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Neurorepair in Spinal Cord Injury

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.

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LIST OF ABBREVIATIONS

BBB – Blood-Brain Barrier
BDNF – Brain-Derived Neurotrophic Factor
bFGF – basic Fibroblast Growth Factor
BMSC – Bone Marrow Stromal Cell
ChAT – Choline Acetyltransferase
CNS – Central Nervous System
CNTF – Ciliary-derived Neurotrophic Factor
DNA – Deoxyribonucleic Acid
EPO – Erythropoietin
EPO-R – Erythropoietin Receptor
ESC(s) – Embryonic Stem Cell(s)
GDNF – Glial cell line-Derived Neurotrophic Factor
HA – Hyaluronic Acid
HAMC – Hyaluronan-methylcellulose
hESC(s) – human Embryonic Stem Cell(s)
hIPSC(s) – human Induced Pluripotent Stem Cell(s)
IPSC(s) – Induced Pluripotent Stem Cell(s)
LIF – Leukemia Inhibitory Factor
MAG – Myelin Associated Glycoproteins
MSC(s) – Mesenchymal Stem Cell(s)
NGF – Nerve Growth Factor
NK – Natural Killer
NSC(s) – Neural Stem Cell(s)
NT-3 – Neurotrophin-3
OEC(s) – Olfactory Ensheathing Cell(s)
OPC(s) – Oligodendrocyte Progenitor Cell(s)
PGA – Poly (Glycolic Acid)
PHEMA – Poly (2-Hydroxyethylmethacrylate)
PHPMA - Poly N-(2-Hydroxypropyl)-Methacrylamide
PLA – Poly (Lactic Acid)
PLGA – Poly (Lactic-co-Glycolic Acid)
PNS – Peripheral Nervous System
SC(s) – Schwann Cell(s)
SCI – Spinal Cord Injury
Trk – Tropomyosin-related kinase receptor
VPA – Valproic Acid

Neurorepair in Spinal Cord Injury

K.O.Rojek

Summary

Spinal cord injury (SCI) is a result of central nervous system trauma and can be characterized by massive damage of the neural tissue and spinal cord disintegrity. Patients suffering from SCI are devoid of sensory and motor functions below the place of injury and are usually not able to handle basic daily life activities. Life-long disability of the patients results in the burden to the entire family and raises enormous costs of medical care. The currently available therapeutic measures are mostly focusing on reducing the local inflammation and stabilizing the injured spine. However, the ultimate goal in treating SCI is to restore spinal cord continuity and recover the locomotor functions of the patients. Recently, better understanding of SCI pathology and CNS inhibitory mechanisms resulted in many novel therapeutic approaches. The preclinical studies of cell transplantation, exogenous neurotrophic factor and biomaterials have shown very promising results. Current actions should attempt to translate these preclinical findings into human practice as soon as possible.

1. Introduction

Spinal cord injury (SCI) represents an important health issue associated with massive damage of the central nervous system (CNS) tissue and has been classified as an incurable condition. Annually only in the United States approximately 10 000 of individuals are affected by SCI which is a leading cause of life-long disability among adults [1]. The majority of cases come from motor vesicle accidents and injuries after diving [2]. Since the average age of the patients is relatively low, 20 – 30 years old, the economical and social costs of life-long care are staggering [3]. The SCI patients are partially or completely devoid of sensory or/and motor functions below the place of injury. Hence cervical injuries will result in full or partial tetraplegia with impaired legs, hips, arms, hands, neck and possible loss of breathing whereas lumbosacral injuries leading to the paralysis of the lower part of the body, usually do not affect arms, hands or breathing function. Furthermore, loss of motor and sensory neurons may lead to additional health problems including: cardiac function impairment, inability to control blood pressure or body temperature and urinary tract infections. The majority of SCI patients cannot handle basic daily life activities and require non-stop care.

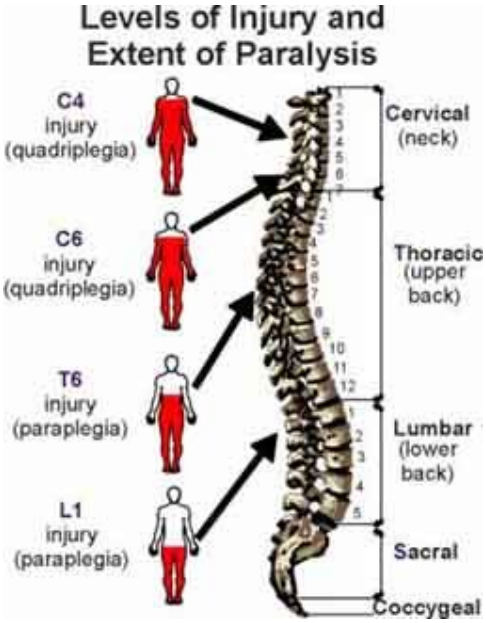

Figure 1. Levels of injury and extent of paralysis. The degree of disability following SCI strictly depends on the place of injury. Arrows indicate the places where spinal cord continuity was disrupted. To visualize the extent of disability, paralyzed parts of the body were depicted in red. [TexasTrailLawyer.con]

The currently available treatments mostly aim to prevent further post-injury damage which can be done by: 1) reducing the local inflammation and 2) stabilizing the injured spine. Methylprednisole administrated shortly after the injury has been shown to have anti-inflammatory effect and reduce cerebral edema [4-7]. However, to be effective the treatment requires high doses of methylprednisole which carries the risk of serious side effects including: gastrointestinal bleeding or pancreatitis. On the other hand, surgical decompression and spinal column stabilization have been shown to reduce the risk of additional neurological post-injury damage [8-11]. The secondary tissue damage is a serious problem in SCI patients which often leads to additional post-injury complications and longer hospitalization time, therefore delaying patient's rehabilitation. Nevertheless, however beneficial the treatment, only less then 2% of the patients regain their ability to walk [12].

Over the last two decades there has been an emerging number of investigation aiming to develop a therapeutic strategy to increase the functional recovery following SCI. To be successful the potential therapy should: 1) replace damaged due to the injury tissue 2) restore neuronal circuits and 3) promote remyelization and neovascularization at the lesion site eventually leading to the formation of functional bridge and spinal cord (SC) continuity. The recently proposed approaches include use of neurotrophic molecules, stem cell or biopolymers (reviewed in: [13-17]). Some of them show promising results in preclinical studies and might soon or have already entered the clinical stage.

In this paper I will present an overview of current stages of SCI research discussing benefits, challenges and risks of possible treatments. The review will be preceded with a brief summary of SCI pathology explaining the reasons of neuroregenerative failure following CNS injury.

