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Building the Regenerative Microenvironment with Functional Biomaterials for Spinal Cord Injury Repair

Zhifeng Xiao#, Bing Chen# and Jianwu Dai*

State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, PR China
#These authors contributed equally

Abstract

Spinal cord injury (SCI) is a devastating injury resulting in changes in the spinal cord's motor, sensory, or autonomic functions. Following SCI, an inhibitory environment develops at the injury site for neural regeneration. In this review, we summary the strategies to rebuild the regenerative microenvironment with functional biomaterials for SCI repair mainly based on our research. We have developed a functional biomaterial consisting of collagen scaffolds and biologically active molecules (neurotrophic factor or the antagonists to myelin-associated inhibitor), and stem cells to rebuild a nerve regeneration microenvironment. Specifically, (1) the linear ordered collagen scaffold (LOCS) was used to guide the neural regeneration along its fibers and decrease the formation of glial scars, (2) collagen binding neurotrophic factors were incorporated into the scaffolds to promote neuronal survival and neural fiber regeneration, (3) antagonists to myelin-associated inhibitors were added to the scaffold to direct the neuronal differentiation of the native or transplanted neural stem cells at the injury site, (4) mesenchymal stem cells (MSCs) were also added to the scaffold to reduce the acute inflammatory response due to SCI. These strategies were found to promote neural regeneration and functional recovery in SCI animals. In addition, the endogenous neural stem cells (NSCs) or implanted NSCs could be differentiated into neurons, which re-established the neuronal circuits to improve SCI repair under the favorable environment.

Keywords: Spinal cord injury; Collagen scaffold; Functional biomaterial; Regenerative microenvironment

Introduction

Spinal cord injury (SCI) is a devastating injury to the spinal cord resulting in the damage of the cord's motor or sensory functions [1]. Currently, repair after SCI is still a huge challenge because of the inhibitory regenerative environment at the injury site. SCI is the most often traumatic, mainly caused by trauma such as car accidents, falls, and sports injuries. Traumatic SCI often initiates a cascade of biochemical reactions. First, following the initial traumatic insult, a variety of inflammatory and cytotoxic mediators is released at the injured site, resulting in secondary damage to the spinal cord. This will lead to continued and pervasive cell death and tissue damage [2,3]. Second, the residing astrocytes become hypertrophic in response to SCI, and the reactive astrocytes produce chondroitin sulfate proteoglycans (CSPGs) and form a dense scar at the injury site. The glial scar can protect intact neural networks from further damage; however, it also serves as an impediment for regenerating axons attempting to reach their distal targets [4-6]. Third, following injury, the myelin is disrupted locally where the axons degenerate, leaving large amounts of inhibitory materials. The myelin-associated inhibitors, such as Nogo-A, MAG, and OMgp, inhibit neural regeneration through Nogo-66 Receptor-1 (NgR1) and Paired-Immunoglobulin-like-Receptor-1(PirB). In addition to myelin-associated inhibitors, several members of the axon guidance molecules expressed by oligodendrocytes have also been implicated to play adverse roles in CNS axon regeneration, such as ephrinB3 and semaphrin4D [7,8]. Taken together, these factors make up an inhibitory environment following SCI for neural regeneration. As a result, the nerve connections between the brain and the spinal cord are interrupted, which result in the loss of sensation and movement function of the body. Thus, building a regenerative microenvironment is essential for spinal cord injury repair. In this review, we summary the strategies to rebuild the regenerative microenvironment with functional biomaterials for SCI repair mainly based on our research. Functional biomaterials consisting of collagen scaffolds and biologically active molecules (neurotrophic factor or the antagonists to myelin-associated inhibitors), as well as stem cells, are then being designed to rebuild a nerve regenerative microenvironment.

