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Spinal Cord Injury: Pathophysiology, Neural Stem Cell Treatment and Its Combination with Other Strategies

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Abstract

Spinal cord injury is associated with the damage to neural circuits and disruption of neural pathways, leading to irreversible long-term complications in terms of physical and mental health. Traditional treatments mainly include surgical decompression and pharmacotherapy whereas none of them could attenuate or even alleviate long-term complications following SCI. This is largely due to complicated pathophysiological process in SCI which includes primary injury and secondary injury. Primary injury is defined as direct mechanical damage on the spinal cord in the initial time course of injury. Secondary injury occurs a few hours after primary injury and it could persist for longer time, promoting a catastrophe of degenerative pathophysiological process in spinal cord and related tissues. Although currently there is no effective cure strategies for SCI, some recent studies have shown that stem cell therapy combined with other strategies is able to enhance neurofunctional recovery and neural stem cell line is one of the stem cell lines which show convincing therapeutic effects (Huang, L., Fu, C., Xiong, F., He, C. & Wei, Q., 2021). Biomaterials can work as carriers which help deliver nutrient biomolecules to help NSC survive and differentiate to more functional cells or as scaffolds which provide structural bridges to lesion site for NSC migration and tissue regeneration (Vismara, I., Papa, S., Rossi, F., Forloni, G. & Veglianese, P., 2017). In addition, the basic principles of magnetic stimulation and electric stimulation are similar and they all involve producing electrical current on brain or spinal cord to modulate brain or spinal cord activity. It has been revealed that electric stimulation and repetitive magnetic stimulation is associated with neuroplasticity which may reduce inappropriate lateral sprouting resulting from NSC therapy, making them become potential complementary strategies to NSC therapy as well (Kricheldorff, J. *et al.*, 2022).

Keywords: spinal cord injury, neural stem cell treatment, magnetic stimulation, biomaterials, electric stimulation

1. Introduction

Spinal cord injury (SCI) is one devastating neurological condition that triggers a complex series of cellular and molecular changes, resulting in cell death and tissue destruction in terms of motor, sensory and autonomic function, currently with no curative treatment. In the United States it has been reported that nearly 17,730 new patients diagnosed with SCI each year and approximately 291,000 people are suffering severe disability and permanent morbidity caused by SCI, with a range from 294,000 to 363,000 persons living with long-term complications after SCI (Quadri, S. A. et al., 2020; Jain, N. B. et al., 2015; Lasfargues, J. E., Custis, D., Morrone, F., Carswell, J. & Nguyen, T., 1995). The most common cause of SCI within the United States is trauma among which motor vehicle accidents (38%) constitute most cases, followed by falls (30%), violence (13%), sports injuries(9%) and iatrogenic damage (5%) (Bennett, J., J, M. D. & Emmady, P. D., 2022). The pathophysiology phase consists of primary and secondary injury phase, which include a cascade of adverse events like edema, oxidative stress in the primary injury phase of SCI caused by mechanical damage to spinal cord and ischemia, demyelination, fibrotic scar formation in the secondary phase of SCI triggered by the onset of biomolecular and pathophysiological changes following primary injury (Anjum, A. *et al.*, 2020; Jin, Y., Bouyer, J., Shumsky, J. S.,

Haas, C. & Fischer, I., 2016). Despite multiple therapeutic strategies such as surgical decompression, therapeutic hypothermia and drug treatment have achieved different degrees of success, these current treatments can only solve one single aspect or a few aspects of events and curative effect is still elusive (Gazdic, M. *et al.*, 2018). Over the past decades, various stem cell lines like neural stem cells (NSC) have been applied to preclinical models and clinical trials, and each cell line has its own pros and cons (Bonosi, L. *et al.*, 2022). Thus, in recent years more and more researchers focus on combination therapy which involves stem cell transplantation and other strategies like biomaterials, magnetic stimulation or electric stimulation, to discover a reliable and effective treatment for SCI (Zheng, Y., Mao, Y. R., Yuan, T. F., Xu, D. S. & Cheng, L. M., 2020; Zeng, Y. S. *et al.*, 2022; Chen, X. *et al.*, 2021). This review summarizes the up-to-date findings on fundamental pathophysiology of SCI and highlights recent research on the mechanism of NSC in neurofunction recovery following SCI and combination therapy involving NSC and other strategies including biomaterials, electric stimulation and magnetic stimulation.