2. Why do CNS axons not regenerate? The repair failure following Spinal Cord Injury.

Before developing a successful treatment for SCI one must first understand the reasons of CNS regeneration failure. Unlike peripheral nervous system (PNS) CNS has a very low regenerative capacity with a few studies demonstrating short distance axonal sprouting following SCI in experimental animal models [18, 19]. Limited regenerative potential of CNS neurons and a highly inhibitory environment at the lesion site contribute to the failure of the long-distance axonal regeneration and make the CNS incapable of restoring damage due to the injured tissue [20, 21].

To better understand the role of inhibitory mechanisms SCI has been divided into three phases: acute, secondary and chronic. The initial phase is a direct consequence of the mechanical damage of the spinal cord and can be characterized by massive cell death. Within the next 24 hours tissue damage triggers a local inflammatory response which results in the influx of cytokines, monocytes and neutrophils which subsequently recruit glial cells to the lesion site [23]. The glial cells consist of: astrocytes, oligodendrocytes and their progenitors and microglia. The interactions between the inflammatory and glial cells have been suggested to be critical in SCI pathology [23]. Arriving reactive astrocytes together with surrounding connective tissue form a glial scar,

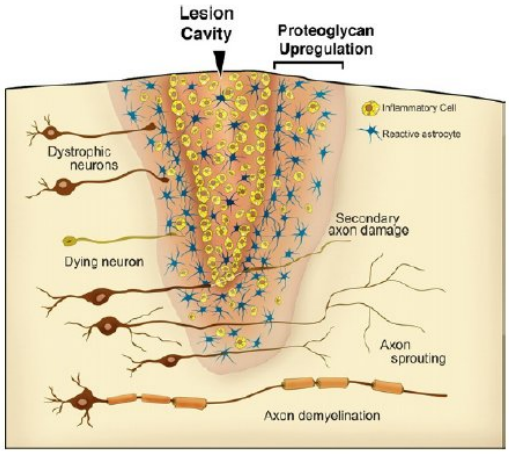

Figure 2. Glial scarring following SCI. Immediately after injury the spinal cord cavity is invaded by inflammatory cells which subsequently recruit reactive astrocytes and other glial cells to the lesion site. Surrounding the cavity cells produce various inhibitory molecules, for example proteoglycans, which create a non-permissive environment for regrowing axons. CNS regenerative failure is later followed by axon demyelination and secondary tissue damage [22].

a physical obstacle for regrowing axons [24]. Furthermore, recently glial scar has been suggested to act not only as mechanical barrier but also to contribute to regeneration failure by synthesizing numerous inhibitory molecules [22, 25]. A suppressing environment of the glial scar has been shown to arrest axon growth and remyeliation of demyeliated axons due to injured axonal tracts [26] pinpointing the critical role of the lesion environment in CNS regenerative failure.

The processes of glial scarring are known as a secondary response to the injury and take approximately a few weeks [26] until the final structure is built up. The inhibitory cascade starts with influx of macrophages and microglia cells to the lesion site, which is followed later by recruitment of oligodendrocytes precursors. The last cells to arrive are astrocytes and they are the main component of the final structure. The majority of the cells contributing to the glial scar formation have been shown to be inhibitory to the regenerating axons (reviewed in [26]). Neurite growth inhibitory molecules produced by oligodendrocytes include myelin associated glycoproteins (MAG) and terascin R. Terascin-R has already been shown to arrest axonal growth *in vitro* by interacting with cell surface F3/11 molecules [27] and restrict functional recovery after SCI in vivo [28]. The mechanism of MAG-dependent inhibition are not yet fully understood [26]. Furthermore, the CNS injury triggers overexpression of Nogo-66 receptors which is known for its growth cone collapsing activity [29]. A second major type of CNS inhibitory molecules are proteoglycans. Produced mostly by reactive astrocytes they are highly upregulated within the scar. Numerous studies have already demonstrated their neurite outgrowth inhibitory properties with chondroitin sulphate proteoglycans as the main players [26].
Although glial scarring sacrifice the CNS capacity for long distance functional regeneration there is increasing evidence suggesting that scar tissue formation following SCI injury is critical for survival [30, 31]. According to the studies performed by Faulkner et al. [30] glial scarring is indispensable for the blood-brain barrier (BBB) repair and significantly limits cellular degradation and inflammation following the injury therefore preventing uncontrolled tissue damage.

Eventually after the glial scarring is completed SCI enters the chronic phase. The lesion site is stabilized and the patients can start the rehabilitation process.

Table 1. Key cellular and molecular mechanisms following SCI

Phase	**Events**	**Cell types**	**Underlying pathological mechanisms**
Acute	Necrosis, inflammation	Cytokines, monocytes, neutrophils	Massive CNS tissue damage, disruption of BBB, edema, oxidative stress (free radicals, nitric oxide)
Secondary	Apoptosis, demyelination, glial scar formation, axon degeneration	Macrophages, microglia cells, oligodendrocytes and their precursors, reactive astrocytes	Secondary tissue damage, failure of endogenous repair, activation of apoptotic pathways, oligodendrocyte loss, secretion of various inhibiting molecules (MAG, proteoglycans)
Chronic	Glial scar stabilization, demyelination, axon degradation	Reactive astrocytes, oligodendrocytes	Oligodendrocyte loss, Wallerian degradation (destruction of the distal ends of severed axons)

3. Neuroregenerative approaches

The changing microenvironment of the lesion site seems to strictly define the therapeutic time window for any SCI therapy to be successful. During the acute and chronic phase one can encounter completely different mechanisms of damage and repair which might promote or suppress the potential beneficial effects of individual treatments. For example the time before glial scarring is completed seems to be optimal for treatments aiming to modify extracellular matrix and promote the endogenous repair. For instance arresting the inhibitory cascade could reduce the secondary tissue damage and lead to locomotor function improvement. On the other hand playing with the unstabilized spinal cord carries a risk of additional damage which could deteriorate patient condition.
Over the last two decades an emerging number of researchers aimed to develop a successful and safe treatment for SCI. Currently the three main therapeutic approaches focus on: intraspinal application of growth promoting factors, cell replacement therapies and use of biomaterials for neural tissue engineering.

3.1. Growth promoting molecules

The balance between growth promoting and inhibitory cues plays a key role in CNS regeneration [32]. Thus, it can be postulated that intrinsic regenerative capacity of mature neurons can be improved by either stimulating the promoting cues or inhibiting suppressive cues therefore modulating the lesion environment. Here we present an overview of neuronal growth promoting molecules and discuss their potential use in SCI.

