Application of Regenerative Therapy in the Treatment of Spinal Cord Injury (SCI): Repair and Regeneration

By

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A project submitted to the school of pharmacy in partial

fulfillment of the requirements for the degree of

Bachelor of Pharmacy (Hons.)

School of Pharmacy

BRAC University

November, 2022

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

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Approval

The project titled "Application of Regenerative Therapy in the Treatment of Spinal Cord Injury (SCI): Repair and Regeneration" submitted by Umma Habiba Rine (18346003) of Summer, 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.).

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Ethics statement

This paper is a review article and did not involve any use of animal or human experiments.

Abstract

Spinal cord injury (SCI) is one of the main factors contributing to world disability resulting in irreversible functional impairment, severe morbidity, and financial difficulties with no effective clinical treatments. Regardless of varying degrees of success, it has remained a challenging area for scientists due to the complex healing mechanism and the adverse nature of its pathophysiology. Although the results of clinical trials employing regenerative therapy specifically cell-based therapies have proved safe, their efficacy has not been successfully demonstrated. In this paper, I will review previously explored therapy strategies including cell-based therapies, biomaterial scaffolds, nanopharmaceuticals, and a combination of all of these therapies while highlighting current challenges in the research and clinical trials which may fill the gap between the brain and regenerative therapeutics. Therefore, this review article aims to provide insight into the most promising restoration methods and future directions to promote further research of regenerative therapies in the treatment of SCI.

Keywords: Spinal cord injury, regenerative therapy, cell-based therapies, scaffolds.

Dedication

This project is dedicated to my family members, respected faculty members and friends for their continuous support and guidance.

Acknowledgement

All praises to Almighty Allah. I would like to convey my sincere gratitude to my supervisor Kazi Fatema Rahman, Lecturer, School of Pharmacy for her invaluable feedback, patience and guidance to complete my project.

Then, I would also like to express my gratitude towards our Honorable Dean, Professor Dr. Eva Rahman Kabir, School of Pharmacy, BRAC University for her cooperation and support.

Finally, I would like to thank my family, friends and other respected faculty members for the motivation and support throughout my work.

Table of contents

List of Tables

List of Figures

List of Acronyms

SCI	Spinal Cord Injury
ECM	Extracellular matrix
TSCI	Traumatic spinal cord injury
GFAP	Glial fibrillary acidic protein
ROS	Reactive oxygen species
AAD	Acute axonal degeneration
CSPGs	Chondroitin sulfate proteoglycans
ESCs	Embryonic stem cells
NSCs	Neural stem cells
MSCs	Mesenchymal stem cells
iPSCs	Induced pluripotent stem cells
OECs	Olfactory ensheathing cells
IGF-1	Insulin-like growth factor 1
HGF	Hepatocyte growth factor
BDNF	Brain-derived neurotrophic factor
BSCB	Blood-spinal cord barrier

Chapter 1

Introduction

The yearly incidence of spinal cord injury (SCI) ranges from 10 to 80 per million individuals, making it a significant financial and administrative burden on healthcare systems across the world (Barbiellini Amidei et al., 2022; Thompson et al., 2015). Injury to the vertebrae, ligaments, or disks of the spinal column or the spinal cord itself can cause significant dysfunction and disability, including partial or complete paralysis, loss of sensation at the site of injury, and little chance of recovery, and is most commonly caused by vehicle accidents especially motor vehicles, followed by falls, sports injuries, and violent events (Van et al., 2020). Due to the adult spinal cord's poor regenerative capacity, there are currently no standard therapeutic options. This is primarily due to the development of a glial scar and the buildup of extracellular matrix (ECM) proteoglycans, which together form an impenetrable barrier that stops axons from regenerating over the site of damage, resulting in impaired nerve impulse conduction and decreased neural growth (Daniell, 2012). Pharmacotherapy, physical treatment, and surgical decompression are the main therapeutic options for SCI, all of which aim to restore neurological capabilities lost after the injury. Although some improvement is shown with each of these treatments, their efficacy is poor and their effects are very transitory (Alishahi et al., 2020; Khorasanizadeh et al., 2019). There is an immediate need for novel therapeutic methods to the management of SCI, given the scarcity of current therapy choices and the difficulties inherent in those that do exist. There are currently no viable therapies that can regenerate the spinal cord after damage, but regenerative medicine is a promising therapeutic alternative that uses bioactive signals to encourage cells to repair and renew(Ashammakhi et al., 2019). Research is needed to establish a successful regenerative therapy, but the development of cell-based therapies has already been seen as innovative. However, a combination of biomaterials

and nanocarriers following a combinatorial treatment strategy might prove more significant and long-lasting improvements in sensory and motor outcomes (Hiemstra et al., 2015).

The purpose of this paper is to explore all the possible treatment strategies for SCI focusing on regenerative therapy. This paper will emphasize on combination treatment strategy and also discuss the obstacles and challenges encountered in the development of regenerative therapies with an overall aim to prompt the scientific to accelerate research efforts in this field.

Chapter 2

Methodology

To write an impactful review paper on the “application of regenerative therapy in the treatment of spinal cord injury: repair and regeneration”, current and pertinent research papers and articles from journals with high-impact factor have been used to gather information and relevant theoretical evidences. To conduct quality literature review, databases were gathered using the following search engines such as Research Gate, Science Direct, Google Scholar, PubMed, Elsevier, etc.

Chapter 3

Spinal cord injury overview

3.1. Prevalence

An increasing number of people suffer from spinal cord injuries each year, making it one of the most devastating neurological and pathological conditions. Mortality risks and the frequency of traumatic spinal cord injury (TSCI) remained unchanged in 2022, with 26.5 cases per 1,000,000 people with an average age of 59.2 years, a focus on the fact that most cases were males (68.3%), and a focus on the fact that most TSCI was due to cervical lesions (52.1%), with traffic accidents accounting for 29.9% of all causes of injury (Barbiellini Amidei et al., 2022). When comparing emerging and developed nations, TSI was shown to be significantly higher in low and moderate income nations (13.69 per 100,000 people) than in high income nations (0.5 per 100,000 people) (8.72 per 100,000 persons). The true number and worldwide impact of TSCI are difficult to ascertain since, despite greater incidence rates in poorer nations, information registration in these countries is less precise and often incorrect.

