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Bone Marrow Stromal Cells for Repair of the Injured Spinal Cord

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1. 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" (Cushing 1927). 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 are affected (Ditunno & Formal, 1994; Ackery et al., 2004). In contrast to the developed countries, in the less developed countries a shift of etiology can be observed towards falls (Hoque et al., 1999) and violence (Da Paz 1992, Dincer 1992, Hart 1994).
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 clinical consequences, assessments, and treatments of SCI and will then focus on stem cells and in particular BMSC as a therapy for SCI.

2. Clinical consequences of SCI

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; Blight 1992; Hagg & Oudega, 2006). 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 (Dietz & Harkema, 2004; Dobkin et al, 2007). 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 (Fiefler et al., 1999; Ackery et al, 2004). 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 (Boekamp et al., 1996; Widerström-Noga et al., 1999/2007). 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 (Boekamp et al., 1996). 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 (Widerström-Noga et al., 1999).

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 (Winslow et al., 2002). 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 (McKinley et al., 1999).

2.1 Clinical assessment of SCI

The American Spinal cord Injury Association (ASIA) impairment scale is widely used and provides clinicians with a standard way of grading the functional severity of a spinal cord injury. The scale has one grade for complete injuries (ASIA A), three other grades for incomplete injuries (ASIA B-D), and another for no impairment from the injury (ASIA E). To categorize the grades for incomplete injuries, clinicians determine the degree of muscle strength, being active movement against full resistance, of the key muscles below the neurological level of the injury. The assignment is based on the extent to which more than half of the key muscles have a muscle strength grade of 3 or higher. Classifications of SCI can also be achieved by implementing tests that measure functional ability (Ditunno et al., 2005). A widely used scale for such measurements is the Functional Independence Measure (FIM), which uses a 7-point scale to measure 18 items concerning mobility, locomotion, self-

care, bowel and/or bladder function, communication, and social cognition (McKinley et al., 2004; Ditunno et al., 2007). Other functional assessment scales are the Quadriplegic Index of Function (QIF), Modified Barthel Index (MBI), Walking Index for SCI (WISCI), Capabilities of Upper Extremity Instrument (CUE), Spinal Cord Independence Measure (SCIM), and the Canadian Occupational Performance Measure (COPM). These functional tests become more important in the rehabilitation phase of the patient when it is important to analyze the limitations in daily life.

2.2 Treatment of SCI

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 (Botel et al., 1997). Depending on the type of injury, surgical interventions should be considered to decompress the spinal cord and or stabilize the spinal column (Brodkey et al., 1980; Fehlings & Perrin, 2006). Decompression surgeries may accelerate functional improvements and result in shorter hospitalization and rehabilitation periods (Papadopoulos et al., 2002; McKinley et al., 2004). However, it does not result in an improved functional outcome (Chen et al., 1998). 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 (Fehlings & Perrin, 2006). 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 (Bracken et al., 1990/ 1997/ 2002). 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; Bracken et al., 1990/ 1997/ 2002). 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 (Nesutherai, 1998/ 2001; Lee et al., 2007). 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 (Qian et al., 2005; Lee et al., 2007). 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 (Widerstrom et al., 1999/ 2004), decreased fertility (Patki et al., 2007), and autonomic dysreflexia with loss of bladder and bowel control (Weaver et al., 2006). 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 (Mariano, 1992; Sawatzky et al., 2008). 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 (Kennedy et al., 2003; Strauss et al., 2006).

3. A stem cell; the origin of life?

Stem cells are defined by their capacity to divide in one cell that remains the stem cell without signs of ageing (self-renewal), and another cell that begins the process of differentiation, thus providing an unlimited source for implantation. Moreover, a stem cell is able to differentiate into different lineages (pluripotency). It may also be possible for a stem cell to cease proliferation, entering a quiescent phase, in which it is not an actual stem cell, but still has the potential since it can re-enter the cycle of self-renewal (Potten et al., 1990).

3.1 The origin of life

Aristotle (384-322 BC) developed the concept of spontaneous generation, which was first mentioned during ancient Roman times, and described that earth, air, fire, and water (the four elements) mixed with an essence known as "quintessence" or "ether" would give 'life' after it was brought in contact with 'pneuma' (or `soul`). In the middle ages it became generally believed that the concept of living was based on "spontaneous generation", hypothesizing that the embryo was derived from the mother's menstrual blood, based on the concept that living animals arose from slime or decaying matter. It was not until 1855 that zoologist and comparative anatomist, Leydig pronounced that life could only arise from preexisting life *(omne vivum ex vivo)*. Virchow extended this and postulated that all cells in an organism are derived from preexisting cells *(omnis cellula e cellula)*; all cells of the human body arise from the fertilized egg. The theoretical proof for his hypothesis was provided by the French chemist and biologist, Louis Pasteur (1822-1895) in his essay "Mémoire sur les corpuscules organisés qui existent dans l'atmosphère" of 1861 in which he described a series of experiments showing that germs do not form spontaneously in sterilized flasks. Nineteenth century pathologists first hypothesized the presence of stem cells in the adult as

"embryonal rests" to try to explain the cellular origin of cancer cells. Cancer results from an imbalance between the rate at which cells are produced and the rate of their apoptosis. Nowadays, it is generally agreed that stem cells reside in the adult body as well, possibly in a quiescent state. Certain cues can cause them to re-enter the cycle of cell proliferation and differentiation (Sell, 2004).