2. Pathophysiology of SCI

The pathophysiology process consists of two major events, primary injury and secondary injury (Anjum, A. *et al.*, 2020). Primary injury, as the name suggested, is defined as the direct mechanical damage on the spinal cord in the initial time course of injury (Figure 1). Despite of different types of primary injury such as laceration or compression, the cascade of cellular and molecular changes is similar which includes damage to neurons and axons, disruption of neurovascular structures and glial membrane (Anjum, A. *et al.*, 2020), glutamate excitotoxicity (Hellenbrand, D. J. *et al.*, 2021). Consequently, this focal damage to spinal cord immediately initiate a sustained secondary injury cascade. Secondary injury occurs a few hours after primary injury and it could persist for longer than 6 months. According to the time from damage secondary injury can be categorized into 3 stages, acute, subacute (intermediate) and chronic phases (Slater, P. G., Dominguez-Romero, M. E., Villarreal, M., Eisner, V. & Larrain, J., 2022). Acute secondary injury phase lasts from 2 hours to 48 hours following primary injury and its main manifestation is hemorrhage and edema caused by spinal cord ischemia which can be divided into 3 subgroups, cytotoxic, ionic and vasogenic. Then events of spinal cord injury gradually transit into subacute and chronic secondary injury stage, leading to substantial apoptosis, axonal surviving demyelination, Wallerian degeneration and formation of glial scar at the injury site (Alizadeh, A., Dyck, S. M. & Karimi-Abdolrezaee, S., 2019). Apoptosis is defined as one programmed cell death pathway which plays one pivotal role in SCI. Astrocytes are activated initially following SCI and migrate to the lesion site to participate repairing tissue whereas they promote glial scar formation in chronic secondary phase of SCI via a reactive cellular mechanism (Okada, S., Hara, M., Kobayakawa, K., Matsumoto, Y. & Nakashima, Y., 2018). One recent paper found that the inflammatory response to the SCI may be involved in the cystic cavity formation which includes the confinement of inflammation within the localized are separated from the rest of the spinal cord, transfer the edema fluid from a larger area in the spinal cord around the site of necrosis into the forming of cavity and the eventual development of a layer of astrogliosis around the cavity to isolate cavity region from the rest of the spinal cord so as to preserve the homeostasis (Escartin, C. & Bonvento, G., 2008; Karimi-Abdolrezaee, S. & Billakanti, R., 2012).

3. Stem Cell Therapy in SCI

Stem cells are defined as a population of undifferentiated cells which have features of the ability to self-renewal and to differentiate into different types of cells and tissue, usually arising from one single cell (Kolios, G. & Moodley, Y., 2013) and stem cell therapy has been studied as one potential treatment for at least decades in preclinical studies and clinical studies. The underlying mechanisms involving a) renew themselves sufficiently to provide appropriate level of cells and differentiate into mature neurons and glia to achieve tissue repair and neurofunctional recovery; b) produce factors to build a favorable microenvironment for spinal cord regeneration and neurofunctional restoration (Martin-Lopez, M., Fernandez-Muñoz, B. & Canovas, S., 2021). There are different cell lines of stem cells which include human embryonic stem cells (hESC), neural stem cells (NSC), mesenchymal stem cells (MSC) and other different stem cells (Figure 2). hESC are pluripotent cells derived from the inner cell mass of human embryos, which are able to differentiate into tissue from all 3 germ layers (endoderm, mesoderm, and ectoderm). Mesenchymal stem cells are multipotent stem cells developing into tissue derived from mesoderm, which include bone cells (osteoblasts), cartilage cells (chondrocytes), muscle cells (myocytes) and fat cells that give rise to marrow adipose tissue (adipocytes). Neural stem cells are also multipotent somatic cells producing all neural lineages such as neurons, astrocytes and oligodendrocytes. NSC are present in both embryo and adult brains (subventricular zone of the lateral ventricle) (Huang, L. & Zhang, L., 2019). Neural progenitor cells are the progenitor cells of the brain and spinal cord which are committed to generate only one category of neural components such as regional and spatially distinct neurons or glial cells (Homem, C. C., Repic, M. & Knoblich, J. A., 2015). Each typical type of stem cell treatment has its own pros and cons (Bonosi, L. *et al.*, 2022). NSC can secrete neuroprotective cytokines like brain-derived neurotropic factors (BDNF) and enhance cell proliferation as well as myelination (Bonosi, L. *et al.*, 2022). In addition it can

modulate the inflammatory response by decreasing proinflammatory cytokines production like TNF-alpha, IL-6 and IL-12 (Cheng, Z. *et al.*, 2016).

However, the neurofunction recovery is still limited, which might be attributed to failure of migration of NSC to lesion sites and difficulties for NSC to survive and differentiate into functional neurons which decrease glial scar formation (Bonosi, L. *et al.*, 2022). NSC, multipotent CNS cells, are capable of differentiating into neurons and glia like astrocytes and oligodendrocytes which play important roles as neural building blocks (Jin, Y., Bouyer, J., Shumsky, J. S., Haas, C. & Fischer, I., 2016). NSCs, known as endogenous NSCs, stay in a relatively 'quiescent' state which can be activated by some injuries or certain factors like VEGF. NSCs can reenter the cell cycle to replicate themself and develop into different cells including neurons (only in the neurogenic areas), astrocytes and oligodendrocytes, producing multiple possibilities for restore neurofunction in SCI on the basis of large scale of tissue cells (Zheng, Y., Mao, Y. R., Yuan, T. F., Xu, D. S. & Cheng, L. M., 2020). Exogenous NSC refers to the NSCs which are derived from cell sources in vitro such as pluripotent stem cells including ESCs and iPSCs and somatic tissue including skin fibroblast, urine cell and blood cell (Tang, Y., Yu, P. & Cheng, L., 2017).

