Mini review

Research advances in stem cell transplantation combined with fibrin scaffold for treating spinal cord injury in an aging society

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Abstract

Older patients with spinal cord injury (SCI) had higher injury severity scores, longer hospital stays, and were significantly more likely to be discharged to an institution rather than younger patients. Treating SCI and promoting neural tissue regeneration is a major challenge for current medical technologies in an aging society. Tissue engineering scaffolds can provide a microenvironment suitable for cell survival and promote nerve tissue regeneration, and thus have become a promising therapeutic intervention. Among them, fibrin scaffold has become one of the most promising scaffolds for spinal cord regeneration due to its excellent biocompatibility, biodegradability, and high degree of integration with tissues. This article reviews the characteristics of an ideal fibrin scaffold and the role of fibrin scaffolds in the treatment of SCI in aging patients. Meanwhile, we summarize the current applications of different types of stem cells combined with fibrin scaffolds in the treatment of SCI. Through extensive and in-depth studies, this review will be a practical guide for the development of applications of fibrin scaffold combined with stem cell transplantation and provide a direction for possible future work.

Keywords: fibrin scaffolds, stem cells, spinal cord injury, tissue engineering scaffolds, aging

1. Introduction

Spinal cord injuries (SCI) are often caused by motor vehicle collisions. The occurrence of SCI not only causes severe physical and psychological damage to the patients themselves, but also imposes an enormous economic burden on families and society [1]. When the spinal cord is damaged, the connection between the brain and the body is forced to be interrupted, resulting in severe sensory and motor dysfunction of the limbs below the damaged segment. The characteristics of SCI patients have been changed as the population has aged. In a previous report from the United States, older patients in the acute phase had higher injury severity scores, longer hospital stays, lower Glasgow Coma Scale scores, and were significantly more likely to be discharged to an institution rather than younger patients [2]. According to the new standard of 7% of the total population aged 65 years, 91 countries in the world have now entered into an aging society, including Japan, Italy, and Germany. Therefore, how to treat SCI in an aging society becomes a very delicate issue.

The pathophysiological mechanisms after SCI include primary and secondary injury. The initial mechanical trauma causes a primary injury, such as tissue hemorrhagic edema and neuronal cell necrosis in the injured spinal cord, which subsequently triggers severe secondary injury, such as tissue ischemia and necrosis, nerve conduction fiber fragmentation, demyelination, and glial scar proliferation. This cascade of pathological changes leads to endogenous spontaneous repair processes that contribute little to the recovery of spinal cord function [3, 4]. In addition, a large amount of tissue loss occurs after severe SCI, resulting in a glial/fibrotic scar cavity at the center of the SCI site that continues to expand over time, leading to further tissue injury [5].

Currently, there are medical symptomatic, physiotherapeutic rehabilitation, stem cell transplantation, and surgical intervention modalities used to treat SCI in aging people, but there is no single treatment modality that can completely restore the injured spinal cord structure and function. The reasons for the failure of spinal cord regeneration are mainly attributed to the following two points: poor axonal regeneration ability and the inhibitory hostile microenvironment at the injured site after trauma [6]. When the

central nervous system is injured, a large number of glial cells, macrophages, and stromal cells are accumulated at the injury site, and then molecules that inhibit the axon growth, such as chondroitin sulfate proteoglycan, are accumulated, which eventually leads to the formation of glial scar, the lack of neurotrophic factors, the destruction of myelin, and the demyelination of axons and other secondary injuries. This series of secondary injuries impeded the growth of axons toward synapses, which formed a physical and chemical barrier to spinal cord regeneration, leading to the failure of spinal cord regeneration [7-9] (**Figure 1A**). Currently, the focus of the research hotspot for SCI in the elderly is focuses on the mechanistic aspects of spinal cord regeneration, including biological stem cell transplantation, tissue engineering, gene therapy, and drug screening, among which stem cell transplantation is one of the high-potential approaches [10].

Stem cells, as the cells used for transplantation, have the function of self-renewal. It can differentiate into glial cells or neural progenitor cells (NPCs) along the specific neural lineage under the appropriate environment, so as to replace and renew the necrotic or apoptotic cells in the injured area, and finally achieve the purpose of repairing the damaged spinal cord [11]. However, because the stem cells are not preferentially oriented before transplantation, axonal growth disorders occur after transplantation of stem cells into the injured area. Studies have found that tissue engineering scaffold technology can provide support and guidance for newly generated axons while preventing newly generated axons from being damaged by scar tissue, thereby improving the survival rate of transplanted cells [12].

Tissue engineering scaffolds can provide a suitable microenvironment for cell survival, limit local inflammatory responses, inhibit apoptosis, promote nerve regeneration, and promote axon myelination [13]. Among them, fibrin scaffold has become one of the most promising scaffolds for spinal cord regeneration in the elderly due to its excellent biocompatibility, biodegradability, and ability to combine with tissues [14].