3.2 Determination

The ultimate stem cell is the fertilized egg, a totipotent cell, which can differentiate in each cell type. After a few divisions, when the dividing cells are called blastomeres (32 to 64 cells), the cells are determined to become specific for one of the three germ layers; the ectodermal layer, which will give rise to skin and neural tissue, the mesodermal layer, which will give rise to connective tissue, muscle, bone and blood cells, and the endodermal layer, which will give rise to gastrointestinal tract and internal glandular organ cells. In classic embryology, this 'determination' of stem cells is thought to be an irreversible process. As the cell mass develops, the daughter cells begin to acquire properties different from one another, so that different regions destined to become different components of the embryo are formed. Recently, it has been suggested that the determined stem cell is in fact phenotypically plastic and is able to give rise to cells from different germ layers, a process known as transdifferentiation (Morrison et al., 1997; Johansson et al., 2003). The process of transdifferentiation can also result in the formation of abnormal phenotypes having no counterpart in the normal body (dysplasia). These dysplastic cells may be the first step in carcinogenesis (Slack, 2007). The possibility of transdifferentiation has raised the interest in using of easy to harvest (adult) stem cells and try to transdifferentiate them into a more desirable cell type.

3.3 Embryonic versus adult

Embryonic stem (ES) cells are obtained from the undifferentiated inner cell mass of the blastocyst, which in humans forms 4 to 5 days after fertilization. At this stage the inner cell mass consists of around 50-150 cells. ES cells are pluripotent cells and can develop into more than 200 cell types of the adult body when given the appropriate stimuli for differentiation (Morrison et al., 1997). They are known to proliferate rapidly in culture. Because of this plasticity some people prefer to use embryonic stem cells for research purposes. On the other hand, it recently has been shown that the ES cells need to be genetically modified and extensively manipulated in vitro before they can be transplanted safely. Direct transplant of ES cells are known to give rise to teratomas and uncontrollable cell proliferation (Slack, 2007). To avoid immune rejection a life-long use of immunosuppressive drugs after transplantation is necessary or the ES cells have to be tissue-matched from a bank of stem cells created from 'spare' human embryos; a procedure prone to failure and morally objectionable to many, including scientists.

By contrast, adult stem cells can be transplanted directly without genetic modification or pre-treatments. There is no problem with immune rejection because the cells can readily be isolated from the patient's bone marrow, skin, fat, umbilical cord blood and other sources *(autografting)*. Although the adult stem cells show less plasticity in vitro, they do show high degrees of genomic stability during culture. They have the potential to differentiate in response to cues from the surrounding tissues and do not show uncontrollable proliferation resulting in tumors. Finally, since it is not necessary to take cells from embryos there are less ethical concerns for the use of this kind of stem cell.

3.4 Ethical and social perspectives

The involvement of human cells gives rise to ethical and social concerns, mostly concerning the status of the human embryo. Although there is a general consensus that the early human embryo is worthy of some measure of respect as an organized assembly of human cells, opinions vary as to the measure of respect deserved. The Roman Catholic Church teaches that "from its conception, the child has a right to life" and "it must be treated from conception as a person" (Roman Catholic Church, 1994). On the other hand, there are those that consider the early human embryo as merely an assembly of human cells and not a human individual at all at this stage. The embryo should be considered an individual once it becomes viable. Over the last few decades this viability of the embryo has shifted as a result of advanced medical techniques to the 20th week of gestation (Goldenring, 1985; Peterfy, 1995). The use of human embryo's for research or therapeutic purposes is still the subject of controversy.

The conversion of stem cells into gametes (sperm or egg cells) in itself presents, if any, few ethical hurdles (Testa & Harris, 2005). On the positive side, stem cells as a potential source of oocytes for clinical applications, such as in vitro fertilization to induce pregnancy in childless couples, have proven successful. Many of the ethical concerns arise from the potential for misuse rather than the use of such gametes (Westpal, 2003). The ability to derive oocytes from stem cells of male origin, inducing X and Y chromosomes and fertilized oocytes would raise the possibility of in vitro fertilization of such oocytes and the production of a child with two male biological parents (Hubner et al., 2003; Geijsen et al., 2004; Azim, 2004). Most extreme, the possibility of producing oocytes from the male stem cells and fertilizing these oocytes with his own sperm would open the avenue for single-parent families and in theory would be the most incestuous relationship. Obviously, there still has to be a female surrogate mother to bring any offspring to term. The offspring can be expected, if it survived, to exhibit many developmental defects resulting from acquisition of pairs of (recessive) genes for anomalies (Hubner et al., 2003, Whittaker, 2007).

Therapeutic cloning and genetic manipulation of stem cells have ethical and social concerns. Cloning of cells, genetically matched for the host can contribute to the field of organ transplantation, where a shortage of donor organs and rejection of not perfectly matched grafted organs by the host are major concerns. However, the destruction of human embryos for this application opposes obvious problems in itself and is in general considered unethical. Genetic manipulation may perhaps be one of the most significant applications of converting embryonic stem cells into gametes, opening the possibility for germ line gene therapy (GLGT) (Newson & Smajdor, 2005). GLGT offers the possibility for eliminating the transfer of a gene mutation from a parent to their children and their children's children, and as a result eliminating the aberrations in the gene pattern. Genetic manipulation could, even more controversially, open the possibilities for inserting 'desirable' genes into the germ line, resulting in the so-called "designer babies".

Stem cell-based therapies are still at a very early stage and the associated risks are still unclear. On the other hand, when a patient is suffering from a disabling and sometimes life-threatening disease, a case might be made for lowering the ethical barriers to enable treatment. As with any medical interventions, the questions to be asked are whether this approach is the most likely to achieve success and whether the potential benefits outweight the potential risks.

4. Stem cells for central nervous system repair

The promise of neural stem/progenitor cells (NSC) for repair of traumatic injuries of central nervous tissue lies in their ability to differentiate into the three neural cell types; neurons, astrocytes, and oligodendrocytes (Fig. 1). Besides NSC, other types of stem cells may also be beneficial for replacement-based repair approaches if they can be successfully manipulated into the neural lineage (i.e., transdifferentiation).

Although, following an injury to the central nervous system, endogenous NSC are typically recruited, they generally do not lead to significant repair. A potential approach to benefit more from NSC for neural repair is to isolate the cells from donor tissue and prepare them ex vivo for transplantation. The advantages of ex vivo isolation of cells are the large numbers of cells that can be cultured, and the opportunity of committing the cells towards a certain cell type. Experimental evidence has demonstrated that neural stem cells after transplantation into damaged nervous tissue are more likely to differentiate into astrocytes than into neurons or oligodendrocytes. In some cases this may be the objective, but most likely there is a larger need for neurons or oligodendrocytes. Therefore, it is more effective to transplant cells already committed to become a certain neural cell type such as oligodendrocyte-precursor cells (OPCs) and glial-restricted precursor cells (GRPCs).