4. Combination Therapy

Although stem cell therapy provides some promise to restore neurofunction in SCI in preclinical studies, there is still huge gap existing between mice and human beings (Harding, J., Roberts, R. M. & Mirochnitchenko, O., 2013). Two main obstacles for clinical translation are as follows. Firstly, small number of stem cells can survive and fewer viable cells can be retained around the lesion site. It has been noted that some cells moved out from the parenchyma or are removed into blood stream mechanically, resulting in the low retention of cells (Nie, Y. *et al.*, 2017; Dow, J., Simkhovich, B. Z., Kedes, L. & Kloner, R. A., 2005). The pathophysiological changes in SCI lead to damage to the microenvironment which is suitable for the survival of neurons, glia and other tissues, thus it is significantly difficult for stem cells to proliferate and differentiate in such unfavorable microenvironment and even many cells go die in vivo (Choumerianou, D. M., Dimitriou, H. & Kalmanti, M., 2008). Secondly it is still elusive about the mechanisms of differentiation processes in endogenous and exogenous NSCs, and in fact, majority of viable stem cells differentiate into astrocytes other than neurons under pathological conditions, impeding the functional neuronal relay formation and efficient synaptic connections establishment. Hence incorporating stem cell therapies with other strategies might provide a potential treatment to facilitate stem cells transplantation and enhance functional neural and synaptic connections in SCI.

4.1 Biomaterial

Some decades ago, biomaterials were mainly performed to delivery drugs and carry bioactive factors to promote NSC survival and boost axonal growth through a variety of growth factors cocktail interaction in SCI animal models (Wang, Y. *et al.* 2011; Gao, M. *et al.*, 2013; Robinson, J. & Lu, P., 2017). Furthermore, biomaterials can function as one stiffness matching design to CNS tissue, which encourages cell adhesion, immunosuppression response, cell survival and differentiation in the pathological microenvironment of SCI (Leipzig, N. D. & Shoichet, M. S., 2009; Khaing, Z. Z. *et al.*, 2011; Aurand, E. R., Lampe, K. J. & Bjugstad, K. B., 2012). Meanwhile, different components and structures of biomaterials convey different signals to cells in order to help them achieve different roles during tissues repair process in SCI. For example, several pivotal parameters in biomaterials need to be reviewed so as to decide differentiation direction of cells, including topography, chemistry and physical properties. Previous studies have displayed that stem cell survival, proliferation, attachment and differentiation could be modulated by the topography and chemical composition of biomaterials (Alvarado-Velez, M. *et al.*, 2021; Martino, S., D'Angelo, F., Armentano, I., Kenny, J. M. & Orlacchio, A., 2012).

Overall biomaterials can be divided into two categories: natural and synthetic. ECM proteins such as collagen hydrogels and laminin hydrogels (Yuan, T. *et al.*, 2014; Masand, S. N. *et al.*, 2012; Stabenfeldt, S. E., García, A. J. & LaPlaca, M. C., 2006) and non-ECM materials such as alginate and chitosan can be the source of natural biomaterials (Tummino, M. L., Magnacca, G., Cimino, D., Laurenti, E. & Nisticò, R., 2020). ECM proteins can integrate into injured tissue well whereas they degrade rapidly. The degradation time of non-ECM materials can be manipulated whereas they cannot have good interaction with mammalian cells due to the non-mammalian source (Kean, T. & Thanou, M., 2010; Rowley, J. A. & Mooney, D. J., 2002). Synthetic biomaterials include polylactic acid (PLA), poly lactic-co-glycolic acid (PLGA), polycaprolactone (PCL) and other materials (Shahriari, D., Koffler, J. Y., Tuszynski, M. H., Campana, W. M. & Sakamoto, J. S., 2017). One advantage of synthetic biomaterial is easy to tune compared to natural biomaterial whereas they need to be bound to ECM or other surface protein due to the lack of integrin-binding molecules (Kaplan, B. *et al.*, 2020; Führmann, T., Anandakumaran, P. N. & Shoichet, M. S., 2017). There are mainly three different types of biomaterial implants including hydrogel (Klouda, L. & Mikos, A. G., 2008), hollow tube conduit (Saltzman, E. B. *et al.*, 2019) and porous scaffold (Shahriari, D., Koffler, J. Y., Tuszynski, M. H., Campana, W. M. & Sakamoto, J. S., 2017). Injectable hydrogels are commonly used in SCI where injury sites are small and irregular with covered by spinal cord meninges, because they can conform to fit in different shapes of lesion sites appropriately (Lu, P. *et al.*,

2012; Lu, P. *et al.*, 2014; Kadoya, K. *et al.*, 2016). Hollow tube conduits can work as a bridge between damages nerve tissues thus they are more commonly used in peripheral nerve injury (Saltzman, E. B. *et al.*, 2019; Shapira, Y. *et al.*, 2016). Porous scaffolds can be used in SCI and the inner microarchitecture of pores encourage axons to grow without disorganized extension (Kaplan, B. *et al.*, 2020; Stokols, S. *et al.*, 2006).