Fibrin is a blood clotting protein synthesized by the liver. It is one of the most important proteins for promoting blood coagulation, accelerating wound healing, and promoting tissue regeneration [15]. Fibrin is formed by thrombin cleavage of

fibrinogen *in vivo* and can spontaneously condense into multimers with sponge-like structures in three dimensions. Based on this property, fibrin can be injected into the human injury site in liquid form and transformed into a solid-state scaffold after entering the body [16], which may be useful for improving the microenvironment of the injury site, aiding in cell transplantation as well as neuroprotection [17, 18]. The results of a previous study showed that neural stem cells (NSCs) expressing green fluorescent protein, when embedded in a growth factor-rich fibrin matrix, were transplanted into the injured spinal cord. The results showed that the transplanted stem cells differentiated into neurons and helped the elongated axons connect with the host cells to form rich synapses, which ultimately promoted the recovery of spinal cord function [19].

In this review, we focused on the advantages and roles of fibrin scaffolds in promoting neurological functional recovery in the elderly. Meanwhile, we summarized the current applications of different types of stem cells combined with fibrin scaffolds in the treatment of SCI, and then elaborated on the mechanisms of stem cells combined with fibrin scaffolds for the treatment of SCI, especially in the elderly. Finally, the challenges and prospects of stem cell transplantation combined with fibrin scaffold application in SCI were pointed out.

2. Connections of fibrin scaffolds to SCI

As a fibrous biopolymer, fibrin can stop bleeding and promote wound healing by forming a temporary matrix around the lesion [20]. Fibrin is a component of the extracellular matrix, which can promote the repair of damaged parts by combining cells, extracellular matrix proteins and various growth factors [21]. Because of its high biocompatibility, fibrin can be used as a carrier for stem cell transplantation or as an injectable biomaterial to promote nerve regeneration. After SCI, although axons in the central nervous system cannot regenerate, they can promote axon healing by expanding new axon buds in the peripheral nerves [22]. According to this property, Tsai *et al.* [23] used fibrin matrix as a hollow nerve conduit to span the nerve defect area to promote axon regeneration and growth. King *et al.* [18] injected

fibrin/fibronectin into the injured part of the human spinal cord and observed new axon growth in the scaffold one week later. In addition, Lu *et al.* [19] found that the fibrin scaffold containing growth factors can also well support the long-distance axon growth and interconnection of NSCs implanted with severe SCI in rats. It can be seen that fibrin, as a new natural scaffold material, has a good research prospect for the repair of SCI.

2.1 Biological characteristics of suitable fibrin scaffolds

Scaffold materials are the foundation of tissue engineering scaffolds, and an ideal fibrin scaffold must have the following properties.

2.1.1 Biocompatibility

Fibrin scaffolds play a role by directly contacting peripheral nerves and tissues, and therefore scaffolds with excellent biocompatibility are needed to meet the requirements of nerve regeneration. Good biocompatibility facilitates cell adhesion, does not cause inflammation, provides a good microenvironment for cell growth, and can be used safely in the human body. In addition, appropriate biocompatibility can facilitate axonal growth by incorporating biomolecules such as full-length proteins or shorter peptide chains that mimic the native extracellular matrix [24].

2.1.2 Biodegradability

The ideal biological scaffold should have a good affinity with tissues in the process of organism degradation. To provide space for nerve regeneration after injury, avoiding the injury caused by the secondary procedure of scaffold removal [13]. In addition, the biodegradation rate should match the nerve regeneration rate, which can be artificially regulated or self-regulated to avoid scaffold degradation too fast or too slow to provide adequate supporting effects for SCI.

2.1.3 Physical property

The biological scaffold material should have the mechanical properties that match the spinal cord lesions: adequate strength, hardness, and elasticity [25]. A good fibrin scaffold can not only promote axonal regeneration, but also withstand the force generated during the movement of the spine and surrounding muscle tissues, so as to

provide adequate protection for spinal cord regeneration. Ideally, SCI biomaterials can support axon growth with appropriate stiffness and provide space for axons to pass through or enter the scaffold.

2.1.4 Three-dimensional solid structure

A good fibrin scaffold can promote wound healing by establishing a three-dimensional spatial architecture, and provide enough space for stem cells to adsorb on the scaffold surface. In addition, the three-dimensional porous structure can help cells interact by simulating the extracellular matrix, which is important for cell survival and growth [26].

2.2 The role of fibrin scaffolds in SCI

2.2.1 Creating a microenvironment for cell regeneration

Fibrin provides a loose microenvironment for cell adhesion and axonal regeneration by adhering to other embedded proteins. The fibrin-based scaffold material has great potential as a matrix for the delivery of growth factors or cell therapy after SCI. In particular, studies have shown that fibrin scaffolds can be used as carriers of neurotrophic factors to support regeneration of injured nerves [27]. In addition, the axial holes, channels, and arranged fibers are conducive to controlling the growth direction of the nerve structure [28]. The porosity of a well-designed fibrin scaffold is conducive to better cell adhesion, which is critical for providing a larger bridge distance.

2.2.2 Providing a platform for cell attachment

Fibrin is a natural nano scaffold that provides a platform for stem cell transplantation to promote cell activity and extracellular matrix deposition [29]. Due to its high biocompatibility, fibrin is used to support stem cell transplantation for attachment and growth to promote nerve regeneration. Previous studies have shown that fibrin scaffolds are widely used to support the differentiation of embryonic stem cell-derived NPCs into neurons and oligodendrocytes, and to develop an effective cell delivery platform for neurons derived from pluripotent stem cells [30]. Willerth *et al.* [31] confirmed through research that fibrin scaffold can be used as a platform for neural

tissue engineering to treat SCI. Other studies have shown that mesenchymal stem cells (MSCs) cultured in fibrin glue have strong vitality and can improve the differentiation ability and promote MSCs to differentiate into neurons [32, 33].