3.2. Types of Spinal cord injury

3.2.1. Primary injury

It is common for sudden spinal injury to cause fractures and vertebrae dislocation, followed by the characteristics of bone fragments and torn spinal ligaments into the tissue, but in most cases the injury does not completely sever the spinal cord, and this stage is known as primary injury or acute SCI(Katoh et al., 2019; Ohnmar et al., 2009). The most prevalent kind of primary damage is caused by impact and persistent compression, however other types of injury caused by impact alone,

impact with transitory compression, laceration/transection, and distraction have also been discovered (Dumont et al., 2001; Rowland et al., 2008). Primary injuries to the spine cause systemic hypotension, spinal shock, ionic imbalance, ischemia, and neurotransmitter buildup because of direct damage to the spinal cord's ascending and descending pathways as well as blood vessels and cell membranes (Alizadeh et al., 2019; M. G. Fehlings & Sekhon, 2002).

3.2.2 Secondary injury

Secondary injury refers to afterwards occurring chemical and mechanical damage to spinal structures .and it is caused due to neuronal excitotoxicity and high buildup of calcium ions within cells, increased reactive oxygen concentrations, and elevated glutamate levels, all of which contribute to neurological dysfunction. There are several pathological processes involved, and they don't end until weeks after the initial damage. Increased cell permeability, apoptotic signaling, ischemia, edema, excitotoxicity, vascular damage, ionic dysregulation, inflammation, lipid peroxidation, demyelination, free radical production, fibroglial scar, etc. are all clinical manifestations (Anjum et al., 2020; Dimitrijevic et al., 2015). Acute, sub-acute, and chronic injury are the three stages of secondary injury.

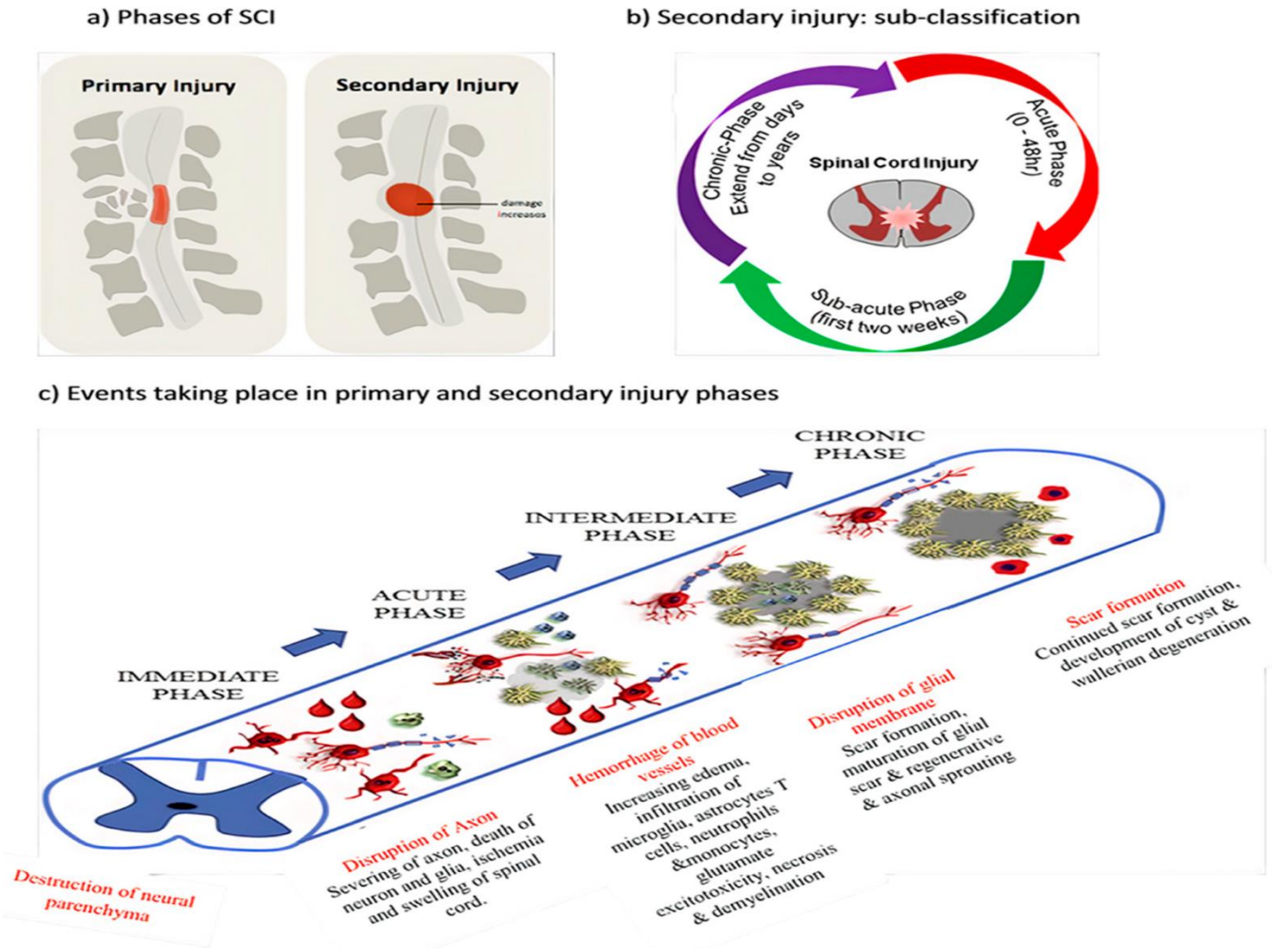

Figure 1: (a) Types of SCI, (b) Duration time of secondary injury, and (c) manifestation of primary and secondary injury with clinical futures (Anjum et al., 2020).

3.3. pathophysiology

To find the best regenerative medicinal approach for spinal cord injury, it is very important to fully understand the pathophysiology and have a clear knowledge of how disease systems and cellular interactions take place to design a therapeutic strategy for the highest yield.