Collagen Scaffolds for Neural Regeneration

A neuronal regenerating scaffold should provide a physical support for axon extension and should be nontoxic and nonirritating. Collagen has low antigenicity and excellent biocompatibility and biodegradability. Different types of collagen scaffolds have been tested to repair SCI in animal models, including collagen tubes, fibers, membranes, and gels. They showed that collagen scaffold was a suitable biomaterial for guiding neural regeneration [9-11]. Yoshii reported that when collagen filaments were grafted to the axis of the spinal cord, it supported the axonal regeneration and the restoration of function in adult SCI rats [10]. Liu used collagen tube to guide axonal regrowth, they found that the spinal axons could regrowth into the caudal sectioned to reconnect ventral roots in hemisected adult rat spinal cord [11]. Recently, Liu developed electrospun collagen nanofibers and demonstrated the potential use of these scaffolds for SCI repair in rat hemi-section model [12]. We prepared a novel type of collagen nerve guidance material from the bovine aponeurosis, which mainly consists of ordered collagen fibers. The processed material could guide the neurite outgrowth along its fibers [13]. The linear ordered collagen scaffold was named LOCS. When LOCS was transplanted into hemisected or transected SCI animal models (rats and canines), the neural fibers grew along the direction of the LOCS [14-16]. It is well known that a dense glial scar was formed at the injured site following SCI, and the glial-derived chondroitin sulfate proteoglycans (CSPGs) within the glial scar form a barrier to axonal regrowth and sprouting after SCI [4-6]. It was reported that antagonizing the CSPG signaling pathway could induce

***Corresponding author:** Jianwu Dai, Institute of Genetics and Developmental Biology, ChineseAcademy of Sciences, 3 Nanyitiao, Zhongguancun, Beijing 100190, PR China, Tel: 86-10-82614426; Fax: 86-10-82614420; E-mail: jwdai@genetics.ac.cn

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the regeneration of serotonergic system, and promote the functional recovery of locomotor and urinary systems [17]. However, we found when only LOCS was transplanted into complete transected SCI model in rat, LOCS could induce a significant decrease in the density of astrocytes (GFAP staining) surrounding the lesion site [15]. The same result was also found in canine SCI, the accumulation of CSPGs at the injury site decreased significantly in LOCS-treated group (unpublished data). The results from the rat and canine SCI models suggest that LOCS could decrease the formation of glial scar. Accordingly, LOCS transplantation alone could promote functional recovery after SCI [14-16]. These data suggest that LOCS is a suitable neural scaffold to build regenerative microenvironments for guiding neural regeneration.

Collagen Binding Neurotrophic Factors to Promote SCI Repair

Neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3), and neurotrophin 4/5 (NT- 4/5), have been proved to promote neuronal survival or regeneration of neural fiber in the central nervous system [18-20]. However, when growth factors were used at the injury site in practice, it is difficult to maintain the therapeutic concentrations because these factors rapidly diffuse away from the application site. Different method was used to inhibit the diffusion of growth factors. Yang incorporated NGF into the conduit by mixing or encapsulating the protein with microspheres, a sustained release was detected and NGF retained its bioactivity [21]. Chang reported that nerve growth factor was sucked into polycaprolactone (PCL) conduits by using genipin as a crosslinking agent. The NGF showed pulse releasing and steadily releasing, which promoted nerve regeneration in a 15-mm rat sciatic nerve defect model [22]. Zhao produced the sustained-release microspheres using the polylactic-co-glycolic acid copolymer, which contained NGF, NT-3 and BDNF, the microspheres could sustain the release of neurotrophic factors *in vitro* and promote sciatic nerve repair after injury [23]. In our study, a collagen-binding peptide was fused with them, and the fusion proteins acquired the ability to bind specifically to collagen, which could prevent the rapid diffusion of growth factors at the target sites [24,25]. BDNF has positive effects on neuroprotection and neural regeneration. As one of the best characterized growth factors, BDNF plays an important role in CNS development and repair, such as in early-phase long-term potentiation, neural survival, differentiation, and synaptogenesis [26]. Using the rat hemisaction SCI model, we found that when LOCS loaded with CBD-BDNF was transplanted into the injury site, it significantly improved the functional recovery of locomotion system and induced axonal regeneration along with the collagen scaffold [14]. In addition, when the functional biomaterials were implanted into completely transected canine SCI, LOCS + CBD-BDNF transplantation significantly promoted locomotion and sensory functional recovery, some dogs could stand unassisted and walk transiently. Furthermore, the transplantation of LOCS + CBD-BDNF induced the significant functional recovery by reduction of lesion volume, decreasing of the scar deposits, inducing neural regeneration and improving axonal myelination. In summary, LOCS + CBD-BDNF transplantation showed a striking therapeutic effect on completely transected canine SCI models, and this is the first report of such a great progress in a completely transected large animal model with long-term (38 weeks) observation [16].