3.1.1. Neurotrophins

Neurotrophin family members which include: brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3) and glial cell line-derived neurotrophic factor (GDNF) are known to promote axonal growth during mammalian development [33]. They act via the tropomyosin-related kinase receptor (Trk) family with NGF activating TrkA, BDNF interacting with TrkB and NT-3 binding to TrkC [34]. Recent studies have shown that spinal cord injury triggers the expression of endogenous neurotrophins leading to their upregulation at the lesion site [35, 36]. However, their role in regeneration still seems to be limited. The failure of axon regrowth despite the upregulation of endogenously produced neurotrophins lies in highly suppressive environment of the lesion.
Changing the gradient between promoting and inhibiting cues by administrating exogenous neurotrophins might help to overcome this problem and induce axonal regrowth.

3.1.1.1. Brain-derived neurotrophic factor

The neuroregenerative properties of brain-derived neurotrophic factor (BDNF) are based on its affinity for the tropomyosin-related kinase receptor type B (TrkB) [34] which has been postulated to modulate neuroplasticity following SCI [37]. The majority of recent studies focused on application of genetically modified BDNF expressing fibroblasts [38-40] which have been shown to have some neuroprotective effects and induce modest axonal regrowth when applied shortly after the injury [38]. Liu et al. [38] have demonstrated that the engraftment of BDNF-expressing fibroblasts into the acute cervical hemisection cavity results in significant regeneration of rat rubrospinal axons leading to the partial functional recovery of the animals. The delayed grafting (6 weeks delay) provided highly limited axon regeneration. Nevertheless, the neuroprotective properties of BDNF-expressing fibroblast were comparable in both cases and included: prevention of the atrophy or death of axotomized neurons [39], and axon remyeliation [41]. It has been postulated that by inducing proliferation of oligodendrocytes BDNF can promote the remyeliation of the injured axons [41].
Furthermore, BDNF when co-applied with NT-3 has been shown to have increased regenerative properties [42]. Nevertheless, the co-application of BDNF and NT-3 is still questionable. There is considerable evidence suggesting that the combination of exogenous BDNF and NT-3 may lead to some adverse effects therefore limiting its clinical use.

3.1.1.2. Neurotrophin 3

Neurotrophin 3 (NT-3) acts via tropomyosin-related kinase receptor type C (TrkC) [34] and is known for its contribution to neurogenesis in developing CNS [33] and to enhance axonal growth of the mature neurons *in vitro* [43, 44]. Lately, there is an increasing evidence that exogenously delivered NT-3 can facilitate neuronal regeneration in SCI. Numerous studies showed that the engraftment of genitically modified fibroblasts expressing NT-3 into injured spine promotes axonal regrowth and remyeliation improving lokomotor activity of treated animals [39, 41, 45, 46]. Grill et al. [45] have shown that cellulary delivered NT-3 into acute spinal cord lesion induces sustained growth of corticospinal axons and promotes functional recovery. Furthermore, Schnell et al. [47] have demonstrated that a single dose of NT-3 administrated into spinal cord cavity is enough to induce sprouting of the corticospinal axons. Nevertheless, the regenerative effect is usually restricted to the place of engraftment, meaning that axons will regenerate only within the graft, not beyond it.

3.1.1.3. Nerve growth factor

Nerve growth factor (NTF) is a tropomyosin-related kinase receptor type A (TrkA) dependent neurotrophin [34] of putative use for SCI. *In vivo* studies have shown that exogenous NGF induces robust neurite outgrowth in unlesioned [48] , acute [49] and chronically [50] injured spinal cord. Tuszynski et al. have reported robust sprouting of sensory axons after the engraftment of NGF-expressing fibroblasts into acute spinal cord [49]. However, not all axonal population response to NGF grafts. Growth of corticospinal, and local motor axons seem not to be regulated via TrkA receptor, hence be NGF-independent [48-50]. Nevertheless, despite its selective application NGF still can be seen as a putative target in treating SCI.

3.1.1.4. Glia cell line-derived neurotrophic factor

Glia cell line-derived neurotrophic factor (GDNF) has been proposed to promote robust neuronal outgrowth and survival with GDNF continued administration leading to hyperinnerviation [51] and GDNF depletion resulting in reduced axonal growth [52]. Since many neuronal populations projecting to the spinal cord express GDNF receptors it can be hypothesized that GDNF administration into the injured spinal cord will promote its regeneration. Blesch and Tuszynski [53] have demonstrated that administration of GDNF-expressing fibroblasts into the spinal cord lesion promotes axonal regeneration ,including the regrowth of motor and dorsal column sensory axons and by recruiting Schwann cells contributes to their remyeliation. However, no functional improvement was observed. It has been suggested that lack of functional recovery lies in inability of newly generated axons to extend beyond the graft and bridge across the lesion site.

3.1.2. Neurotrophic cytokines

Neurotrophic cytokines represent the family of interleukin 6 class cytokines and are known to regulate growth and differentiation of different cell types. The leukemia inhibitory factor (LIF) and ciliary-derived neurotrophic factor (CNTF) have been shown to have the greatest therapeutic potential in SCI. Both LIF and CNTF act via LIF receptor and by inducing the JAK/STAT pathway can regulate the expression of various prosurvial molecules [54] (including the Bcl2 family of apoptosis regulatory proteins [55]), thus promote cell survival.

3.1.2.1. Leukemia inhibitory factor

Leukemia inhibitory factor (LIF) is a pleiotropic cytokine which has been shown to be upregulated following the CNS injury [56] and by promoting oligodendrocytes survival contribute to the secondary damage reduction [57-60].

Azari et al. [58] have demonstrated that IP administration of exogenous LIF limits the spread of oligodendrocyte death and reduces demyeliation after spinal cord injury. Other studies performed in acute SCI mice model [61] have showed that exogenous LIF when administrated within 24 hours post injury

promotes oligodendrocytes survival and enhances functional recovery of the animals. Furthermore, there is an increasing evidence that LIF can promote neuronal outgrowth of sensory and motor neurons *in vivo* [62, 63] hence contribute to the restoration of neuronal circuits.
Furthermore, interestingly Blesch et al. [64] suggested an alternative, JAK/STAT-independent mechanism via which LIF can facilitate regeneration after the injury. According to these studies exogenous LIF can upregulate the expression of endogenous NT-3 in the spinal cord lesion which has already a well established function in CNS regeneration.