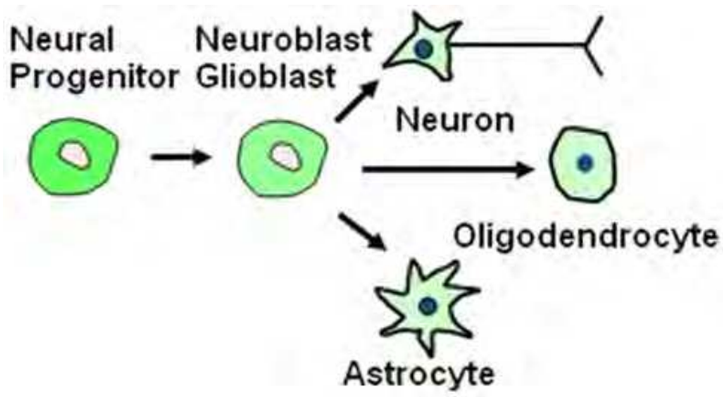

Fig. 1. Neural lineage differentiation: Neural stem cells differentiate into neuroblasts, which give rise to neurons, and in glioblasts, which give rise to astrocytes and oligodendrocytes.

Replacement of lost neurons with NSC-derived neurons may provide the cellular foundation for restoring axonal connections between affected regions in the nervous system (Fig. 2). The prerequisite for such repair is that the transplanted neurons grow their axon towards the original (or new) target cells and form functional synaptic connections. Replacing lost oligodendrocytes by ones generated from stem/progenitor cells may benefit remyelination of axons that have survived the initial and secondary damage but have lost their insulating myelin sheaths due to oligodendrocyte death (Fig. 2). In addition, oligodendrocyte replacement may help myelination of newly generated axons from the transplanted neurons as well as regenerated axons from original neurons. Research has demonstrated that oligodendrocyte replacement using OPCs is a powerful tool to elicit (re)myelination and as a result improve functional recovery. Currently, OPCs are being tested in a clinical trial for their potential to repair the spinal cord. Replacement of lost astrocytes can also be of great value. Astrocytes play a crucial role in a number of events such as neuronal functioning, blood-spinal cord-barrier maintenance, and more. Astrocyte

replacement is often undervalued mainly because astrocytes are known to be an integral component of the glial scar that forms at an injury site. This glial scar presents a molecular and cellular barrier for axon growth and thus for functional restoration.

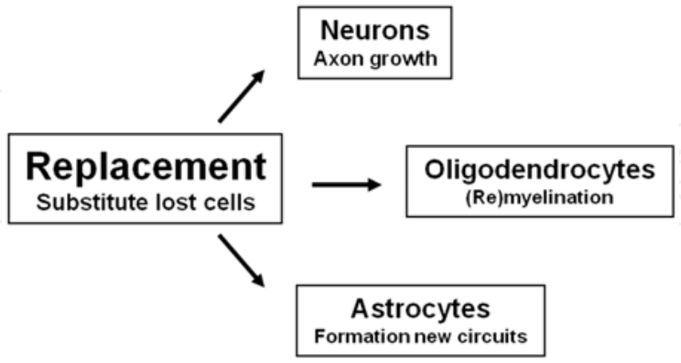

Fig. 2. Replacement strategies for nervous tissue repair. Neural progenitor cells could be employed to replace lost cells. This may result in formation of new neurons, oligodendrocytes or astrocytes.

The cellular consequences after SCI in time can generally be characterized in 5 stages, starting with 1) immediate neural cell death and axonal damage accompanied by motor and sensory function loss due to the trauma; 2) progressive loss of tissue resulting in the development of cystic cavities; 3) infiltration of macrophages to remove cellular debris; 4) formation of a glial scar surrounding the injury epicenter limiting and/or preventing endogenous axonal sprouting; 5) dieback of damaged axon stumps away from the injury.
In general, there are three therapeutic platforms for spinal cord repair (Fig. 3); neuroprotection (i.e., limiting additional loss of nervous tissue; *rescue tissue*), regeneration (i.e., promoting axonal growth and/or myelination, nervous tissue formation/modeling; *restore tissue*), and plasticity (i.e., exploiting axonal circuits that were left intact; reuse *tissue*).

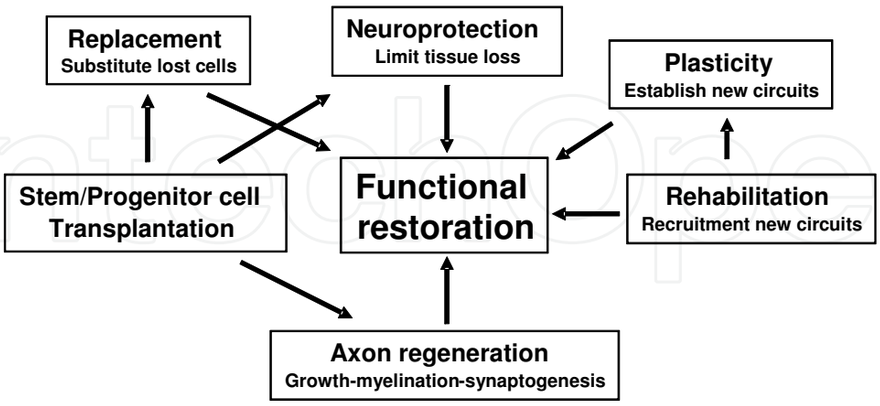

Fig. 3. Repair platforms for the injured spinal cord. Spinal cord repair may be achieved through a variety of opportunities that involve tissue rescue (neuroprotection), tissue restoration (axonal regeneration/myelination), and tissue reuse (plasticity). Stem cells may influence all of these platforms as well as replace lost cells, which has its own repair effects. All can lead to functional recovery.