With the emergence of 3D printing and its rapid development, it has been applied to many aspects especially tissue engineering. In terms of regenerative medicine, 3D bioprinting is one of the latest trending printing technologies which holds potential to be applied in SCI treatment. Basically, 3D printing refers to a process of constructing 3D solid objects from a digital file using raw materials such as polymeric resins, plastic, metal or rubber whereas the materials used in 3D bio printing are biomaterials or bioinks. Bioinks consist of living animal cells and other growth factors or biomaterial, which have capacity to deliver bioactivity of scaffolds and mimic the ECM environment so as to promote stem cells adhesion and differentiation. The combination application of 3D bioprinting and NSCs has gradually become a hotspot issue during recent years and several research has explored and analyzed the treatment outcome of several types of hydrogels and biomaterials in SCI (Lin, C. *et al.*, 2021) and the number of live cells contained in the biomaterials can be controlled by adjusting synthetic parameters including shear stress, pressure, temperature and the properties of bioinks. One study on combination therapy of collagen scaffold and NSPCs has revealed that the collagen scaffold is able to offer support to induce cell proliferation and differentiation and play one important role in guiding axonal growth as well as help remyelination and neuro-regeneration (Zou, Y. *et al.*, 2020). One latest paper has shown there are significantly favorable effects using combination therapy of NSCs and a 3D bioprinted collagen/silk fibroin scaffold in rat SCI models and they found plentiful axonal extension, less formations of glial scars and large enhancement in neurological scores (Jiang, J. P. *et al.*, 2020). 3D bioprinting technology has shown advances to become the ideal printable biomaterials that mimic the natural complicated structure of the ECM and the biomaterials or bioinks on 3D solid objects help axonal regrowth and reconstruct the neural circuitry. Bioinks consist of living animal cells and other growth factors or biomaterial, which have capacity to deliver bioactivity of scaffolds and mimic the ECM environment so as to promote stem cells adhesion and differentiation.

4.2 Electric Stimulation

Over half a century ago electrical stimulation has been implemented after spinal cord injury to help neurofunction restoration and improve patients' life quality (Thrasher, T. A., Flett, H. M. & Popovic, M. R., 2006). It is generally believed that some neural circuits are spared even under the condition of complete SCI although these circuits are not efficient to achieve a sufficient output to activate motor neurons distal to the lesion site. One study demonstrated that electromyographic activity can be voluntarily produced in two dependent muscles from paralyzed limbs in all 12 patients with complete spinal cord injury, suggesting the existence of spared pathways in nearly all patients even with clinically complete damage (Moss, C. W., Kilgore, K. L. & Peckham, P. H., 2011). Accordingly electric stimulate is considered to be one potential therapy for induction of neuroplastic changes at synapses within the spinal cord and brains, helping synapses and pathways reorganize and adapt to the new microenvironment following SCI. According to the anatomical site, current electric stimulation can be basically subdivided into three classes, cortical, deep brain and spinal cord stimulation. Cortical brain stimulation is commonly studied in brain injury models which is able to stimulate specific cortical regions and it includes epidural electrical cortical stimulation (eECS) and transcranial direct current stimulation (tDCS) (Kim, W. S., Lee, K., Kim, S., Cho, S. & Paik, N. J., 2019; Moisset, X. & Lefaucheur, J. P., 2019). Deep brain stimulation (DBS) in subthalamic nucleus and the internal globus pallidus is one effective treatment for eligible patients with Parkinson's disease whereas DBS on the midbrain locomotor center has also been suggest as one potential strategy to help motor function recovery following SCI (Bachmann, L. C. *et al.*, 2013). Spinal cord stimulation (SCS) is one of the most frequently studied electrical stimulation in treating SCI and it can be further subdivided into four types, intraspinal, transcutaneous, epidural stimulation and electroacupuncture (EA). Among of them, epidural SCS has been focused most and firstly it was emerged as a potential treatment of chronic pain in the late 1960s and early 1970s (Shealy, C. N., Mortimer, J. T. & Hagfors, N. R., 1970; Shealy, C. N., Mortimer, J. T. & Reswick, J. B., 1967). One previous paper stimulated the spinal cord using one single electrode placed on the dura of the patient with chronic chest and abdominal pain, which caused a 'buzzing' sensation at the frequency of 10-50 Hz and reduced the pain for 5-15 mins (Shealy, C. N., Mortimer, J. T. & Reswick, J. B., 1967). One recent paper demonstrated that motor function and bladder function was enhanced following the treatment of epidural SCS, which might be explained by the increase in excitability of baseline level of spinal cord resulting from the electrical stimulation (Grahn, P. J. *et al.*, 2017). For instance, other low levels of inputs such as proprioceptive inputs is able to activate the motor circuits for a given task (Edgerton, V. R. *et al.*, 2008). Transcutaneous stimulation improves spasticity and enhances restoration of stepping function within the stimulation periods following SCI (Hofstoetter, U. S. *et al.*, 2015; Hofstoetter, U. S. *et al.*, 2014; Minassian, K. *et al.*, 2016). Some paper found that ES with high frequency produced higher current transcutaneous current over the skin above spinal cord with little discomfort and the current generated is able to