3.1.2.2. Ciliary-derived neurotrophic factor

Ciliary-derived neurotrophic factor (CNTF) is a neuropoietic cytokine which has been shown to promote neuronal survival both: *in vitro* [65, 66] and *in vivo* [67-69] and induce oligodendrocyte progenitor cells (OPCs) proliferation and differentiation possibly contributing to remyeliation of the damage due to the injured axons [70, 71]. Unfortunately, more recent studies conducted by Talbott et al. [72] reviled that although CNTF induces OPC proliferation and differentiation *in vitro* it fails to promote remyelination *in vivo*. Furthermore, studies performed by Bregman et at. [42] questioned neurorestorative properties of CNTF. The authors showed that exogenous CNTF administrated into the injured spinal cord together with NSCs graft has no effect on axonal growth. These finding challenge the potential therapeutic use of CNTF for SCI.

3.1.3. Growth factors

To the growth factors with neurotrophic properties belong basic fibroblast growth factor and erythropoietin.

3.1.3.1. Basic fibroblast growth factor

Basic fibroblast growth factor (bFGF) alike neurotrophins [36] has been found to be upregulated at the site of spinal cord lesion [73, 74] which suggests its potential role in neurorestorative processes following SCI. *In vitro* bFGF has been shown to promote neuronal survival and differentiation of various CNS neurons [75, 76], hence display neurotrophic-like behavior. *In vivo* studies performed by Baffour et al. [37] have demonstrated that co-administration of bFGF and methylprednisole enhances functional outcome in SCI rats. Later, Rabchevsky et al. [77] have investigated whether exogenous bFGF can promote recovery after severe SCI in rats. In his studies Rabchevsky implanted osmotic minipumps secreting bFGF into acute spinal cord, delivering exogenous recombinant human bFGF to the lesion site over a period of 1 week. Already after two weeks a significant improvement in locomotor activity has been observed albeit that the functional recovery was not accomplished with any significant changes in the lesion site histology. Even though the other studies also showed beneficial effect of bFGF in treating different CNS insults [78-80], the mechanism via which exogenous bFGF promotes functional recovery still remains elusive. It can be postulated that intrinsic bFGF properties to 1) suppress the apoptosis of oligodendroglial lineage [81], 2) remyelinate chemically demyelinated axons [82] and 3) promote restoration of blood-brain barrier [83] can contribute to the positive outcome.

3.1.3.2. Erythropoetin

Erythropoietin (EPO) is a hematopoietic growth factor with a well-established function in regulating erythrocyte cell lineage proliferation and differentiation. Recent studies have suggested that EPO might have a critical role in neurogenesis. Both EPO and its receptor (EPO-R) have been found to be highly expressed in fetal CNS tissue [84]. Furthermore, EPO has been suggested to regulate the production of neuronal progenitor cells in hypoxic conditions [85]. It has been shown that a hypoxic environment which is often a result of CNS trauma triggers upregulation of EPO [86, 87] and enhances neuronal differentiation and proliferation [88, 89]. Shingo et al. [85] have shown that exogenous EPO when administrated in hypoxic conditions results in increased production of neuronal progenitor cells by NSCs both *in vitro* and *in vivo*. Numerous

studies performed in experimental models of SCI revealed that exogenous recombinant human EPO when administrated shortly after the injury has a remarkable effect on functional recovery [90, 91]. Gorio et al. [91] have demonstrated that EPO given one hour after the injury prevents oligodendrocyte death and preserves white matter tracts therefore counteracting the secondary damage. Other studies performed by Celik et al. [90] revealed that intravenously administrated EPO immediately after the injury, by inhibiting the inflammatory response, arrests motor neurons apoptosis. All these data together suggest that via its neuroprotective, neurorestorative, anti-apoptotic and anti-inflammatory function EPO can make a significant contribution to neurorepair in SCI.

3.1.4. Progesterone

Progesterone is a steroid hormone with important functions in female menstrual cycle and pregnancy. During the last two decades an emerging list of publications point out the value of progesterone in treatment of various CNS insults emphasizing its neuroprotective and promyelinating properties in SCI [92-97]. Progesterone has been shown to recruit and promote proliferation and differentiation of OPCs *in vivo*, hence contribute to remeyliation of demyelinated due to the injured axonal tracts [95]. Recently Labombarda et al. [95] have studied the mechanism how progesterone regulates proliferation of OPCs. It has been shown that progesterone can trigger the expression of Olig2 and Nkx2.2 transcription factors which subsequently induce OPCs proliferation. Furthermore, there is considerable evidence that progesterone can contribute to neuronal function restoration by modulating the expression of the following molecules: 1) choline acetyltransferase (ChAT), 2) growth associated protein GAP-43, 3) Na, K-ATPase subunits [97] and 4) BDNF [96]. Neurorestorative and neuroprotective effects of BDNF in SCI have already been discussed in this review. ChAT, GAP-43 and Na, K-ATPase subunits are responsible for basic neuronal functions including: neurotransmitters synthesis [98], axonal growth [99] and maintenance of ion transport [100], respectively. All these functions are disturbed following SCI injury [98-102].

3.2. Cell replacement therapies

Replacing damage due to the injured CNS tissue is an ultimate goal in treating SCI. Transplanted cells can restore damage due to the injured neuronal circuits and promote the functional recovery of the patients. Furthermore, transplantation of myelin producing cell could support axonal remyelinating. Numerous studies have already demonstrated the beneficial use of various stem cells, schwann cell or olfactory ensheathing cells in animal models of SCI. Here we present an in-deep overview of exogenous cell replacement therapies for SCI and briefly discuss the neurorestorative potential of endogenous spinal cord stem cells.