The consensus in the field of spinal cord injury/repair is that different interventions addressing the different repair platforms need to be combined in an approach to obtain significant functional restoration. The components of such combination strategies will need to be applied simultaneously and/or successively depending on each individual purpose thereby creating optimal conditions for histological and functional repair. Intensive rehabilitation to develop and stabilize new axonal circuits will be necessary either as one of the components in the repair strategy or as an 'anchoring'-mechanism of the outcomes obtained by neuroprotection- and regeneration-based interventions.

5. Bone marrow stromal cells

Mesenchymal stem cells from bone marrow (BMSC) have therapeutic potential for the injured spinal cord (Nandoe Tewarie et al., 2006). BMSC were shown to differentiate into bone, fat, tendon and cartilage cells (Pittenger et al., 1999). Although still debated, it has been reported that BMSC can transdifferentiate in vitro into liver cells (Petersen et al., 1999), skeletal cells (Wakitani et al., 1995), cardiac muscle cells (Orlic et al., 2001), and neural cells (Petersen et al., 1999, Mezey et al., 2000). 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 all consequences of spinal cord injury and support anatomical and functional repair.

6. Rodent models of SCI

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 4 shows the rat model system for spinal cord contusive injury.

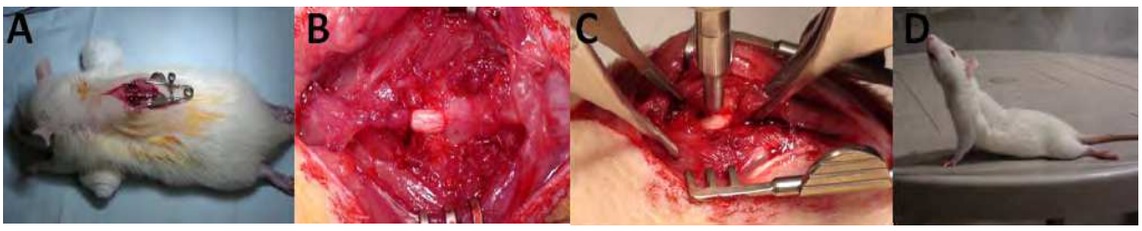

Fig. 4. Rat spinal cord contusion model. A. A laminectomy is performed exposing the underlying spinal cord. **B.** Enlarged view of the exposed spinal cord segment. **C.** A computerized impactor is used to contuse the spinal cord. The piston is attached to a sensor to record velocity, force and displacement to ensure consistency. **D.** A moderate contusion results in loss of function at and below the level of injury and loss of bladder function.

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.

7. BMSC preparation and 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 (Ohta et al, 2004), or transplanted acutely into the cervical (Lu et al, 2004) or thoracic spinal cord (Hofstetter et al, 2002; Ankeny et al, 2004) or into the chronically injured cord (Zurita and Vaquero, 2004).

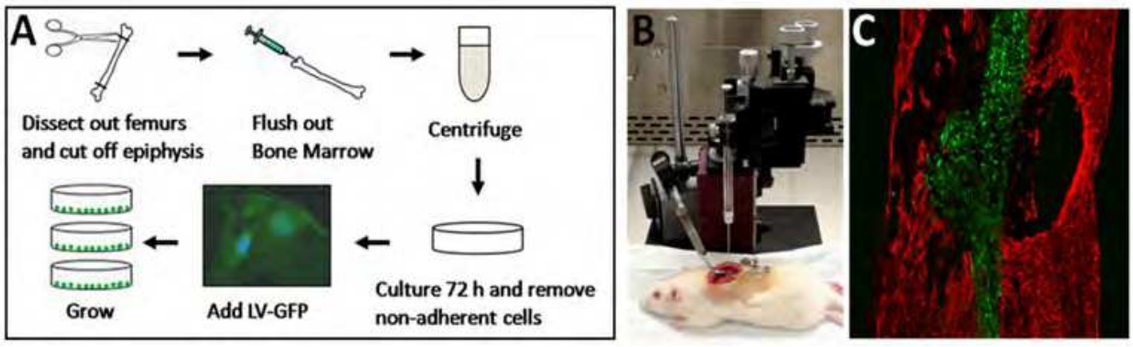

Fig. 5. Transplantation of BMSC. **A.** BMSC are isolated from femurs of rats by cutting off the epiphyses and flushing out the bone marrow. Cells are plated onto plastic culture dishes. Non-adherent hematopoietic stem cells are removed and the plastic-adherent BMSC are infected with LV-GFP. **B.** Cells 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.

8. BMSC transplantation improves function after spinal cord contusion

BMSC have been transplanted into rodent models of spinal cord injury by various research groups and with generally promising results. Considerable variation exists between injury models, treatments paradigms, and analytical methods used by the various investigators, and consequently, different results have been found by different groups. The injection

paradigms vary considerably in time, number, location, survival times, etc. Given these differences between the approaches, it is difficult to compare the respective results and thus to properly value the regeneration-promoting abilities of BMSCs.
However, it was observed by most groups and it is clear that some histological and/or functional beneficial effects can be expected after transplantation of BMSC into the injured spinal cord. These effects include improvements in locomotion, sensorimotor function, sensory function, promotion of axonal regeneration, and preservation of neural tissue (reviewed by Nandoe Tewarie et al., 2006).
Improvements in hind limb locomotor recovery have been reported after BMSC transplantation in the acutely, subacutely, and chronically injured spinal cord. Hind limb function is typically evaluated using the open field BBB-test, which scores for joint movements, paw placement, weight support, and coordination between fore limbs and hind limbs. Although a valid way to test hind limb function, the BBB test has limitations; the scoring is subjective and difficult for observation of coordination. This may affect the assessment of hind limb motor performance. Other sensorimotor tests such as foot print, grid and beam walking, and analysis of gait using the CatWalk® provide a more complete measurement of hind limb function (Figure 6). Recently, Ritfeld et al. (2011) performed an extensive battery of functional tests after BMSC transplantation into a spinal cord contusion injury rat model, including BBB open field testing, performance on horizontal ladder, footprint analysis, mechanical allodynia and thermal hyperalgesia. Improvements were found in the BBB-subscore, horizontal ladder performance, base of support, angle of paw rotation, and mechanical and thermal hypersensitivity. No improvements were found in BBB open field locomotion, illustrating the importance of using multiple functional outcome assays to detect treatment-mediated effects on functional recovery.

