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Review

Scaffold-facilitated locomotor improvement post complete spinal cord injury: Motor axon regeneration versus endogenous neuronal relay formation

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ABSTRACT

Complete transected spinal cord injury (SCI) severely influences the quality of life and mortality rates of animals and patients. In the past decade, many simple and combinatorial therapeutic treatments have been tested in improving locomotor function in animals with this extraordinarily challenging SCI. The potential mechanism for promotion of locomotor function relies either on direct motor axon regeneration through the lesion gap or indirect neuronal relay bridging to functionally reconnect transected spinal stumps. In this review, we first compare the advantages and problems of complete transection SCI animal models with other prevailing SCI models used in motor axon regeneration research. Next, we enumerate some of the popular bio-scaffolds utilized in complete SCI repair in the last decade. Then, the current state of motor axon regeneration as well as its role on locomotor improvement of animals after complete SCI is discussed. Last, the current approach of directing endogenous neuronal relays formation to achieve motor function recovery by well-designed functional bio-scaffolds implantation in complete transected SCI animals is reviewed. Although facilitating neuronal relays formation by bio-scaffolds implantation appears to be more practical and feasible than directing motor axon regeneration in promoting locomotor outcome in animals after complete SCI, there are still challenges in neuronal relays formation, maintaining and debugging for spinal cord regenerative repair.

1. Introduction

Spinal cord injury (SCI) refers to spinal tissue damage that causes temporary or permanent irreversible sensory and motor functional impairments below the level of the injury [1]. Traumatic SCI is generally triggered by an external physical impact including motor vehicle crashes, sports-related injuries, falls, and violent acts. Acutely, the external insult results in primary spinal tissue damage and neural cell death, but also initiates a secondary injury cascade including ischemia, inflammation, substantial neuronal and oligodendrocyte death, and glial scar and cystic cavity formation [2]. Over the last few decades, studies of traumatic SCI animal models have confirmed that primary and secondary injury cascades after adult mammalian traumatic SCI gradually change the cellular components and structural architecture of injured spinal tissue, ultimately forming a non-permissive microenvironment within the lesion site that prevents axonal regeneration. Additionally, many studies have shown that apart from an adverse regenerative microenvironment, poor intrinsic growth potential of spinal neurons leads to poorer spontaneous recovery potential in the spinal cord, resulting in permanent neurological deficits in SCI animals [3–6]. Nonetheless, in recent years, manipulating the intrinsic growth potential and certain inhibitory substrates in the lesion microenvironment (either in isolation or combination), has shown that some adult spinal neurons and axons have regenerative competence [7–12].

Animal studies have provided new concepts and alternatives for treating traumatic SCI. However, despite numerous neuro-regenerative therapies developed from animal studies have shown promising clinical potentials, clinical treatment for SCI still primarily focuses on

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stabilizing the lesion area, preventing further damage to adjacent spinal tissue, and improving patients long-term functional disabilities [13,14]. Thus, at present, there is no effective clinical treatment for traumatic SCI, and repair of complete transected SCI is still regarded as one of the most challenging problems in clinical research [15]. Moreover, the direct costs of SCI are reported to be approximately 1–4 million US dollars per injured patient over a lifetime [16]. As young adults are the primary SCI population, and if treated in time, SCI does not significantly influence lifelong mortality rate in patients [17]. Hence, SCI not only results in a physical and emotional impact on injured individuals, but also leaves a financial burden and devastating social consequences for the patients' families and society.

2. Complete spinal cord injury model

In past decades, many traumatic animal SCI models (including contusion, compression, transection, distraction, and chemical injury models) have been developed for both basic biological research and therapeutic assessment of treatments designed for SCI repair [18,19]. These different animal SCI models have their own advantages and drawbacks, for example, contusion and compression models simulate real situations of clinical cases, but do not cause uniformity in degree of injury of experimental animals, which consequently results in individual differences. Moreover, it is difficult to determine whether axons observed below the lesion site represent regeneration of transected axons or sprouting from spared axons (Fig. 1). Hence, these models cannot show genuine axonal regeneration due to spontaneous sprouting of spared axon fibers. Yet less than 5% of spared fibers can result in significant locomotor recovery in adult rats with incomplete SCI [20]. The left or right semi-transection model also has disadvantages associated with model uniformity of injured animals, but is a suitable model for investigating sprouting of uninjured axons in re-crossing the midline from the contralateral injury side. The complete transection model reflects real axonal regeneration and can solve the sprouting/regeneration unpredictability problem of contusion or crush injury models, because the transected spinal cord is completely separated without any spared axons in the lesion site (Figs. 1 and 2). Therefore, the complete transection SCI model has emerged in the last decade, and been gradually developed and improved from rodents to large animals in the past few years [21]. As a caveat, complete transection of the spinal cord abolishes axon fiber and propriospinal spinal neuron connectivity at the lesion site, which usually results in permanent paralysis and a series of secondary medical complications such as pressure ulcers, osteoporosis, deep vein thrombosis, urinary tract infections, muscle spasms, and respiratory complications [21]. Thus, the complete transection SCI model severely affects quality of life and mortality rates of animals compared with other milder SCI models. Meanwhile, the arduous postoperative care of animals that have received complete transection injury makes this severe SCI model particularly challenge [22–24]. Although severe SCI model by complete transection of spinal tissue with a gap is rarely seen, this injury model excludes the possibility of sprouting from spared axons and provides the most optimal platform for investigating axonal regeneration. During the past decade, many researchers have regarded the complete transection SCI model as the gold standard for validating axonal regeneration. Recently, we pioneered acute and chronic complete transection SCI models in dogs, of which the clinical signs, poor prognosis, and rehabilitation training are more relevant and accurate than rodent models for the study of severe SCI repair with regard to human patients [25].

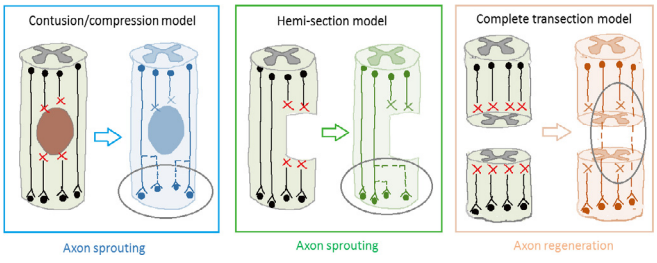

Fig. 1. Axon sprouting could be widely observed in spinal cord contusion/compression and hemi-transection injury models. Only complete spinal cord transection model could verify the real regeneration of the cut axons.

