

# Schwann cells for spinal cord repair

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## Abstract

The complex nature of spinal cord injury appears to demand a multi-factorial repair strategy. One of the components that will likely be included is an implant that will fill the area of lost nervous tissue and provide a growth substrate for injured axons. Here we will discuss the role of Schwann cells (SCs) in cell-based, surgical repair strategies of the injured adult spinal cord. We will review key studies that showed that intraspinal SC grafts limit injury-induced tissue loss and promote axonal regeneration and myelination, and that this response can be improved by adding neurotrophic factors or anti-inflammatory agents. These results will be compared with several other approaches to the repair of the spinal cord. A general concern with repair strategies is the limited functional recovery, which is in large part due to the failure of axons to grow across the scar tissue at the distal graft-spinal cord interface. Consequently, new synaptic connections with spinal neurons involved in motor function are not formed. We will highlight repair approaches that did result in growth across the scar and discuss the necessity for more studies involving larger, clinically relevant types of injuries, addressing this specific issue. Finally, this review will reflect on the prospect of SCs for repair strategies in the clinic.

## Key words

- Spinal cord injury
- Implantation
- Regeneration
- Neurotrophic factors
- Clinical trial
- Rehabilitation
- Gene profiling

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Presented at the  
XIX Annual Meeting of the  
Federação de Sociedades de  
Biologia Experimental,  
Águas de Lindóia, SP, Brazil,  
August 25-29, 2004.

Publication supported by FAPESP.

Received May 27, 2005  
Accepted February 24, 2005

## Introduction

Shortly after injury to the adult mammalian spinal cord, polymorphonuclear granulocytes including neutrophils and, later on, lymphocytes, macrophages, and Schwann cells (SCs) invade the damaged area and local resident microglial cells become activated (1). These inflammatory events in concert with other cytotoxic events result in progressive loss of spinal tissue, i.e., secondary injury (2). In the case of a contusion injury, the resulting cavity often extends across the diameter of the cord leaving only a rim of spinal white matter (3-5). Damaged axons die back from the injury site (6,7) and

those that are spared may become demyelinated due to death of oligodendrocytes (8). Cellular and molecular changes at the injury site result in the formation of a growth-inhibitory scar (9), which frustrates the axonal regeneration response often seen immediately after injury (10). Consequently, axons do not grow beyond the lesion to form new synaptic connections with target neurons. Injury-induced partial or complete paralysis is permanent.

The primary injury to the spinal cord usually results in progressive tissue loss. Neuroprotective strategies applied soon after injuries may limit but not prevent further tissue loss. Clearly, repair of the (sub-)chroni-

cally damaged cord will require implants to fill (bridge) the injury gap. Such implants will likely contain cells that need to be selected for any of the following criteria: compatibility with spinal tissue, replacement of lost neurons or glial cells, promotion of axon regeneration, provision of a substrate/guidance for these axons, myelination of new sprouts and demyelinated axons, and ability to migrate into spinal tissue. The choice of implant will likely be determined by the nature and extent of the injury at the time of grafting. Cellular implants that have been explored include peripheral nerve, olfactory ensheathing glia (OEG), genetically engineered fibroblasts, fetal spinal tissue, stimulated macrophages, stem cells, and SCs. For

clarity and focus we will review here mainly the repair potential of SCs and compare their effects with some key approaches that involved other types of cells or tissue.

### Spinal cord injury models

Human spinal cord injuries are very heterogeneous (3-5). About 27% of human spinal cord injuries are lacerations caused by penetrating objects that tear the dura ('open' injuries) and spinal tissue, resulting in a discontinuity of the cord. This usually causes massive tissue loss, cyst formation, and a significant invasion of meningeal cells. The majority of the clinical cases are the result of a temporary compression of the cord that leaves the cord surface intact ('closed' injuries; 73%). Three types of compression injuries are described: massive compression, contusion, and solid cord injury. A massive compression (44% of all compression injuries) causes substantial destruction/loss of spinal tissue. A contusion injury (31%) results in the gradual formation of a central fluid-filled cyst and a minimal invasion of connective tissue. In case the contusion cyst progressively enlarges, it is referred to as syringomyelia, which is present in a limited number of cases. With a solid cord injury (25%) the shape of the cord is largely retained and there is no central hematomyelia and cyst formation, and mostly white matter tracts are damaged. Anatomically, compression injuries are typically incomplete and present clinically as "central cord syndrome" with variable sensory and partial motor loss. Often anatomically incomplete compression injuries do result in complete paralysis.