3. Fibrin scaffold combined with stem cell transplantation for spinal cord repair

Although various medical technologies have changed with each passing day, there is no effective way to solve the problem of nerve regeneration. Stem cell transplantation shows the potential of neuroprotection and nerve regeneration in SCI, with various targets and responses to stimuli, such as regulating inflammatory response, providing nutritional support, and improving plasticity [34]. The therapeutic principles of stem cells for SCI include replacement of damaged neurons and glial cells, secretion of trophic factors, inhibition of glial scar formation, and promotion of axon regeneration. Among these, it is more important that stem cell transplantation can prevent or replace damaged glial cells, especially oligodendrocytes, which can promote the remyelination of surviving axons [35] (**Figure 1B-C**). The fibrin scaffold technology can provide support and guide the growth of stem cell-derived axons, provide protection for new axons, avoid scar tissue invasion, reduce the apoptosis of transplanted stem cells, and improve the survival rate of transplanted cells. The following are some of the hot research topics in recent years on the combination of stem cells and SCI (**Table 1**).

3.1 Embryonic stem cells

Embryonic stem cells (ESCs) can differentiate into a variety of neuronal cell types, which has great potential as a cell replacement therapy after SCI. It has been shown that an appropriate fibrin scaffold can enhance the activity of ESC-derived NPCs [36, 37]. Johnson *et al.* [38] embedded ESC-derived NPCs containing growth factor and heparin-binding delivery system (HBDS) and found that the combination of neurotrophin-3 (NT-3), platelet-derived growth factor (PDGF) and fibrin scaffold could increase the number of NPCs at the site of SCI. The results prove that the application of fibrin scaffold can help to increase the survival and differentiation of ESC-derived NPCs at the injured site, and this discovery will help to increase the

feasibility of fibrin scaffold combined with stem cell transplantation in the treatment of SCI. In addition, Willerth *et al.* [31] experimentally determined the culture conditions suitable for proliferation and differentiation of ESCs in fibrin scaffold. The optimal concentration for fibrin scaffold polymerization is 10 mg/mL of fibrinogen and 2 NIH units/mL of thrombin. After 14 days of continuous culture in the scaffold, ESCs were found to differentiate into neurons and astrocytes by immunohistochemistry.

3.2 Induced pluripotent stem cells

Induced pluripotent stem cells (iPSCs) reprogram terminally differentiated somatic cells into pluripotent stem cells by introducing specific transcription factors into adult somatic cells [39, 40]. After reprogramming under specific conditions, the differentiated somatic cells returned to the totipotent state. Montgomery *et al.* [41] designed two systems to investigate whether iPSCs could increase the number of iPSCs differentiated into neural cells after combination with fibrin scaffold. The results confirmed that the differentiation ability of iPSCs based on the fibrin scaffold platform was improved, and the promotion of neurons generated from murine iPSC-derived EBs seeded in fiber could be maximized using the 2-/4+ differentiation protocol. This experiment verified that 3D fibrin can improve the survival rate of transplanted stem cells and the degree of transformation into neural cells. At the same time, it provides ideas and references for stem cell transplantation combined with fibrin scaffold for the treatment of SCI.

3.3 Mesenchymal stem cells

The combination of MSCs and fibrin scaffold can promote the migration and differentiation of the patients' mesenchymal cells into neurons, and inhibit the glial scar tissue that blocks axon regeneration at the SCI site [29, 42]. The most commonly used MSCs in combination with fibrin to treat SCI are bone marrow mesenchymal stem cells (BM-MSCs) and adipose-derived mesenchymal stem cells (AD-MSCs). MSCs promote angiogenesis by expressing angiogenic factors, such as vascular endothelial growth factor (VEGF) [43]. In addition, MSCs can be used to promote axonal regeneration of the myelin sheath and myelination of the myelin sheath in

nervous system injury, which is helpful in promoting nerve regeneration [33, 44, 45]. Garc á Elisa *et al.* [46] observed mechanical retraction, increase of axonal fibers and recovery of motor and sensory function in rats after implantation of fibrin scaffold and BM-MSCs into injured spinal cord. Mukhamedshina *et al.* [47] investigated the effect of the combination of AD-MSCs and fibrin matrix on the post-traumatic response of the spinal cord in rats. The experiment showed that the application of AD-MSCs reduced the expression of astrocytes in the SCI area, and the combination of AD-MSCs and fibrin matrix significantly increased the expression of platelet-derived growth factor receptor (PDGFR) and HSPA1b mRNA, and decreased the expression of ionized calcium-binding adapter molecule 1 in the central canal. Liu *et al.* [48] randomly divided the rat model of SCI into four groups: control group (laminectomy group), SCI group (laminectomy + spinal cord transection group), fibrin group (fibrin transplantation immediately after SCI), fibrin cell group [fibrin scaffold containing ectodermal mesenchymal stem cells (EM-SCs) implanted after SCI]. In comparison, it is proven that the combination of ectodermal mesenchymal stem cells and fibrin scaffold in the treatment of SCI in rats is higher than that of fibrin scaffold alone in terms of cell apoptosis rate, number of nerve fibers, myelin sheath thickness, and motor score. In addition, a large number of experiments support the conclusion that the fibrin niche contributes to the stable differentiation of rat MSCs into neural progenitor cells and promotes nerve tissue regeneration [49-51].

