

Review

Combined Approaches Leading to Synergistic Therapeutic Effects in Spinal Cord Injury: State of the Art

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Abstract

Cell-based regenerative medicine approaches and motor rehabilitation are currently being used to overcome the consequences of spinal cord injury (SCI). However, their success in preclinical studies does not always translate into successful implementation in clinical practice. Recent work suggests that modern neuromodulation approaches hold great therapeutic promise. Despite these advances, the complete resolution of functional deficits caused by SCI is impossible, especially in cases of severe injury. Therefore, combined approaches based on cell transplantation and neuromodulation are needed to enhance the neuroregenerative effect. The additional inclusion of a dosed locomotor load in the overall therapeutic plan and against a background of combined approaches can have a significant supportive effect. The aim of this review is to evaluate studies that use combinations of different approaches, thereby advancing our current understanding of the mechanisms that underlie their therapeutic effect. This review will consider mostly the effects and limitations of regenerative approaches, as well as the effects of locomotor load and neuromodulation on molecular and cellular changes in the spinal cord.

Keywords: spinal cord injury; cell therapy; locomotor load; neuromodulation

1. Introduction

To overcome the consequences of spinal cord injury (SCI) and restore function, the main goals are neuroprotection [1–3], the neutralization of endogenous inhibitors of axon growth, chondroitin sulfate proteoglycans and some myelin proteins [4–9], maintenance of extended axonal growth [10,11], stimulation of remyelination [12–14], formation of a new intraspinal neural network [15], and activation of the central pattern generator [16,17]. The effectiveness of pharmacotherapy, electrical stimulation, locomotor load, and of gene, cell and gene-cell therapy at solving these problems is being actively investigated. So far, the research results show these approaches are insufficient to achieve full recovery of function when implemented separately. Recently, it has become clear that a comprehensive program involving a combination of various approaches will be needed to more effectively overcome the consequences of traumatic SCI. With this in mind, results from numerous clinical and experimental studies indicate that it will also be essential to include regular and dosed locomotor load in the general therapeutic plan of procedures.

The aim of this review is therefore to evaluate studies on the various approaches used for SCI therapy in order to find the most effective combination of regenerative therapy method and locomotor load, with or without neuromodulation. The review will primarily consider the efficacy and limitations of regenerative approaches, as well as the effects of locomotor load and neuromodulation on molecular and cellular changes in the spinal cord.

2. Cell Therapy as the Most Common Regenerative Approach in Spinal Cord Injury

Over the past two decades, various cell types have been tested as the choice of cell therapy for transplantation in SCI [18–23]. Neural stem cells (NSCs), cord blood cells, olfactory ensheathing cells (OECs), Schwann cells (SCs), and mesenchymal stem cells (MSCs) derived from different sources provide significant support to the injured spinal cord, both individually and in combination [24–26]. They also enhance the low neuroregenerative potential of central nervous tissue, which is further weakened by SCI.

Many studies have reported on the characteristics, origin, and differentiation potential of NSCs [27–30]. NSC transplantation after SCI can result in favorable therapeutic outcomes due to anti-inflammatory and neuroprotective effects, as well as by supporting the remyelination and stimulation of axon growth [31–33]. The sourcing of NSCs from adult nervous tissue is problematic, however, and NSCs obtained from iPS cells therefore offer the most promise. The latest iPS-cell technology does not require much time for induction of pluripotency and neuronal differentiation. Nevertheless, iPS cells have yet to be implemented for autologous transplantation in the early stages of human SCI due to safety issues involving their possible contribution to tumor formation [34,35].

Human umbilical cord blood (hUCB) is an ethically acceptable source of stem and progenitor cells with high regenerative potential. Obtaining hUCB is a simple pro-



cedure with no risks for the donor. Especially significant is the fact that hUCB cells have a lower probability of immune rejection, as they are more tolerant to human leukocyte antigen differences [36]. hUCB contains various types of stem and progenitor cells, some of which are often used to stimulate neuroregeneration. These include hematopoietic stem cells, MSCs, endothelial progenitor cells, and non-restricted somatic stem cells [37,38]. A separate area of research is the mononuclear hUCB fraction, which is used in most of the preclinical studies that assess the role of these cells in stimulating regeneration of neural tissue [39–42]. Due to its unique cellular composition, hUCB has properties that allow it to partially overcome the factors that limit neuroregeneration, but without resulting in significant restoration of neurological functions in SCI, while also contributing to neuropathic pain following their clinical application [43].

