Journal of Neurorestoratology

Volume 7 | Number 4 Article 3

2019

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Journal of Neurorestoratology
DOI 10.26599/JNR.2019.9040024

2019, 7(4): 196–206
ISSN 2324-2426

REVIEW ARTICLE

Progress in research into spinal cord injury repair: Tissue engineering scaffolds and cell transdifferentiation

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ARTICLE INFO

Received: 19 September 2019
Revised: 27 November 2019
Accepted: 9 December 2019

KEYWORDS

spinal cord injury;
stem cells;
olfactory ensheathing cells;
hydrogel;
transdifferentiation

ABSTRACT

As with all tissues of the central nervous system, the low regeneration ability of spinal cord tissue after injury decreases the potential for repair and recovery. Initially, in spinal cord injuries (SCI), often the surgeon can only limit further damage by early surgical decompression. However, with the development of basic science, especially the development of genetic engineering, molecular biology, tissue engineering, and materials science, some promising progress has been made in promoting the repair of central nervous system injuries. For example, transplantation of neural stem cells (NSCs), olfactory ensheathing cells (OECs), and gene-mediated transdifferentiation to repair central nervous system injury. This paper summarizes the progress and prospects of SCI repair with tissue engineering scaffold and cell transdifferentiation from an extensive literatures.

1 Introduction

Spinal cord injury (SCI) is a worldwide medical problem characterized by severe morbidity, a high disability rate and high mortality [1–3]. SCI includes primary and sequential spinal cord injuries, and the main component of the former is traumatic spinal cord injury after which about 45% of patients will remain paralyzed with a significantly reduced quality of life [4]. Currently, there are 2 to 3 million patients with SCI around the world, a number that is increasing annually by 130,000, on average. One of the principal areas of research is to protect the remaining axons and neurons from secondary damage, and promote the regeneration of the downlink conduction system, using drugs, stem cells, or other cell-transplantation techniques. Recently, the technology of cell transdifferentiation has made it possible to transform a type of differentiated cell into nerve cell, which challenges the traditional idea that the developmental path of terminally differentiated cells cannot be changed and opens up new possibilities for the repair of SCI. To provide some references for basic research and the clinical treatment of SCI repair, this review

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mainly focuses on the application of tissue engineering scaffolds and cell transdifferentiation.

2 Factors affecting axonal regeneration after SCI

After SCI, there is a series of traumatic cellular reactions including astrocyte differentiation into "scarred" glial cells. These glial cells proliferate and migrate into the injured area and produce many important inhibitory factors, including myelin-associated blockers NI-35, NI-250, myelin-associated glycoprotein (MAG), inhibitory proteoglycan 2, and related toxins, killing damaged nerve cells, and axons. In addition, acid-base imbalance, disruption of blood supply, the inactivation of related enzymes and the production of many inflammatory factors further inhibits the regeneration of axons in the injured area. Therefore, the repair of SCI is affected by a variety of inhibitory factors. In general, among the main factors affecting axonal regeneration is the ability to express axon regeneration-related genes. These control effective trophic factors that promote axonal regeneration, inhibitory factors that inhibit axonal growth and elongation, and effective matrix molecules that guide elongation of axon regeneration, and isolation from glial scarring.

3 Strategies for promoting axonal regeneration after SCI

Most scholars recognize that the repair of SCI should be based on reducing inflammation and scarring, blocking the influence of inhibitors, promoting and supporting axonal regeneration, and encouraging the connection between damaged axons and target organs. At present, there are two main strategies for the promotion of axon regeneration: (1) To change the inhibitory environment of central nervous tissue regeneration. For example, antibody neutralization reduces the formation of inhibitors and scars, and increases the number of cells that are conducive to axonal regeneration. (2) To improve the axonal regeneration ability of central nervous cells, including the application of exogenous nerve growth factors (NGFs) and neurotrophic factors (NTFs), and regulation of the expression of related regenerative genes.

4 The main researches of SCI repair

4.1 Nutrient factors and inhibitory factor blockers

Reliable experiments have shown that NGFs and NTFs can promote axon growth and protect nerve cells from necrosis. Similarly, gangliosides can stabilize and protect nerve cell function, activate related enzyme activities, and promote axonal regeneration. NTF-3 also plays an important role in enhancing synaptic plasticity, promoting neuroprotection and axon regeneration after SCI [5]. The mRNA sequencing has shown that the expression of NTF-3 is inadequate in the injured area after SCI [6]. In a SCI model, the combination of monoclonal antibody IN-1 with NTF-3 and brain-derived neurotrophic factor has been shown to promote axonal regeneration in the cortical spinal cord. There is much relevant in-depth research being carried out in this area.