3.2.1. Endogenous Spinal Cord Neural Stem Cells and their progenitors

Endogenous stem cells populations rest in mammalian adult CNS in both brain and spinal cord with high concentration in the hippocampus [103, 104] and the central canal of the spinal cord [105, 106]. Theoretically in case of an injury endogenous neural stem cells should be able to proliferate and facilitate neural repair. Resent studies have shown that spinal cord endogenous neural stem cells or their progenitors can proliferate in response to the injury and migrate to the place of the lesion [107, 108]. However, there is limited evidence that they could facilitate neural repair following SCI. Firstly, according to the studies performed by Yamamoto et al. [107] the parenchymal neural progenitors isolated from the injured spine can differentiate into neurons *in vitro* but not *in vivo* which suggest that there is a suppressive mechanism that inhibits formation of new neurons *in vivo*, hence makes the CNS incapable of restoring neuronal networks destroyed during the injury. In further studies, Yamamoto confirmed presence of some suppressive environmental factors and suggested cell-surface receptor Notch signaling to mediate the inhibition of neurogenesis [109]. Secondly, the majority of neural stem cells recruited to the site of the injury proliferate into astrocytes [107, 108]. Newly generated astrocytes invading the lesion area become hypertrophic and immediately contribute to the glial scar formation, hence

inhibiting putative axonal regrowth [108] (in physiological conditions astrocytes secrete neural growth factors and neurotrophins which promote axonal growth).
On the other hand, oligodendrocyte progenitor-derived cells are capable to remyelinate injured axons *in vivo* [110]. Nevertheless, it seems a process of minor significance.
Taken all together, endogenous neural stem cells are capable to response to the injury and proliferate *in vivo* into astrocytes and oligodendrocytes but not into neurons. The highly suppressive environment of the lesion site inhibits local neurogenesis, hence limits the possibility to replace damaged neurons and restore the neuronal connections. Furthermore, newly generated astrocytes do not promote axonal regrowth by secreting various growth factors as they do in the intact CNS; on the contrary, they inhibit axonal regeneration by forming scar tissue. All in all, without supportive environment endogenous repair cannot be successful. The alternative approach includes use of exogenous cells which, transplanted into the injured spinal cord, could facilitate its repair.

3.2.2. Exogenous cell transplantation

Considering the failure of endogenous CNS repair exogenous cell transplantation seems to be a good solution. Currently researchers offer a broad spectrum of possible candidates starting with pluripotent embryonic stem cells through partially differentiated neural stem cells up to fully specialized mature cell lines.

3.2.2.1. Embryonic Stem Cells

Embryonic stem cells (ESCs) have a great potential in a wide range of cell replacement therapies [111, 112]. They can be easily obtained from blastocyst stage embryos and proliferate into all possible somatic cells [113-115]. ESCs have been shown to successfully differentiate *in vitro* into multiple CNS cell types including mature neurons, astrocytes and oligodendrocytes [116-122]. Recent studies in rodents have shown that transplanted ESCs can survive, differentiate *in vivo* and promote recovery of the injured spine [123]. Furthermore, ESCs-derived oligodendrocytes can remyelinate previously demyelinated axons [124] therefore partly restore locomotion after the injury [125, 126]. Nevertheless, despite the promising results human ESCs based therapies still rise ethical issues and have an increased risk of host rejection and tumorigenesis.

3.2.2.2. Induced Pluripotent Stem Cells

Human induced pluripotent stem cells (hIPSCs) provide a promising alternative to hESCs since alike hESCs they do not entail bioethical concerns and lower the risk of possible immunogenicity. IPSCs are generated from adult somatic cells by ectopic expression of specific reprogramming factors that can restore the pluripotency [127-130]. The initial technology involved use of viral vectors [131-133]. However due to possible increased risk of tumorigenesis new alternative methods have been developed including plasmid transfection [134], use of episomal vectors [135], recombinant proteins [136], chemicals [137, 138] or more recently miRNA [139, 140]. IPSCs have been shown to be capable to differentiate *in vitro* towards main neural lineage cells (neurons, oligodendrocytes and astrocytes) [141-143]. Transplanted into injured spine, IPSCs survived, migrated towards the place of lesion and differentiated into neurons [144, 145]. In a study Nori et al. [144] observed that transplantation of IPSCs in mice promotes motor functional recovery after the spinal cord injury. IPSCs-derived neurons were capable of making new synaptic connections between grafted and host neurons. Furthermore, the treatment resulted in enhancement of axonal regrowth and angiogenesis.
IPSCs open a new window in neural tissue engineering. However, there is still much to be done. The lack of immunogenicity of IPSCs have been already questioned by Zhao et al [146]. Furthermore, there is increasing evidence that wrongly selected so-called "unsafe" IPSCs may form deadly teratomas [145] which raises a need for detailed safety testing systems before any clinical use of IPSCs.

3.2.2.3. Neural stem cells

Transplantation of exogenous neural stem cells (NSCs) represents another approach of cell-derived therapy for SCI. NSCs are believed to be programmed to differentiate into neural cell lineages, hence to show reduced neoplasticity when compared to the ESCs. They can be successfully isolated from both embryonic and adult CNS tissue [147], with the brain [148, 149] and spinal cord [150] as the most common source. Transplantation of fetal CNS tissue has already been shown successful in human patients with Parkinson's disease and results in some clinical improvement [151, 152]. However promising the results, the use of high number of fetuses required to obtain sufficient tissue for the transplantation of CNS tissue rises practical and ethical issues. *In vitro* expansion of neural progenitor cells seem to overcome these problems and opens a new window for NSCs transplantation. However, with a higher degree of differentiation comes a lower frequency of cell divisions which could generate difficulties to produce large numbers of NSCs required for clinical applications [153].

Currently, numerous studies using transplanted NSCs for spinal cord repair have been performed in both rodents [154-156] and primates [157]. NSCs were able to differentiate *in vivo* into functional neurons, astrocytes and oligodendrocytes [156-158] and naturally secrete neurotrophic factors including: NGF, BDNF and GDNF [155]. NSCs-derived neurons formed synaptic connections with host axons and were able to generate axon potentials presumably contributing to improvement in locomotor activity in treated animals [156-158]. Remyelination of the injured axons have been reported in rodent [156] but not primate [157] studies. However, the authors suggest that lack of remyelination is caused by the late time of the transplantation and it is not species-dependent [157].

NSCs transplantation shows a great potential in treating SCI, however some aspects still need further investigation before going into clinic. The main aim in SCI treatment is to restore the neural connections in the area of the injury and build a functional bridge between two sites of the lesion which require a huge number of newly generated neurons. Unfortunately, usually transplanted NSCs are able to proliferate into large amounts of astrocytes and oligodendrocytes but not neurons [158-160]. This problem has been recently studied by Abematsu et all. [154] who demonstrated that NSCs when injected into the spinal cord together with valproic acid (VPA) can generate significantly increased proportions of neural progeny than NSCs alone which is consistent with previous findings where VPA has been shown to induce neural differentiation but suppress astrocyte and oligodendrocyte differentiation of NSCs [161]. Therefore the beneficial effect of NSCs alone is mostly caused by extensive growth of the host axons (secreted by NSCs-derived cells neurotrophic factors promote neural growth) and their remyelination.