3.4 Neural stem cells

Neural stem cells (NSCs) are a group of cells that can self-renew and have the ability to differentiate into neurons and various types of glial cells. The transplanted neural stem cells replace the injured neurons and glial cells through renewal, thus reconnecting the injured spinal cord. Lu *et al.* [19] implanted NSCs into the fibrin matrix containing the growth factor cocktail, and found that a large number of axons extended in the injured spinal cord and formed neuronal relay, which improved the electrophysiological function of rats. Then, Lu *et al.* [52] separated fresh NSCs from transgenic Fischer 344 rat embryos expressing green fluorescent protein (GFP) and embedded them in fibrin matrix containing growth factors. The results showed that

the graft completely filled the lesion cavity and differentiated into two types of neurons (the axons extended very far from the host spinal cord) and glial cells. Later, Robinson *et al.* [53] experimentally verified the conclusions of Lu *et al.* [52]. Arulmoli *et al.* [54] reported a new composite scaffold composed of fibrin, hyaluronic acid, and laminin that can support the function of human neural stem/progenitor cells (HNSPCs). This composite biomaterial scaffold has physical properties suitable for HNSPCs and the central nervous system, supports the proliferation and differentiation of human neural stem/progenitor cells, and attenuates the rapid cell-mediated scaffold degradation. This has set a benchmark for the treatment of central nervous system injuries with biomaterials.

4. Summary and outlook

In this review, we have briefly discussed different types of stem cell transplantation combined with fibrin scaffold for the treatment of SCI. SCI is a devastating neurological injury, and there is no single treatment that can completely restore the injured spinal cord structure and function. Currently, the focus of SCI research is on stem cell transplantation and tissue engineering scaffolds, but each has its own limitations. It is difficult to control the direction and amount of differentiation of stem cells when they are transplanted into the injured spinal cord alone, and it is difficult to ensure the healthy survival of transplanted stem cells in a poor microenvironment. Therefore, the use of tissue engineering scaffold technology can provide support and guidance for the newborn axons, and at the same time can avoid the characteristics of the newborn axons being invaded by scar tissue. It is a very potential research direction to combine the two technologies in the treatment of SCI.

However, at present, our research on fibrin scaffold combined with stem cell transplantation is still in the preliminary exploration stage, and there are still many directions worthy of exploration and development. Therefore, the following is our outlook for the future of this topic. First, we need to search for more types of stem cells and compare their ability to differentiate into neurons to find the most suitable type for transplantation. Second, in the future research, we can focus on the

morphology and physical properties of fibrin scaffold. Update the existing fibrin scaffold technology, improve the mechanical properties of fibrin scaffold, and provide the best transplantation site for stem cells to protect them for better proliferation and differentiation. Third, in the future, we can broaden our perspective and combine fibrin scaffold with stem cell transplantation and growth factors for research. The advantages of different substances should be fully exploited to better reduce neuronal damage after SCI, promote axon growth and rebuild the damaged spinal cord tissue.

5. Conclusion

In a word, for SCI, especially for elderly SCI, the current clinical treatment effect is not satisfactory. Based on the understanding of the pathological changes of SCI, with the deepening of research on stem cell transplantation combined with fibrin scaffold, this treatment strategy may be a promising choice for the regenerative treatment of patients with SCI.

Declarations

Author contributions

TJ and ZC jointly drafted the manuscript and prepared and revised the figures and tables. YH participated in the critical revision of the manuscript for intellectual content. XT and YL identified and reviewed the relevant literature. GG drafted the outline and revised the manuscript. All authors have read and approved the final version of the manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest related to this work.