Incorporation of Antagonists to Myelin-associated Inhibitors to the Scaffolds

Following injury, the myelin is disrupted locally, leaving large amounts of inhibitory materials. The inhibitory activity of MAG, OMgp, and the extracellular domain of Nogo-A (Nogo-66) is mediated by the common receptor complexes, the receptor complexes were composed of NgR (ligand-binding Nogo-66 receptor) and its signaling co-receptors p75/TROY and Lingo-1 [7,8]. Signals induce elevation of the intracellular calcium level and then activate epidermal growth factor receptor (EGFR). Thus, EGFR was transactivated by myelin-associated inhibitors, which is the downstream of NgR receptor signaling [27]. Erschbamer reported that local infusion of an irreversible EGFR inhibitor, PD168393, onto the damaged area could lead to functionally recovery of hindlimb function accompanied by improved sensory function in contusion SCI rats [28]. Li demonstrated that EGFR inhibitor, PD168393, decreased reactive astrogliosis and proinflammatory cytokine secretion of reactive astrocytes *in vitro*. When PD168393 was used in the injured area of a traumatic SCI, it suppressed CSPGs production and glial scar formation, resulting in hindlimb motor function and bladder improvement [29]. A monoclonal antibody, 151IgG, which was a direct competitive inhibitor of EGFR kinase activity, was used to block EGFR in our research. 151IgG was cross-linked to LOCS, and then CBD-BDNF was added to make a triple-functional biomaterial to induce neural regeneration, which could bridge the gap, neutralize the growth inhibitor and promote the neural growth. When tested in a 6-mm transected SCI model, most NF-positive fibers were consecutive and parallel in the direction along with LOCS, and the length of most fibers was above 500 μm. Spinal somatosensory evoked responses (SSERs) were significantly restored [15].

In addition to myelin-associated inhibitors, several axon guidance molecules expressed by oligodendrocytes have also been implicated to play adverse roles in CNS axon regeneration, such as ephrinB3 and semaphrin4D.CBD-EphA4LBD and CBD-PlexinB1LBD were produced to bind specifically to the collagen scaffold and neutralize the inhibitory effect of the axon guidance molecules ephrinB3 and sema4D. Their effect on promoting neurite outgrowth of cerebellar granular neurons and dorsal root ganglion neurons were tested *in vitro*. Subsequently, when functionalized collagen scaffolds, consisted of LOCS, NEP1-40, CBD-EphA4LBD and CBD-PlexinB1LBD, were transplanted into T10 complete removal SCI model, results showed that rats transplanted with the functional collagen scaffold displayed great therapy effects on SCI by inducing neuronal regeneration and locomotion recovery [30].

Directing Neuronal Differentiation of Neural Stem/Progenitor Cells

Neural stem/progenitor cells (NPCs) are a valuable cell source for the therapy of injuries in the central nervous system (CNS). However, when NPCs are transplanted into the adult mammalian spinal cord, they rarely differentiate into neuronal lineage. The results have also been detected for endogenous NPCs during spinal cord injury [31-33]. We have first identified that myelin protein and Nogo-66 could inhibit the differentiation of NPCs into the neuronal lineage and promote its differentiation into the glial lineage. The NgR and mTOR-Stat3 pathways were involved in this process [34]. An epidermal growth factor receptor (EGFR) neutralizing antibody, cetuximab, was used to inhibit the downstream signaling activated by myelin-associated inhibitors, the collagen loaded with cetuximab was found to antagonize the effect of myelin-associated inhibitors. When NPCs exposed to myelin proteins *in vitro,* it significantly enhance the neuronal differentiation of NPCs. Furthermore, when functional biomaterials consisted with LOCS, cetuximab and NPCs were implanted into the 4-mm-long hemisaction lesion of rats, it induced neuronal differentiation significantly and decreased astrocytic differentiation of NPCs and eventually promoted functional recovery. Thus, a well-functionalized scaffold was developed,