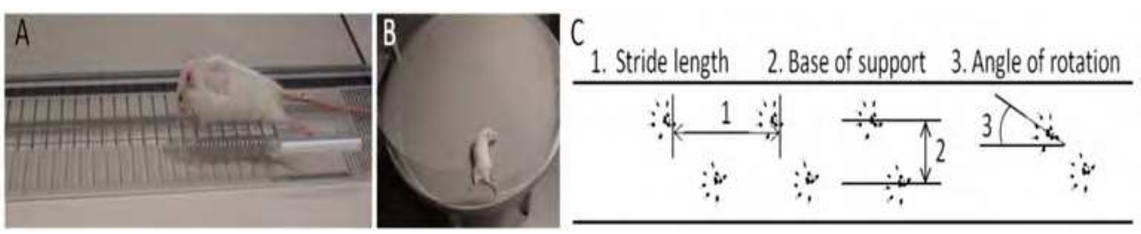

Fig. 6. **A.** Performance on a horizontal ladder is used as an indicator of sensorimotor function. The number of slips made on the rungs is counted by playing the videotaped runs back in slow motion. **B.** Testing of BBB open field locomotion includes scoring for joint movements, paw placement, weight support, and fore/hind limb coordination. **C.** In footprint analysis stride length, base of support and angle of paw rotation are determined.

The finding that BMSC transplantation into the contused spinal cord leads to improvements in functional restoration holds promise for future BMSC-based repair strategies for the injured spinal cord. However, knowledge of the underlying mechanisms is essential to increase the overall outcomes. Which repair-mechanisms are positively influenced by the BMSC transplant? Which molecules and receptors are involved? How do BMSC transplant-mediated events elicit functional recovery? Initial reports on BMSC-initiated functional improvements suggested that beneficial effects could be due to replacement of lost cells by BMSC. The first study that provided evidence that BMSC can differentiate into neural-like cells *in vivo* was from Mezey and colleagues (2000). Using a mouse model they transplanted male BMSC into the peritoneal cavity of female recipients. The grafted BMSC preparation

did not contain neuron- or glia-like cells at the time of transplantation, although it should be noted that about 18% of the cells expressed the neural precursor cell marker nestin, when cultured for several weeks. Using in situ hybridization techniques, Y chromosome-containing neurons were located in the brain of the host, suggesting that the grafted BMSC had crossed the blood-brain barrier and formed neurons within the CNS.
Interestingly, Cogle and colleagues (2004) also demonstrated Y chromosome-containing neurons that were nicely integrated in the hippocampus of three female humans that had received transplants of male BMSC up to six years earlier. It should be mentioned that a fusion between a grafted BMSC and a host cell could provide false-positive results. In several studies it has been reported that BMSC can spontaneously fuse with other cells in vitro. Whereas this is a real possibility, Cogle and coworkers (2004) used fluorescence in situ hybridization techniques to reveal the presence of only one X chromosome, concluding that the neurons found in their study had not fused with BMSC. The Y chromosome containing, transgender cells accounted for approximately 1 % of all neurons and 1 -2 % of all astrocytes and microglial cells in the hippocampus. These studies have provided exciting data suggesting that BMSC can differentiate into neural cells in the mature CNS. However, to date this possible transdifferentiation of BMSC is intensely debated and conclusive evidence of this possibility is lacking. The data showing functional and histological improvements after BMSC transplantation into spinal cord contusion injury models point to repair mechanisms other than cell replacement of lost cells by BMSC. Within spinal cord injury models, few investigators have shown expression of neural markers by BMSC transplanted in the spinal cord. Functionality of BMSC-derived neurons (e.g. synapse formation, firing of action potentials, release of neurotransmitters, myelination) and astrocytes has not been demonstrated. Thus whether transplanted BMSC can transdifferentiate into neural cells in the damaged spinal cord is highly questionable. Whether BMSC-derived neural cells are involved in the observed functional improvements after BMSC transplantation in the injured spinal cord is even more improbable. This raises the question in what manner transplanted BMSC contribute to the functional improvements.

9. BMSC and neuroprotection

One possible repair mechanism that could be influenced by a BMSC transplant is neuroprotection. Neuroprotection aims to protect neural cells from loss due to pathophysiological events secondary to the initial injury resulting in more spinal cord nervous tissue which may lead to functional gains. Neuroprotective approaches are considered the first line of defense against the devastating life-debilitating consequences of spinal cord injury. The sooner neuroprotective strategies are implemented after injury the more effective they will be. If functional recovery is not achieved with neuroprotective strategies there may still be important benefits because a larger volume of nervous tissue is present to serve as a substrate for regenerative or plasticity-promoting interventions. In general, stem/progenitor cell transplants may elicit neuroprotection through different direct and indirect mechanism. After transplantation the cells may secrete molecules that prevent/limit the loss of neural cells due to apoptosis (direct mechanism), decrease the invasion of macrophages and thus macrophage-mediated cell death (indirect mechanism), or prevent the breakdown of blood vessels leading to blood-spinal cord-barrier permeability (indirect mechanism).
Our current understanding of BMSC-mediated repair points towards a mechanism that involves secretion of trophic factors by BMSC resulting in decreased loss of neural tissue

(i.e., neuroprotection). Ritfeld and colleagues (2011) demonstrated a strong correlation between improved function and volumes of spared tissue in rats with BMSC transplanted in their contused spinal cord at three days post-injury. The mechanisms underlying BMSC-mediated tissue sparing in the injured spinal cord are still under investigation. A possible mechanism is that BMSC transplants positively influence blood vessel presence in the injury site either by blood vessel stabilization/rescuing or formation which results in tissue sparing. This in turn may involve sparing of descending and ascending axons mediating those functions that were improved (Ritfeld et al., 2011).