Glial cells, most often SCs and OECs, are also used to stimulate regeneration after SCI. The rationale for using these cells in neuroregeneration include: (1) the possibility of myelin formation when transplanted into the central nervous system (CNS); (2) their ability to produce neurotrophic factors and adhesion molecules; and (3) their ready availability and the possibility of obtaining autologous cells [44–46]. Nevertheless, the lack of reliable OEC-specific markers and hence of a robust method to identify OECs makes it impossible to differentiate them from other cell types in human olfactory cultures. This in turn prevents the production of highly enriched populations of human OECs for transplantation [47]. A further issue is the limited migration of SCs due to their inability to interact with other glial cells [48–50]. Clinical trials using SCs and OECs have not shown efficacy in SCI patients [51,52].

MSCs are the most promising cell type for SCI stimulation due to their high biosafety, immunomodulatory properties, and ability to synthesize neurotrophic and proangiogenic factors [23,53,54]. The therapeutic effect of these cells is due to their paracrine mechanism of action [55,56]. Therefore, special attention must be paid to the preservation of MSC viability and to the optimal delivery of therapeutic molecules secreted by these cells [57]. To date, however, the use of MSCs in clinical practice has not shown any noticeable effects. Their use is limited by the need to establish the safety of MSC administration in phase I clinical trials, and also because only partial improvement of neurological functions have been reported [58–60].

Cell therapy has long been considered one of the most promising methods for the repair and replacement of damaged nerve tissue following SCI. However, many studies have shown poor survival and differentiation of stem and/or progenitor cells after transplantation [61–63], thereby requiring repeat applications to maintain the therapeutic effect. This in turn creates limitations associated with the high cost of maintaining cell cultures and the complexity of repeated cell isolation. Over the past two decades, a

large number of studies have tried to evaluate the effectiveness of cell therapy and to identify the molecular and cellular mechanisms that mediate the regeneration of injured spinal cord. So far, however, there has been no agreement among researchers on the best choice of cell type to stimulate neuroregeneration following transplantation for SCI. Moreover, it is widely accepted that it is often not possible to overcome a severe functional deficit in SCI under clinical conditions. Therefore, the application of several therapeutic tools should be considered, and any approach should not be limited to the use of regenerative medicine only.

3. The Effect of Locomotor Load on Post-Traumatic Processes of the Spinal Cord

Evaluation of the recovery of neurological function after SCI using locomotor load has been performed since the late 1970s and is still carried out to reveal the fundamental processes involved in the regeneration of damaged spinal cord [64–66]. In the modern concept of neurorehabilitation, locomotor load is considered to be an effective, easily reproducible, and dosed method of motor function recovery stimulation [67–69]. By acting at different levels along numerous neural pathways, the locomotor load increases the force of contraction of paralyzed muscles. It also influences the mechanism of brain plasticity, activates remodeling processes, and influences the microenvironment of motoneurons and supports their function by reorganizing synaptic input. Locomotor load also promotes the restoration of alpha motoneuronal soma size and of synaptophysin expression and Na^+ , K^+ -ATPase activity [70–72]. This mode of rehabilitation has been thoroughly investigated in the clinic at the phenomenological level, but research into the underlying molecular and cellular mechanisms has only begun recently.

Several studies have reported an increased level of brain-derived neurotrophic factor (BDNF) in the injured spinal cord of animals against a background of locomotor load [73–75]. Important results that support the action of BDNF have been published [76]. The reduced expression of myelin-associated glycoprotein (MAG) induced by locomotor load was shown to be mediated by increased BDNF expression, which overcomes the inhibitory effect of MAG on axonal growth. An increased level of protein kinase A associated with BDNF expression was also observed, while the positive effects of locomotor load can be attenuated by blocking BDNF. Recent work has revealed another pathway of BDNF action related to the tropomyosin tyrosine kinase receptor (TrkB) [74,77]. The blocking of BDNF-TrkB signaling inhibited functional recovery after exercise [77].