Schwab et al. injected IN-1, a monoclonal antibody that neutralizes the central nervous system inhibitor NI-250, into the SCI area and found that some axons could regenerate up to 10 mm. This suggests that the application of NGFs could promote axonal growth and improve the innervation and mobilization of some lower limbs [7]. In another experiment, IN-1 was injected into an area of complete corticospinal tract injury, and it was found that the contralateral corticospinal tract could flank the spinal cord and cross the midline, and replace the partially damaged

corticospinal tract, indicating that monoclonal antibody IN-1 promotes collateral growth of axons. Another important inhibitory factor, MAG, has been discovered, but no corresponding antibody has been developed. After knocking out the *MAG* gene of SCI mice, it has been found that axon regeneration after corticospinal injury is higher than that of mice retaining the *MAG* gene, but this has not yet translated into the improvement of motor function. There have been no reports on the neutralization of proteoglycan so far.

4.2 Tissue engineering scaffold

The characteristics of low survival and non-directional growth of transplanted cells underlies the inefficiency of neural structure integration and reconstruction. With the development of tissue engineering materials, especially materials with specific degradation and release characteristics, this deficiency has been significantly improved. There are many reports on research into biological matrix carriers. These include silk fibroin (SF) scaffolds, fibrin, and hyaluronic acid hydrogels, bio-protein electrospinning, and prefabricated shaped scaffolds, especially in combination with 3D printing, to produce biocompatible hybrid scaffolds. The controlled release of drugs such as NTF could effectively promote the survival, proliferation, and differentiation of transplanted cells in an injured area. Contact-directed or chemically guided transplanted cells can be made to grow in a directed and orderly distribution and we can induce the directional extension of new nerve fibers, and promote axonal myelination, and synapse reformation [8, 11, 22, 25].

Hydrogel is a water-rich and porous biomaterial. The application of hydrogel in an SCI area could promote local substance exchange, reduce the damage cavity, promote angiogenesis, contact and guide cell attachment and growth extension, and inhibit glial scar formation. Its degradable characteristics support the production and release of factors which provide space for the regeneration of nerve tissue and promote the demyelination of damaged axons [9, 10]. Boido et al. consider that the high biocompatibility of chitosan (CS) based hydrogels guarantees a proper environment for the survival of transplanted cells and for their ability to provide anti-inflammatory and antioxidant cues. CS-based hydrogels were developed to host mesenchymal stem cells (MSCs), thus limiting the formation of glial scarring and reducing cell death at the injured site. Preliminary *in vivo* tests on SCI mice revealed good handling of the CS solution loading MSCs during implantation, and higher survival rate of the encapsulated MSCs after 7 days [11]. Dcumens et al. implanted OECs in a biological matrix guiding bridge in SCI rats and observed that the injured corticospinal tract extended significantly further between the host and graft junction [12]. Cigognini injected RADA16-4GBMHP1 and Ac-FAQ-LDLK12 hydrogel into SCI rats and found that both hydrogels can promote hemostasis after injury, significantly reducing local hematoma formation. They observed axon regeneration and obvious recovery of the hind limb movement and coordination [13]. Ghosh et al. injected hydrogel-based minocycline hydrochloride (MH) into the intrathecal space of the injured cervical spinal cord for local delivery of high concentrations of MH without damaging spinal cord tissue, and demonstrated that it preserved the critical phrenic motor circuitry in cervical SCI models. MH hydrogel also decreased lesion size and degeneration of cervical motor neuron somata [14]. After applying QL6 hydrogel to a rat SCI model, Liu found that QL6 hydrogel can significantly reduce post-traumatic neuronal apoptosis, inhibit local inflammation and later astrocyte activation, and increase axon regeneration [15]. Studies have shown that hyaluronic acid (HA)