which could promote the neuronal differentiation of neural progenitor cells and improve the recovery of SCI *in vivo* [35]. Lu P reported that when NSCs were transplanted to sites of severe SCI combined with fibrin matrices containing growth factor cocktails, grafted cells differentiated into neurons, which extended large numbers of axons to form abundant synapses with host cells. Grafted neurons supported long-distance connectivity, resulting in functional recovery. The same results also observed when human induced pluripotent stem cells were transplanted into the injured site after spinal cord injury in rats [36-37].

Using Mesenchymal Stem Cells to Improve the Regenerative Microenvironment

Mesenchymal stem cells (MSCs) are self-renewing, multipotent progenitor cells with the capacity to differentiate into several distinct mesenchymal lineages. MSCs have recently been considered as a promising source for cellular repair after SCI. MSCs can synthesize a number of neurotrophic cytokines, including brain-derived neurotrophic factor, NGF, and vascular endothelial growth factor (VEGF), which have neuroprotective and growth-promoting effects after SCI; Furthermore, there is increasing evidence that MSCs may be immunosuppressive. The immunosuppressive properties may combine to reduce the acute inflammatory response to SCI and hence reduce cavity formation and decrease astrocyte and microglia/macrophage reactivity [38,39]. It was reported that MSCs could play positive immunomodulatory and neurotrophic effects to promote SCI repair. MSCs could exert its effect on both immune cells and neural cells simultaneously. It prevented macrophage-mediated axonal dieback, and promoted neural regrowth to overcome the negative effects of inhibitory proteoglycans [40]. Thus, MSCs may promote axonal regeneration or encourage functional plasticity by establishing a regenerative environment. Transplantation of MSCs has been reported to promote successful functional outcome in animal models of SCI, and several small clinical trials were performed to investigate the efficacy and safety of MSCs in SCI. Our recent work showed that LOCS with MSCs implants strikingly promoted locomotion and functional recovery after completely transected SCI in the canine with multisystem rehabilitation. Further histological analysis showed that the transplantation of LOCS with MSCs decreased scar formation. In addition, when LOCS with MSCs were implanted into SCI canine, there are more neurons and synapse formation in the lesion sites in the LOCS with MSCs group than in the control group at 36 weeks after SCI (unpublished data). It suggests that the newborn neurons could form new synaptic connections in the lesion area and contribute to the functional recovery after SCI.

Mechanisms of Spinal Cord Injury Repair

It is widely accepted that the existence of regeneration inhibitory environments such as myelin-associated inhibitors and reactive glia scars in the lesion site, which impeded neural regeneration after SCI (Figure 1A and 1B). It has also long been recognized that axonal regeneration is the main way to restore function after severe SCI in which the long descending and ascending tracts were interrupted. Many efforts have been made to induce long axonal regeneration, but there were few evidences to support corticospinal tract (CST) growth into grafts or transplants [41]. Beside reconnection of the injured pathway by guiding axonal regrowth, we reported that the functional biomaterial consisted of the collagen scaffold and cetuximab, an antagonist to myelin-associated inhibitor, markedly promoted neuronal differentiation and decreased astrocytic differentiation of transplanted NSCs in SCI rats [29]. Our recent work discovered that the collagen scaffold loaded with cetuximab or MSCs increased neuronal differentiation of the endogenous neural stem cells to produce different types of neurons throughout the lesion area (unpublished data). It suggest that these newly generated relay neurons may further rebuild the synaptic connections with each other or with the host spinal neurons to improve locomotion outcomes in the completely transected SCI canine. We thus propose a model that under a favorable environment, the endogenous neural stem cells or implanted neural stem cells could be differentiated into neurons, form functional neuronal circuits to improve spinal cord injury repair (Figures 1C and 1D). We believe that rebuilding neuronal relays would be a more efficient way to repair SCI comparing with inducing axonal regrowth.