Increased production of BDNF promotes dendrite growth and synaptogenesis in the SCI region. Several studies have also found that locomotor load increases axon growth and the total length of neurites of lumbar motoneurons, while reducing dendrite atrophy [78,79]. Researchers

have attributed the growth of axons to induction of the Erk1/2 pathway, which is an important mediator for signal transmission from the damage site to the cell body. Suppression of MEK1 by appropriate inhibitors reduced the collateral growth of descending corticospinal axons due to inactivation of the Erk1/2 pathway [80]. Other workers showed increases in the expression of NGF, IGF-1 and neurotrophin NT-3 growth factors, which are normally suppressed following SCI. Jung *et al.* [81] observed increased PI3K expression, increased pAkt/Akt and Bcl-2/Bax ratios, and suppression of cleaved caspase-3 expression. These authors linked improved motor functions in a background of locomotor load to the suppression of apoptosis and to increased expression of neurotrophic factors through activation of the PI3K/Akt pathway. Hayashibe *et al.* [82] reported a more marked locomotor improvement and tissue recovery in their study, including axonal extension, by forced plantar placement of hind paws during treadmill training and a focus on the importance of properly organized training.

The study of gene function is important for understanding the molecular mechanisms of neuroplasticity. In this regard, studies showing significant variability in the expression of individual genes against a background of locomotor load are important. Microarray analysis of injured spinal cords in mice undergoing treadmill rehabilitation identified 82 upregulated and 297 downregulated differentially expressed genes [83]. Also using microarrays, Shin *et al.* [84] showed restoration of the expression of genes related to metabolism and biosynthesis under the influence of training, which had been reduced following injury. They also showed increased expression of genes involved in neuroplasticity and angiogenesis.

Despite the advances mentioned above, the relationship between intensity of an applied locomotor load and severity and type of SCI remains uncertain. However, it is becoming clear that locomotor load induces changes at each level of organization, thus contributing to a more complete recovery of motor function in mild SCI. A partial neuroregenerative effect is a limitation in clinical practice and hence this method is insufficient. Additional measures must be included to enhance the reparative effect of locomotor load in a background of SCI.

4. Mechanisms for the Therapeutic Effect of Neuromodulation in Spinal Cord Injury

Neuromodulation has been tried as a stimulating method of therapeutic action on the injured spinal cord since the last century. It is a promising method and is actively used in clinical practice. To date, there is no clear evidence regarding the mechanism of action of neuromodulation methods, but studies on motor function recovery showed a marked effect with such rehabilitation interventions [85,86]. The most common neuromodulation method includes electrical stimulation at different stages of SCI.

Electrostimulation, including various direct and indirect effects on the injured spinal cord [86], changes the excitability of nerve fibers by altering the plasticity of neurons. It has a restorative effect on the locomotor system and autonomic functions by promoting neurogenesis, sprouting and regeneration of axons, and reorganization of spinal circuits [87]. Transcutaneous spinal cord stimulation (tSCS) is used as a noninvasive electrostimulation method, but this intervention is less precise. Studies using tSCS have shown decreased spasticity in patients with SCI [88]. This is associated with the activation of inhibitory circuits and hence with a reduction in the amplitude of H-reflexes and F waves [89–91]. tSCS has also been shown to improve upper limb function through an excitatory effect at the spinal level and an inhibitory effect at the cortical level [92]. The recovery effect can persist for a long time [93], contributing to improved connectivity of brain and spinal cord descending-ascending networks and reorganization of supraspinal-spinal networks. Consolidation of the effect at the end of tSCS therapy is associated with changes in the amplitude of spinally evoked motor responses and with a significant increase in grip strength of the hand [94]. Considering the change of lower limb functions against a background of tSCS in people with varying degrees of injury, the results of the appearance of involuntary leg movements like locomotor movements were cited [95], as well as significant improvement in the quality, speed of movements and endurance in cases of incomplete damage [96]. Furthermore, a high level of muscle activity was observed in chronic paralysis under tSCS conditions, allowing patients to stand upright independently [97].