can reduce inflammatory cell infiltration and astrocyte proliferation in surrounding tissues after SCI, and alleviate local inflammation and glial cicatrix. The neurotrophin-modified HA-methylcellulose hydrogel, which can achieve sustained release of supporting factors in the specific location, was implanted into the SCI area of rats and it was able to reduce scar formation, and promote axonal extension across the damaged area. In the region, HA-methylcellulose-NTF3 also inhibited the proliferation of astrocytes, the inflammatory response, and significantly alleviated syringomyelia after SCI. Li et al. implanted an NTF3-SF scaffold into an SCI canine model, which significantly inhibited glial scars, narrowed the lesion cavity, and improved the hindlimb motor function and nerve conduction in the experimental dogs [5]. Ranch et al. combined vascular endothelial cells (ECs) with NSCs in a hydrogel implant and found that this composite scaffold could induce angiogenesis in a rat SCI model [16]. Arulmoli found that hydrogel materials such as fibrin and HA have an angiogenic effect [17] and in *in vitro* 3D culture, HA has shown the ability to induce ECs to form capillary structures [18] and this vascularization is associated with cell adhesion and migration. Collagen can inhibit endothelial cell tube formation when added to a fibrin matrix *in vitro* [19]. In another rat model, after implanting collagen matrix with VEGF, angiogenesis and recovery of neurological function were observed. Chedly et al. designed a scaffold material for SCI treatment containing only CS and water as fragmented physical hydrogel suspension (Chitosan-FPHS), with a defined degree of acetylation, polymer concentration, and mean fragment size. It is found to promote reconstitution of spinal tissue and vasculature. Growing axons were myelinated or ensheathed by endogenous Schwann cells, whose survival was prolonged, that migrated into the lesion site [20]. Prefabricated shaped scaffolds are mainly suitable for severely damaged or semi-transverse injury models. Their advantages are stable mechanical structure, slower degradation than hydrogel, and longer structural support and drug release. The pore ratio and modification of its chemical composition can be precisely regulated, providing mechanical support for cell transplantation, axonal regeneration and reconstruction of synapses, as well as chemical orientation information [21]. In another experiment by Zeng et al., induced pluripotent stem cells (iPS cells) were transplanted into three-dimensional gelatin prefabricated scaffold wrapped with polylactic-co-glycolic acid copolymer, and implanted into the injured spinal cord. The cell scaffold was found to reduce inflammation at the injury site, motivate angiogenesis, support long-term survival of transplanted cells, promote axonal regeneration, and extend along the scaffold morphology [22]. Jalali Monfared et al. used MiR-219 overexpressed human endometrial stem cell-derived oligodendrocyte progenitor cells encapsulated in fibrin hydrogel, as an injectable scaffold. After it was injected to the injury site, the rate of myelination was observed to be significantly higher, but it did not cause recovery of motor function [23]. Duan et al. added the transplanted cells and NTF to a prefabricated degradable scaffold and placed it in the injured spinal cord region. After the scaffold degradation, the regenerated axons were seen passing through the damaged area and were distributed in an ordered longitudinal structure [24]. Nowadays, the strategy of combining tissue engineering materials with cell transplantation to repair nerve injury is becoming a viable research field.

4.3 Gene therapy

The regenerative capacity of the central nervous system gradually reduces with age, mainly due to decreased expression of nerve growth-related

genes [25, 26]. Micro RNA (miRNA), as a key molecule regulating cell biological processes, plays a key role in the nervous system by regulating neuronal internal signaling pathways. miRNA could be widely used as a diagnostic and therapeutic target in clinical and basic research [27]. miRNA-signal transducer and activator of transcription 3 (STAT3) signaling pathway is involved in the complex molecular biological process such as differentiation of nerve cells, maturation, and release of neural mediators [28, 29]. STAT 3 as an upstream transcription factor, promotes the expression of growth-associated protein 43 (GAP43) in neurons, thereby promoting regeneration of axons [30–32]. Up-regulation of miRNA-21 expression is associated with prolonged cell survival, promotes cell growth, proliferation, and reduces apoptosis. Hu et al. used miRNA-21 antagonists to inhibit the expression of miRNA-21 in neurons, resulting in decreased recovery of hindlimb motor function in SCI rats, increased range of lesions and decreased normal tissue range. Compared to the negative control group, miRNA-21 antagonists significantly increased apoptosis [33]. The proapoptotic genes fas ligand (*FasL*), phosphatase and tensin homolog (*PTEN*) and programmed cell death protein 4 (*PDCD4*) have been shown to be the direct target of miRNA-21. And miRNA-21 antagonist could increase the expression of FasL and PTEN *in vivo* but does not affect the expression of PDCD4, indicating that miRNA-21 can attenuate the occurrence of secondary apoptosis after SCI, which may be related to the regulation of apoptosis-promoting genes [34]. Bhalala et al. overexpressed miRNA-21 in transgenic mouse astrocytes, and the results show that the overexpression reduces secondary apoptosis after SCI and increases the axonal density in the damaged area [35]. Song et al. transplanted miR-124-modified bone marrow mesenchymal stem cells (BMSCs) into SCI rats and found that overexpression of miRNA-124 inhibited the expression of pyridoxal kinase (PDXK) and accelerated the differentiation of BMSCs into neural cells [36]. Dergham validated that the reduced expression of RhoA by miRNA-133-b could promote the repair of the corticospinal tract [37]. Conrad et al. found that miRNA-133-b expression was significantly up-regulated in the adult zebrafish SCI model, and further, inhibition of RhoA promoted repair of the corticospinal tract [38]. Jee et al. infused miRNA486 into the spinal cord of healthy mice and found the expression of neurogenic differentiation 6 (NeuroD6) was significantly inhibited. The motor function of the mice decreased and neuronal death increased. In contrast, knocking out miRNA486 revealed enhanced expression of NeuroD6 and improved recovery of hind limb function in the mice [39]. NeuroD6 is an important protein for neuronal differentiation and oxidative stress. As a target, it could inhibit miRNA486 by up-regulating NeuroD6 expression, thereby reducing apoptosis and improving functional recovery after SCI. Currently, miRNA-21, miRNA-l24, miRNA-219, miRNA-338, miRNA-133-b, miRNA-486, and miR-20a-3p are known to target STAT3, thereby regulating the extension ability and regeneration of neuronal axons after SCI.