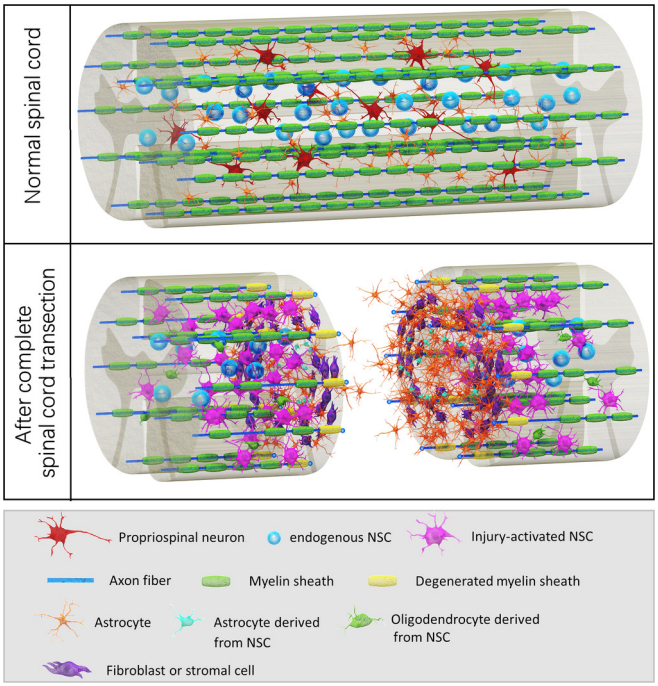

Fig. 2. Pathophysiology of axons and endogenous neural stem cells (NSCs) in adult spinal cord with complete spinal cord injury (SCI). Uninjured spinal cord contains endogenous NSCs (brilliant blue) which remain quiescent under normal physiological condition. Sensory and motor axons (blue) wrapped in myelin sheath (green) are also existed in the intact spinal cord. Additionally, propriospinal neurons (red) and astrocytes (yellow) are presented throughout the intact spinal cord. At the subacute stage (about 1–2 weeks after injury), complete spinal cord transection with a gap induces considerable loss of neurons, die back of axons, and degeneration of myelin sheaths (turn yellow from green). Moreover, astrocytes around the lesion gap become reactive after injury and along with fibrotic cells (purple) forming a scar tissue in the lesion site. As for the endogenous NSCs, complete SCI can also result in robust activation and proliferation of endogenous NSCs (bright red) near the lesion site. In addition, the activated NSCs are able to give rise to new astrocytes (bright blue cells) and oligodendrocytes (green cells) but rare neuron post injury. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In the chronic stage after severe SCI, densely formed scar tissue extends throughout the injury site of animals and patients [26–29]. Indeed, many studies have revealed that scar tissue not only acts as a physical barrier to impede axonal regeneration, but that chondroitin sulfate proteoglycans (CSPGs) expressed by scar tissue also become chemical obstacles for axon regrowth [30,31]. Enzymatic digestion or proteins antagonizing CSPG signaling have therapeutic effects in attenuating the inhibitory nature of densely formed scar tissue on axonal regeneration and motor function recovery of injured animals [32–36]. We recently developed a surgical scar resection approach using intraoperative neurophysiological monitoring to precisely distinguish and remove scar tissue (ranging from 0.5 to 4.5 cm before functional scaffold implantation) in five chronic complete SCI patients [37]. Our study firstly showed that direct removal of scar tissue by surgery is an effective and safe alternative for eliminating the inhibitory effect of scar tissue on functional recovery after severe SCI. Further, scar tissue removal at the chronic stage in patients with complete SCI is another type

Table 1
Summary of treatments with their effectiveness on promoting locomotor recovery, motor axon regeneration and endogenous neuronal generation throughout the lesion site in complete spinal cord transection animal models in the last decade.

Complete SCI model	Scaffold utilized	Spontaneous locomotor recovery	Scaffold (Treatment) improved locomotor recovery	Motor axons regeneration across the lesion site	Endogenous neurogenesis in the lesion site
2007. Ibarra et al. [44]	No	Yes, BBB score 2-3	No, BBB score 2-3	No corticospinal axon	N.D.
2008. Guest et al. [46]	No	Yes, BBB score 2-4	Yes, BBB score 7-8	No corticospinal axon	N.D.
2008. Yang et al. [47]	No	Yes, BBB score 1-2	Yes, BBB score 5-8	A few corticospinal axons	N.D.
2010. Abematsu et al. [51]	No	Yes, BBB score 2-3	Yes, BBB score 8-10	No corticospinal axon	N.D.
2010. Fan et al. [38]	Yes	Yes, BBB score 1-2	Yes, BBB score 6-8	A few corticospinal axons	N.D.
2011. Han et al. [39]	Yes	N.D.	N.D.	N.D.	N.D.
2011. Fan et al. [64]	Yes	Yes, BBB score 1-2	Yes, BBB score 3-5	N.D.	N.D.
2011. Du et al. [59]	Yes	Yes, BBB score 1-2	Yes, BBB score 3-5	No corticospinal axon	N.D.
2012. Lu et al. [48]	No	Yes, BBB score 1-2	Yes, BBB score 7-8	Some reticulospinal axons	N.D.
2012. Lu et al. [49]	Yes	Yes, BBB score 1-2	Yes, BBB score 7-8	N.D.	N.D.
2012. Gao et al. [54]	Yes	N.D.	N.D.	Some reticulospinal, serotonergic axons	N.D.
2012. Guo et al. [53]	Yes	Yes, BBB score 1-2	No, BBB score 3-4	No corticospinal axon	N.D.
2013. Hou et al. [52]	Yes	N.D.	N.D.	N.D.	N.D.
2014. Sharp et al. [76]	Yes	Yes, BBB score 1-2	No, BBB score 1-2	No reticulospinal axon	N.D.
2015. Li et al. [40]	Yes	Yes, BBB score 1-2	Yes, BBB score 4-6	N.D.	N.D.
2015. Han et al. [21,56]	Yes	Yes, Olby score 3-5	Yes, Olby score 6-8	N.D.	N.D.
2015. Yang et al. [60]	Yes	Yes, BBB score 0-2	Yes, BBB score 8-10	Some corticospinal axons	Yes
2016. Li et al. [41]	Yes	Yes, BBB score 1-2	Yes, BBB score 6-8	N.D.	Yes
2016. Li et al. [62]	Yes	Yes, BBB score 1-2	Yes, BBB score 6-8	N.D.	Yes
2016. Han et al. [68]	Yes	Yes, BBB score 2-4	Yes, BBB score 6-9	N.D.	N.D.
2016. Li et al. [45]	Yes	Yes, Olby score 3-5	Yes, Olby score 6-8	No Corticospinal axon	Yes
2016. Fan et al. [42]	Yes	Yes, BBB score 2-4	Yes, BBB score 6-9	N.D.	Yes
2017. Xu et al. [43]	Yes	Yes, BBB score 2-4	Yes, BBB score 6-9	N.D.	Yes
2017. Li et al. [58]	Yes	Yes, Olby score 3-5	Yes, Olby score 6-8	N.D.	Yes
2017. Tian et al. [73]	Yes	Yes, BBB score 1-2	Yes, BBB score 8-13	N.D	Yes.
2017. Ganz et al. [55]	Yes	Yes, BBB score 1-2	Yes, BBB score 6-15	N.D.	Yes
2017. Han et al. [57]	Yes	Yes, Olby score 3-5	Yes, Olby score 6-8	N.D.	Yes
2018. Yin et al. [63]	Yes	Yes, Olby score 3-5	Yes, Olby score 6-8	No corticospinal axon	Yes
2018. Li et al. [78]	Yes	Yes, BBB score 2-3	Yes, BBB score 6-9	N.D.	N.D.
2018. Chen et al. [137]	Yes	Yes, BBB score 2-3	Yes, BBB score 6-9	N.D.	Yes

BBB and Olby scoring systems were utilized to assess locomotor recovery levels of rats and dogs, respectively, post complete SCI. N.D. indicates the item is not detected in the corresponding reference.

of severe SCI model, as scar tissue removal before therapeutic intervention always leaves a gap.