In the laboratory, a complete/partial discontinuity of the spinal cord can be modeled using a surgical microknife or microscissors (Figure 1A,B,D). A complete transection causes major damage to spinal tissue and its blood supply, as well as the formation of a relatively large growth-inhibitory scar, which includes meningeal fibroblasts (11). Consid-

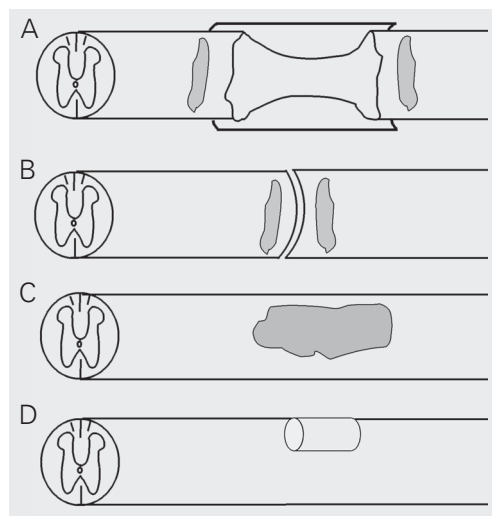


Figure 1. Schematic representation of various spinal cord repair models. In all cords rostral is on the left and dorsal is up. *A*, Completely transected spinal cord with tissue removed and a Schwann cell cable encased by a polymer tube implanted between the cord stumps. Note that only the back half of the tube is visible and that the cord ends are within the polymer tube (model used in Ref. 16). The dark gray areas close to the rostral and caudal graft-host spinal cord border represent injected olfactory ensheathing glia (model used in Ref. 28). *B*, Completely transected spinal cord without removal of tissue and with olfactory ensheathing glia (dark gray) implanted in the rostral and caudal cord ends (model used in Ref. 29). *C*, Contused spinal cord with implantation of cells (darker gray; model used in Refs. 15,19,35,36,40,42). *D*, Dorsal column transection and implantation of a peripheral nerve bridge as used in Ref. 57.

erable practice of the surgery techniques and a well-organized and skilled animal maintenance team are essential before reliable scientific outcome and a tolerable survival rate can be achieved. A complete transection model allows for an unambiguous reading of the axonal regeneration response.

A contusion injury (Figure 1C) can be modeled by temporarily compressing the spinal cord (12,13), for which the New York University device is most frequently used. With this device a blunt 10 g weight is dropped onto the exposed cord from different heights resulting in injuries of graded severity. The electromagnetic spinal cord injury device from the Ohio State University (13) is also available; an impounder placed onto the cord compresses the cord over a precise distance in a short time period. If used appropriately both devices result in reproducible contusion injuries. With a contusion injury, the analysis of the axonal regeneration response is complex due to spared axons and their collateral sprouts.

### Schwann cells in transection injuries

Early in the last century, Santiago Ramon y Cajal (14) documented the axonal growth-promoting abilities of peripheral nerve grafts in the injured central nervous system. Over the last decades many studies have explored the use of SCs, the major cellular component of peripheral nerve, for spinal cord repair either focusing on axonal regeneration (15-19) or remyelination (20-22).

Acute implantation of an SC/Matrigel cable contained within a polymer tube in the completely transected adult rat spinal cord (Figure 1A) promoted regeneration of propriospinal and sensory axons of which typically about 25% were surrounded by SC myelin (16). The contribution of endogenous SCs that invade the implant from nearby roots (16) on the axonal growth/myelination response has not yet been properly determined.

In this particular model, without additional interventions, supraspinal axons did not regenerate into the SC bridge. Also, axons that grew into the implant failed to exit and grow into the spinal tissue beyond. Both these responses are essential for restoration of motor recovery controlled by the brain (23). It was clear that SC implantation needs to be combined with interventions to modify the permissiveness of the graft and/or graft-spinal cord interfaces that would then elicit supraspinal growth into and beyond the SC bridge. This was confirmed, at least in part, by increasing the levels of the neurotrophic factors, brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) within the SC graft environment (24) and by systemic administration of a high dose of the corticosteroid and anti-inflammatory agent, methylprednisolone shortly after SC graft implantation (25), which both resulted in supraspinal growth into the SC bridge. The elevation of neurotrophic levels caused a specific supraspinal response, i.e., the responding axons derived from neurons that express tyrosine kinase receptor B and/or tyrosine kinase receptor C, the high affinity receptors for BDNF and NT-3, respectively (24).