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References

1. Chay W, & Kirshblum S. Predicting Outcomes After Spinal Cord Injury. *Phys Med Rehabil Clin N Am*, 2020, 31(3): 331-343. https://doi.org/10.1016/j.pmr.2020.03.003
2. Hiremath SV, Karmarkar AM, Kumar A, Coffman DL, & Marino RJ. Factors associated with post-acute functional status and discharge dispositions in individuals with spinal cord injury. *J Spinal Cord Med*, 2022, 45(1): 126-136. https://doi.org/10.1080/10790268.2021.1888023
3. Jiang T, & He Y. Recent Advances in the Role of Nuclear Factor Erythroid-2-Related Factor 2 in Spinal Cord Injury: Regulatory Mechanisms and Therapeutic Options. *Front Aging Neurosci*, 2022, 14: 851257. https://doi.org/10.3389/fnagi.2022.851257
4. Liu X, Zhang Y, Wang Y, & Qian T. Inflammatory Response to Spinal Cord Injury and Its Treatment. *World Neurosurg*, 2021, 155: 19-31. https://doi.org/10.1016/j.wneu.2021.07.148
5. Tran AP, Warren PM, & Silver J. The Biology of Regeneration Failure and Success After Spinal Cord Injury. *Physiol Rev*, 2018, 98(2): 881-917. https://doi.org/10.1152/physrev.00017.2017
6. Pang QM, Chen SY, Xu QJ, Fu SP, Yang YC, Zou WH, *et al*. Neuroinflammation and Scarring After Spinal Cord Injury: Therapeutic Roles of MSCs on Inflammation and Glial Scar. *Front Immunol*, 2021, 12: 751021. https://doi.org/10.3389/fimmu.2021.751021
7. Khaing ZZ, Thomas RC, Geissler SA, & Schmidt CE. Advanced biomaterials for repairing the nervous system: what can hydrogels do for the brain? *Materials Today*, 2014, 17(7): 332-340. https://doi.org/https://doi.org/10.1016/j.mattod.2014.05.011
8. Papastefanaki F, & Matsas R. From demyelination to remyelination: the road toward therapies for spinal cord injury. *Glia*, 2015, 63(7): 1101-1125. https://doi.org/10.1002/glia.22809
9. Tian DS, Dong Q, Pan DJ, He Y, Yu ZY, Xie MJ, *et al*. Attenuation of astrogliosis by suppressing of microglial proliferation with the cell cycle inhibitor olomoucine in rat spinal cord injury model. *Brain Res*, 2007, 1154: 206-214. https://doi.org/10.1016/j.brainres.2007.04.005
10. Zipser CM, Cragg JJ, Guest JD, Fehlings MG, Jutzeler CR, Anderson AJ, *et al*. Cell-based and stem-cell-based treatments for spinal cord injury: evidence from clinical trials. *Lancet Neurol*, 2022, 21(7): 659-670. https://doi.org/10.1016/s1474-4422(21)00464-6
11. Mothe AJ, & Tator CH. Review of transplantation of neural stem/progenitor cells for spinal

cord injury. *Int J Dev Neurosci*, 2013, 31(7): 701-713. https://doi.org/10.1016/j.ijdevneu.2013.07.004

12. Ansorena E, De Berdt P, Ucakar B, Simón-Yarza T, Jacobs D, Schakman O, *et al*. Injectable alginate hydrogel loaded with GDNF promotes functional recovery in a hemisection model of spinal cord injury. *Int J Pharm*, 2013, 455(1-2): 148-158. https://doi.org/10.1016/j.ijpharm.2013.07.045

13. Shrestha B, Coykendall K, Li Y, Moon A, Priyadarshani P, & Yao L. Repair of injured spinal cord using biomaterial scaffolds and stem cells. *Stem Cell Res Ther*, 2014, 5(4): 91. https://doi.org/10.1186/scrt480

14. Itosaka H, Kuroda S, Shichinohe H, Yasuda H, Yano S, Kamei S, *et al*. Fibrin matrix provides a suitable scaffold for bone marrow stromal cells transplanted into injured spinal cord: a novel material for CNS tissue engineering. *Neuropathology*, 2009, 29(3): 248-257. https://doi.org/10.1111/j.1440-1789.2008.00971.x

15. Domingues MM, Carvalho FA, & Santos NC. Nanomechanics of Blood Clot and Thrombus Formation. *Annu Rev Biophys*, 2022, 51: 201-221. https://doi.org/10.1146/annurev-biophys-111821-072110

16. de la Puente P, & Ludeña D. Cell culture in autologous fibrin scaffolds for applications in tissue engineering. *Exp Cell Res*, 2014, 322(1): 1-11. https://doi.org/10.1016/j.yexcr.2013.12.017

17. Chen Q, Zhang Z, Liu J, He Q, Zhou Y, Shao G, *et al*. A fibrin matrix promotes the differentiation of EMSCs isolated from nasal respiratory mucosa to myelinating phenotypical Schwann-like cells. *Mol Cells*, 2015, 38(3): 221-228. https://doi.org/10.14348/molcells.2015.2170

18. King VR, Alovskaya A, Wei DY, Brown RA, & Priestley JV. The use of injectable forms of fibrin and fibronectin to support axonal ingrowth after spinal cord injury. *Biomaterials*, 2010, 31(15): 4447-4456. https://doi.org/10.1016/j.biomaterials.2010.02.018

19. Lu P, Wang Y, Graham L, McHale K, Gao M, Wu D, *et al*. Long-distance growth and connectivity of neural stem cells after severe spinal cord injury. *Cell*, 2012, 150(6): 1264-1273. https://doi.org/10.1016/j.cell.2012.08.020

20. Park CH, & Woo KM. Fibrin-Based Biomaterial Applications in Tissue Engineering and

Regenerative Medicine. *Adv Exp Med Biol*, 2018, 1064: 253-261. https://doi.org/10.1007/978-981-13-0445-3_16

21. Yu Z, Li H, Xia P, Kong W, Chang Y, Fu C, *et al*. Application of fibrin-based hydrogels for nerve protection and regeneration after spinal cord injury. *J Biol Eng*, 2020, 14: 22. https://doi.org/10.1186/s13036-020-00244-3

22. Schmidt CE, & Leach JB. Neural tissue engineering: strategies for repair and regeneration. *Annu Rev Biomed Eng*, 2003, 5: 293-347. https://doi.org/10.1146/annurev.bioeng.5.011303.120731