An appropriately created complete transection SCI model is regarded as the gold standard for axonal regeneration. Accordingly, in the past decade many researchers have used this severe SCI model, mostly complete thoracic SCI (which completely severs both descending and ascending axons to the hindlimbs), to investigate axonal regeneration as well as lower limb locomotor recovery. In these studies, many simple or combinatorial therapeutic treatments including growth factors [38,39], bioactive antagonists or peptides [39–43], clinical drug delivery [44,45], exogenous cell transplantation [46–53], and implantation of different functional bio-scaffolds [40–43,45,54–60] were designed and performed on thoracic complete SCI animal models. Most treatments promoted neural regeneration or improved locomotor function of animals in this extraordinarily challenging transection model (Table 1). Overall, there are two main mechanisms that can alone or simultaneously explain locomotor improvement in these complete SCI animals: direct motor axon regeneration through the lesion gap or an indirect bridge by neuronal relay formation to functionally re-connect transected spinal stumps.

3. Bio-scaffolds for complete SCI

With the development of the material science in the past decade, a plethora of bio-scaffolds have been developed and applied into various SCI repair [38–40,43,49,55,57,59–64]. Some of the well-designed bio-scaffolds have also shown clinical potentials for the development of operative therapies in various SCI models. Most bio-scaffolds developed for SCI repair are natural polymers composed of repeating units due to their diverse advantages including easily obtain, low antigenicity, excellent biocompatibility, and good biodegradability [65]. Currently, some general issues should be considered when designing an ideal bio-scaffold for SCI repair. First, bio-scaffold needs to provide a structural support for the regrowth and migration of damaged axons and newly generated neurons into the lesion site. Second, the biochemical, physical properties of the bio-scaffold should be adjustable that matches mechanical characteristics of the spinal tissue and ensures proper presentation of guidance cues [66]. Third, the bio-scaffolds should be easily modified or functionalized with some biologically active peptides or proteins for specific repair effects. Fourth, bio-scaffolds need to be degrade within a suitable window of opportunity and replaced by regenerating nerve tissue. Last, the scaffolds as well as their breakdown products should also be non-cytotoxic and avoid unnecessary immune response.

The prominent bio-scaffold used for addressing complete SCI model in the last decade include collagen, chitosan, fibrin and PLGA (Table 1). Bio-scaffolds derived from natural sources are benefit from their abundant existence, low antigenicity, and good biodegradability when utilized for spinal cord repair. Whereas some synthetic bio-scaffolds like PLLA and PLGA are distinctly advantageous in modification, tunability of mechanical properties and gel formation after derivatization. Generally, these scaffolds showed different shapes (fibers, sponge-like, gel-like), characteristics, and modifications (combined with different kind of cells and/or drugs), but they all performed potential roles in recovering the lost hindlimb locomotor functions following complete SCI (Tables 1 and 2). Although long descending motor axons (like corticospinal tracts, CSTs) regeneration is considered as an important motor function repair mechanism for the completely transected spinal cord, very few bio-scaffold based treatment showed robust therapeutic effects on promoting motor axons especially CSTs regeneration across the

Table 2
Summary of composition, structure and modification of various bio-scaffolds utilized in complete spinal cord transection animal models in the last decade.

Complete SCI model	Injured length	Scaffold		
		Composition	Structure	Modification
2010. Fan et al. [38]	2 mm at T8	Collagen	Linear ordered fibers	Collagen binding NT3
2011. Han et al. [39]	6 mm at T8	Collagen	Linear ordered fibers	Collagen binding BDNF and IgG151
2011. Fan et al. [74]	2 mm at T8	PLGA	Linear conduit	NT3
2011. Du et al. [59]	1 mm	PLGA	Macroporous scaffold	NSCs and TrkC expressing NSCs
2012. Lu et al. [49]	2 mm at T3	Fibrin	Fibrin/thrombin gel	Dissociated E14 spinal cord cells and a cocktail containing 10 different growth factors
2012. Gao et al. [54]	1.8 mm at T3	Agarose	Fiber bundle templates	Bone marrow stromal cells secreting BDNF
2012. Guo et al. [53]	2 mm at T8	Chitosan	Channels	Adult brain derived NSCs
2013. Hou et al. [52]	1 mm at T4	Fibrin	Fibrin/thrombin gel	E14 brainstem-NSC and spinal cord-NSC
2014. Sharp et al. [76]	2 mm at T3	Fibrin	Fibrin/thrombin gel	Dissociated E14 spinal cord cells and a cocktail containing 10 different growth factors
2015. Li et al. [40]	3 mm at T10	Collagen	Linear porous sponge	Collagen binding EphA4 and PlaxinB1, NEP1-40
2015. Han et al. [21,56]	5 mm at T11 (dog)	Collagen	Linear ordered fibers	Collagen binding BDNF
2015. Yang et al. [60]	5 mm at T8	Chitosan	Linear tube with chitosan particles	NT3
2016. Li et al. [41]	3 mm at T10	Collagen	Linear porous sponge	Collagen binding EphA4 and PlaxinB1, NEP1-40, Collagen binding BDNF and NT3
2016. Li et al. [62]	3 mm at T8	Collagen	Linear porous sponge	Collagen binding EphA4 and PlaxinB1, BDNF and NT3, NSCs
2016. Han et al. [68]	3 mm at T10	Collagen	Porous sponge	BMSCs
2016. Li et al. [45]	5 mm at T8 (dog)	Collagen	Linear ordered fibers	Cetuximab
2016. Fan et al. [42]	3 mm at T8	Collagen	Linear ordered fibers	Collagen binding EGFR Ab
2017. Xu et al. [43]	3 mm at T8	Collagen	Linear ordered fibers	NSCs and Collagen binding EGFR Ab
2017. Li et al. [58]	5 mm at T8 (dog)	Collagen	Linear ordered fibers	hUC-MSCs
2017. Tian et al. [73]	2 mm at T8	Acellular nerve	Acellular sciatic nerves	MSCs
2017. Ganz et al. [55]	2 mm at T10	PLLA/PLGA	Porous sponges	human oral mucosa stem cells hOMSC
2017. Han et al. [57]	5 mm at T8 (dog)	Collagen	Linear ordered fibers	hUC-MSCs
2018. Yin et al. [63]	1 cm at T8 (dog)	Collagen	Linear ordered fibers	Taxol liposome
2018. Li et al. [78]	3 mm at T8	Collagen	Porous sponges	NSCs, Taxol liposome
2018. Chen et al. [137]	3 mm at T8	Poly (Propylene Fumarate)-Collagen	Multichannel	Collagen binding NT3

lesion site post-complete SCI in the past decade (Table 1). Alternatively, some motoneurons including serotonergic (5HT), choline acetyltransferase (ChAT), and tyrosine hydroxylase (TH)-positive neurons were reported to have generated in the lesion center by functional bio-scaffolds implantation [42,45,49,57]. This indicates that functionally modified bio-scaffold-based therapies are more beneficial to promote neurogenesis than CSTs regeneration in the lesion site.