The use of high doses of methylprednisolone following spinal cord injury has shown promise in the laboratory, but clinically its benefits have been a subject of disagreement (26). One of the concerns is that the reported functional improvements do not outweigh the possible secondary clinical complications. The search for effective and clinically relevant neuroprotective agents is still going on.

Although these combination treatments elicited supraspinal growth into the graft, axons failed to grow beyond the graft. This is due, at least in part, to the presence of 'scar' tissue at the graft-cord interface (9, also see below). Elevation of the neurotrophin levels within the graft milieu increases growth into the SC graft (24) but the axons do not grow

further into the spinal cord. When an SC bridge in the completely transected cord was combined with adeno-associated viral vector-mediated elevation of the levels of BDNF and NT-3 just caudal to the implant, axons still failed to grow into the caudal spinal tissue (27). This failure may reflect the fact that the levels of neurotrophic factors were not high enough to lure the axons through the graft-cord interface.

A different type of manipulation of the interfaces proved to be effective in promoting axon growth into the caudal cord. Grafting of OEG into the graft-cord interfaces combined with implantation of an SC bridge (Figure 1A) (28), and also in a transection only model (Figure 1B) (29), caused axons to exit the caudal bridge-cord interface and extend caudally. Why does OEG grafting into the cord near an injury result in such a response? At present, the underlying mechanisms are not fully known, but it has been proposed that the OEG prevent ('mask') growing axons from recognizing inhibitory molecules in the scar tissue. Also, OEG are able to migrate within spinal nervous tissue (28,29), i.e., mingle with astrocytes (30), thereby accompanying growing axons. The migratory ability of OEG has been disputed (31) but it may set them apart from SCs, which fail to mingle with astrocytes.

There are several other strategies, following a complete transection of the spinal cord, that result in growth of supraspinal axons into and/or axons out of the graft. And, similar to the experiments described above that involved SC grafting, all of these approaches have in common the fact that this response could only be achieved when grafting of cells/tissue into the injury site was accompanied by interventions that were designed to increase the levels of neurotrophins in the graft and/or to modify the graft-cord interfaces. Delayed (2-4 weeks) grafting of fetal spinal tissue promoted supraspinal growth, and, with the addition of BDNF and NT-3 at the implant site, axons grew beyond

the implant (32). Another study showed that bridging white to gray matter in a transection gap with peripheral nerves, which were stabilized using fibrin glue with acidic fibroblast growth factor and compressive wiring of the nearby vertebrae, caused growth of corticospinal axons across the grafts and into the caudal cord (33).

In general, implantation of cells/tissue alone into an injury site is not sufficient to promote an axonal response that would lead to biologically significant functional recovery. Additional treatments are needed. It is difficult if not impossible to compare different existing combinatorial strategies for their axonal growth promoting abilities. Different groups perform the assessment of these responses and of the functional improvements differently. Only a direct comparison between strategies by one group would be satisfactory but this has not been done. Arguably, grafting of OEG has resulted in an impressive growth response of supraspinal axons beyond the injury site, which at 7 months after implantation had resulted in improved functional outcome (29). With the combination of an SC bridge and OEG implantation (28) hind limb motor improvements were not evaluated at earlier times. It is important to consider that for clinical use the harvest of OEG for grafting in the injured spinal cord does involve complicated and delicate surgical techniques. The results obtained by Cheng et al. (33) have been difficult to reproduce by other groups, perhaps for technical reasons. Ethical issues complicate the use of fetal spinal tissue (32) in the clinic.

One important aspect of using SCs in spinal cord repair strategies has not been mentioned yet, namely their ability to myelinate central axons (20-22), besides peripheral axons. This sets the SC apart from other types of cells/tissue that have been explored for repair of the spinal cord. Also, central axons that have been myelinated by SCs demonstrated conduction of impulses

(34). As injury sites in the spinal cord can be extensive it will be an advantage if the grafted tissue can not only promote regeneration but also myelination of axons. This would improve conduction, i.e., functioning, of the regenerated axons. OEG naturally are ensheathing cells and do not form myelin *in situ*, although following grafting of purified cultures of OEG a low degree of myelination is observed in the implantation site (35). For these myelination-related reasons, and other reasons mentioned above, SCs are a strong candidate for future surgical cell-based strategies to repair the spinal cord.