23. Tsai EC, Dalton PD, Shoichet MS, & Tator CH. Matrix inclusion within synthetic hydrogel guidance channels improves specific supraspinal and local axonal regeneration after complete spinal cord transection. *Biomaterials*, 2006, 27(3): 519-533. https://doi.org/10.1016/j.biomaterials.2005.07.025

24. Straley KS, Foo CW, & Heilshorn SC. Biomaterial design strategies for the treatment of spinal cord injuries. *J Neurotrauma*, 2010, 27(1): 1-19. https://doi.org/10.1089/neu.2009.0948

25. Zhang Q, Shi B, Ding J, Yan L, Thawani JP, Fu C, *et al*. Polymer scaffolds facilitate spinal cord injury repair. *Acta Biomater*, 2019, 88: 57-77. https://doi.org/10.1016/j.actbio.2019.01.056

26. Zamani F, Amani-Tehran M, Latifi M, Shokrgozar MA, & Zaminy A. Promotion of spinal cord axon regeneration by 3D nanofibrous core-sheath scaffolds. *J Biomed Mater Res A*, 2014, 102(2): 506-513. https://doi.org/10.1002/jbm.a.34703

27. Jeon O, Ryu SH, Chung JH, & Kim BS. Control of basic fibroblast growth factor release from fibrin gel with heparin and concentrations of fibrinogen and thrombin. *J Control Release*, 2005, 105(3): 249-259. https://doi.org/10.1016/j.jconrel.2005.03.023

28. Madaghiele M, Sannino A, Yannas IV, & Spector M. Collagen-based matrices with axially oriented pores. *J Biomed Mater Res A*, 2008, 85(3): 757-767. https://doi.org/10.1002/jbm.a.31517

29. Blando S, Anchesi I, Mazzon E, & Gugliandolo A. Can a Scaffold Enriched with Mesenchymal Stem Cells Be a Good Treatment for Spinal Cord Injury? *Int J Mol Sci*, 2022, 23(14)https://doi.org/10.3390/ijms23147545

30. Willerth SM, Rader A, & Sakiyama-Elbert SE. The effect of controlled growth factor delivery

on embryonic stem cell differentiation inside fibrin scaffolds. *Stem Cell Res*, 2008, 1(3): 205-218. https://doi.org/10.1016/j.scr.2008.05.006

31. Willerth SM, Arendas KJ, Gottlieb DI, & Sakiyama-Elbert SE. Optimization of fibrin scaffolds for differentiation of murine embryonic stem cells into neural lineage cells. *Biomaterials*, 2006, 27(36): 5990-6003. https://doi.org/10.1016/j.biomaterials.2006.07.036

32. Yousefi F, Lavi Arab F, Nikkhah K, Amiri H, & Mahmoudi M. Novel approaches using mesenchymal stem cells for curing peripheral nerve injuries. *Life Sci*, 2019, 221: 99-108. https://doi.org/10.1016/j.lfs.2019.01.052

33. Zhao Z, Wang Y, Peng J, Ren Z, Zhang L, Guo Q, *et al*. Improvement in nerve regeneration through a decellularized nerve graft by supplementation with bone marrow stromal cells in fibrin. *Cell Transplant*, 2014, 23(1): 97-110. https://doi.org/10.3727/096368912x658845

34. Huang L, Fu C, Xiong F, He C, & Wei Q. Stem Cell Therapy for Spinal Cord Injury. *Cell Transplant*, 2021, 30: 963689721989266. https://doi.org/10.1177/0963689721989266

35. Muheremu A, Peng J, & Ao Q. Stem cell based therapies for spinal cord injury. *Tissue Cell*, 2016, 48(4): 328-333. https://doi.org/10.1016/j.tice.2016.05.008

36. McCreedy DA, Wilems TS, Xu H, Butts JC, Brown CR, Smith AW, *et al*. Survival, Differentiation, and Migration of High-Purity Mouse Embryonic Stem Cell-derived Progenitor Motor Neurons in Fibrin Scaffolds after Sub-Acute Spinal Cord Injury. *Biomater Sci*, 2014, 2(11): 1672-1682. https://doi.org/10.1039/c4bm00106k

37. Willerth SM, Faxel TE, Gottlieb DI, & Sakiyama-Elbert SE. The effects of soluble growth factors on embryonic stem cell differentiation inside of fibrin scaffolds. *Stem Cells*, 2007, 25(9): 2235-2244. https://doi.org/10.1634/stemcells.2007-0111

38. Johnson PJ, Tatara A, Shiu A, & Sakiyama-Elbert SE. Controlled release of neurotrophin-3 and platelet-derived growth factor from fibrin scaffolds containing neural progenitor cells enhances survival and differentiation into neurons in a subacute model of SCI. *Cell Transplant*, 2010, 19(1): 89-101. https://doi.org/10.3727/096368909x477273

39. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, *et al*. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*, 2007, 131(5): 861-872. https://doi.org/10.1016/j.cell.2007.11.019

40. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, *et al*. Induced

pluripotent stem cell lines derived from human somatic cells. *Science*, 2007, 318(5858): 1917-1920. https://doi.org/10.1126/science.1151526