Another important effect of noninvasive neuromodulation, in conjunction with conservative treatment methods in the form of catheterization and pharmacological substances, is the restoration of normal gastrointestinal tract function by reducing intraluminal pressure and increasing anal sphincter pressure [98,99], of the urinary bladder by regulation of detrusor hyperreflexia [100], and of the genital system when dysfunction occurs after SCI [101]. Besides, an earlier and more established method of minimally invasive sacral neuromodulation, in particular used for patients refractory to behavioral and pharmacological treatment [102], shows clinical improvement of more than 50% [103–106]. Non-invasive percutaneous tibial nerve stimulation with minimal side effects demonstrates efficacy in the treatment of overactive bladder, fecal incontinence, and pelvic pain, but is limited by the short-term preservation of the therapy effect [107]. Other percutaneous methods include interferential electrostimulation, that shows potential as a novel and more economical means of treating gastrointestinal dysfunction such as constipation and for improved bladder management [108,109].

Epidural electrostimulation (EES) that controls reflex and locomotor activity is used to achieve a more precise effect on spinal circuits [110]. There is also the possibility of

applying various levels of intensity and frequency of EES to reduce pain in SCI patients at high exposure frequencies [111], improve upper limb control [112], restore voluntary movements of lower limbs in complete paralysis [113], and restore supraspinal control for some leg movements after prolonged therapy [114]. However, in the absence of supraspinal input against SCI, control could come from afferents from the moving lower limbs [115]. Implantation of electrodes in the sacral nerve roots is one of the invasive methods used for therapy of lower urinary tract dysfunction when standard catheterization is ineffective. This includes acral anterior root stimulation combined with rhizotomy of posterior sacral roots to further stimulates afferent and efferent pathways of lower urinary tract [107,116]. Observed in patients with motor-complete SCI, epidural stimulation of the spinal cord acutely modulates the lower urinary tract and intestinal function [117]. In turn, experimental data on site-specific areas show that activation of detrusor occurs during the stimulation of the L1 and L5-L6 spinal segments and external urethral sphincter was activated by sacral stimulation [118]. The presence of several disadvantages of invasive neuromodulation, probability of infection during electrode implantation and pain in the place of injection, the temporary effect of the general procedure [116,119], in general, can worsen the patient's condition. Thus, further which further involves understanding of the neuromodulatory therapy, possibly using methods of optogenetics to enable precise and minimally invasive neuromodulation [120]. Also, the problem with changes in the gut microbiome, plays vital role of the individual, after SCI cannot be solved by neuromodulation, it will require parallel therapy with probiotics, fecal microbiota transplantation, and oral short-chain fatty acid to improve gut-brain communication [121].

In addition to the electrostimulation methods already being used in clinical practice, other experimental approaches have been tested in animal models whereby the mesencephalic locomotor region (MLR), the medullary raphe, and the periaqueductal gray act as targets for neurostimulation, along with the spinal cord. Interventions to the MLR lead to improved locomotion and swimming to near baseline performance, as well as to restoration of movement in the paralyzed legs of rats with subtotal SCI. The authors of this work suggested that activation of the major supraspinal motor control pathway from the MLR to the medial brainstem, and from there via reticulospinal fibers to the lumbar spinal cord, may underlie their results [122]. Stimulation of the medullary raphe in the acute phase of SCI persistently improved motor activity in experimental animals. Further analysis showed an increased number of myelinated axons in perilesional white matter and of serotonin-containing terminals in gray matter. Similar findings of an increased number of myelinated axons and improved locomotion were observed when exposed to periaqueductal gray, thus demonstrating impor-

tant restorative feedback between neurons of the medullary raphe and the spinal cord after SCI [123]. Restorative feedback was also seen with vagus nerve stimulation, which contributed significantly to improved upper limb conditions in rats by enhancing the reorganization of synaptic connections [124,125]. In general, electrostimulation that exploits the plasticity of residual neural circuits and the regenerative potential of damaged neurons has positive effects on tissue organization.