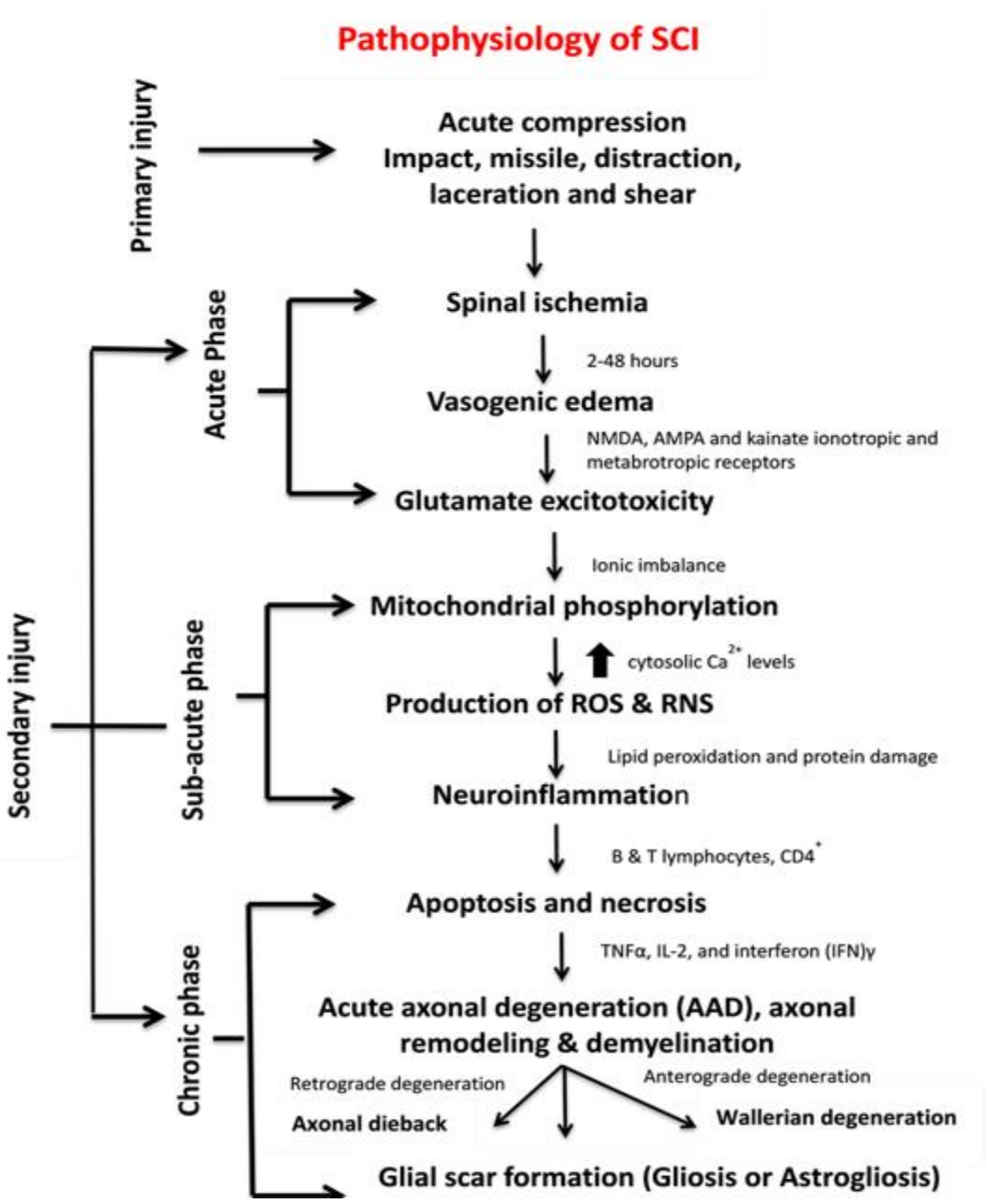

Figure 2: Schematic illustration of clinical manifestation of SCI(Anjum et al., 2020)

The first thing that happens following an injury is spinal cord ischemia due to the lack of blood flow to the area and the subsequent hypotension, bradycardia, and neurogenic shock that results from it. Necrosis causes free radical generation, glutamate-mediated excitotoxicity, and neurotoxicity (figure 1c), and the presence of structural indicators such glial fibrillary acidic

protein (GFAP) or interleukin 6 (IL-6) in CSF fluid increases both of these risk factors(Vanzulli & Butt, 2015). NMDA and AMPA receptor hyper-activation, mechanical stress, lipid peroxidation, 4-hydroxynonenal synthesis, and the excessive increase in glutamate excitation all play a role in inducing necrotic cell death via dysregulation of intracellular ions. Ca2+ and Na+ ion inflow increased, but intracellular K+ ion concentrations decreased. Ca2+ excess causes mitochondrial malfunction and ultimately cell death(Cao et al., 2013). Multiple cell types, including neutrophils, microglia, astrocytes, macrophages, B and T lymphocytes, dendritic cells, and other molecular components that cause neurotoxic or neuroprotective effects, are involved in the complicated inflammatory response associated with SCI known as neuroinflammation(Hall et al., 2016; Miron & Franklin, 2014). Both apoptosis and necrosis play important roles in mediating cell death following SCI. As the chronic phase of spinal cord injury (SCI) progresses, acute axonal degeneration (AAD) plays a crucial role by inducing Wallerian degeneration and the cysteine protease calpain. And last, axonal demyelination and subsequent axonal dysfunction are caused by oligodendrocyte injury and death(Almad et al., 2011; Domingues et al., 2016).