4.4 Cell transdifferentiation

In 2002, Tosh et al. defined transdifferentiation as the process by which a differentiated cell is irreversibly transformed into another differentiated cell [40], i.e., the gene of one somatic cell is directly reprogrammed into another cell type without transition into a cell pluripotency state [41]. Wada et al. converted fibroblasts expressing the single transcription factor MyoD into myoblasts for the first time [42]. To date, many studies have reported that a somatic cell can be transdifferentiated to another functional cell [43–46]. Methods

for transdifferentiating cells into neurons include the inducible neurons (iNs) technique, induced pluripotent stem cells (iPSCs) technique and indirect lineage conversion (ILC) technique.

To obtain the neural cells, iPSCs technique involves directly reprogramming genes to obtain induced neural stem cells (iNSCs) or induced neural progenitor cells (iNPCs). But iPSCs technology has low induction efficiency, poor proliferation ability, and unstable epigenetic and biological characteristics, which greatly increase the degree of degeneration or malignancy of cells. The iNs technique directly reprogrammes a gene to transdifferentiate cells to iNs without intermediate steps such as the creation of pluripotent stem cells [47]. iNs has a corresponding physiological function. Liu et al. injected the transcription factor ASCL1 into the dorsal midbrain of mice by the adeno-associated virus carrier with GFAP and found AST is transdifferentiated into functional neurons [48]. Bonilla transdifferentiated human MSCs into neurospheres and neuron-like cells within 3 to 7 days [49]. However, this method can only be applied to the transformation of certain lineage cells, and the efficiency is still very low, which limits cell proliferation making it difficult to obtain enough cells for clinical use. Since the emergence of iPSCs and iNs techniques, they have been used to transdifferentiate cells into blood cells, hepatocytes, cardiomyocytes and nerve cells [46, 50].

ILC is a technique in which differentiated cells are dedifferentiated and lose their initial phenotype, allowing cells to exhibit a plastic transitional state and finally induce differentiation into new types of cells. For the first time, Kurian et al. used the ILC method to transdifferentiate human fibroblasts into progenitor vascular cells [51]. These new cells not only proliferate but also differentiate into ECs and vascular smooth muscle cells. Lim et al. used the co-expression of myelin transcription factor 1 (Myt 1) and the inhibitor of apoptosis gene Bcl-xL to transdifferentiate fibroblasts into iNPCs with better proliferative capacity, and then added nuclear receptor-related factor (Nurr) of dopamine-associated transcription factor and forkhead box protein A2 (FoXA2), which is beneficial to neuronal growth. The result was iNPCs being converted into neurons with dopaminergic neurological function [52]. Subsequently, Doeser et al. induced the protooncogene OSKM from the skin wound of transgenic mice to transdifferentiate fibroblasts into myofibroblasts, thereby improving tissue healing, reducing scar tissue, and reducing the risk of tumor [53]. Compared with iPSCs or iNs technology, ILC is a simple and effective technology, which can rapidly generate stem cells, and has cross-line differentiation potential. It shortens the operation time and reduces the risk of tumorigenesis. Therefore, it is a promising candidate to meet the needs of clinical application in the future with regard to both cell types and required quantities.