41. Montgomery A, Wong A, Gabers N, & Willerth SM. Engineering personalized neural tissue by combining induced pluripotent stem cells with fibrin scaffolds. *Biomater Sci*, 2015, 3(2): 401-413. https://doi.org/10.1039/c4bm00299g

42. Mortazavi MM, Harmon OA, Adeeb N, Deep A, & Tubbs RS. Treatment of spinal cord injury: a review of engineering using neural and mesenchymal stem cells. *Clin Anat*, 2015, 28(1): 37-44. https://doi.org/10.1002/ca.22443

43. Chun SY, Lim JO, Lee EH, Han MH, Ha YS, Lee JN, *et al*. Preparation and Characterization of Human Adipose Tissue-Derived Extracellular Matrix, Growth Factors, and Stem Cells: A Concise Review. *Tissue Eng Regen Med*, 2019, 16(4): 385-393. https://doi.org/10.1007/s13770-019-00199-7

44. Gardin C, Vindigni V, Bressan E, Ferroni L, Nalesso E, Puppa AD, *et al*. Hyaluronan and fibrin biomaterial as scaffolds for neuronal differentiation of adult stem cells derived from adipose tissue and skin. *Int J Mol Sci*, 2011, 12(10): 6749-6764. https://doi.org/10.3390/ijms12106749

45. Wu X, Ren J, & Li J. Fibrin glue as the cell-delivery vehicle for mesenchymal stromal cells in regenerative medicine. *Cytotherapy*, 2012, 14(5): 555-562. https://doi.org/10.3109/14653249.2011.638914

46. García E, Rodríguez-Barrera R, Buzoianu-Anguiano V, Flores-Romero A, Malagón-Axotla E, Guerrero-Godinez M, *et al*. Use of a combination strategy to improve neuroprotection and neuroregeneration in a rat model of acute spinal cord injury. *Neural Regen Res*, 2019, 14(6): 1060-1068. https://doi.org/10.4103/1673-5374.250627

47. Mukhamedshina YO, Akhmetzyanova ER, Kostennikov AA, Zakirova EY, Galieva LR, Garanina EE, *et al*. Adipose-Derived Mesenchymal Stem Cell Application Combined With Fibrin Matrix Promotes Structural and Functional Recovery Following Spinal Cord Injury in Rats. *Front Pharmacol*, 2018, 9: 343. https://doi.org/10.3389/fphar.2018.00343

48. Liu J, Chen Q, Zhang Z, Zheng Y, Sun X, Cao X, *et al*. Fibrin scaffolds containing ectomesenchymal stem cells enhance behavioral and histological improvement in a rat model of spinal cord injury. *Cells Tissues Organs*, 2013, 198(1): 35-46.

https://doi.org/10.1159/000351665

49. Chandrababu K, Sreelatha HV, Sudhadevi T, Anil A, Arumugam S, & Krishnan LK. In vivo neural tissue engineering using adipose-derived mesenchymal stem cells and fibrin matrix. *J Spinal Cord Med*, 2023, 46(2): 262-276. https://doi.org/10.1080/10790268.2021.1930369

50. Ibarra A, Mendieta-Arbesú E, Suarez-Meade P, García-Vences E, Martiñón S, Rodriguez-Barrera R, *et al*. Motor Recovery after Chronic Spinal Cord Transection in Rats: A Proof-of-Concept Study Evaluating a Combined Strategy. *CNS Neurol Disord Drug Targets*, 2019, 18(1): 52-62. https://doi.org/10.2174/1871527317666181105101756

51. Rodríguez-Barrera R, Flores-Romero A, Buzoianu-Anguiano V, Garcia E, Soria-Zavala K, Incontri-Abraham D, *et al*. Use of a Combination Strategy to Improve Morphological and Functional Recovery in Rats With Chronic Spinal Cord Injury. *Front Neurol*, 2020, 11: 189. https://doi.org/10.3389/fneur.2020.00189

52. Lu P, Graham L, Wang Y, Wu D, & Tuszynski M. Promotion of survival and differentiation of neural stem cells with fibrin and growth factor cocktails after severe spinal cord injury. *J Vis Exp*, 2014, (89): e50641. https://doi.org/10.3791/50641

53. Robinson J, & Lu P. Optimization of trophic support for neural stem cell grafts in sites of spinal cord injury. *Exp Neurol*, 2017, 291: 87-97. https://doi.org/10.1016/j.expneurol.2017.02.007

54. Arulmoli J, Wright HJ, Phan DTT, Sheth U, Que RA, Botten GA, *et al*. Combination scaffolds of salmon fibrin, hyaluronic acid, and laminin for human neural stem cell and vascular tissue engineering. *Acta Biomater*, 2016, 43: 122-138. https://doi.org/10.1016/j.actbio.2016.07.043