activate the lumbar spinal cord in spinally injured (Gad, P. *et al.*, 2017; Gad, P. N. *et al.*, 2015) and intact subjects (Gerasimenko, Y. *et al.*, 2015; Gerasimenko, Y. *et al.*, 2016), eliciting appropriate movements. EA refers to applicating pulsed current to acupuncture needles on the skin and it has shown to have better effects when combining with NSC derived neural network scaffold transplants (Jin, H. *et al.*, 2019). EA can secrete neurotrophic-3 (NT-3), activate downstream TrkC/AKT/mTOR signaling pathway and promote neuron survival and differentiation in transplanted neural network tissues as well as enhance synaptic connections and improve neurofunctional integration of stem cells with the host spinal cord neural network (Jin, H. *et al.*, 2019).

Compared to other electric stimulation, EA has been demonstrated to promote transplanted tissue survival and provide appropriate microenvironment to achieve cell synthesis (Jin, H. *et al.*, 2019). Intraspinal stimulation is defined as electrical stimulation within the spinal cord and there are many animal studies however intraspinal cord stimulation in humans are rare. Intraspinal stimulation can elicit various appropriate movements including steeping, reaching and grasping (Saigal, R., Renzi, C. & Mushahwar, V. K., 2004). Intraspinal stimulation can activate motoneurons or ventral root axons and can stimulate intermediate lamina and interneurons, eliciting single joint movement or coordinate motor patterns in complicated neural network (Ranck, J. B., Jr., 1975). There is no much paper focusing on NSC therapy with electric stimulation on treating SCI whereas electric stimulation is always combined with exercise to help animals and patients with SCI recover sensorimotor function and reduce spasticity and pain, which shows synergistic and non-interfering therapeutic effects compared to task specific rehabilitation only (Kumru, H. *et al.*, 2020; Jo, H. J., Richardson, M. S. A., Oudega, M. & Perez, M. A., 2021; Awad, B. I., Carmody, M. A., Zhang, X., Lin, V. W. & Steinmetz, M. P., 2015; Naro, A. *et al.*, 2017). However one paper points out that some combination approaches involving rehabilitation have failed to achieve higher levels of recovery than those with independent individual treatments and it might be explained by the inherent plasticity of nervous system produce competing neural changes which are incompatible with complementary strategies (Maier, I. C. *et al.*, 2009). Accordingly, more research should be done to explore the therapeutic effects of different combination therapy.

4.3 Magnetic Stimulation

Magnetic stimulation refers to one diagnostic and therapeutic technique which offers an exogenous electric pulse on certain electrically conducting tissues with magnetic coils over tissues (Rossini, P. M. *et al.*, 2015) and it can be divided into two different types including transcranial magnetic stimulation (TMS) and trans-spinal magnetic stimulation (TSMS). From 1985 when TMS as the noninvasive method of brain stimulation firstly has been applied to activate human cortex (Barker, A. T., Jalinous, R. & Freeston, I. L., 1985), to this point when a large number of research have revealed the property of TMS and its effects on treating CNS and PNS disease, we have already known that TMS is able to induce electric potential and muscle contraction if given appropriate magnetic stimulation over the mammalian motor cortex (Kremer, K. L. *et al.*, 2016). Furthermore, repetitive TMS or TSMS has been studied in some research later and it is defined as a serious of frequent magnetic stimulation on cortex regions or local spinal cord level, respectively. It is believed that repetitive magnetic stimulation is associated with synaptic plasticity and int turn causes long-term therapeutic effects following TMS or TSMS, which involves long-term potentiation and long-term depression resulting from the modifications of activity of the NMDA receptors (Hunanyan, A. S., Petrosyan, H. A., Alessi, V. & Arvanian, V. L., 2012; Duan, H. *et al.*, 2015; Shang, Y. *et al.*, 2016). According to different stimulation parameters including stimulation pattern, frequency, location and instrument, neural activity could be enhanced or suppressed (Fernandez, L., Major, B. P., Teo, W. P., Byrne, L. K. & Enticott, P. G., 2018; Ross, J. M., Iversen, J. R. & Balasubramaniam, R., 2018). Upper motor neurons within motor cortex are activated by the current induced by TMS over cerebral cortex and the action potentials are sent down to spinal cord via descending conduction tract such as CST, encouraging metabolism and growth in neural circuits (Duan, H. *et al.*, 2015). TSMS produces a stimulation in central pattern generators (CPGs), pivotal neural networks in the spinal cord which generate rhythmic movement such as walking and swimming, which motivates the functional restoration of neural circuits and promotes the motor activity recovery (Diaz-Ríos, M., Guertin, P. A. & Rivera-Oliver, M., 2017). One recent paper has found that applying focal rTSMS paradigm as treatment of SCI could help endogenous neural stem cell proliferation and promote differentiation of ependymal cells to oligodendrocytes and astrocytes to modulate glial scar formation (Chalfouh, C. *et al.*, 2020). Moreover, rTSMS is able to induce axonal regrowth and neural branching via upregulating the expression of myelin and microtubule proteins such as MBP (Myelin Basic Protein), MAPT (Microtubule-Associated Protein Tau), MOG (Myelin Oligodendrocyte Glycoprotein) (Chalfouh, C. *et al.*, 2020). It is additionally shown that neuronal function is enhanced by rTSMS which is associated with the release of neurotrophic factors including BDNF and VEGF (Grehl, S. *et al.*, 2015; Zhang, Z. C. *et al.*, 2015).