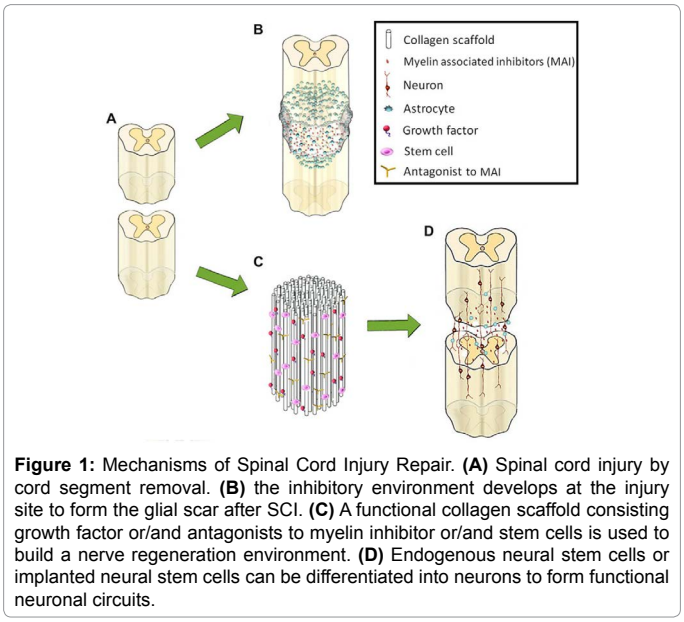

Figure 1: Mechanisms of Spinal Cord Injury Repair. **(A)** Spinal cord injury by cord segment removal. **(B)** the inhibitory environment develops at the injury site to form the glial scar after SCI. **(C)** A functional collagen scaffold consisting growth factor or/and antagonists to myelin inhibitor or/and stem cells is used to build a nerve regeneration environment. **(D)** Endogenous neural stem cells or implanted neural stem cells can be differentiated into neurons to form functional neuronal circuits.

Conclusion

Following SCI, an inhibitory environment for neural regeneration develops at the injury site. A regenerative microenvironment was made by the functional biomaterial to promote neural regeneration and functional recovery after SCI. The functional biomaterial may decrease glial scar formation, guide neural fibers regenerating along the direction of the LOCS, and induced neuronal differentiation of neural stem cells. Rebuilding neuronal relays by newborn neurons induced from endogenous neural stem cells or implanted neural stem cells could be a major mechanism for SCI repair.

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Competing Interest

The authors have no potential conflicts of interest.

References

1. Tator CH (2006) Review of treatment trials in human spinal cord injury: issues, difficulties, and recommendations. Neurosurgery 59: 957-982.
2. Hausmann ON (2003) Post-traumatic inflammation following spinal cord injury. Spinal Cord 41: 369-378.
3. Singh PL, Agarwal N, Barrese JC, Heary RF (2012) Current therapeutic strategies for inflammation following traumatic spinal cord injury. Neural Regen Res 7: 1812-1821.

4. Cregg JM, DePaul MA, Filous AR, Lang BT, Tran A, et al. (2014) Functional regeneration beyond the glial scar. Exp Neurol 253: 197-207.

5. Silver J, Miller JH (2004) Regeneration beyond the glial scar. Nat Rev Neurosci 5: 146-156.

6. Lukovic D, Stojkovic M, Moreno-Manzano V, Jendelova P, Sykova E, et al. (2015) Concise review: reactive astrocytes and stem cells in spinal cord injury: good guys or bad guys? Stem Cells 33: 1036-1041.