Despite some therapeutic success for neuromodulation methods in SCI, electrostimulation is not widely used in clinical practice. This is because of the need for expensive equipment and additional training of medical personnel, as well as the need to validate neuromodulation protocols with clear instructions regarding the intensity, frequency and time of influence, as well as the potential targets for stimulation. Experimentation in this area has already begun, with the possibility of obtaining synergistic neuroprotective effects from using combinations based on regenerative approaches (cell therapy) and neuromodulation with active motor rehabilitation (Fig. 1).

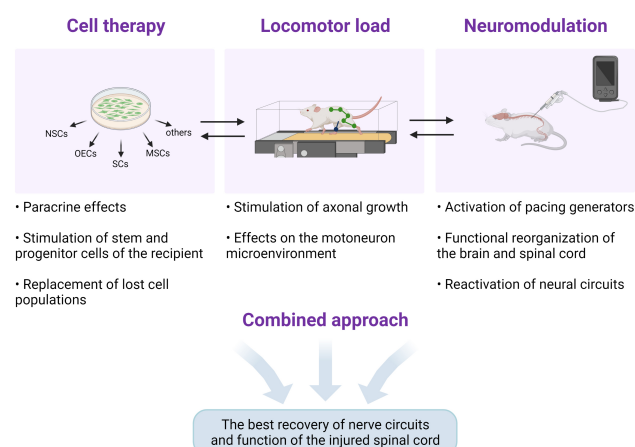


Fig. 1. Benefits of a combined approach for therapy of spinal cord injury.

5. Integrated Approach Leading to a Synergistic Therapeutic Effect

5.1 Neuroregeneration and Locomotor Load

Combinations of known SCI therapy methods can be divided into several groups depending on the choice of pharmacotherapy approach, cell or gene therapy, motor rehabilitation, etc. This chapter will consider the efficacy of combined approaches based on stem cell therapy and locomotor load in animal models of SCI (Table 1, Ref. [126–132]).

Table 1. Preclinical *in vivo* trials with combined approaches based on stem cell therapy and locomotor load.

Organism/Type of SCI	Cell therapy and route of administration	Locomotor load/training period	Treatment effect of combination method	References
Female Sprague–Dawley rats; contusion SCI at T10.	Olfactory ensheathing cells and Schwann cells (OEC+SC); Intraspinal injection at 2 weeks.	Treadmill training 20 ± 10 min 5 days per week, for 10 weeks.	Axonal growth, remyelination, decreased GFAP immunoreactivity density, increases neuronal plasticity below the lesion.	[126]
Female C57BL/6J mice; contusion SCI at T9.	Neural Stem/Progenitor Cell (NS/PC); Intraspinal injection at 49 days.	Treadmill training 20 min 5 days per week, for ~8 weeks.	Improved neuronal differentiation and maturation of CPG activity along with trophic support.	[127]
Male Wistar rats; compression SCI at T9.	Stem cells from human exfoliated deciduous teeth (SHEDs); Intraspinal injection at the day of the SCI.	Treadmill training 20 min 5 days per week, for 6 weeks.	Ineffective motor exercise, SHEDs affected decreased TNF- α , cystic cavity and glial scar levels.	[128]
Male Sprague-Dawley rats; contusion SCI at T9–10.	Bone marrow stromal cells (BMSCs); Intraspinal injection at 1 week.	Treadmill training 30 min 6 days per week, for 6 weeks.	More potent decrease of Bax and p-JNK expression, more potent increase of Bcl-2, p-ERK1/2 and p-c-Jun expression.	[129]
Female C57Black6 mice; compression SCI at T9.	Bone marrow mesenchymal stem cells (MSCs); Intraspinal injection at 1 week.	Treadmill training 10 min 3 days per week, for 8 weeks.	Better preservation/regeneration of tissues with an increased number of preserved myelinated fibers and better preservation of white matter, high expression of neurotrophin 4.	[130]
Male Sprague-Dawley rats; compression SCI at T6-T7.	Neural precursor cells (NPCs); Intraspinal injection at 4 weeks.	Treadmill training 20 × 2 min 5 days per week, for ~11 weeks.	Relief of neuropathic pain, reduction of IL1 β and TNF α markers.	[131]
Female Wistar rats; contusion/compression SCI at C6.	Neural precursor cells (NPCs); Intraspinal injection at 10 days.	Treadmill training 20 × 2 min every day, for 8 weeks.	Improvement of myelination and regeneration of descending nerve fiber pathways, tissue preservation. NPCs survival, as well as increased differentiation into neurons and oligodendrocytes.	[132]