4. Motor axon regeneration in the complete SCI model

As we have already described, the complete transected SCI model avoids the likelihood of spared axons sprouting below the lesion site because this injury results in two spinal cord stumps separated by a gap, typically millimeters in length. Since last century, researches have shown that after SCI, most cut axons are unlikely to spontaneously extend into or across the lesion gap without an effective intervention, not to mention form connections with its original or another target [67]. Whereas various therapeutic strategies were able to promote recovery of locomotor function of animals after complete SCI (Table 1). Hence, many studies turn to determine if improvements in locomotor function are dependent on regeneration of motor axons (or long tracts) post-complete SCI including corticospinal, raphespinal, reticulospinal, and rubrospinal axons. These descending systems mediate motor functions that are important for improved motor function outcomes in animals with complete SCI. Among these axons, the corticospinal tract is the most studied axon in regeneration of complete spinal cord transection animal models, as this tract is considered critical for recovery of voluntary motor function. Consequently, many investigators regard regeneration of CST as the most likely way of restoring locomotor function of fore and hind limbs after complete SCI. In this review, we will mainly summarize research progress on CST regeneration after complete SCI from the last decade.

We reviewed publications from the past ten years related to animal assays on motor axonal regeneration, endogenous neuronal generation in the lesion site and locomotor recovery or improvement post-complete SCI [38–60,62–64,68–81], and found that more than half of these studies reported significant locomotor improvements after implementation of various treatments (Table 1). Common treatments used in studies of complete spinal cord transection research in general include implanting artificial neural scaffolds or matrices modified with drugs, proteins, numerous types of exogenous cells (e.g., olfactory ensheathing glia, neural stem cells, mesenchymal stem cells, and human oral mucosa stem cells), and/or other substances. These studies support the growth of specific transected motor axons including 5HT, ChAT, and TH-positive axons after injury (Table 1). However, most treatments post-complete SCI in the past ten years have either failed to promote robust CST axon growth into and across the lesion site or else do not report regeneration profiles of CST axons (Table 1). Early results indicate that only fetal spinal cord grafted into the lesion site results in modest supportive regeneration of CST fibers [82]. Recently, Kadoya et al., found that a small fraction (less than 20%) of CST fibers can regenerate at most 2 mm (Fig. 3D) into neural progenitor cell (NPC) grafts derived from embryonic day 14 (E14) rat spinal cord primordia at the site of T3 complete spinal cord transection [71]. However, the regenerated CST fibers could not across the lesion site and reach the caudal stump (Fig. 3D). In addition to fetal spinal cord grafts, a study from Yang et al. [60], claimed that a few CST fibers entered the lesion area from the rostral end, traversed the 5-mm lesion gap, and reentered the host caudal spinal cord at 1 month after the operation. Moreover, the same group recently reported that when the above mentioned NT3-loaded chitosan scaffold was inserted into a 1-cm gap of hemisectioned thoracic spinal cord of adult rhesus monkey, a few CST axons were detected to regenerate across the 1-cm-long lesion gap and re-enter into the caudal spinal cord [83]. However, more specific and solid labeling results need to be provided by different labs for better validation of credible and repeatable regeneration of CST fibers, especially such long

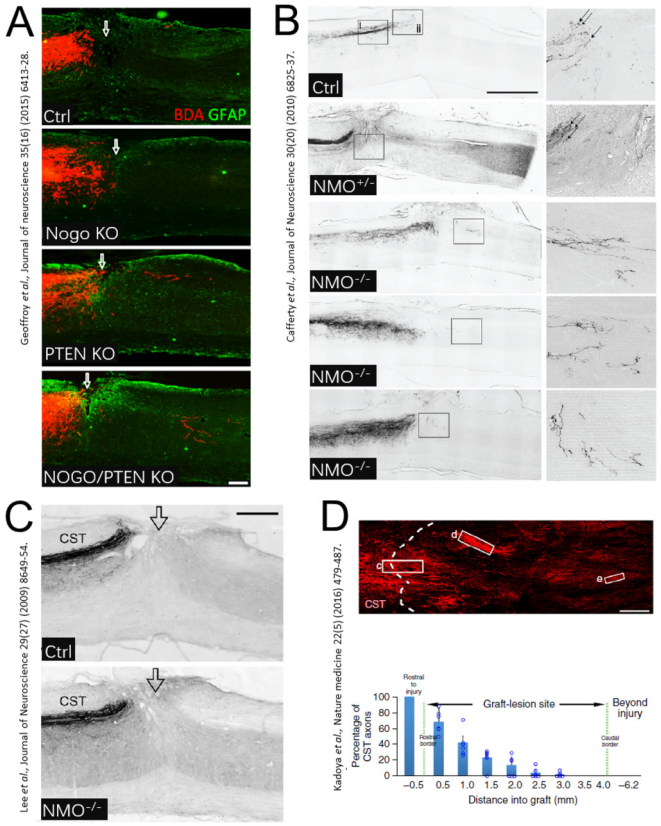

Fig. 3. Corticospinal tracts (CSTs) sprouting or regeneration profiles in rodents with incomplete and complete spinal cord injury (SCI) from different labs. (A) Geoffroy et al., re-assessed the effects of PTEN and Nogo single or codeletion on CST sprouting after a T8 dorsal hemisection. Their result showed that genetic deletion of either Nogo or PTEN promoted CST sprouting and codeletion of PTEN and Nogo could not further enhance CST sprouting [97]. Arrows indicate the lesion sites. Scale bar, 200 μm. (B) Cafferty et al., showed that the nogoabtrap/trapmag_/_omgp_/_ mice displayed more BDA labeled CST fibers in the lesion site than wild-type mice after a T6 dorsal hemisection [90]. Scale bar, 2 mm. (C) Lee et al., showed that deleting any one inhibitor of Nogo, MAG, and OMgp in mice enhanced CSTs sprouting but no synergistic effect of deleting all three inhibitors. Furthermore, triple-mutant mice failed to exhibit promoted CSTs regeneration following a dorsal hemisection injury [89]. Arrows indicate the lesion sites. Scale bar, 500 μm. (D) Kadoya et al., found that some CSTs were detected to regenerate 3 mm at least into neural progenitor cell (NPC) grafts derived from embryonic day 14 (E14) rat spinal cord primordia at the site of T3 complete spinal cord transection [71]. Scale bars, 240 μm. Quantification of the proportion of CSTs into the lesion site. The regenerated CSTs could not detected to reach the caudal stump in complete SCI model.

distance regeneration that never reported ever. To date, the current situation of CST regeneration research is still as reviewed by Tuszynski and Steward in 2012 [67]. A few studies claimed the observation of CST regeneration after complete transection have not stood the test of time and replication. Hence, CST regeneration is extremely difficult and there is little evidence from the literature supporting CST fiber regrowth through grafts or transplants following complete SCI transection. So far, we still believe that regeneration of CST axons across a complete spinal cord transection site remains a key goal of spinal cord regeneration research. Therefore, preparation or modification of ideal scaffolds that enable CST fiber regeneration into and across the lesion site is the immediate priority in SCI research to assess the exact role on fine motor movement recovery.