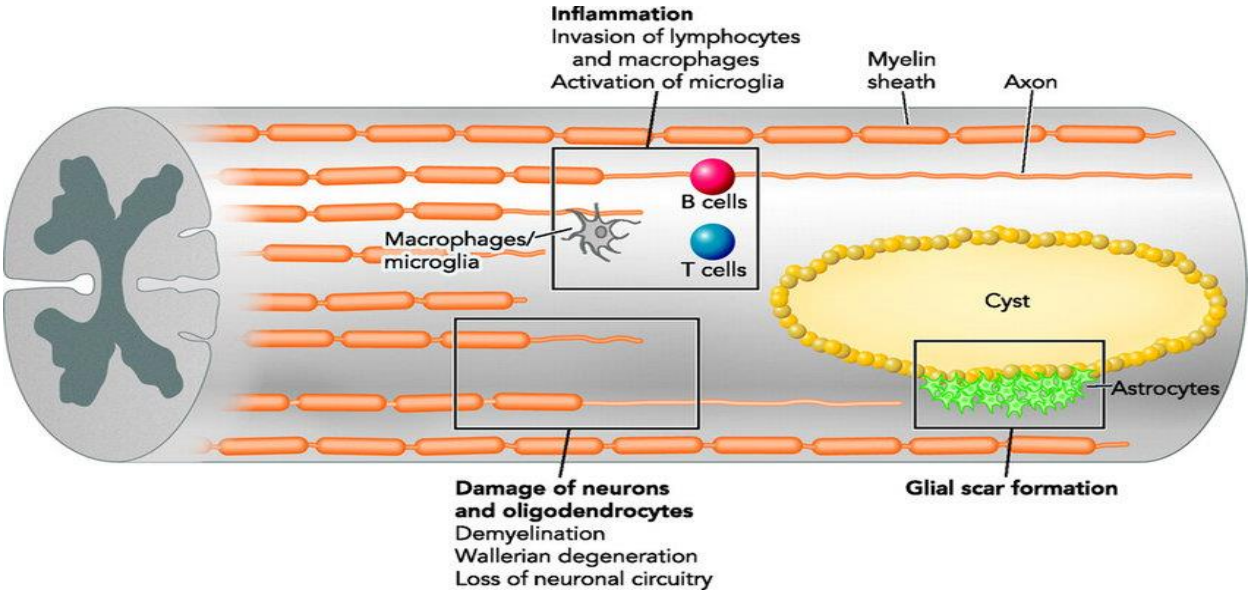

Figure 3: Glial scar formation at the site of SCI (Tsintou et al., 2015)

Figure 3 schematically presents the formation of gliar scar mediated by astrocytes which are considered the primary cause behind spinal cord degeneration (T. Yang et al., 2020). The formation of a lentiform-shaped cyst in the central part of the spinal cord during the chronic phase of secondary injury is the hallmark feature of SCI. Extracellular matrix and inhibitory factors like chondroitin sulfate proteoglycans (CSPGs) are formed by astrocytes and other cells, leading to the glial scar formation (Toy & Namgung, 2013; Yuan & He, 2013). This evidences that neurons cannot regrow due to the cyst and continuous enlargement of the lesion site and glial scar formation (Tsintou et al., 2015).

Chapter 4

Definition of regenerative therapy in SCI

The term “Regenerative medicine” stands for engineering, replacing, or regenerating human cells, tissues, or organs to restore the functional capacity of the injured tissues and organs, has been applied in the field of the central nervous system for decades(Sudhakar et al., 2015). The clinical presentation of SCI makes clear that axon renewal is blocked by glial scar formation, axon regeneration inhibitor factors, and demyelination, which in turn leads to cell death. To restore the injured neural network to its pre-injury state as closely as possible has therefore been the main objective of SCI research. state as possible through restoration or repair. Therefore, regenerative medicine can be proven to be an effective technique for restoring normal function to the injured spinal cord by promoting the reconnection of descending neural fibers with their original destinations, such as spinal interneurons and motor neurons in the caudal spinal cord (Katoh et al., 2019). There are three main ways that regenerative therapies are thought to achieve this goal: a) 1) replacing injured or lost neurons with healthy ones, b) 2) giving environmental enrichment via growth factors generated by freshly transplanted cells, or c) mending damaged neural tissue or organs (Nemeth et al., 2019). Some people with severe spinal cord injuries have benefited from stem cell transplantation procedures, which aim to replace damaged cells and repair damaged neural circuitry (Katoh et al., 2019). Many efforts have been made in the field of SCI research to produce a viable regenerative medicine, including the use of cell-based therapy, biomolecule delivery techniques, and scaffold-based treatment strategies(Tsintou et al., 2015).

Chapter 5

Treatment strategies

5.1. Current management

Although clinical therapies for SCI can be discouraging, it is essential that SCI be effectively addressed before becoming more severe after a traumatic incident in order to achieve a rapid restoration of neurological functions and prevent additional damage to the spinal cord. There is presently no therapy that can replace the destroyed tissue's functionality. Therefore, the current approaches to SCI care center on preserving the function of the surviving neurons and avoiding both short-term and long-term consequences such infections and pressure ulcers(Ashammakhi et al., 2019). The first treatment for spinal cord injury patients is nonpharmacological and consists of resuscitation, stabilization, decompression, rehabilitation, control of non-motor symptoms, and critical care in specialist centers to enhance functional results (Saremi et al., 2022; Venkatesh et al., 2019). Even while the general prognosis has improved because to advances in therapeutic care, the resulting neurologic impairments in SCI patients are still not being addressed. To restore the injured tissue, neuroregeneration is facilitated, and cell death is minimized, since this has been the primary focus of therapeutic care (Wilcox et al., 2012). However, these approaches do not significantly alleviate SCI-related neurological impairments. Thus, researchers in the field of SCI therapy have focused for decades on regenerating injured axons and myelin and reconnecting efferent and afferent pathways(Hyun & Kim, 2010).