3.2.2.4. Mesenchymal stem cells

Mesenchymal stem cells (MSCs), often referred as bone marrow stromal cells (BMSCs), share multiple traits with stem cell populations. They can be easily obtained from a patient (give the possibility of autologous transplantation) and are free of ethical concerns. It has been shown that MSCs are capable to differentiate into multiple cell types [162, 163] including neurons, astrocytes and oligodendrocytes [164-168]. However, the functionality of MSCs-derived neurons is still questionable. Recent studies performed by Hofstetter et al. [169] showed that neurons derived from MSCs display neuron-like morphology and express typical neuronal markers but are not able to generate axon initial potential. However, the mechanism how MSCs-derived cells could facilitate neural repair in SCI remains elusive notwithstanding that numerous studies have been performed. In many cases MSCs when injected into injured spine promoted functional recovery of the animals [169-171].

Furthermore, beside replacing damaged tissue, MSCs are suggested to contribute to the spinal cord repair via different indirect mechanisms. Firstly by suppressing expression of T cells and NK and changing the cytokine secretion profile, MSCs create more anti-inflammatory and are hence more favorable for axonal regeneration [172]. Secondly, MSCs are claimed to produce numerous growth factors that could promote the recovery after the injury [173, 174]. Lastly, MSCs have been shown to promote differentiation of NSCs *in vitro* which

suggests that MSCs could activate endogenous NSCs and induce their proliferation *in vivo* [171].

3.2.2.5. Schwann Cells

The potential of exogenous Schwann cells (SCs) transplantation for spinal cord repair lies in their ability to secrete numerous growth factor including: NGF, BDNF, NT-3 and produce myelin *in vivo* [175, 176]. Engrafted SCs promote axonal growth and remyelinate demyelinated axons [177-180]. Studies performed in rodents showed substantial functional improvements after SCs transplantation into the injured spine [176, 181, 182]. Furthermore, unlike ESCs, NSCs or MSCs, SCs are terminally differentiated before the engraftment hence they do not carry the risk of tumorigenesis. Nevertheless, the beneficial effect of exogenous SCs is usually restricted to the place of engraftment since SCs show limited ability to migrate within CNS tissue [183] and are not able to enter the astrocytic environment surrounding the lesion [184].

3.2.2.6. Olfactory Ensheathing Cells

Olfactory ensheathing cells (OECs) represent an alternative approach of autologous cells transplantation for SCI. They can be easily obtained for the patient by performing nasal biopsy. OECs are known for their exceptional properties to support and facilitate axonal growth during the lifetime of the organism [185-187]. Their exceptional plasticity together with ability to promote neurogenesis [185] and remyelinate injured axons [188, 189] make them a prime candidate for cell-mediated repair following SCI. Several studies have shown beneficial results of OECs transplantation into injured spine including long axon regeneration, remyelination and improvements in locomotor activity [190-192]. Nevertheless, the mechanism how OECs promote recovery after SCI still remains elusive. Recent studies have suggested that beneficial effects of OECs engraftment might lie not in OECs ability to support axon regrowth and myelination in adults but in their ability to recruit SCs which can facilitate above mentioned processes [193, 194].

3.3. Biomaterials

Biopolymers are natural or synthetic materials which have a great potential in regenerative medicine including neural tissue engineering. Numerous animal studies have already shown their beneficial effects in treating SCI [14, 15, 195]. Application of injectable scaffolds into the spinal cord lesion alone or in combination with cellular therapies or growth factors have been shown to support the functional recovery of the treated animals. Below we discuss the therapeutic potential of selected natural and synthetic polymers and their putative application in treating SCI patients. The combinational approach of using cell or neurotrophin loaded biopolymers will be presented later (see *Combine therapies*).

3.3.1. Natural polymers

Naturally-derived polymers are biodegradable, usually native to the human body materials with very high biotolerability. To the natural polymers with potential use for SCI belong collagen, fibrin and hyaluronic acid. Their main role is to stabilize injured spine by filling in the cavity and provide a structural foundation for regrowing axons. Furthermore, some of them have been shown to have a suppressing effect on glial scar formation.

3.3.1.1. Collagen

Collagen type I is the main extracellular matrix protein which has been already applied in numerous medical devices [196]. Its intrinsic property to form a gel at physiological temperature makes it attractive to use for tissue engineering application. *In vitro* collagen type I has been shown to inhibit DNA synthesis in glial cells [197], hence arrest their proliferation which could play a significant role in suppressing glial scaring following CNS injury. *In vivo* studies performed in rat [198, 199] and rabbit [200] models of SCI showed that application of collagen scaffolds into spinal cord lesion creates a favorable environment for axonal regeneration and

promotes functional recovery of the animals. Collagen-filaments have been suggested to form a foundation for regrowing axons and support their regeneration along the filaments. In both: rat [198, 199] and rabbit [200] studies the authors observed axonal growth through the implant. Collagen-filaments have been shown to bridge the two sites of the lesion therefore restoring the structural continuity of the spinal cord. After administration collagen type I is subsequently degraded by endogenous proteases secreted at neuronal growth cones.

3.3.1.2. Fibrin

Fibrin is a plasma derived natural polymer which is known for its role in wound repairing. Under physiological conditions by activating proteolytic enzyme, thrombin, fibrin rapidly polymerizes. Its degradation is maintained by endogenous plasmin enzymes. Being extremely elastic and having the ability to bind different neural cells and growth factors [201], fibrin is a good candidate for neural tissue engineering. Fibrin-based injectable scaffolds have been shown to delay the accumulation of reactive astrocytes at the lesion site, therefore diminishing the secondary damage following the SCI [202]. Furthermore, fibrin has been demonstrated to promote neural fiber sprouting and support axonal regrowth [202, 203]. Nevertheless, the axonal regeneration usually has not been followed by functional recovery of the animals [203, 204] which might be due to inability of regrowing axons to growth beside the implant site [204]. Another drawback of fibrin scaffolds is their potential immunoreactivity.
All in all, although fibrin shows some promising results there is still a great need of further research to confirm its therapeutic use for SCI.

3.3.1.3. Hyaluronic acid

Hyaluronic acid (HA) is a non-immunogenic glycosaminoglycan naturally present through-out connective, epithelial and neural tissue. Unlike collagen and fibrin, HA is not cell adhesive and due to its high water solubility does not form a gel under physiological conditions. Therefore for tissue engineering applications HA must be used in combination with other polymers from which methyl-cellulose (MC) has been of greatest use [205, 206]. HAMC injectable scaffolds have been shown to reduce inflammation and scarring in rat models of SCI by providing a favorable environment to promote the functional recovery [206]. The anti-inflammatory properties of HA are believed to be an effect of its ability to interact with inflammatory cells which has been suggested due to the high molecular weight of HA [207].