Besides the frustrating result of CST regeneration in regenerative medicine research field, basic researches focusing on CST regeneration by using the same animal background and injury model always show inconsistent, sometimes conflicting results. Earlier research found that Nogo-directed inhibitory signals exert significant influences on CSTs sprouting or regeneration [84,85]. As a result, many different laboratories have used Nogo-knockout mice to study CST regeneration and sprouting with a dorsal hemisected SCI model [86–89]. Unexpectedly, Nogo-deficient mice in different labs have shown various results including robust [86], moderate [87] to no obvious [88] CST regeneration. These varied results may be due to inconsistencies of injury severity in SCI models from different labs, which may lead to variable degrees of axonal fiber sprouting from uninjured CST [30]. Hereafter, two independent studies on CST regeneration in Nogo-A, B/myelin-associated glycoprotein (MAG)/oligodendrocyte-myelin glycoprotein (OMgp) triple mutant mice both observed CST sprouting to varying degrees (Fig. 3B and C) [90,91]. Nonetheless, improvements in motor function were not consistently observed between the two labs, and only Cafferty and coauthors reported locomotor recovery in triple mutant mice after injury.

Aside from Nogo, Liu and coauthors first discovered that specifically upregulating mechanistic target of rapamycin (mTOR) activity in corticospinal neurons by conditional deletion of phosphatase and tensin homolog (PTEN), a negative regulator of mTOR, promoted both compensatory sprouting of uninjured CST axons and successful regeneration of injured CST axons in a T8 crush injury model. Furthermore, these regenerating CST axons formed synapses in spinal segments below the lesion [92]. Thereafter, many studies confirmed that modulation of intrinsic PTEN/mTOR activity in corticospinal neurons may be an effective therapeutic strategy for promoting regenerative growth of CST axons and functional recovery after adult SCI [93–96].

Geoffroy et al., re-assessed the effects of PTEN and Nogo codeletion on CST sprouting and regeneration and found that genetic deletion of PTEN indeed promoted CST sprouting and regeneration and codeletion of PTEN and Nogo further enhanced CST regeneration but not sprouting (Fig. 3A) [97]. Additionally, their results also showed that the promoted CSTs sprouting was related to little or temporary improvement in locomotor recovery. Jin et al., found that conditional knockout of cortical suppressor of cytokine signaling 3 (SOCS3), a negative regulator of a cytokine-activated pathway, in the sensorimotor cortex promoted sprouting of uninjured CST axons to the denervated spinal cord. Further, CST sprouting quantity as well as skilled locomotion was significantly improved by synergetic PTEN deletion [98]. These discrepancies in locomotion have questioned the role that CST plays in rodent locomotor function, although differing locomotor recovery with different mutants might be related to inadequate CST fiber sprouting and connection with caudal targets.

Additionally, more regulatory proteins including the axon guidance molecule, ephrinB3, and its receptor EphA4 [99,100], Krüppel-like Factor 7 (KLF7) [101], signal transducer and activator of transcription 3 (STAT3) [102], conventional protein kinases C (cPKC) [103], transcriptional factor Sox11 [104], and the mTOR substrate, S6 Kinase 1 (S6K1) [105] were shown to influence CST axonal sprouting. Again, the amount of CSTs axons detected in the lesion site is very low (Fig. 3D), which indicate that robust regeneration of CSTs to across the lesion site were hard to realize by the existing methods or treatments. Next, the staining figures provided in a few studies [38,47,60] which claimed the observation of CST regeneration by implantation of various functional bio-scaffolds after complete SCI are far less convincible comparing to the above basic studies (Fig. 3). Therefore, the conclusion of long distance regeneration of CST fibers should be made with caution. Moreover, although the above researches have shown that CST axons can regrow through the lesion site after incomplete SCI in rodents, it is hard to conclude that there is regeneration of transected CST axons because in these assays, the SCI models used were not complete transection models but a crush model and dorsal hemisection injury model. Even though a spinal cord complete crush model was used to investigate CST regeneration, the possibility of spared CST tracts post-injury (especially ventrally located CST axons) still attracted much criticism and debate. Thus, the complete transection SCI model is still required to show

actual regeneration of CST, which is responsible for locomotor improvements.

5. Bio-scaffolds aided endogenous neuronal relays formation in the complete SCI model

Many therapies for the repair of complete SCI in rodents and dogs in the past decades have resulted in improved locomotor recovery (Table 1). More and more studies have hypothesized that instead of inducing motor axon regeneration, graft or endogenous neuronal relays formed within the injury site may play a major role in reconnecting transected long tracts and locomotor improvements [41–43,45,49,50,60,62,63,78,106]. Accordingly, it has been proposed that grafted or endogenous neural stem cell (NSCs) can be programmed to differentiate into multiple types of neurons to form neuronal relays in the lesion site, ultimately resulting in new synaptic connections, circuit formation, and improvements in motor function in animals with severe SCI [45,49,60].

For years, exogenous NSC/NPCs have been extensively used to treat SCI [107–112]. Accordingly, animal trials have confirmed that exogenous NSC/NPCs can differentiate into neural cells after transplantation in the injured spinal cord, and thereby compensate for neuronal loss, provide trophic support, restore connectivity, and improve functional recovery [49,113–115]. However, clinical translation of exogenous NSC/NPC therapy for SCI still faces enormous challenges. Firstly, despite the ethical concerns, the risk of neoplasm and teratoma formation with NPCs derived from human fetal tissue sources cannot be completely excluded. Secondly, autologous sources of adult NPCs are unavailable, thus patients receiving exogenous human NPCs will face immune rejection and require immunosuppression. Moreover, expanding adult human NPCs is more problematic regarding yielding sufficient numbers of cells for translation [116]. Last, but by no means least, the optimal source and dose of NPCs, route of administration, and style of graft are crucial but not at all clear [116]. The advantages, disadvantages, progress, perspectives, and problems of exogenous NSC/NPC grafts for neurological and locomotor recovery post-SCI have been well-documented in many reviews [106,108,109,111,117,118]. Therefore, here we only focus on the advances, perspectives, and challenges of directing endogenous NSCs by some well-designed bio-scaffolds for improvement of motor function in rodents and dogs post-complete transected SCI.