5.2. Regeneration strategies for spinal cord injury

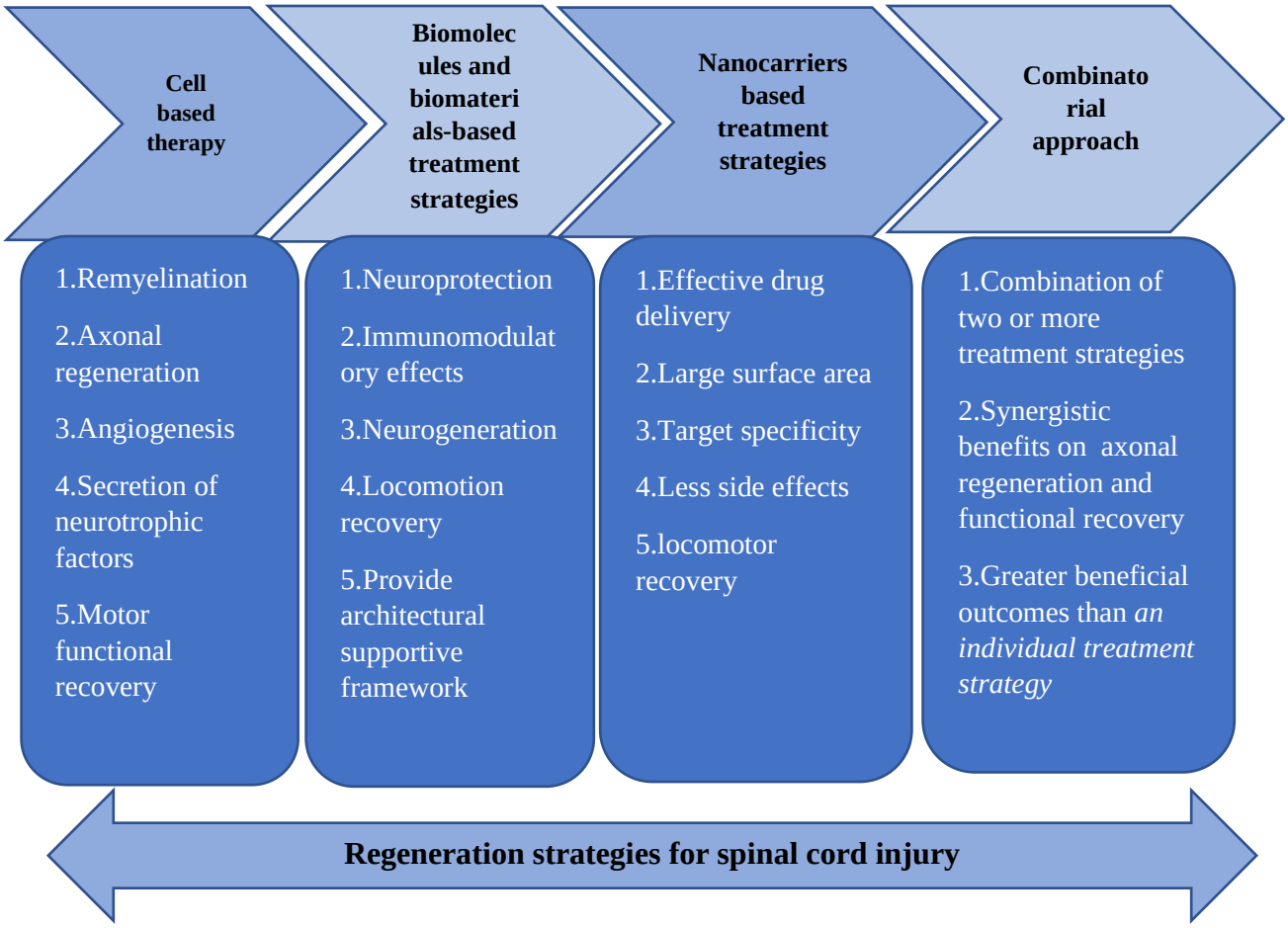

Figure 4: Regeneration therapeutic strategies for spinal cord injury. Inspired by (Ashammakhi et al., 2019)

5.2.1. Cell-based therapy

Figure 4 represents all the regenerative treatment strategies that have been explored in regenerative research. One of the most popular regeneration techniques in the neurogenerative area in recent years has been the transplantation of certain cell populations into damaged tissue in an effort to discover efficient and effective therapeutics for treating SCI. Neuroprotection, immunological regulation, angiogenesis, and the production of new central nervous system (CNS) cells are just a few of the potential advantages of cell-based treatments that might lead to functional results (Ahuja et al., 2020; M. Fehlings et al., 2020; Sabapathy et al., 2015). Stem cells, such as embryonic

(ESCs), neural (NSCs), or mesenchymal (MSCs), induced pluripotent (iPS) stem (iPS) cells, and glial cells, such as Schwann cells, olfactory ensheathing (OEC) cells, are among the most promising and investigated cell types for the regenerative purpose (OECs). Transplantation of many types of stem cells has been examined for its potential to repair neural circuitry and promote nerve regeneration(Silva et al., 2014). However, the effectiveness of the treatment can be jeopardized by a number of factors, including the immune reaction to allogenic or xenogeneic cells, which may cause cell rejection, and the implantation of fragmented cells, which are not organized into a functional network.

Embryonic stem cells (ESCs)

Since the capacity to repair and regenerate mechanically injured nerve tissue in SCI has been the focus of several stem cell treatment techniques, it is not surprising that these tactics have been tried out across a spectrum of disorders (Ashammakhi et al., 2019). This cell type is one of the most attractive choices for cell replacement treatments since it can provide a source of differentiated oligodendrocytes and motoneurons (Lee et al., 2007; Nistor et al., 2005). For the treatment of SCI, a variety of therapeutic methods based on embryonic stem cells (ESCs) have been investigated (SCI) in animal models of SCI. Animal studies have shown that transplanting oligodendrocytes produced from human embryonic stem cells (hESCs) might improve functional outcomes by activating brain-derived neurotrophic factor and interleukin-6 signaling pathways (Glazova et al., 2009). Reconstitution of white matter by differentiation of ESCs into oligodendrocytes improves motor capabilities in a rat model of spinal cord injury (All et al., 2015).