### Schwann cells in contusion injuries

Purified adult rat SCs injected into the contused adult rat spinal cord (Figure 1C) limited injury-induced tissue loss (15,19,34). In addition, SC grafting into a contusion lesion promoted myelination and supraspinal and spinal axon sparing/regeneration, and improved hind limb motor function (35). Many different cell types have been grafted into the contused spinal cord, among them OEG (35,36), genetically modified fibroblasts (37), stem cells (38), and macrophages (39), but only seldom have cell types been properly compared for their regenerative effects. In one such comparative study, implantation of OEG was found to result in similar but slightly less strong improvements compared to those observed (and mentioned above) after SC grafting in the contused rat thoracic spinal cord (35). The main difference between the groups was the superior ability of SCs to myelinate the responding axons.

In general, cell implantation into the contusion lesion results in a neuroprotective effect, which could indirectly be responsible for the observed improvements in the axonal and behavioral response. Recently, in an effort to profit more from neuroprotective effects, in the moderately contused spinal cord acute administration of two well-known

neuroprotective agents, methylprednisolone and interleukin-10, was combined with a 7-day delayed implantation of SC and/or OEG (40). This study demonstrated that the combination improved tissue sparing over the individual treatments but the overall functional improvements were largely similar between treatments or in some cases worse. In this study, it was not determined whether the approaches individually or in combination caused either axonal sparing and/or axonal regeneration. The incompleteness of contusive injuries makes it difficult to distinguish between spared and regenerated axons. The only reliable approaches ought to involve double-neuronal tracing techniques or time course studies, which both are technically demanding and labor intensive.

The survival of cells implanted within a contusion environment may be compromised because of the ongoing immune, inflammatory, excitotoxic, proteolytic, and anoxic events (1,2). The optimal time for implantation is not known and may be different for different types of cells. Grafting of SCs has been delayed up to 7-10 days, mainly to avoid the first wave of the inflammatory response (15,19,35). It is imperative to investigate the fate of each type of cell after grafting into a contused spinal cord, and to determine the best time of grafting, to optimally benefit from the regeneration promoting effects of the implanted cell, especially when these cells have been genetically altered to secrete regeneration-supporting molecules.

It is clear from the studies mentioned above that cell grafting into the contused spinal cord will result in several regenerative effects that together may be at the basis of the significant, albeit modest, behavioral improvements seen after treatment. It also is apparent that in the contused spinal cord cell grafting alone is not adequate for achieving biologically significant restoration of function. Similar to the repair models involving a transection of the spinal cord, additional

interventions need to accompany cell grafting to increase, through either sparing or true regeneration, the number of supraspinal axons involved in motor control present in the caudal cord. A promising avenue to increase the overall regeneration response is the addition of cyclic adenosine monophosphate (cAMP) analogs and/or preventing cAMP breakdown with phosphodiesterase inhibitors, such as Rolipram, which has been explored alone (41) or combined with cell transplantation (42-44) in various spinal cord repair models. It has been known for some time that increasing cAMP levels promote axonal extension (45), but the mechanisms are not fully known. Increased cAMP levels block axonal growth inhibition exerted by myelin-associated inhibitors, possibly through the protein kinase A/Rho pathway (46). It was demonstrated *in vitro* that the cAMP-mediated axonal growth response can be caused by a direct effect on axons rather than by the environment (47). With an SC graft into a moderately contused spinal cord, administration of cAMP and Rolipram enhanced tissue sparing, axonal regeneration and functional outcome. Overall, the regenerative response was larger than in any other combination approach that involved SCs. Further studies are necessary to elucidate the mechanisms behind these improvements.