5.1. Endogenous neural stem cell sources for repair of complete spinal cord injury

Numerous studies have shown that ependymal cells (ECs), which line the central canal of the adult spinal cord, have NSC-like potential [119,120]. These cells remain quiescent in the normal state, but can be activated in response to mild and complete SCI, whereby they migrate to the lesion epicenter [120–123]. After cerebral ischemia or stroke, NSCs in brain show neuronal differentiation potential and can replace damaged neurons and repair neuronal connectivity [120,124–128]. However, most activated ECs tend to differentiate into glial fibrillary acidic protein $(GFAP)^-$ astrocytes, which account for 53% of new astrocytes generated at 4 weeks post-injury, and not neurons [124]. Further, 47% of newly-generated astrocytes are $GFAP^+$ and derived from activation of naive astrocytes, which accumulate around the epicenter and gradually transform into astrocytes that form dense glial scars, the main impediment to axonal regeneration over 2 weeks post-injury in mice [129,130]. Mechanistic information on the rare neuronal differentiation of ECs represents a lack of adequate interpretation. A possible reason may be that the complex injured microenvironment formed post-SCI interferes with neuronal lineage potential of proliferated ECs. Therefore, understanding altered environmental interactions on neuronal differentiation potential of ECs is an urgent problem that needs solved. However, it is also necessary to determine whether the intrinsic potential of ECs to generate neurons is already low.

Recently, nestin/brain lipid-binding protein (BLBP)-positively stained NSCs were identified in the central canal as well as normal spinal cord. Meanwhile, we defined the activation timeline of endogenous spinal cord-derived NSCs in rats with complete removal of the thoracic spinal cord at T8 [45]. Following complete transected SCI, nestin-positive NSCs were massively increased and peaked at 5 days post-injury (dpi) in adjacent uninjured spinal segments. The amount of nestin-positive NSCs at 5 dpi gradually decreased with distance from the lesion epicenter. Moreover, the characteristics of NSCs in the central canal and spinal cord were different. Injury-activated NSCs in the central canal were positively stained by the NSC markers, nestin and BLBP only, but not sex determining region Y-box 2 (Sox2). In contrast, ECs located in the central canal were nestin/BLBP/Sox2 triple-positive. If NSCs in the spinal cord are derived from proliferation of ECs in the central canal, this result indicates altered expression profiles of EC progeny during their migration out of the central canal towards the lesion epicenter. While if nestin/BLBP double-positive NSCs are not derived from nestin/BLBP/Sox2 triple-positive ECs, then the source of endogenous NSC/NPCs is diverse. The concrete lineage relationship between these NSCs and ECs still needs further verification, with definite lineage tracing assays performed.

Both our recent study and that by Yang et al., found that after complete transection SCI, a small fraction of nestin-positive NSCs spontaneously differentiate into neurons, with the neuronal differentiation rate of NSCs further promoted by drug application [45,60]. Altogether, if properly regulated, it is likely that endogenous spinal cord NSCs might be different from ECs and provide a promising cell source for repair of complete transection SCI.

As mentioned before, motor axon regeneration is hardly achieved post complete SCI transection. However, some purposely-designed bio-scaffolds could effectively facilitate neurogenesis in the lesion to form neuronal relays, which is considered as an important repair mechanism for the complete transected SCI. Therefore, we next discuss recent studies using specifically prepared functional bio-scaffolds for modification of endogenous NSCs to form neuronal relays reconnecting the lesion gap for locomotor and functional improvements in animals following complete transected SCI.

5.2. Tuning endogenous NSCs with different functional bio-scaffolds for functional and locomotor recovery post-complete SCI

At 5 days post-complete T8 removal in rat, most activated NSCs clustered in spinal tissue adjacent to transected stumps, and only some scattered NSCs were observed in the lesion center [45]. This is reasonable as the complete spinal cord transection model always leaves a gap of variable length, and the absence of specific guidance cues within the gaps is detrimental for migration as well as survival of injury-activated NSCs. As such, we designed a series of functional collagen-based bio-scaffolds and implanted them into the lesion gap to reconnect the transected stumps. These functional bio-scaffolds act not only as a physical platform for bridging the lesion gap after complete spinal cord transection or scar tissue resection, but also provide guidance cues for programming endogenous NSCs and their progeny to participate in regeneration, with functional and locomotor improvements.

As activated NSCs post-complete SCI express EGF receptor (EGFR), we modified collagen scaffolds with the clinical drug, cetuximab (an antibody of EGFR), and implanted these functional scaffolds into the lesion site of rats. At 15 dpi, although approximately equal amounts of NSCs were observed in both rostral and caudal stumps of differently treated groups, NSC density was significantly greater in the lesion center of the functional scaffold implantation group compared with the other groups [45]. This indicates that injury-activated NSCs do not spontaneously migrate into the lesion center, and implantation of optimally modified bio-scaffolds are capable of directing specific migration routes of endogenous NSCs by integrating bioactive factors within

a reasonable time–space distribution and specific release patterns.

Through implantation with a cetuximab-modified collagen scaffold after complete T8 removal injury in rat, we not only identified increased neurogenesis from endogenous injury-activated NSCs within the lesion site but also substantiated that endogenous neurogenesis can be achieved in a complete SCI transection model of a large animal (dog). Likewise, more mature neurons were observed within the lesion area of dogs at 9 months post-cetuximab-modified scaffold implantation [45]. Our results in rats and dogs convincingly demonstrate that implantation of cetuximab-functional collagen scaffolds can enhance neuronal differentiation in endogenous injury-activated spinal cord NSCs in complete transected SCI models. Moreover, in dogs at 9 months post-implantation, differentiated neurons that regenerated in the lesion area matured into distinct functional neurons (such as serotonergic, cholinergic, and dopaminergic neurons) in lesion sites exhibiting myelinated axon fibers and synaptic structures. Meanwhile, canines with cetuximab-functional collagen scaffold implantation showed partial restoration of motor evoked potential responses and significantly improved hindlimb locomotor function [45]. Thus, we proposed a hypothesis that differentiated neurons from endogenous injury-activated NSCs might form neuronal relays, which re-bridge the lesion gap and may eventually lead to improved motor function after complete transected SCI.

Coincidentally, the study of Yang et al. (2015) also showed that neurotrophin-3 (NT-3) administration can successfully lead to neuronal relay generation in the lesion site, with subsequent recovery of motor function from nestin$^+$ endogenous NSCs [60]. They found that implantation of NT3, but not EGFR inhibitor-coupled chitosan biomaterial (and not collagen scaffold) into a 5-mm gap of a complete transected rat SCI model effectively caused NSCs to migrate into the lesion area, differentiate into neurons, and result in recovery of sensory and motor behavior. Nevertheless, the staining methods of these two studies only show neuronal differentiation ability of injury-activated spinal cord NSCs, and failed to determine the precise ratio of NSC-derived neurons to native spinal neurons in the lesion site. Thus, a cell fate mapping study is needed. Consequently, we generated nestin–cre reporter mice to address this question in our recent study. This study also verified our results and postulation that injury-activated NSCs can differentiate into neurons after complete SCI in adults, and the quantity of neuronal lineage from NSCs can be effectively regulated by external growth factor application. Altogether, these studies collectively demonstrate that programming endogenous neuronal relay formation in the lesion gap from injury-activated NSCs via functional collagen scaffold implantation for regenerative repair and locomotor improvement post-severe SCI is more feasible and practical than facilitating axon (or CST) regeneration.