Neural stem cells (NSCs)

Experimental data suggests that axon myelination triggered by NSC-derived oligodendrocytes and neural signal transition by NSC-derived neurons integrating into the severed neural circuit are the two most important pathways for recovery following NSC transplantation (M. Fehlings et al., 2020). Experimental research using NSC transplantation into SCI site has added to the evidence of the cells' favorable benefits. It has been demonstrated, for instance, that these stem cells may survive, differentiate into neurons and glial cells, and also encourage the influx of anti-inflammatory M2 macrophages at the lesion site to help functional restoration(Parr et al., 2007). Functional recovery was shown after NSCs were grafted into a contused adult rat spinal cord SCI model, where they generated neurons that dispersed both rostrally and caudally (Kakulas, 2004).

Mesenchymal stem cells (MSCs)

Bone marrow-derived mesenchymal stem cells (MSCs), fat- and muscle-derived MSCs, and other easily-harvested MSCs all have a low risk of immunoreactivity upon allogenic transplant (Silva et al., 2014). Transplantation of MSCs for the treatment of spinal cord injury has been shown in preclinical investigations to reduce demyelination, decrease neuroinhibitory molecules, promote axonal regeneration, and induce functional improvement (Čížková et al., 2006; Urdzíková et al., 2006). Some proposed mechanisms include the regulation of local immune response, remyelination, axonal regeneration, apoptosis inhibition, and scarring inhibition (Assunção-Silva et al., 2015). Therefore, rat bone marrow mesenchymal stem cells (BMMSCs) were transplanted into a spinal cord injury (SCI) rat model, and the results showed a significant improvement in motor function restoration and an attenuation of the inflammatory response, but only a modest improvement in neural regeneration (Sabapathy et al., 2015). Clinical trials involving MSCs for SCI have been conducted extensively, and more study is needed; for example, the Mayo Clinic is

currently in the midst of a phase I study, adipose-derived MSCs administered intrathecally to patients with American Spinal Injury Association (ASIA) Impairment Scale (AIS) grade A/B/C injuries from 2 weeks to 1 year prior to transplant; the study is expected to be completed by 2023 (Ahuja et al., 2020).

Induced pluripotent stem cells (iPSCs)

For autologous transplantation, these pluripotent stem cells can be generated directly from adult tissues by "reprogramming" through the transduction of pluripotency genes and subsequent differentiation to the target cell lineage(Assunção-Silva et al., 2015). Because iPSCs may be derived from adult tissues, they provide an inexhaustible source of autologous cells without the risks and limitations of traditional stem cell therapies, such as rejection by the immune system, ethical considerations, and the need for tissue donation (Shi et al., 2017). The transplantation of these cells into SCI models, however, has been linked to unchecked cell growth and even tumor formation. Therefore, before to transplantation and clinical application, oncogenic capacity must be carefully screened. Itakura and colleagues observed that immunoregulation might eradicate tumors that formed after transplantation; this is likely due to the infiltration of inflammatory cells like lymphocytes and microglia (Itakura et al., 2015). In contrast, human induced pluripotent stem cell (hiPSC)-derived neuroepithelial-like stem cells (hiPS-lt-NES cells) were shown to develop into neural lineages and increase functional recovery of hind limb motor function following transplantation in a rat model of spinal cord injury(Fujimoto et al., 2012). Human induced pluripotent stem cell (iPSC) neural precursors (iPSC-NPs) transplanted into rats 1 week after spinal cord injury (SCI) led to tissue sparing and motor function recovery, according to another study using the same animal paradigm(Shi et al., 2017). It's worth noting that in December 2021, Keio University Hospital made history by becoming the first hospital in the world to successfully

transplant human iPS cell-derived neural stem/progenitor cells (iPSC-NS/PCs) into a patient with subacute spinal cord injury. This represents a major breakthrough in regenerative research and the field of SCI neuroscience(Sugai et al., 2021).

Schwann cells

Schwann cells, a kind of glial cell in the PNS, are involved in myelination of axonal structures and also provide a structural framework that acts as a conduit to direct the growth of axons (Ahuja et al., 2020; Sabapathy et al., 2015). Cell adhesion molecules like N-cadherins, integrins, N-CAM, L1, and contactins are upregulated, and neurotrophic factors like nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), and extracellular matrix proteins (mostly laminin and collagens) are secreted, creating an optimal microenvironment for neuronal survival and development (Pierucci et al., 2009). Schwann cell transplantation into animal models of SCI was found to boost tissue sparing, enhance CNS axon regeneration, minimize cystic cavitation, and remyelination, and enhance endogenous SC myelination, leading to recovery of sensorimotor function (Ahuja et al., 2020). In addition, it was shown that scar formation is reduced and neural functional potential is restored after SCs are cotransplanted with mesenchymal stem cells and neural stem cells (Sabapathy et al., 2015).

Olfactory ensheathing cells (OECs)

In mammals, a subset of glial cells known as olfactory ensheathing cells (OECs) is responsible for ensheathing olfactory axons and providing ongoing support for the effective regeneration of olfactory axons throughout the animal's lifetime(Gordon Boyd et al., 2005). Successful functional recovery and brain tissue repair following transplantation of rat and mouse OECs into SCI models (Liu et al., 2010; Tharion et al., 2011). A recent phase I experiment using autologous mucosal

OECs and olfactory fibroblasts in patients with AIS grade A injuries demonstrated sensorimotor improvements after transplant; however, bigger sample size and longer follow-up will be needed to verify safety and effectiveness(Ahuja et al., 2020).

5.2.2. Biomolecules and biomaterials-based treatment strategies

Soluble bioactive molecule-based therapies

Since the trophic microenvironment plays a critical part in the SCI damage, soluble bioactive molecules including growth factors and medicines have been implicated in neuroregeneration pathways for SCI treatments for their neuro regenerative, neuroprotective, angiogenic, and immunomodulatory activities(Ashammakhi et al., 2019). Neurotrophins 3 (NT-3), 4 (NT-4), and 5 (NT-5) are examples of growth factors that have been seen to protect injured neurons and promote neuron proliferation and differentiation (Saremi et al., 2022; B. Yang et al., 2020). For instance, chitosan-based implant for NT-3 release in the totally transected spinal cord of rats has shown neural tissue development (Oudega et al., 2019). Another study found that oligodendrocyte autophagy might be reduced by intraspinal injection of NT-3 following SCI, leading to improved motor function recovery (Cong et al., 2020). However, it has also been observed that brain-derived neurotrophic factor (BDNF) significantly affects 5-serotonin, cholinergic, dopaminergic, and GABA neurons by promoting neuron development, regeneration, axon sprouting, and axon remyelination(Costăchescu et al., 2022). Stem cell-derived factors may potentially be useful in SCI therapy. Intrathecal injection of MSC-conditioned medium, which may contain regenerative factors such as insulin-like growth factor 1 (IGF-1), vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF), was found to improve axonal regeneration and locomotor recovery in experimental rats with SCI (Kanekiyo et al., 2018).