### Schwann cell - spinal cord interfaces

Regardless of the type of injury, the graft-host spinal cord interfaces are obstructive to axonal regeneration. Following an injury, reactive glial fibrillary acidic protein-positive astrocytes, meningeal cells, and microglial cells form the glial scar, a structural and chemical barrier for axon growth (8). The scar contains axonal growth inhibitory molecules such as chondroitin sulfate proteoglycans (CSPGs) (48) and semaphorins (11,49) and other myelin-associated proteins (50). So far, it is largely unknown how much different cell types contribute to the forma-

tion of the scar following injury/implantation. Several observations suggest that actual implantation of cells may increase the non-permissiveness of the interface. With an SC/Matrigel graft within a PAN/PVC tube implanted in the transected spinal cord an increased CSPG staining was found in both interfaces, but more so in the caudal one, at 3 weeks post-implantation (51). After a moderate contusion injury, a rim of CSPGs can be observed around the contused area, which persists for many months post-injury (35). Implantation of SCs one week after contusion injury increased the CSPG immunostaining intensity at 8 weeks post-injury compared to control culture medium injections (35), but this has not been properly quantified yet.

### Overcoming the inhibitory nature of the graft-spinal cord interface

It is imperative to develop strategies to obtain axonal growth from grafts into the adjacent spinal nervous tissue. Approaches to obtain such a response in the injured adult spinal cord include: 1) decreasing the inhibitory nature of the scar, 2) preventing axons from recognizing inhibitory molecules, and 3) enhancing the intrinsic growth ability of axons. The permissiveness of the scar for axons can be increased by preventing receptor-ligand (inhibitor) binding (52), by obstructing the synthesis of inhibitors (53), or by degrading biologically active components of the inhibitors (54). The intrinsic growth ability of axons can be increased by targeting molecules downstream in the intracellular pathways that promote neurite extension or prevent growth cone collapse (55).

In injuries that could be considered as 'smaller size' injuries, i.e., with less formation of scar tissue, it was demonstrated that the neutralization or enzymatic destruction of inhibitory molecules resulted in axonal growth across and beyond the injury/grafted area (52,56). So far, with a complete transec-

tion/SC bridge model, a 'larger size' injury, with more severe scar formation, such approaches have had no success in promoting axonal growth beyond the implant. As mentioned above, grafting of OEG close to a large injury with (28) or without (29) an SC graft promoted axonal growth into the distal cord.

In several 'smaller size' injury/repair models, continuous infusion of neurotrophic factors a short distance away from a graft-spinal cord interface using an osmotic minipump resulted in axonal growth through scar tissue (Figure 1D) (57). However, combining an SC/fibrin bridge in the completely transected cord with adeno-associated viral vector-mediated elevation of the levels of BDNF and NT-3 just caudal to the bridge did not result in axonal growth beyond the graft, despite the observation that local spinal cells were infected with virus up to 16 weeks post-injury/treatment (27). With an SC/Matrigel implant placed in the laterally hemisectioned spinal cord, continuous infusion with a minipump of BDNF and NT-3 caudally to the graft resulted in axonal growth into the caudal cord segments (58). In this model, some of the responding axons displayed bouton-like structures (58), but it remains to be investigated whether this implied actual synaptic contacts that exhibit normal electrophysiological properties and/or whether these axons were responsible for the behavioral improvements.

Delivering cAMP to dorsal root ganglia promotes growth of sensory axons beyond a dorsal column lesion (41). Also, with a dorsal column injury, sensory axons were shown to exit a stromal cell implant, but only when combined with elevation of the levels of cAMP within dorsal root ganglia and administration of NT-3 just rostral to the implanted area (43). The axonal regeneration responses seen after administration of cAMP, or elevation of cAMP levels through inhibition of their breakdown, have generated enthusiasm about its application in repair strategies for

the spinal cord (42-44). Although in some models the responses were interesting and promising, it is clear that more studies are necessary to fully understand and then profit from the actions of this drug. One aspect that needs attention is whether systemic delivery would result in unwanted side effects. Also, will this approach be successful in more severe injuries or, importantly, in chronic injuries?

The ability of grafted cells to promote central nervous system repair ultimately depends on the molecules they express after grafting. Similarly, the ability of a neuron to regenerate an axon into cellular environment depends on the molecules expressed. One may therefore envisage the possibility of engineering transplants and/or neurons to induce specific interactions, for example, to promote regeneration of specific axons into the transplants. Laser capture microdissection and microarray profiling (59) now allow profiling of transcripts and proteins expressed by the grafted cells and neurons after spinal injury and transplantation. Specific neuron-transplant interactions can be engineered to remedy deficiencies using appropriate knock-up or knock-down genetic or pharmacological therapies. The concept of "tract-targeted repair" is attractive because "magic bullet" therapies could induce undesired side effects, and therapies could be tailored to specific injuries.