BMSC have been shown to secrete various growth factors, including brain-derived neurotrophic factors (BDNF), glial-derived neurotrophic factor (GDNF), vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF-2), nerve growth factor (NGF) and neurotrophin-3. NGF and BDNF increase survival and decrease apoptotic death of neurons and oligodendrocytes. BDNF also increases oligodendrocyte proliferation. GDNF has been implicated in the rescue of motor neurons, possibly by activating MAP kinase and Bcl-2, an anti-apoptotic regulator. FGF-2 is known to positively affect tissue sparing and promote neuronal survival and angiogenesis following spinal cord injury. VEGF is a potent angiogenic factor and as such could positively affect tissue preservation. Thus, based on the secretion profile, BMSC may contribute to neuroprotection in a direct manner (rescuing neural cells) and/or in an indirect manner (promoting angiogenesis).

The secretion of these molecules is thought to be the main factor behind the observed functional improvements after BMSC transplantation in spinal cord injury models (Figure 7). However, which factors or combinations of factors are specifically necessary and the precise mechanisms by which these factors elicit their effect remain to be elucidated in future investigations. Such mechanistic information is imperative as it can provide us with valuable insights on how to increase the overall reparative effects of BMSC transplants. For

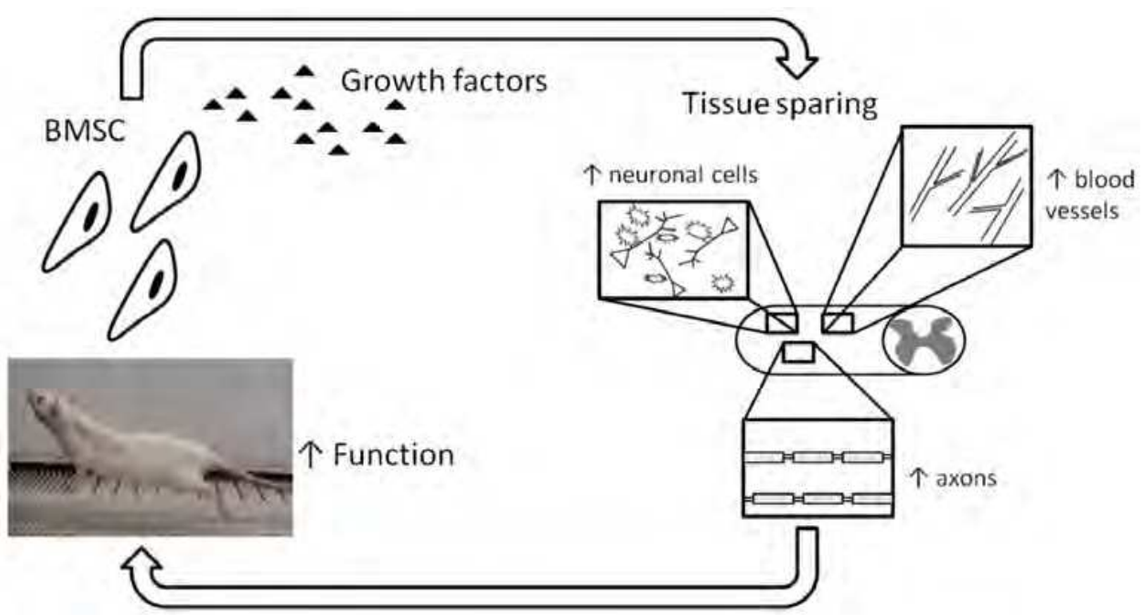

Fig. 7. BMSC secrete various growth factors, including BDNF, VEGF, NGF and NT-3. These factors are thought to limit the loss of tissue in the injured spinal cord, contributing to the increased functional outcomes after BMSC transplantation.

example, increasing the production and secretion of certain growth factors by viral transduction of the cells before transplantation is currently used to gain mechanistic insights and to optimize the therapeutic effect of BMSC.

10. BMSC and axonal regeneration

BMSC transplantation may also lead to improved functional outcomes by promoting axonal regeneration. Spinal cord injury leaves axons cut and/or demyelinated which prevents or limits the conduction of information to their target cells, including signals essential for the generation and control of motor behaviors. Eliciting axonal growth after injury may lead to the reestablishment of previously existing axonal circuits that were involved in motor function or in the formation of new axonal circuits that could be recruited for motor functions. Remyelination is an integral part of axonal regeneration and crucial for proper functioning of axons. A major obstacle in axonal regeneration after spinal cord injury is the distance between injury, where the proximal end of the cut axon is located, and the target area, where synaptic connections need to be established. Many axonal regeneration therapies have shown to promote axonal regeneration but fail to elicit lengthy regeneration. The relative short regenerated sprouts may in the best scenario form synaptic connections with neurons close to the injury site, but not with original target neurons or with neurons nearby or in the target area. As a result, the resulting behavioral effects may be limited or absent, despite the axonal regeneration response.

In general, stem/progenitor cell transplants could promote axonal regeneration (i.e., growth and/or (re-)myelination) by producing and secreting molecules that initiate the growth-process in damaged neurons and promote oligodendrocytes to form myelin sheaths around new or existing undamaged but demyelinated axons. Secreted molecules that promote the differentiation of local oligodendrocyte progenitor cells into mature oligodendrocytes could also contribute effectively to the axonal regeneration process. Alternatively, stem/progenitor cells may elicit an axonal growth response by diminishing the growth-inhibitory nature of the injury environment especially that of the glial scar. This latter effect could set the stage for successful axon growth initiated by the stem/progenitor cells and/or endogenous axon sprouting.