7. Fawcett JW (2006) Overcoming inhibition in the damaged spinal cord. J Neurotrauma 23: 371-383.

8. Akbik F, Cafferty WB, Strittmatter SM (2012) Myelin associated inhibitors: a link between injury-induced and experience-dependent plasticity. Exp Neurol 235: 43-52.

9. Stang F, Fansa H, Wolf G, Keilhoff G (2005) Collagen nerve conduits-assessment of biocompatibility and axonal regeneration. Biomed Mater Eng 15: 3-12.

10. Yoshii S, Oka M, Shima M, Taniguchi A, Taki Y, et al. (2004) Restoration of function after spinal cord transection using a collagen bridge. J Biomed Mater Res A 70: 569-575.

11. Liu S, Said G, Tadie M (2001) Regrowth of the rostral spinal axons into the caudal ventral roots through a collagen tube implanted into hemisected adult rat spinal cord. Neurosurgery 49: 143-150.

12. Liu T, Houle JD, Xu J, Chan BP, Chew SY (2012) Nanofibrous collagen nerve conduits for spinal cord repair. Tissue Eng Part A 18: 1057-1066.

13. Lin H, Chen B, Wang B, Zhao Y, Sun W, et al. (2006) Novel nerve guidance material prepared from bovine aponeurosis. J Biomed Mater Res A 79: 591-598.

14. Han Q, Sun W, Lin H, Zhao W, Gao Y, et al. (2009) Linear ordered collagen scaffolds loaded with collagen-binding brain-derived neurotrophic factor improve the recovery of spinal cord injury in rats. Tissue Eng Part A 15: 2927-2935.

15. Han Q, Jin W, Xiao Z, Ni H, Wang J, et al. (2010) The promotion of neural regeneration in an extreme rat spinal cord injury model using a collagen scaffold containing a collagen binding neuroprotective protein and an EGFR neutralizing antibody. Biomaterials 31: 9212-9220.

16. Han S, Wang B, Jin W, Xiao Z, Li X, et al. (2015) The linear-ordered collagen scaffold-BDNF complex significantly promotes functional recovery after completely transected spinal cord injury in canine. Biomaterials 41: 89-96.

17. Lang BT, Cregg JM, DePaul MA, Tran AP, Xu K, et al. (2015) Modulation of the proteoglycan receptor PTPÏƒ promotes recovery after spinal cord injury. Nature 518: 404-408.

18. Friedman B, Kleinfeld D, Ip NY, Verge VM, Moulton R, et al. (1995) BDNF and NT-4/5 exert neurotrophic influences on injured adult spinal motor neurons. J Neurosci 15: 1044-1056.

19. Tuszynski MH, Gabriel K, Gage FH, Suhr S, Meyer S, et al. (1996) Nerve growth factor delivery by gene transfer induces differential outgrowth of sensory, motor, and noradrenergic neurites after adult spinal cord injury. Exp Neurol 137: 157-173.

20. Schnell L, Schneider R, Kolbeck R, Barde YA, Schwab ME (1994) Neurotrophin-3 enhances sprouting of corticospinal tract during development and after adult spinal cord lesion. Nature 367: 170-173.

21. Yang Y, De Laporte L, Rives CB, Jang JH, Lin WC, et al. (2005) Neurotrophin releasing single and multiple lumen nerve conduits. J Control Release 104: 433-446.

22. Chang CJ (2009) The effect of pulse-released nerve growth factor from genipin-crosslinked gelatin in schwann cell-seeded polycaprolactone conduits on large-gap peripheral nerve regeneration. Tissue Eng Part A 15: 547-557.

23. Zhao Q, Li ZY, Zhang ZP, Mo ZY, Chen SJ, et al. (2015) Polylactic-co-glycolic acid microspheres containing three neurotrophic factors promote sciatic nerve repair after injury. Neural Regen Res 10: 1491-1497.

24. Fan J, Xiao Z, Zhang H, Chen B, Tang G, et al. (2010) Linear ordered collagen scaffolds loaded with collagen-binding neurotrophin-3 promote axonal regeneration and partial functional recovery after complete spinal cord transection. J Neurotrauma 27: 1671-1683.