Figure legends

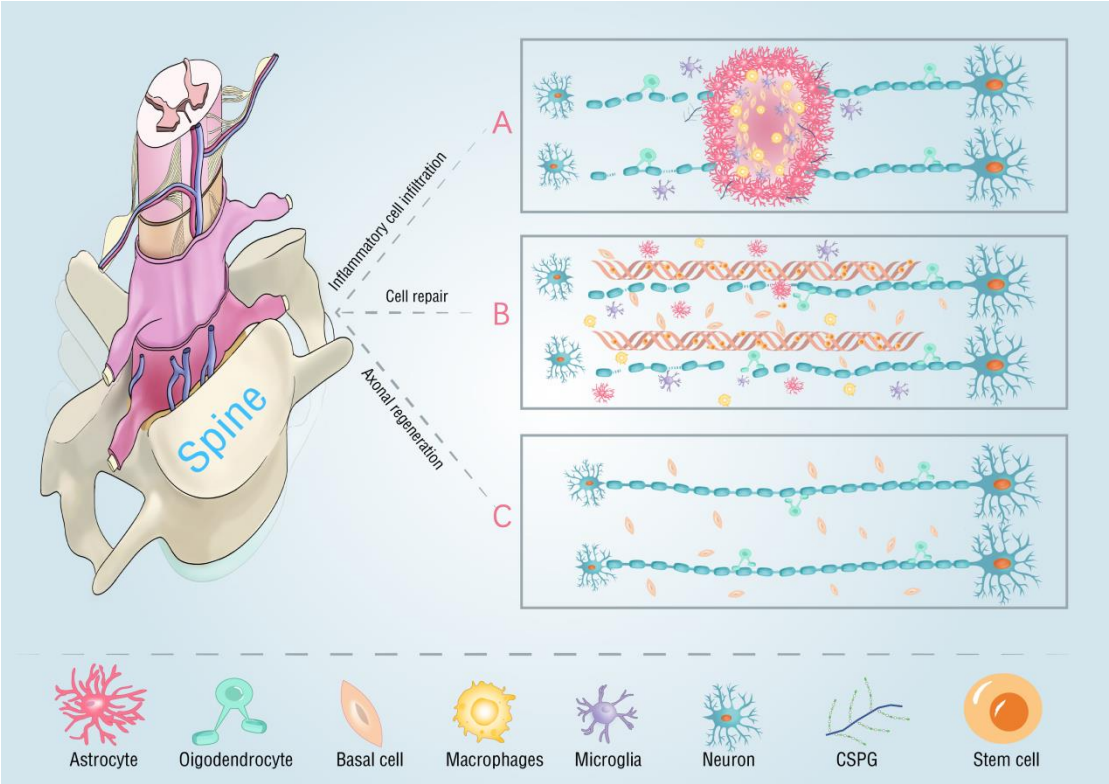

Figure 1. (A) After SCI, inflammatory cells infiltrated and wrapped around the damaged axons, forming a glial scar that impedes axon regeneration. (B) Some repair mechanisms after transplantation of fibrin scaffold containing stem cells into the injured spinal cord. The number of neurons and oligodendrocytes differentiated from the transplanted cells increased, and the number of other inflammatory cells decreased. (C) The axon regeneration and neural circuit recovery after transplantation of fibrin scaffold and stem cells.

528 **Table 1**. Fibrin scaffold combined with different types of stem cells for treatment of
529 SCI.

Stem cell types	Experimental methods	Outcome	Ref.
ESC-derived NPCs	NPCs + fibrin scaffold containing HBDS, NT-3, and PDGF.	NPCs↑ and neuron↑	[38]
ESCs	Retinoic acid was added to EBs to induce mouse ESCs to become NPCs and implanted into fibrin scaffolds of different concentrations.	Optimal concentrations for scaffold polymerization were 10 mg/mL of fibrinogen and 2 NIH units/mL of thrombin. The optimal aprotinin concentration was determined to be 50 μg/mL for dissociated EBs (2D) and 5 μg/mL for intact EBs in 3D fibrin scaffolds.	[31]
iPSCs	an 8-day 4-/4+ protocol using soluble retinoic acid in the last 4 days and a 6-day 2-/4+ protocol using soluble retinoic acid and the small molecule sonic hedgehog agonist purmorphamine.	In iPSCs and ESCs, the proportion of neurons generated by EBs generated by 2-/4+ protocol is higher.	[41]
BM-MSCs	BM-MSCs were affixed with fibrin glue and injected inside or around the graft.	Repaired rat sciatic nerve.	[33]
BM-MSCs	For this purpose, female adult rats were subjected to SCI, 60 days after lesion, rats were randomly distributed in four groups: (1)	Treatment with INDP alone significantly increased motor recovery, anti-inflammatory cytokines,	[51]

	Rats immunized with complete Freund' s adjuvant + PBS (vehicle; PBS-I); (2) Rats with SR + FGM-MSCs; (3) Rats with SR + INDP + FGM-MSCs; (4) Rats only with INDP.	regeneration-associated molecules, axonal regeneration, and neurogenesis.	
AD-MSCs	The derived progenitors, tagged with fluorescent tracker dye were delivered in rat T10 contusion SCI using fibrin hydrogel.	Fibrin niche aided stable differentiation of rat ADMSCs into neural progenitors.	[49]
NSCs	NSCs were implanted into fibrin matrix containing growth factor cocktails.	The injured spinal cord extended a large number of axons and formed neuronal relay, and the electrophysiological function of the rats recovered at the same time.	[19]
NSCs	Fresh NSCs were isolated from transgenic Fischer 344 rat embryos expressing GFP and embedded in fibrin matrix containing growth factors.	The graft completely filled the lesion cavity and differentiated into two types of neurons (axons extended very far from the host spinal cord) and glial cells.	[52]