Although TMS and TSMS can potentially result in improvement in neuronal function and motor function, we still do not have a complete understanding of mechanism of combined therapy of magnetic stimulation and stem cell therapy. One reason for it is lack of enough research on animal studies and another reason is mice studies are different from human studies. The biological similarity between mice and human being and the differences of

technology used to evaluate and monitor the effects of stem cell therapy are the two main factors for differences between mice and human studies. Mice have shorter life span so it will be difficult to know about long-term effects of stem cell therapy on SCI. Mice have different physiological parameters such as those about immune system compared to those of human beings (Harding, J., Roberts, R. M. & Mirochnitchenko, O., 2013). Mice have less number and types of stem cells that can be sufficient to manipulate and analyze in stem cell therapies. The technologies used in mice and human beings to evaluate and monitor neurofunction recovery is different (Barbagianni, M. S. & Gouletsou, P. G., 2023). In addition, the safety of stem cell applications cannot be evaluated in the same way because the dosages of stem cells and the route of administration are very different in mice and human with huge differences in size (Plews, J. R., Gu, M., Longaker, M. T. & Wu, J. C., 2012). Furthermore, although there are a large number of papers studying the effects of magnetic stimulation on treating neuropsychiatric disease (Concerto, C. *et al.*, 2015; Val-Laillet, D. *et al.*, 2015; Ren, J. *et al.*, 2014), there is far more less paper exploring the effects of neural stem cell transplantation combined with magnetic stimulation after SCI. One latest study has found that the combination therapy of rTMS and MSC transplantation diminished apoptosis of SCI-induced neural stem cell and attenuated motor dysfunction in rat models (Guo, M., Wu, L., Song, Z. & Yang, B., 2020).

5. Conclusion

As the rapid development of people's living standards and increasing life expectancy, society and government demand of restoration and recovery of functional spinal activity and attenuation in complication following spinal cord injury is growing and till now there is still no cure treatment of SCI even a large amount of research studying the traditional treatment such as surgery treatment and pharmacology therapy. Stem cell therapy has been applied and tested in multiple preclinical animal studies whereas the treatment outcomes are not satisfying in clinical trials. The aberrant structures, glial scars and fluid filled cystic cavities, form during the pathophysiological progress during SCI and impede the axonal growth towards the lesion site. Furthermore, the glial scars and the inhibitory extracellular matrices surrounding it inhibit the migration and neurites growth of NSC into injury site (Huang, L., Fu, C., Xiong, F., He, C. & Wei, Q., 2021). In addition, NSCs remain undifferentiated or mostly differentiate into astrocytes, which impede the neurofunction recovery and reduce normal neuronal network reestablishment (Cao, Q. L., Howard, R. M., Dennison, J. B. & Whittemore, S. R., 2002). Thus, more attention and focus has been transferred to combination therapy involving stem cell cells and other strategies such as biomaterials, magnetic stimulation or electric stimulation. Neural stem cells possess the capability to differentiate into three lineages, neurons, oligodendrocytes and astrocytes, thus NSCs therapy seem to be one potential choice that could regenerate cells in spinal cord and regain function, without inducing teratomas (Hosseini, S. M. *et al.*, 2018). However, more preclinical and clinical research should be implemented to test the combination therapy effects of neural stem cells with other strategies. The combination of NSCs therapy with other strategies might be one vital future research and clinical practice topic, providing a promising outcome in terms of SCI treatment opening a new window for SCI patients.