3.3.2. Synthetic polymers

Using artificial materials for neural tissue engineering gives the possibility to modify the physical and chemical properties and create highly specialized implants for SCI. The implant optimization can increase axonal sprouting within the scaffold and improve axonal guidance. On the other hand implantation of synthetic materials into the spinal cord carries an increased risk of the immune reaction to the graft and additional tissue scarring. To the most promising synthetic scaffolds belong poly ά-hydroxy acid polymers and methacrylate and methacrylamide-based hydrogels.

3.3.2.1. Poly ά-hydroxy acid polymers

Poly ά-hydroxy acid polymers are biodegradable, well tolerated by neuronal tissue [208-210] synthetic biomaterials that have been already approved for clinical use including their application in peripheral nerve repair . To the most studied belong poly (lactic acid) (PLA), poly (glycolic acid) (PGA) and their co-polymer poly (lactic-co-glycolic acid) (PLGA) [195]. In SCI their key role is to provide a structural foundation for regrowing neurons. As mentioned above one of the main advantages of synthetic polymers over the natural scaffolds is the possibility to modify their architecture which gives an opportunity to develop highly optimized candidates for axonal regrowth materials [210, 211]. Cai et al. [210] have shown that PLA foam implants with longitudinal inner channels promote axonal regeneration into the graft much more efficient then amorphous implants. The authors

observed spontaneous formation of well-organized SC cables along the channels which have been suggested to create a favorable environment for regrowing axons. More recent studies showed that aligned PLA microfibers induce significantly greater axonal regeneration then when organized randomly [211]. These findings make poly ά-hydroxy acid polymers promising candidates for SCI. By optimizing their properties we could support cell migration and proliferation and promote axonal growth and guidance.

3.3.2.2. Methacrylate and methacrylamide-based hydrogels

An another group of synthetic polymers of potential use for SCI consists of biodegradable hydrogels based on poly (2-hydroxyethylmethacrylate) (PHEMA) and poly N-(2-hydroxypropyl)-methacrylamide (PHPMA). Both: PHEMA and PHPMA are highly biocompatible and have been suggested to be a good candidate to promote nervous tissue repair [212, 213]. The wide range of possible modifications (including surface charge regulation as great importance) allows to tailor their properties in order to increase cell adherence and axonal extension [214-216]. Positively charged PHEMA/PHPMA hydrogels have been shown to have exceptional cell adhesive and neurorestorative properties [214, 217]. Hejcl et al. [217] have demonstrated that positively charged PHEMA scaffolds implanted into acute or chronic spinal cord cavity integrate with the host tissue and form a bridge across the lesion with numerous axons, SC cables and blood vessels crossing the implant. Furthermore, more interestingly Kubinova et al. [215] by introducing cholesterol methacrylate and ethylene dimethacrylate groups into PHEMA increased scaffold's softness. Modified PHEMA has been shown to minimize additional post-transplational scarring.

3.4. Combined therapies

Combining different therapeutic approaches is a promising strategy for SCI. Wisely conjugated therapies can increase the positive outcome of the treatment. The synergetic effects of stem cells co-transplantation, growth factors overexpressing cell grafts or biopolymers loaded with neurotrophins and cells have been already described in the literature.

3.4.1. Stem cells co-transplantation

Recently there is an emerging number of studies showing the synergetic effects of combinational cell grafts and their potential advantages over single-cell-type transplantations [218-220]. Zeng et al. [220] have shown that SCs co-transplanted with NSCs into injured spinal cord promote NSC survival and increase the rate NSC-derived neurons. One of the critical issues of NSCs transplantation is their very low capacity to proliferate into neurons, the majority of NSCs differentiating into astrocytes and oligodendrocytes [158-160]. The authors postulate that intristic property of SCs to produce various neurotrophins including NGF, BDGF and GDGF [176, 221] can enhance the survival and integration of transplanted NSCs and promote their differentiation into neurons. The other studies performed by Wang et al. [219] have revealed synergetic effects of co-transplantation of OPC and NSCs. OPCs are known to enhance neurogenesis [185] and have the rare ability to migrate through the glial scar hence facilitate axonal regrowth through the lesion site. Indeed, the co-transplantation of OPCs and NSCs increase the number of newly generated neurons, and the authors observed a significantly higher density of myelinated axons at the site of injury in the OPCs +NSCs group then in OPC or NSC transplants alone. Furthermore, animal from the combined group showed the greatest improvement in locomotor function.

The success of combined transplantation lies in the complementarity of biological properties of two different cell types. It is a relatively new approach and needs further investigation; however, it seems to have a great potential in treating SCI.

3.4.2. Genetically modified stem cells

To enhance the therapeutic use of stem cells they can be genetically modified to produce neurotrophic molecules therefore provide the graft with the local source of growth stimulating factors and promote neuronal growth and axon regeneration. Liu et al. [222] have studied the use of NT-3 overexpressing NSCs in intact rat spinal cord. NT-3 modified NSCs were able to survive after the transplantation, differentiate into neurons and glia cells and migrate for long distances. Later studies performed by Blesch et al. [13] revealed that NT-3 modified NSCs transplanted into injured spinal cord results in the extended axonal growth when compared to the control groups. Furthermore, the authors reported partial reconstitution of the cellular matrix attempting to bridge two sites of the lesion.

More recent genetically modified MSC expressing NT-3 transplanted into spinal cord lesion have been shown to be advantageous over the unmodified MSC and result in better locomotor functional recovery [223].

Furthermore, an interesting study was performed by Hamada et al. [224] where researchers transfected ESCs with MASH1, a gene involved in neurogenesis and neuronal fate determination. It has been postulated that MASH1 can direct differentiation of ESCs into spinal motoneuron precursors which have a great neurorestorative potential. Engraftment of MASH1-expressing ESCs derived neuronal progenitors into injured mice spinal cord resulted in exceptional axonal regrowth and functional recovery of the animals. Furthermore, the authors observed that MASH1-transfected cells inhibit the expression of Nogo receptor, which is known to be critical for axonal regeneration [225] and has been suggested to contribute to the positive outcome of this study.

All in all, genetically modified stem cells are a powerful tool for treating SCI. By giving the possibility to modulate the properties of various types of stem cells they can enhance their therapeutic use and contribute to the better outcome of the treatment.