Biomaterials as tissue engineering approach for SCI repair

Clinical trials have shown that biomaterials as a tissue engineering strategy for SCI are an effective tool for restoring tissue continuity and promoting axonal regeneration and vascularization, all of which are important for a full recovery from spinal cord injury. (Costăchescu et al., 2022). To facilitate axonal regeneration after spinal cord injury (SCI), biomaterials are employed to offer mechanical support, fill SCI-associated cavities, interact with host cells, bridge the injury to direct axonal development over the gap, and rebuild extracellular matrix (ECM) (Ashammakhi et al., 2019). It has been shown that delivering biomolecules like growth factors can restore neuronal function and stimulate nerve creation, however the limited permeability of the blood-brain barrier and the blood-spinal cord barrier prevents these molecules from diffuse freely(Pardridge, 2012). As a result, standard administration methods necessitate large systemic dosages, which can lead to systemic cytotoxicity and, in extreme cases, tumor growth (Tsintou et al., 2015). It is for this reason that scientists have proposed enclosing growth factors and transplanted stem cells in nanoparticles or other scaffolds. For neural engineering in SCI, hydrogels and electrospun guiding channels stand out as promising scaffolding solutions.

Recently, hydrogels have gained prominence as 3D scaffolds due to their biocompatibility and physical features that allow them to accurately mimic the milieu of soft tissues and the CNS and to be injected into the body in a noninvasive way (Assunção-Silva et al., 2015). Neuroprotection and axonal development were examined in several hydrogels, including fibronectin, collagen, fibrin, and fibronectin/fibrin (King et al., 2010). Collagen and fibrin gel hydrogels promoted axonal development, whereas fibrin gel, with or without fibrinonectin, integrated properly with the spinal cord and promoted both axonal growth and recovery when injected into a rat model of SCI. Progress was also shown for collagen scaffolds, which were able to greatly boost neurogenesis and

axon regeneration, decrease glial scar formation, and speed up recovery of movement in an animal investigation (X. Li et al., 2018). Han and colleagues took a different approach by creating a matrigel-loaded agarose scaffold to aid in the regeneration of linearly organized axons following SCI, hence facilitating the reconnection of functioning axons and facilitating functional recovery, which in turn led to improved mobility(Han et al., 2018). Since electrospun nanofiber guiding channels may function like natural ECM, they have been showing great promise for use in tissue regeneration, either on their own or in conjunction with a hydrogel. A study confirming this hypothesis used bone mesenchymal stem cells (BMSCs) grown on a porous silk fibroin scaffold to increase transplanted cell survival and promote nerve regeneration. Synthetic polymer conduit that was created and employed in another investigation that implanted pHEMA-MMA into rats with T8-transected spinal cords. Even while scarring was minor in both channels, neurofilament production was more prominent in the group with a greater elastic modulus(Tsai et al., 2004).

It's important to note that bioactive scaffolds that promote greater supramolecular mobility significantly accelerate healing from spinal cord damage. The recent synthesis of supramolecular peptide fibril scaffolds by Alvarez and colleagues has two peptide sequences that stimulate nerve regeneration: one that inhibits glial scarring and another that encourages the development of new blood vessels. Researchers have already begun the process of getting this novel therapy licensed for use in human patients, and it is safe to say that this is a major advance in the field of regenerative medicine (Álvarez et al., 2021).

5.2.3. Nanopharmaceuticals or nanocarriers based treatment strategies

The use of nanopharmaceuticals in SCI therapy studies has recently gained a lot of attention. It is difficult for most therapeutic drugs used to treat SCI to cross the blood-spinal cord barrier (BSCB) and have their desired pharmacological effects in the injured area(Costăchescu et al., 2022). The

large specific surface area reflects their primary benefit, which is the increased delivery of biomolecules such drugs, imaging moieties, enzymes, and cellular components (Zhao et al., 2021). Furthermore, nanocarriers bring the most unprecedented and outstanding advantages in terms of reducing cargo degradation, promoting selective interactions with cell compartments, improving drug absorption, diminishing adverse effects, and increasing the bioavailability of drugs(Costăchescu et al., 2022). Thus, nanocarriers represent an attractive strategy for SCI treatment, since they can improve recovery time by targeting localization, and cellular uptake alongside altering the signaling pathways. Particularly, gold-based nanocarriers are advantageous for their stability, low cytotoxicity, biocompatibility, ability to regulate cell growth pathways, and the possibility of surface functionalization. Fang and collogues biosynthesized gold nanoparticles (Au NPs) from the bark extract of Juglans regia and combined them with zonisamide which showed high stability and solubility ensuring a controlled release of the incorporated drug under room temperature conditions(Fang et al., 2019). Polymer-based nanocarriers, on the other hand, have a number of advantages that set them apart. In rats with spinal cord injuries, the topical administration of PLGA containing glial cell line-derived neurotrophic factor (GDNF) was shown to preserve neuronal fibers, leading to improved locomotor function in the hind limbs(Wang et al., 2008).

5.2.4. Combinatorial approach

There has been a lot of lab work done on the aforementioned SCI therapy techniques, however they have their limitations when used alone. Since each therapy option targets a distinct process and consequence, combining them can provide synergistic advantages for regeneration and functional repair of the damaged spinal cord. In this section, to find an effective and successful cure in the treatment of SCI, the most promising combinatorial therapeutic approaches have been

highlighted. Here, we highlight the most promising combinatorial therapy methods in the search for an effective and successful cure in the treatment of SCI.