Furthermore, we still do not fully understand the specific mechanism of magnetic stimulation and electrical stimulation in circuit restoration and function recovery following SCI and there are not abundant clinical data on the efficacy of magnetic stimulation and electrical stimulation for SCI. More clinical trials are encouraged to gain more data on human beings. A deeper understanding of magnetic stimulation and electrical stimulation in patients with SCI could help explore the mechanism of combination therapy involving stem cell therapy and other strategies, resulting in improvement in treatment effects and prognosis of stem cell therapy. Besides of traditional combination therapy involving stem cell, innovative experiment designs will occur with the progress of neurobiological technology and artificial intelligence. For example, promising advances in neurobiological technology especially those imaging technology which can be used to diagnose and monitor the circuit changes and neuronal recovery in SCI can provide a better track of dynamic neurological reorganization following SCI, which can help locate the target, observe the development of SCI and help guide as well as evaluate treatment of SCI. In addition, more attention is focused on use of artificial intelligence in SCI treatment gradually. Robotic devises are now applied in the field of neurorehabilitation training, which demonstrate promising improvement in neuroplasticity in terms of motor function recovery and sensory function recovery (Stevenson, A. J., Mrachacz-Kersting, N., van Asseldonk, E., Turner, D. L. & Spaich, E. G., 2015) as well as significant decrease in the stress of therapists and physicians (Hussain, S., 2014). Combination therapy involving stem cell therapy and motor rehabilitation may also provide one potential therapy regimen in patients with SCI, especially those with limb palsy. One recent paper shows that robotic-assisted gait training (RAGT) offers many advantages in people with incomplete SCI, including improvement in gait speed, walking distance, strength, range of motion and mobility ability whereas there is still insufficient evidence for the effect on balance, depression, cardiorespiratory fitness and quality of life (Alashram, A. R., Annino, G. & Padua, E., 2021). Brain-machine interface gains a firm foothold in the industry and research field during the last few years, which may benefit from the rapid development in artificial intelligence. Over the past decade, individuals with limb paralysis have

already been capable of rapid on-screen typing and point-and-click control of tablet apps with the help of intracortical brain-computer interfaces which can analyze and decode intended upper limb movements using recorded neural signals via implanted microelectrode arrays with a mountain of cables on the brain. However, in recent years wireless intracranial brain-machine interface is used in some pre-clinical and clinical research, one recent paper pointed out that communication bitrates were equivalent between cabled and wireless transmitters after comparison of bit error rate, packet loss, and the recovery of spike rates and spike wavefroms from the recorded neural signals, showing that wireless multi-electrode recording may be one valuable tool for human neuroscience research and treatment of SCI (Simeral, J. D. *et al.*, 2021). Although wide application of brain-machine interface is still in its infancy, maybe one day it can be explored more and fill in the gap between combination therapy involving stem cell treatment and the function restoration of SCI.

Table 1.

Stem cell	combination	Mechanisms and functions	Ref.
NSC (Neural Stem Cell)	Biomaterials	Induce NSC adhesion, proliferation and differentiation, help axonal regrowth and reconstruct the neural circuitry, less formations of glial scars, large enhancement in neurological scores.	(Zou, Y. *et al.*, 2020; Jiang, J. P. *et al.*, 2020; Chen, C. *et al.*, 2017)
	Magnetic stimulation	Promote synaptic plasticity, help endogenous neural stem cell proliferation, promote differentiation of ependymal cells to oligodendrocytes and astrocytes to modulate glial scar formation, induce axonal regrowth and neural branching.	(Moisset, X. & Lefaucheur, J. P., 2019; Bachmann, L. C. *et al.*, 2013; Shealy, C. N., Mortimer, J. T. & Hagfors, N. R., 1970; Hofstoetter, U. S. *et al.*, 2015)
	Electrical stimulation	Promote synaptic plasticity, improve spasticity and enhance restoration of stepping function, active local cells within lesion site to secrete neurotrophin-3, increase reconstruction of host neural tissue.	(Rossini, P. M. *et al.*, 2015; Hunanyan, A. S., Petrosyan, H. A., Alessi, V. & Arvanian, V. L., 2012; Diaz-Ríos, M., Guertin, P. A. & Rivera-Oliver, M., 2017; Chalfouh, C. *et al.*, 2020; Grehl, S. *et al.*, 2015; Val-Laillet, D. *et al.*, 2015)