There are few studies that have focused on the axonal regeneration promoting abilities of BMSC. Lu and co-workers (2004) demonstrated that transplantation of native BMSCs into the contused spinal cord promoted modest sensory and motor axon regeneration. In another study from Lu and colleagues (2005) it was shown that BMSC modified to produce and secrete higher levels of BDNF also promoted axonal regeneration. BMSCs produce and secrete several growth factors including GDNF, NGF, and BDNF that could positively affect axonal growth and/or myelination. To date, most studies that investigated the effects of BMSC transplants in the injured spinal cord have limited focus on axonal regeneration. In those studies that did focus on axonal regeneration lengthy regeneration was not demonstrated and the involvement of the responding axons in behavioral recovery not proven. Clearly, further investigations are necessary to establish the axonal regeneration-promoting abilities of BMSC transplants in the injured spinal cord. Especially effective in such studies could be techniques that silence the production of particular molecules in BMSC, for instance approaches based on small interfering RNA molecules, to elucidate which of the molecules if any are in fact needed for BMSC to elicit an axonal growth response.

11. BMSC and plasticity

BMSC transplants may lead to functional recovery after spinal cord injury by promoting plasticity within circuits that have been spared. Most spinal cord injuries in humans are anatomically incomplete; connections between brain and below-level spinal cord segments exist but are ineffective. Locomotor patterns can be generated by a complex network of axonal circuits which is present in the lumbar spinal cord segments and known as the central pattern generator (CPG). The existence of ineffective axonal connections and the CPG offers the possibility to elicit plasticity within these circuitries after spinal cord after injury possibly resulting in motor activities. Plasticity may occur at the molecular level resulting in changes in neuronal properties (e.g., excitability), or at the structural level resulting in new synaptic connections. Eliciting plasticity has gained much attention in the last decade as an interventional goal with the potential to elicit functional recovery after spinal cord injury.

Intraspinal stem/progenitor cell transplants may evoke plasticity via secreting molecules that could elicit changes in the functioning of neurons or modulations in synaptic organization. Although plasticity within the damaged spinal cord has been in the spotlight for some time, studies that involve stem/progenitor cells in general or BMSC in particular and address plasticity in the injured spinal cord are sparse. There are, however, many BMSC studies that have demonstrated some degree of functional restoration without clear evidence for underlying mechanisms. Possibly, plasticity was among the BMSC-mediated effects in the damaged spinal cord resulting in improved functional outcomes.

12. BMSC survival in damaged nervous tissue

Although functional and histological improvements are observed after BMSC transplantation in the injured rodent spinal cord, BMSC survival is typically poor, with the majority of cells dying within the first week after transplantation (Nandoe Tewarie et al., 2009). Possible cytotoxic factors include an extensive inflammatory response, free radical accumulation due to initial cell loss, lack of oxygen and nutrients due to the loss of blood vessels, and lack of a survival-promoting substrate. The question arises how BMSC transplant exert their repair effects if their survival is poor. To date, the answer to this question is not known but the most likely answer is that their relative short presence in the injury site is sufficient to bring about repair-supporting processes. This would point at neuroprotection as the main mechanism underlying BMSC-mediated repair because axonal regeneration and plasticity may require their presence in later stages after injury. Evidence that supports this idea is that BMSC transplantation at seven days post-injury or later does not lead to tissue sparing whereas transplantation immediately or three days after injury does (Nandoe Tewarie et al., 2009). An interesting associated question is whether BMSC are in fact necessary during later stages after injury. Future investigations will need to be conducted to obtain the right answers to these questions which will have major impact on BMSC-based spinal cord repair approaches.

The growth factors secreted by BMSCs may affect BMSC survival and/or proliferation *in vivo* through autocrine actions. Poor survival then may reflect inadequate amounts of these factors to positively affect their own survival within an extremely harsh injury milieu with many cells and factors that negatively influence survival. Timing of BMSC transplantation, combination with anti-inflammatory and/or immune-modulatory drugs, and

transplantation within a surviving –promoting scaffold have been studied for their effects on BMSC survival.

13. Timing of transplantation

In an experiment by Nandoe Tewarie and colleagues (2006), 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 8). 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 (Nandoe Tewarie et al., 2009). 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,

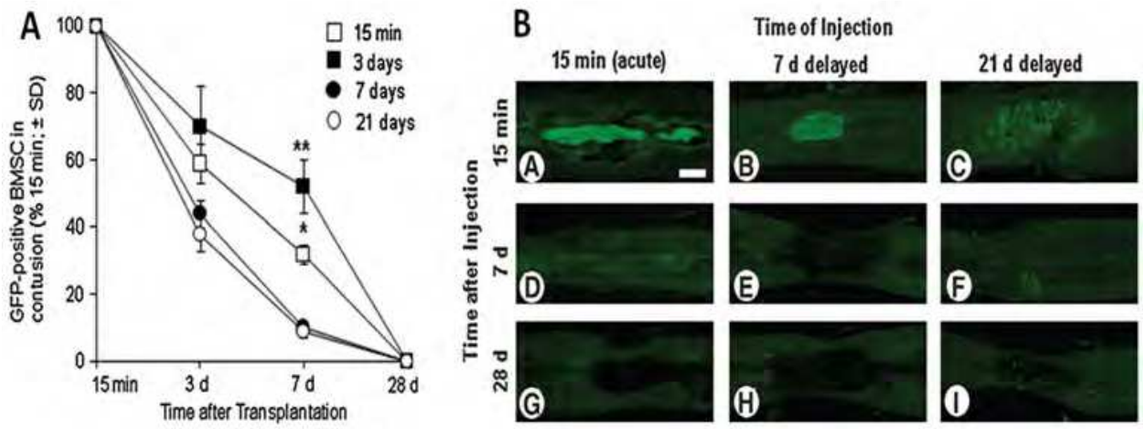

Fig. 8. **A.** BMSC numbers within a moderate contusion in the adult rat thoracic spinal cord decrease during 28 days post-injection. The rate at which cell death occurs is higher when BMSC are transplanted 7 or 21 days post-contusion, compared to BMSC transplantation 15 min or 3 days after contusion. **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.

because of the clinical relevance of delayed treatment, it seems imperative to find strategies to improve BMSC survival in delayed paradigms.
Previously, using a rat contusion injury model, Hofstetter and colleagues (2002) 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 (2009) 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 (2002) 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 (2009) 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.