Except for naturally incubating cetuximab with the bio-scaffold to promote insufficient migration of endogenous NSCs into the lesion center post-complete SCI, we also altered the binding style between drug and scaffold to provide a specific and constant guidance cue and achieve higher migration efficiency. It is reported that in normal and injury cord, ependymal cells as well as their injury-activated progenies are able to express CXCR4 [131]. Stromal cell-derived factor-1α (SDF1α) can enhance recruitment of endogenous CXCR4 positive cells to the lesion site via long-range migration [132]. We thus fused SDF1α with the heptapeptide TKKTLRT (also known as collagen-binding domain [CBD]) to ensure SDF1α protein more specifically binds to the collagen scaffold. Moreover, we also created a continuous gradient of SDF1α on an aligned collagen scaffold which was achieved by adjusting the collector size and collection time during electrospinning, with the entire process performed in a controllable and reproducible fashion [133]. The aligned CBD–SDF1α gradients were maintained on the scaffold for 7 days, indicating long-term presence of CBD–SDF1α on the collagen scaffold. Subsequent *in vitro* NSC culture assay showed that the CBD–SDF1α gradient collagen scaffold could direct efficient NSC migration from the periphery to the center of the culture dish along the aligned electrospun collagen fibers. Our results also show the promising potential of CBD–SDF1α-modified collagen scaffolds for constant induction and guidance for migration of endogenous NSCs after implantation into the lesion gap post-complete SCI.

Successfully inducing directional migration of endogenous spinal cord NSCs into the lesion site is only the first step in programming NSCs to form neuronal relays for locomotor and functional improvements following complete transected SCI. Neuronal differentiation, maturation, and functional connectivity of migrated NSCs and their neuronal progeny in the lesion gap are the subsequent barriers to be overcome. Myelin-associated inhibitors (MAIs) are the main components constituting the injury microenvironment within the lesion gap, and play an inhibitory role in axonal regeneration [134]. We previously discovered that MAIs can influence NPC differentiation rate *in vitro* [61,135]. As MAIs activate EGFR signaling by influencing intracellular calcium influx [8,136], EGFR activation post-SCI might influence both axonal regeneration and NSC differentiation profiles. Our previous study found that addition of an EGFR-neutralizing antibody (cetuximab) reverses the lowered neuronal differentiation rate of cultured NSCs due to addition of myelin proteins *in vitro*. Moreover, cetuximab administration markedly promoted neuronal differentiation and reduced astrocytic differentiation rate of grafted NSCs in a rat hemisection SCI model [61]. As previously described, at 5 days post-complete T8 SCI transection in rats, most activated nestin-positive NSCs also show activated EGFR signaling with phosphorylated EGFR (pEGFR) expression [45]. We isolated these NSCs from the spinal tissue adjacent to the lesion site at 5 dpi and found that the cultured NSCs could generate into multipotent neurospheres, which gave rise to astrocytes and neurons in normal differentiation medium. Accordingly, neuronal differentiation rate of cultured NSCs decreased when CNS myelin extracts were included in the differentiation medium. Cetuximab in the medium successfully rescued the reduced neuronal differentiation rate induced by myelin extract [45].

To achieve long-term sustained released of cetuximab from the collagen scaffold post-implantation, we constructed a collagen-specific binding EGFR antibody (CBD–Fab) by fusing the Fab fragment of cetuximab with a collagen binding domain (CBD), similar to CBD-SDF1α. After confirming that the biological activity of CBD–Fab showed no significant decrease once fused with CBD, the CBD–Fab modified collagen scaffold was implanted into complete transected SCI rats. We found enhanced adhesion, retention, and neuronal differentiation of NSCs, as well as decreased glial scar deposition and improved motor function [42,43]. Moreover, when CBD–Fab-modified collagen scaffolds were seeded with exogenous NSCs and grafted into lesion sites of complete transected SCI rats, both exogenous grafted and endogenous injury-activated NSCs were effectively captured and retained on the functional collagen scaffold. Increased neuronal differentiation of both exogenous and endogenous NSCs was observed [43]. Recently, when collagen binding neurotrophin-3 modified multichannel Poly (propylene fumarate) and collagen dual scaffolds were implanted into a T8 complete transection rat model, the combinatorial treatment could also facilitate neuronal regeneration and functional improvement following SCI [137]. Moreover, one of our soon-to-be-published research demonstrated that when neurotrophin-3 modified collagen scaffolds were utilized to repair the acute thoracic (T9) complete transection model in non-human primate, the enhanced endogenous neurogenesis was also successfully observed in NT3-scaffold treated rhesus monkeys.

Altogether, collagen scaffolds can be functionally modified to facilitate effective neuronal relay formation from endogenous NSCs and reconnect the lesion gap, eventually resulting in improved motor function recovery (Fig. 4).

Our recent clinical trial on implantation of human umbilical cord mesenchymal stem cells (hUC-MSCs) modified collagen scaffolds into acute complete spinal cord injury patients also observed recovery of the sensory and motor functions similar to that in the above-mentioned animal models. The two acute SCI patients, respectively injured at

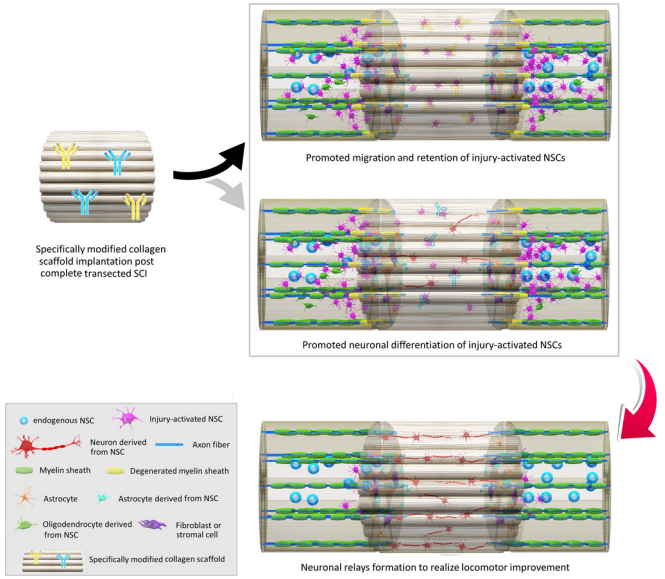

Fig. 4. In situ modulation of neuronal relays formation from injury-activated NSCs by functional bio-scaffolds implantation in promoting locomotor outcome in animals after complete SCI. At the acute and subacute stages post complete spinal cord transection, amount of injury-activated endogenous NSCs are presented in the rostral and caudal stumps of the transected cord. Implantation of specifically modified bio-scaffolds into the lesion gap showed great effects on modulating the directed migration and neuronal differentiation of NSCs. The well-designed bio-scaffolds could eventually facilitate functional reconnection and integration of the neurons derived from injury-activated NSCs with the transected motor axons to realized motor function recovery.

thoracic 11 and cervical 4 level, were firstly judged as functional complete SCI by comprehensive diagnosis including magnetic resonance imaging (MRI), American Spinal Injury Association (ASIA) Impairment Scale, and neuroelectrophysiological examination [138]. Then, hUC-MSCs modified collagen scaffolds were transplanted into the lesion site. During 1 year follow up, both sensory and motor functional improvements of the two patients were observed. The two patients regained the bowel and bladder sensations. Furthermore, the T11 complete SCI patient is able to walk voluntary under the help of brace and the C4 injured patient can raise his lower legs against gravity and shake his toes under conscious control. The sensory and motor functional improvements of the two patients were accompanied by the recovery of the neuronal electrophysiological response. These results suggested that functional scaffolds transplantation is effective for acute complete SCI patients. However, whether the mechanism of functional improvements depends on axonal regeneration or neuronal relays formation remains to be confirmed.