Regeneration of supraspinal axons beyond an intraspinal graft is essential to achieve supraspinal control over motor function after spinal cord injury. Such a specific axonal response has been demonstrated in several experimental implantation paradigms that in general involve smaller size injuries. These approaches need to be tested in models that involve 'larger size' injuries to verify their potential for clinical application. The unraveling of the underlying mechanisms of these effects is vital to further enhance the regenerative response. Whereas we are greatly challenged by the failing axonal re-

generation across scar tissue, we have not yet fully confronted the next crucial element of a successful repair strategy, i.e., the formation of synaptic connections by the regenerating axons with spinal cord neurons.

### **Feasibility of clinical application of Schwann cells**

In the clinic, treatment of spinal cord injury relies on preventing further damage using neuroprotective approaches and surgical interventions such as decompression, stabilization, and detethering. Even under the best circumstances, the time between injury and these treatments is not short enough to prevent the onset of many acute events that result in (progressive) loss of tissue and neurological deficits. Thus, interventions aiming at replacing lost tissue and restoring lost axonal connections and motor and sensory function need to be developed. Could SCs be a component of such repair strategies?

From many experimental studies it has become clear that in case of a compression injury a combination strategy will be needed that includes the implantation of a cellular substrate to fill the cavity in order to promote axonal growth across and beyond the injury towards the lower areas of the cord that contain the neurons involved in motor function. For optimal functioning of these circuits, some regenerated axons may need to be myelinated. It is very likely that these newly formed axonal circuits are not identical to the original ones (60), and additional rehabilitative therapies will be required to obtain biologically significant motor recovery that involves these new connections.

As discussed above, in the experimentally contused spinal cord, implantation of SCs fills the cavity, limits further tissue loss, and promotes regeneration of severed axons (15,19,35). Currently, additional interventions are being explored for their added effects to the SC-mediated regeneration response. SCs are capable of myelinating cen-

tral axons that have grown into the graft. However, it has to be kept in mind that implanted SCs do not migrate into the surrounding adult spinal nervous tissue and can therefore not be expected to myelinate axons that have regenerated through and beyond the graft. It is imperative to also develop strategies that will rescue oligodendrocytes from dying following spinal cord injury. An important advantage of SCs over other cell types for implantation approaches is their ability to myelinate central axons. That plus the neuroprotective and regenerative abilities of SCs have established them as strong candidates for clinical cell-based repair strategies. However, as mentioned above, other cell types have also shown great promise for repair of the spinal cord.

Several issues need careful consideration before SC implantation strategies will be a legitimate option for repair strategies of the injured human spinal cord. The benefits of the procedure should outweigh the risks and the grafting technique should be safe and not exacerbate the neurological deficit. For this, visualization of the shape and dimensions of the lesion using magnetic resonance imaging before implantation could be advantageous. In general, any implantation strategy should not evoke immunological and/or inflammatory reactions. With SCs this can be accomplished since they would permit autologous implantation thereby avoiding such reactions as well as the use of immunosuppressant drugs, such as cyclosporine-A, to prevent graft rejection. Autologous implantation can be performed by harvesting the SCs from a piece of peripheral nerve from the patient. The removal of a piece of a sensory nerve, such as the sural or saphenous nerve, may be preferred over removal of a piece of a motor nerve to avoid additional loss of motor function. Also, the option for autologous implantation avoids ethical issues raised by the use of fetal tissue or embryonic stem cells.

For repair of some cord injuries one could envisage that a large number of SCs will be



necessary. Because SCs can be cultured *in vitro* with the help of mitogens large enough numbers can be obtained for implantation. One should be aware, though, that many divisions can induce the formation of malignant cells. This has not been observed following multiple divisions of SCs *in vitro*. Another concern that has not yet been fully addressed in experimental studies is that mitogen-induced division may change the neuroprotective and regenerative abilities of SCs.

Taking all this in consideration, autolog-

ous implantation of SCs into the injured spinal cord has a future in the clinic. From experimental studies, many of which presented in this review, it has become clear that grafting SCs alone will not result in substantial functional recovery. Additional interventions and/or rehabilitative treatments need to be part of an SC-based repair strategy. These additional treatments need to be identified before autologous SC implantation aimed at repairing the injured spinal cord will become a reality.

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