25. Chen B, Xiao Z, Shi C, Dai J (2012) Collagen-based functional biomaterials for tissue regeneration. Regenerative Medicine in China. In: Sanders S (ed.) A Sponsored supplement to Science. Science/AAAS, Washington, DC.

26. Weishaupt N, Blesch A, Fouad K (2012) BDNF: the career of a multifaceted neurotrophin in spinal cord injury. Exp Neurol 238: 254-264.

27. Koprivica V, Cho KS, Park JB, Yiu G, Atwal J, et al. (2005) EGFR activation mediates inhibition of axon regeneration by myelin and chondroitin sulfate proteoglycans. Science 310: 106-110.

28. Erschbamer M, Pernold K, Olson L (2007) Inhibiting epidermal growth factor receptor improves structural, locomotor, sensory, and bladder recovery from experimental spinal cord injury. J Neurosci 27: 6428-6435.

29. Li ZW, Li JJ, Wang L, Zhang JP, Wu JJ, et al. (2014) Epidermal growth factor receptor inhibitor ameliorates excessive astrogliosis and improves the regeneration microenvironment and functional recovery in adult rats following spinal cord injury. J Neuroinflammation 11: 71.

30. Li X, Han J, Zhao Y, Ding W, Wei J, et al. (2015) A functionalized collagen scaffold neutralizing the myelin inhibitory molecules promoted neurites outgrowth *in vitro* and facilitated spinal cord regeneration in vivo. ACS Appl Mater Interfaces 7: 13960-13971.

31. Cao QL, Zhang YP, Howard RM, Walters WM, Tsoulfas P, et al. (2001) Pluripotent stem cells engrafted into the normal or lesioned adult rat spinal cord are restricted to a glial lineage. Exp Neurol 167: 48-58.

32. Yang H, Lu P, McKay HM, Bernot T, Keirstead H, et al. (2006) Endogenous neurogenesis replaces oligodendrocytes and astrocytes after primate spinal cord injury. J Neurosci 26: 2157-2166.

33. Sabelström H, Stenudd M, Frisén J (2014) Neural stem cells in the adult spinal cord. Exp Neurol 260: 44-49.

34. Wang B, Xiao Z, Chen B, Han J, Gao Y, et al. (2008) Nogo-66 promotes the differentiation of neural progenitors into astroglial lineage cells through mTOR-STAT3 pathway. PLoS One 3: e1856.

35. Li X, Xiao Z, Han J, Chen L, Xiao H, et al. (2013) Promotion of neuronal differentiation of neural progenitor cells by using EGFR antibody functionalized collagen scaffolds for spinal cord injury repair. Biomaterials 34: 5107-5116.

36. Lu P, Wang Y, Graham L, McHale K, Gao M, et al. (2012) Long-distance growth and connectivity of neural stem cells after severe spinal cord injury. Cell 150: 1264-1273.

37. Lu P, Woodruff G, Wang Y, Graham L, Hunt M, et al. (2014) Long-distance axonal growth from human induced pluripotent stem cells after spinal cord injury. Neuron 83: 789-796.

38. Wright KT, El Masri W, Osman A, Chowdhury J, Johnson WE (2011) Concise review: Bone marrow for the treatment of spinal cord injury: mechanisms and clinical applications. Stem Cells 29: 169-178.

39. Dasari VR, Veeravalli KK, Dinh DH1 (2014) Mesenchymal stem cells in the treatment of spinal cord injuries: A review. World J Stem Cells 6: 120-133.

40. Busch SA, Hamilton JA, Horn KP,Cuascut FX, Cutrone R, et al. (2011) Multipotent adult progenitor cells prevent macrophage-mediated axonal dieback and promote regrowth after spinal cord injury. J Neurosci 31: 944-953.

41. Tuszynski MH, Steward O (2012) Concepts and methods for the study of axonal regeneration in the CNS. Neuron 74: 777-791.

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