 28 days (G–I) after an injection at 15 min (acute), 7 days, and 21 days respectively, post-injury. All microphotographs are from horizontal cryostat sections. (A) Scale bar, 600 mm in A–I.

Previously, using a rat contusion injury model, Hofstetter and colleagues\textsuperscript{43} showed that more BMSCs survived when transplanted one week after injury compared to immediately after injury. The surviving cells were located within trabeculae that span the injury site. These data are in disagreement with those from the Nandoe Tewarie study\textsuperscript{46} although long-term results were in agreement with only 1% of the cells (about 3000 total) surviving at 4 weeks after grafting. The difference in early survival between the two studies may be that Hofstetter and co-workers injected the BMSC not only into the contusion but also rostral and caudal thereof into the spinal cord nervous tissue. Possibly, the surviving cells were located nearby but not in the contusion epicenter. Most studies have reported a poor survival of BMSC. Nandoe Tewarie and colleagues\textsuperscript{46} demonstrated that the contusion milieu is less detrimental during the first week after injury than the second and fourth
week after injury. What factors are important for BMSC survival in vivo? BMSCs are cultured in medium containing 10-20% serum. Factors other than present in serum are not essential for their survival and proliferation within the culture dish. In fact, addition of growth factors such as BDNF, FGF-2, or NT-3 instigates differentiation of the BMSCs into neural-like cells rather than affect survival. To date, the factors that may promote BMSC survival in vivo are unknown and further investigations are necessary to reveal them.

CONCLUSION

Stem cells have gained attraction over the last years in the field of neuroscience. In vitro it has been shown, although still disputed, that Bone Marrow Stromal Cells can transdifferentiate into cells of neural lineage. This has made this adult stem cell type interesting for neural transplantation paradigms. After transplantation of BMSC in the injured spinal cord most cells die. Nevertheless, especially in early transplantation, cells have a neuroprotective effect on the host tissue. This effect may well be the result of secretion of growth factors. Further studies are needed to investigate the true potential of BMSC.

REFERENCES