3.4.3. Injectable scaffolds loaded with neurotrophins and cells

Biomaterials with incorporated cell grafts or neurotrophins are a novel therapeutic approach for SCI. Conjugation of cell transplants and growth promoting molecules with biodegradable scaffolds allows to overcome main issues of using cell grafts or neurotrophin alone. The significant problems with the use of neurotrophic factors have been the maintenance of their high concentration within the lesion site and need of the long exposure. However, the application of neurotrophin secreting minipumps seems to provide a solution. Unfortunately their implantation into the spinal cord lesion carries a high risk of infection and additional tissue damage. Neurotrophins loaded with an injectable scaffold are a safe alternative for the controlled long term delivery. Stanwick et al. [226] have proposed PLGA-based NT-3 loaded nanoparticles as a system for enhanced NT-3 delivery in SCI. Other studies performed by Park et al. [227] have demonstrated that HA-based scaffolds containing BDNF promote axonal regrowth following SCI.

When combined with cell grafts, biopolymer scaffolds provide a structural support for the transplant therefore promoting survival and migration of the cells and guiding regrowing axons. Numerous studies showed beneficial effects of application of the stem cells seeded scaffolds [228-230]. Teng et al. [230] have shown that PLGA-based scaffolds containing NSCs promote long-term functional recovery of adult hemisection rats by reducing glial scarring , preventing tissue loss and promoting neuronal repair by NSCs. More recent studies have revealed that a MSC-fibrin matrix significantly improves the survival and migration of the graft therefore promoting neurological recovery of treated animals [229].

Taking all together, using both neurotrophin and cell loaded biomaterials does not only enhance the therapeutic effect of these treatments alone but also makes use of biomaterial neuroprotective properties which include glial scar arrestment and provision of a growth promoting environment. Having a great neurorestorative potential injectable scaffolds seeded with cell grafts or/ and neurotrophins are a very promising approach for SCI.

3.5. Other approaches

Every year an emerging number of SCI studies together with development of new state of art techniques leads to discovery of novel therapeutic targets for SCI. In this review we have already presented three main therapeutic branches and subsequently evaluated their putative clinical application. However, since the field of SCI research is developing very dynamically it seems to be impossible to discuss all new therapeutic approaches. In this chapter we would like to briefly comment on few of them.

To the one of most promising alternative targets for SCI belong neurite growth inhibiting proteoglycans [231, 232]. NG2 proteoglycan antibodies [232] and chondroitin sulfate proteoglycan degradation enzymes [231] have already been shown to have some positive effect on axonal regrowth. Both treatments, by reducing number of inhibitory proteoglycans, aim to modulate a non-permissive environment of the lesion site and make it more favorable for regrowing axons.

Furthermore up to date, approximately 60 different miRNAs have been shown to display altered expression following acute SCI [233]. Many of them have been identified to regulate the expression of inflammatory or apoptotic genes and have been suggested to play an important role in SCI pathogenesis [233]. Thus, one can assume that modulating the expression level of specific miRNAs can have an effect on axons regenerative capacity. Indeed recent findings seem to confirm this hypothesis. *In vivo* studies performed by Jee et al. [234] have demonstrated that silencing miRNA 486 can enhance the expression of NeuroD6 and by downregulating reactive oxygen species contribute to motor function recovery [234].

Last but not least blocking myelin associated neurite growth inhibitory protein, Nogo-A, has been shown to induce axonal regeneration and promote sprouting of the fibre tracts in rat model of incomplete SCI [235].

4. Clinical and scientific challenges

Although the preclinical studies seem to provide promising results and put a bright light upon the future of the SCI patients there is still a long way before translating new therapies into clinical practice. Before going into the clinic one must first provide clear evidence that the proposed treatments are safe and will not cause additional burden to the patient. Since the injured spine is a very delicate place any surgical procedure carries a high risk of post-operational complication and may deteriorate patients condition. Furthermore, when considering cell based therapies one must first carefully examine the possible risk of immunogenicity and graft rejection and make sure to eliminate any risk of tumorigenesis. Uncontrolled cell differentiation and spontaneous tumorigenesis seem to be an important issue in ESC based therapies and quite likely might be the reason to ban ESCs from clinical use. In this case autologous transplantation of somatic (partly or fully differentiated) cells seems to be in a favorable position. Eventually, before starting human studies one must collect compelling evidence of therapeutic effects of the treatment and perform detailed risks vs. benefits analysis. The benefits should overweigh the risks.

Impressive preclinical findings in the field of neurorepair revealed new promising targets for SCI and resulted in a number of clinical trials involving these new neuroregenerative approachs. Most of those studies focus on application on exogenous cell transplants of both neural and non-neural origin including: NSCs [NCT01321333], BMSCs [NCT00816803, NCT01186679, NCT01274975, NTC01325103, NCT01328860, NCT01490242, NCT01694927, NTC01162915, NCT01446640, NCT01730183, NCT00695149, NCT01676441], SCs [NCT01739023] and OECs [NCT01231893], the status of the last one is unknown. The non-cellular neuroregenerative therapies are represented by anti-Nogo-A antibodies with successfully completed Phase I and Phase II in progress [236]. Some of the above mentioned studies have been already completed [NCT00816803, NCT01186679, NCT01274975] but unfortunately no exciting results have been presented. The active clinical trials are in Phase I or II, or still recruiting candidate patients.

The current clinical studies are mostly focusing on evaluating the safety of cell based therapies. Up to date, no serious side effects have been reported. Furthermore a few small Phase II

efficacy study have been conducted. Unfortunately their results seem to be inconclusive. The other approaches (excluding anti-Nogo-A antibodies) are still waiting to enter the clinical stage.

All in all, there is still a long way to go before new neuroregenerative approaches will become part of a common clinical practice for SCI. One must keep in mind that we might never be able to regrow large areas of neuronal tissue and provide a full functional recovery of the patients. Furthermore, based on preclinical findings it seems that the combinational therapies might lead to better functional outcome of the treatment. However, the more complex the therapy gets the more studies are required which delays its putative clinical application.

5. Conclusions

Until recently, the SCI was though to be a life-long sentence with no chances of restoring functional activity after the injury. The patients had to deal with life-long disability inevitably becoming a social and economical burden. Available treatments were mostly focusing on reducing local inflammation and stabilizing the injured spine. Together with the abortive rehabilitation process they were giving a minimal chance of locomotor recovery. Over the last two decades numerous preclinical studies have demonstrated new, promising approaches aiming to restore the spinal cord continuity and enable patients to come back to the life from before the injury. Unfortunately, the successful results of animal studies seem to be lost during the translation into clinic. Current actions should focus on bringing this preclinical findings as soon as possible into the human use.

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