Astrocytes (AST) are rapidly activated to form reactive astrocytes after central nerve injury. This process is called "AST activation". RAS undergoes defensive changes of AST in morphology, gene expression, proliferation, and function to deal with the complex pathology after central nerve injury including SCI [54]. The activation of AST may be related to the expression of the telomerase reverse transcriptase gene. In the early stage of SCI, RAS can inhibit the spread of inflammation, reduce the extent of injury, and promote the repair of wound sites. In the advanced stage, hyperproliferative RAS can form glial scars at the injury site [55], which blocks the regeneration of axons, can also secrete axonal regeneration inhibitory factors. Experiments have shown that nestin, a specific marker of many NSCs, can be seen in the injured central nervous system. Although NSCs proliferate, they have not yet

successfully differentiated into neurons. It has been confirmed that matrix metalloproteinases (MMPs) can be released in RAS by Falo et al. MMP-3 repairs damaged extracellular matrix proteins and helps them to degrade the debris of cell necrosis, establishing a microenvironment that is beneficial for axonal regeneration [56]. As an endogenous repair mechanism, RAS can use the relevant NTFs to provide a material basis for limited axonal regeneration [57–58], however, the numbers of regenerated axons cannot completely replace the damaged neurons. Therefore, RAS is directly transdifferentiated into neurons *in vivo*, replacing the destroyed neurons and integrating those remaining into the neural network. In addition, glial scars can be suppressed, thereby removing the blocking effect on axonal extension. So far, scholars have tried to use transcription factors, signal pathways and other methods to stimulate transdifferentiation. Faiz et al. transdifferentiated RAS into neurons by the forced expression of ASCL1 in the subventricular region of the mouse [59]. Guo et al. injected Neuro D1 into the mouse striatum using a retrovirus and found that overexpression of Neuro D1 can directly reprogram RAS into functional neurons in the mouse cortex *in vivo* [60]. Magnusson demonstrated that blocking Notch signaling can trigger AST to transdifferentiate into neurons in the striatum of normal mice. Therefore, AST can be transdifferentiated into nerve cells under the regulation of Notch signal [61]. RAS was transdifferentiated into pluripotent neurospheres by Sirko et al. via the SHH signaling pathway [62], and Shen et al. demonstrated that VEGF participates in the process of AST transdifferentiation into nerve cells in the striatum [45]. Therefore, the RAS can be stimulated to differentiate into nerve cells by activating relevant signaling pathways, and the spinal neural network may be reconstructed after SCI.

At present, the studies on cell morphological and functional integrity, epigenetic changes, genetic integrity, telomere and telomere, cell memory and immunogenicity are relatively simple. There are few studies on the transdifferentiation of RAS into neurons. The complex microenvironment *in vivo* is still challenging to the current transdifferentiation technology. Although the transdifferentiation techniques are not yet mature enough, they are challenging the traditional idea that the developmental path of terminally differentiated cells cannot be changed, and opening up a promising new future for the repair of SCI.

7 Summary

There are reports of repair methods such as peripheral nerve transplantation and pulse electrical stimulation. However, due to the limited ability of the central nervous system to regenerate and the intricate pathological changes after SCI, a single treatment method will not be adequate. The "combination" of several methods may greatly improve our ability to repair SCI. Cao et al. firstly implanted the glial cell line-derived neurotrophic factor (GDNF) gene into OECs using a retroviral vector, and found that *GDNF* gene-modified OECs not only expressed and secreted biologically active GDNF *in vitro* but also continuously produced GDNF *in vivo*, which significantly promoted axonal regeneration and functional recovery in complete injury of T10 spinal cord in rats. It increased the Basso Beattie Bresnahan (BBB) scale score of the hind limb function from 4 to 9 [63]. Fouad et al. transplanted a chondroitinase preformed scaffold containing OECs into a complete SCI rat and observed the regeneration of axons extending along the scaffold and obvious recovery of motor function [64]. Therefore, a "combined" approach such as transdifferentiated cells combined with tissue engineering materials will become an important field of research.

Conflict of interests

The authors declare that they have no possible conflict of interests.

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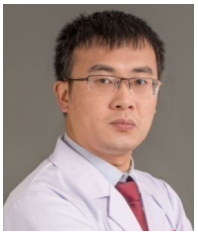

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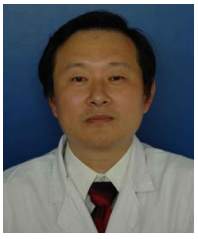

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