SCI, spinal cord injury; CPG, central pattern generator.

One area of regenerative therapy in SCI is to prevent axonal demyelination and to stimulate their myelination by inducing migration of endogenous SCs into the injury area through stem/progenitor cell transplantation and OECs [40,133], and by transplantation of exogenous SCs [134,135]. To achieve the best therapeutic results, attempts have been made to co-transplant SCs and OECs in combination with locomotor load [126]. The experimental results showed axonal growth and remyelination due to both transplanted SCs and OECs. The addition of locomotor load also led to increased neuronal plasticity and a more stable functional recovery during exercise compared to the group in which only cell therapy was performed.

Tashiro *et al.* [127] evaluated the effectiveness of embryonic neural stem/progenitor cell transplantation against a background of locomotor load in SCI. Their results showed improved spinal conduction and maturation of the central pattern generator activity with the combined therapy when compared to cell therapy alone. Furthermore, the combination of cell therapy with locomotor load promoted neuronal differentiation of the transplanted cells, leading to significant restoration of motor activity. In similar work, transplantation of GABAergic neural progenitor cells (NPCs) in combination with intensive locomotor load also had positive functional effects, including the attenuation of neuropathic pain in rats with SCI [131]. In addition to improving the survival of transplanted NPCs, the locomotor load helped to normalize the inhibitory GABAergic function of the remaining neurons that went into the excited state. According to the authors, this was mediated by BDNF-TrkB signaling [136].

Neuronal overexcitation leads to an increase in extracellular glutamate, which in turn causes persistent excitotoxicity and cell death [137]. Dugan *et al.* [131] showed a significant reduction in the pro-inflammatory markers $IL1\beta$ and $TNF\alpha$ after treatment with a combination of early or delayed locomotor load and GABAergic cellular transplantation. Moreover, the lumbar dorsal horn endogenous GABAergic neuronal and process density was restored to almost normal levels. Younsi *et al.* [132] reported similar results after transplanting embryonic stem cells, including NPCs, in combination with locomotor load after SCI at the cervical level. They also found improvement in myelination and regeneration of the rubrospinal and reticulospinal tracts. The effect of locomotor load on the survival and differentiation potential of NPCs towards mature neurons is of practical importance because it increases the effectiveness of cell therapy, thus reducing the need for repeat transplantations.

Mesenchymal stem cells (MSCs) derived from different sources are widely used for neuroregeneration of the injured spinal cord. In this regard, the effect of a combined approach using bone marrow-derived MSCs and locomotor load in SCI has been investigated [129]. The results confirm a synergistic effect for the above-mentioned meth-

ods and showed enhanced axonal regeneration mediated by an increased number of NF-200 positive cells via activation of the BDNF-ERK1/2 pathway. The locomotor load promotes activation of the ERK1/2 pathway while also increasing the expression of BDNF. This work also found a significant change in the expression of factors related to cell survival and neuroplasticity, which in turn led to the enhanced survival of transplanted cells by overcoming apoptosis in association with locomotor load. Massoto *et al.* [130] obtained similar functional results in a study where bone marrow-derived MSCs were transplanted in combination with locomotor load in an SCI model. Morphometric and ultrastructural analysis of animals treated with the combined approach showed an increased number of preserved myelinated fibers and better preservation of white matter, with elevated expression of neurotrophin 4 compared to cell therapy alone.