14. Anti-inflammatory drugs

It has been proposed that one of the mechanisms underlying death of cells transplanted into the spinal cord is injury-induced inflammation. The cellular and molecular components of the inflammatory response could initiate cell death, which would also explain improved survival with delayed grafting paradigms. Application of immune-modulatory molecules could possibly support better transplanted cell survival. A role of macrophages in death of transplanted cells has been suggested for a number of cell types and anti-inflammatory drugs often improve the outcome. A direct relationship between number of macrophages and transplanted cell survival is less clear in case of BMSC. For instance, if macrophage number plays a role in survival of BMSC then delayed transplantation paradigms at time points when macrophage numbers are lower would lead to better survival. This was not demonstrated by Nandoe Tewarie and colleagues (2009). On the contrary, delayed grafting into the adult rat contused spinal cord resulted in decreased survival rates of transplanted BMSC.
Ritfeld and colleagues (2010) studied the effect of pharmacologically decreasing the number of macrophages in the contused rat spinal cord on transplanted BMSC survival. Systemic treatment with the anti-inflammatory agents methylprednisolone, minocycline, or cyclosporine, all showed an effective decrease in macrophage presence in the contused spinal cord at three days post-contusion, the time point of BMSC transplantation. However, the decreased number of macrophages did not significantly improve BMSC survival. A trend towards improved survival was seen, however, for cyclosporine, and accordingly, other studies have reported beneficial effects of cyclosporine on BMSC survival. Conflictingly, BMSC have been reported to have the ability to evade immune responses due to low expression of MHC I and no expression of MHC II molecules. As such, BMSC are being explored for their potential use as immune suppressing agents in combination with other cell transplants. At present, no consensus exists on the necessity, type or dose of immune-suppressing agents for BMSC transplantation.

15. Survival promoting scaffolds

Another means of improving BMSC survival in the injured spinal cord is the use of biomaterials as scaffolds for BMSC transplants. Hydrogels, extracellular matrix-based materials (e.g.fibronectin), agarose scaffolds and fibrin scaffold are among the materials being used as scaffolds for BMSC transplantation. Typically, BMSC survival can be improved by use of such materials, either by providing the cells with a substrate for survival/growth and/or by protecting the cells from detrimental immune responses.

16. Clinical application of BMSC

BMSC have several features that make them appealing candidates for transplantation after spinal cord injury in the human. BMSC can be easily isolated under local anesthetics, rapidly and extensively expanded in cell culture and there is no evidence of tumorigenicity in vivo, even after immortalization to ensure an unlimited source of self-renewal ex vivo. In addition, these cells have demonstrated capacity for tissue repair, and secrete growth factors that enhance histological and functional repair. Clearly, BSMC are a promising candidate for transplantation into the injured spinal cord. However, since considerable variation consists among donors regarding gender, genetics, and general health, specific parameters need to be found that allow rapid and reliable selection of BMSC with therapeutic potential. In addition, it is also clear that our current understanding of BSMC function is not yet sufficient to provide us with the so-called "silver-bullet", the one therapy that will promote regeneration and restore function in the injured spinal cord. In fact, it is generally accepted in the field of spinal cord injury research that such a therapy does not exist. Spinal cord injury results in a particularly complex cascade of histopathological events and a treatment aimed at functional repair requires that these events are dealt with in a timely, most likely sequential, fashion. Despite our incomplete knowledge, several clinical trials are being conducted in Korea, Mexico, Columbia, Brazil, and India. In a Korean study, 35 complete spinal cord injured patients received a BMSC transplant, combined with granulocyte macrophage-colony stimulating factor (GM-CSF) administration within 2 weeks, between 2 weeks and 8 weeks or after 8 weeks of spinal cord injury. Patients were assessed using the American Spinal Injury Association Impairment Scale (ASIA), electrophysiology, and magnetic resonance imaging (MRI). No adverse histological were observed on MRI at four months and no serious clinical adverse effects were reported. In 30.4 % of the acute and subacute treated group ASIA score increased to ASIA B or ASIA C after a median follow-up period of 10.4 months after injury. The chronic treatments group showed no improvements. Although these results are promising, neuropathic pain and tumor formation remain to be evaluated and a long-term and large scale multicenter clinical study is required for more accurate assessment of efficacy. Other studies have reported no adverse effects of BMSC transplants, but efficacy remains to be revealed.

17. 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 BMSC can transdifferentiate into cells of neural lineage. This has made this adult stem cell type interesting for neural transplantation paradigms. We have shown that 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.

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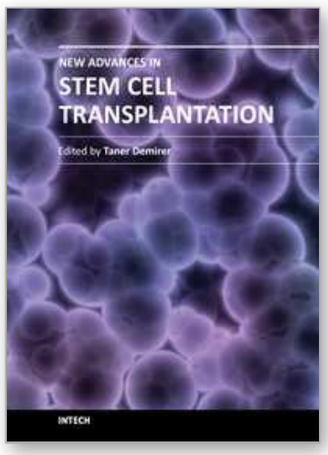

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This book documents the increased number of stem cell-related research, clinical applications, and views for the future. The book covers a wide range of issues in cell-based therapy and regenerative medicine, and includes clinical and preclinical chapters from the respected authors involved with stem cell studies and research from around the world. It complements and extends the basics of stem cell physiology, hematopoietic stem cells, issues related to clinical problems, tissue typing, cryopreservation, dendritic cells, mesenchymal cells, neuroscience, endovascular cells and other tissues. In addition, tissue engineering that employs novel methods with stem cells is explored. Clearly, the continued use of biomedical engineering will depend heavily on stem cells, and this book is well positioned to provide comprehensive coverage of these developments.

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