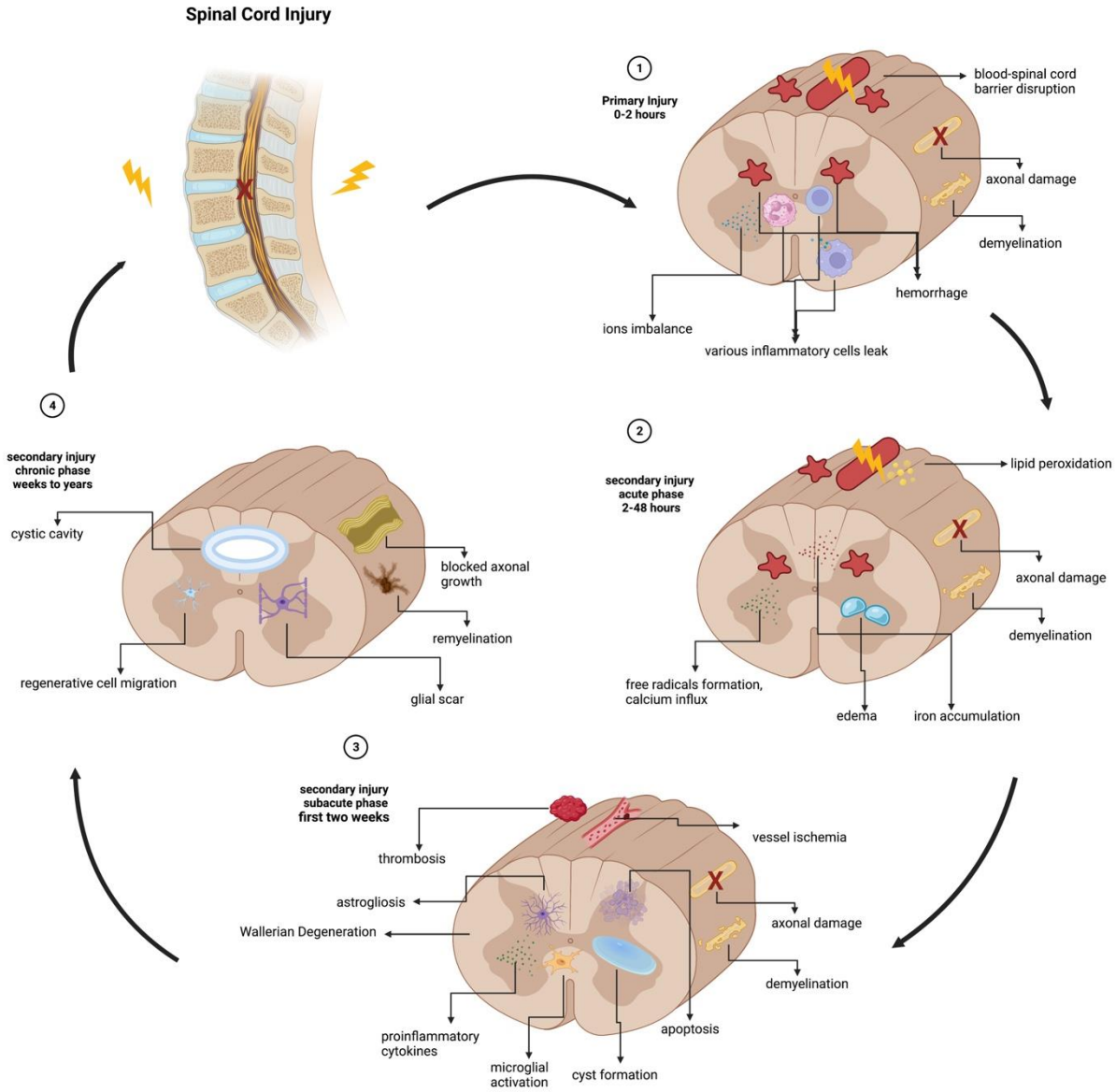

Figure 1. Spinal cord injury pathophysiology

The pathophysiology of SCI is a complicated cascade of biochemical and multimolecular interactions in the spinal cord and other related tissues and basically the pathophysiology process consists of two major events, primary injury and secondary injury (Anjum, A. *et al.*, 2020).

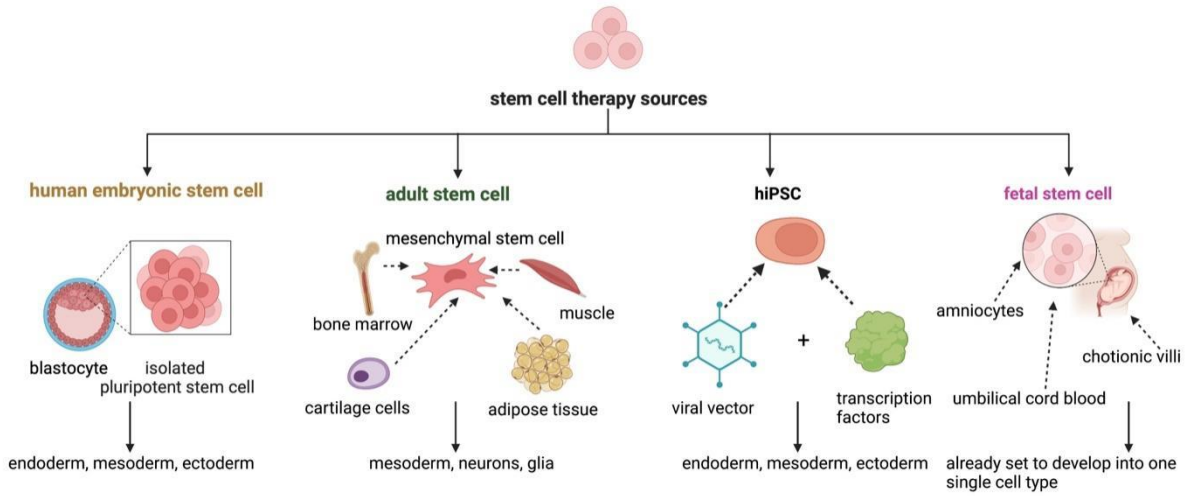

Figure 2. Stem cell therapy sources summary

Note: hiPSC: human induced Pluripotent Stem Cell

Transcription factors: Oct14, Sox2, Klf4, c-Myc, Nanog, Lin28 (Takahashi, K. & Yamanaka, S., 2006)

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