6. Perspectives and challenges

During the last decade, many studies have recognized that blocking extracellular inhibitory influences alone is insufficient for enabling the majority of injured axons or long tracts to regenerate, and alternatively, there are important cellular and molecular mechanisms within neurons that govern axonal regeneration [9,10,12,15,139–142]. By regulating intracellular signaling pathways and networks that control axonal regeneration, some strategies have shown therapeutic effects on promoting axonal regeneration, neuronal circuit assembly, and functional recovery after adult mammalian SCI. However, to our current knowledge, none of the currently available combinatorial strategies perfectly foster long-distance regeneration of motor axons (such as CST) to pass through the lesion gap in a complete transected SCI model. Although CST axons are highly refractory to regeneration, facilitating lengthy regrowth of CST in the complete transection SCI model is difficult to achieve. Thus, long-term efforts should be made to improve the intrinsic growth ability of corticospinal motoneurons and regain reduced or lost regenerative ability of mature CST axons after complete SCI. As CST projections are one of the most important motor projections for human voluntary motor function, it might only be by regenerating enough amount of CST fibers throughout the lesion gap and making accurate reconnections with the original caudal targets that a breakthrough in observed locomotor functional recovery can be achieved. Additionally, any other substitutes developed or fabricated to re-bridge the lesion gap post-complete SCI might not be optimal for fulfilling the role of CST for locomotor recovery.

Currently, instead of only pursuing CST regeneration for recovery of motor function, programming endogenous NSCs might be a practical and feasible alternative strategy. Although complete SCI brings massive neuronal loss, disrupted neural circuitry, and impaired motor function, it also triggers robust activation of diverse, generally dormant, endogenous spinal cord NSCs, which provide a plentiful cell source for functional regeneration if they (and their progeny) are purposely regulated within the lesion site. Therefore, injury-activated endogenous NSCs are a potential therapeutic cell source for SCI repair, with more advantages than any exogenous grafted cells. Thus far, however, the matter is not so straightforward.

It is well documented that endogenous spinal cord NSCs (including ECs) within the adult spinal cord can initiate robust proliferation in response to SCI [143]. Few studies have validated the reasons or inducing factors responsible for activation of adult spinal cord NSCs and their subsequent migration towards lesion borders, let alone the mechanisms underlying activation and migration processes of NSCs. Meanwhile, multiple derivation of NSCs and EC heterogeneity in the adult spinal cord suggests that endogenous spinal cord NSCs are complex and diverse. Furthermore, activation-inducing factors, signaling pathways, and time courses of different endogenous NSCs post-injury might be distinct. Thus, it is crucial to examine and determine the respective proliferation and migration features of stem cells in varied populations, as the characteristics of different spinal cord NSCs will help build, improve, and optimize bio-scaffold-based repair strategies for complete SCI repair.

The differentiation capacities and profiles of different endogenous NSCs post-injury might also vary greatly. It is known that even adult ECs display NSC-like properties in culture, with multipotency to generate astrocytes, oligodendrocytes, and neurons *in vitro* [128]. Nonetheless, they do not give rise to neurons post-SCI. Further, ECs exhibit quite different *in vivo* differentiation profiles compared with the neuronal differentiation capacity-bearing NSCs we discovered [45]. The main purpose for regeneration after complete SCI is reconnection of interrupted neuronal circuits. Consequently, if the element needed to re-bridge the lesion gap is neuronal relays formed throughout the lesion site, more knowledge on the differentiation capacity and profile of different endogenous NSCs post-injury, as well as the factors and signaling pathways directing neuronal differentiation capacity of diverse spinal cord NSCs is an urgent unmet goal. Implanting a modified scaffold into the lesion site to construct a suitable differentiation microenvironment effectively modulates NSCs to produce more neurons and oligodendrocytes after complete SCI, which in turn directly influences neurogenesis, remyelination, and neuronal relay formation in the lesion gap.

Sometimes even when sufficient neuronal relays form within the lesion gap to reconnect transected neural circuits, and after modulating robust neurogenesis and adequate axonal myelination, the locomotor function results are unexpectedly paradoxical. For example, Lu et al. (2012) found promotion of neuronal relays across the lesion site with synapse formation in neurons below the lesion site after acute severe spinal cord complete transection. However, locomotor outcome was surprisingly worsened. Indeed, some studies on facilitating axonal regeneration have shown that improved CST regeneration also directly results in worsened locomotor function [48,144]. Reasons underlying

this paradox of promoted reconnection of the lesion gap resulting in worsened motor function outcome may include formation of incorrect synapses by regenerated axons with downstream targets below the lesion site. Thus, facilitating correct and effective neuronal relays and appropriate axonal reconnections with the original downstream targets should be considered essential when designing treatments for complete SCI animals.

Nowadays, many researchers tend to agree that a multi-disciplinary approach is needed to solve SCI repair. Recently, electrical stimulation has been successful as a treatment approach for SCI [145–148]. Wagner et al. demonstrate recently that epidural stimulation enabled three spinal cord injured patients to regain adaptive control of paralysed muscles. Moreover, two patients were able to walk in their communities using a wheeled walker in the absence of epidural stimulation after a few months [148]. However, these participants are not complete SCI patients with American Spinal Injury Association (ASIA) Impairment Scale at grade C or D. Similarly, Angeli et al. also reported that two ASIA grade B patients achieved over-ground walking after 278 sessions of epidural stimulation of the lower spinal cord and a long period intensive rehabilitation [147]. However, for some complete SCI patients, the story is a little different. There are another two ASIA grade A patients in Angeli et al. study who could only gain some components of independent stepping on the treadmill with body-weight support but not over-ground walking. Unlike the results reported by Wagner et al., all the four participants (two patients with ASIA grade A and B, respectively) in the trial of Angeli et al. fail to continue walking in the absence of stimulation. The results indicated that epidural stimulation showed different effects on performance of standing and stepping with different SCI patients. More importantly, epidural stimulation alone is not enough to achieve motor functional recovery, and intensive physical training is an essential part to enable the above SCI patients to walk again. In summary, the underlying mechanisms for electrical stimulation aided locomotor recovery following complete and incomplete SCI is poorly understood. Meanwhile, whether the mechanism for motor and sensory improvements resulted from electrical stimulation and functional scaffolds transplantation is also largely unclear. Therefore, further research is needed to determine that it is axon regeneration and/or new circuits formation in the lesion site that contribute to the improved functions of complete SCI patients.

Data availability statement

The data are available from the corresponding author on reasonable request.

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