In addition to the efficacy reported in the above-mentioned studies using combined methods, there have also been reports where the locomotor load, due to a poorly chosen training interval, did not support the injured spinal cord either alone or in combination with cell therapy [128]. According to the authors, training in the early stages after SCI did not lead to increased BDNF levels [138], but did increase the expression of toxic substances released by damaged tissue [139].

Studies on the combination of neuroregeneration and locomotor load have mostly demonstrated synergistic therapeutic effects that manifest as significant improvements in the structure and function of the damaged spinal cord. This highlights the importance of a dosed locomotor load that regulates the differentiation and survival of transplanted cells to enhance the neuroregenerative effect with consolidation in the form of long-term preservation of motor activity.

5.2 Neuroregeneration and Neuromodulation

Neuromodulation-activated spinal neural networks require the support of cellular transplants in order to achieve further reconstruction and repair of damaged nerve tissue. Hence, the use of combined approaches based on electrostimulation and cell transplantation can enhance the therapeutic effect, while the inclusion of locomotor load can help to consolidate the restorative effect.

Several studies that transplanted genetically modified cells stand out with regard to the use of cell therapy in combination with neuromodulation and locomotor load. Regenerative therapy using umbilical cord blood mononuclear cells that overexpress recombinant vascular endothelial growth factor, glial neurotrophic factor (GDNF), and nerve cell adhesion molecule, in combination with locomotor load and EES, showed improved motor activity in a pig SCI model [42]. Decreased astrocyte and microglia reactivity and increased expression of stress-induced and synaptic proteins was observed with the above-mentioned com-

bined approach. A similar restorative effect was demonstrated when autologous and genetically-enriched leuco-concentrate was transplanted in combination with EES and locomotor load in a pig SCI model [140]. Improvement of motor functions through the positive reorganization of glial cells and the restoration of neural connections was demonstrated.

Siddiqui *et al.* [141] investigated the implantation of scaffolds seeded with GDNF-producing SCs and rapamycin microspheres in combination with locomotor load and EES. These workers also demonstrated improvement of motor activity. This combined treatment promoted the synaptic reorganization of interneurons and motoneurons against a background of regenerating axons, thus enhancing the restoration of lower limb functions. Moreover, the acquired functional activity was observed even after re-cutting the spinal cord while continuing the combined treatment.

Thus, in contrast to the lack of efficacy by individual approaches, combined therapy results in the best recovery of nerve circuits and improved functions of the injured spinal cord. Nevertheless, a comprehensive study of the cellular and molecular changes involved in the reorganization of nerve circuits is required to better understand the underlying mechanisms of the combined approaches.

6. Conclusions

Despite years of experience in preclinical and clinical trials, the lack of quality cellular preparations for SCI treatment on the market highlights the inefficacy of current regenerative medicine approaches. An overall therapeutic plan that includes a measured locomotor load and neuro-modulation may contribute to the activation and reconstruction of spinal networks under the influence of a trophic support of cell transplants. The early success of such experimental studies provides hope for the successful implementation of combined approaches in clinical practice. Additional preclinical studies should help to elucidate the mechanisms that underlie the combined approaches, thus favoring the development of more effective therapeutic intervention protocols for SCI.

Abbreviations

SCI, spinal cord injury; NSCs, neural stem cells; OECs, olfactory ensheathing cells; SCs, schwann cells; MSCs, mesenchymal stem cells; hUCB, human umbilical cord blood; CNS, central nervous system; BDNF, brain-derived neurotrophic factor; MAG, myelin-associated glycoprotein; TrkB, tropomyosin receptor kinase B; tSCS, transcutaneous spinal cord stimulation; EES, epidural electrostimulation; MLR, mesencephalic locomotor region; NPCs, neural progenitor cells; GDNF, glial cell-derived neurotrophic factor.

Author Contributions

ED and DS contributed to the investigation and writing of the original draft. AR contributed to the formal analysis, reviewing, and editing of the manuscript. DS visualization. YM performed the conceptualization, methodology, supervision and funding acquisition. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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