Combination of cells with biomaterials

Due of their complementary properties, stem cells and scaffolds have been the subject of numerous investigations. Incorporating 3D neural networks created from iPSCs into a custom extracellular matrix-based hydrogel is the highlight of the combinatorial technique examined by Wertheim and colleagues (Wertheim et al., 2022a). This cutting-edge combinatorial method, developed by a team of researchers, has the potential to restore walking in 80% of SCI patients. The cells were converted into iPSCs, and the extracellular matrix (ECM) was processed into a customized hydrogel that wouldn't cause an immune reaction because it was derived from human tissue. This concept is based on the idea that patient-specific iPSCs may be encapsulated in a tailored hydrogel and then differentiated into functional motor neurons inside a 3D implanted neural network that replicates the embryonic development of the spinal cord. There was a decrease in inflammation and glial scar formation, as well as support for cell survival and engraftment, neuroprotection, and neuroregeneration. Incorporating mesenchymal stem cells (MSCs) with a nerve-guided collagen scaffold (CS) is another example of a combinatorial treatment that has been shown to boost activated macrophage polarization and promote neurological recovery. The CS and MSCs were implanted together in a rat model of lateral hemisection spinal cord injury by Peng and colleagues (Peng et al., 2018). Researchers found that the combination implantation lowered cavitation formation and glial scar development at the injury site.

Combination of cells and growth factors

Tissue repair following damage relies on signaling molecules like growth factors to facilitate vital cellular functions including proliferation, migration, and survival. Intrathecal injection of Wharton's jelly-derived mesenchymal stromal cells (WJ-MSCs) and their conditioned media (CM) into the compression lesion in the SCI rat model improved gray and white matter survival and increased expression of genes associated with axonal development, as studied by Chudickova and colleagues(Chudickova et al., 2019). The CM generated from WJ-MSCs has been shown to enhance axon development in neurons, and these cells have been shown to secrete considerable amounts of HGF and other key neurotrophic factors including FGF2, BDNF, or bNGF.

Table 1: Summary of combinatorial approach incorporating different treatment strategies. Inspired (Ashammakhi et al., 2019)

Combinatorial treatment	**SCI Model**	**Advantages**	**Outcomes**	**Reference**
iPSCs and personalized hydrogel matrix	Mice (acute and chronic phase)	• Avoids immune rejection • No ethical restriction • 3D implants of neuronal networks	• Behavioral functional recovery • Locomotion recovery • Eliminates gliosis • Accelerates axonal regeneration	(Wertheim et al., 2022b)
MSCs and nerve-guided collagen scaffold	Rat: Lateral hemisection SCI model	• High survival rate • Favorable microenvironment • Biocompatible	• Promote nerve regeneration • Decrease cavitation formation • Motor recovery	(Peng et al., 2018)

WJ-MSCs and CM (growth factor)	Rat: balloon compression model of SC	• Secret neurotrophic factors • Immunomodulatory • Neuroprotective activity	• Expression of genes associated with axonal growth • Survival rate of gray and white matter • Improve motor sensory functions	(Chudickova et al., 2019)
Sonic hedgehog (Shh) implanted in PLGA microspheres	Adult mice: dorsal over-hemisection and contusion injury model	• Controlled release systems • Avoid multiple injections	• Increase proliferation of oligodendrocyte lineage cells • Decrease astrocytic scar formation • Improve motor function	(Lowry et al., 2012)
GDNF and hUC- MSCs loaded on silk	SCI rat	• Neuronal plasticity • Controlled-release carrier	• Promote axonal regrowth	(Jiao et al., 2017)

fibroin/ alginates (SF/AGs)		Biomimetic platform	• Improve neuronal survival	
MnO2 nanoparticles dotted in the HA hydrogel scaffold seeded with MSCs	Rat: long-span transaction SCI model	• Regulate reactive oxygen species microenvironment • Promote nerve tissue bridging • Structural support for cell adhesion	• Motor function restoration • Highly efficient regeneration of spinal cord	(L. Li et al., 2019)

Combination of growth factors, cells and biomaterials

Given the pathophysiology of SCI, concurrent treatment with biomaterials, cells, and growth factors or pharmacological agents may prove to be a viable strategy for SCI repair. To this end, Jiao and coworkers tested the effects of grafting GDNF and human umbilical cord mesenchymal stem cells (hUC-MSCs) onto silk fibroin/alginates (SF/AGs) composite scaffolds, finding that this combination provided greater therapeutic benefits for spinal cord injury repair in rats than either the administration of SF/AGs or GDNF loaded alone on SF/AGs scaffolds (Jiao et al., 2017). SCI rats transplanted with SF/AGs/GDNF + hUC-MSCs showed dramatic increase in neuronal survival in the injured spinal cord. Further functional healing of the damaged spinal cord was found 8 weeks after therapy began.

Combination of biomaterials and growth factors

Researchers led by Lowry and colleagues found that poly(lactide-co-glycolide) PLGA microspheres can facilitate the transfer of sonic hedgehog (Shh). Oligodendrocyte cell proliferation, axon outgrowth, and gliosis suppression were all outcomes of this sustained dosing (Lowry et al., 2012). During spinal cord development, Shh is essential because it promotes the proliferation of oligodendrocytes and neurons, suppresses the development of astrocytes, and controls proper axon outgrowth. Research on a spinal cord injury (SCI) mouse model including dorsal over-hemisection and a contusion injury showed that bioengineered administration of sonic hedgehog (Shh) can stimulate substantial behavioral recovery.

Combination of cells, biomaterials, and nanoparticles

The inclusion of several cell types and nanoparticles into biomaterial scaffolds has shown promising outcomes in several studies, making it a potential combinatorial treatment method for spinal cord regeneration. Li and coworkers, for instance, produced a peptide-modified hyaluronic acid scaffold with distributed MnO2 nanoparticles and MSCs(L. Li et al., 2019). The hydrogel promotes the adhesive development of the seeded cells, giving structural support, and the nanoparticles can lessen the oxidative environment, both of which increase MSCs' survivability. Implanting MSCs into a rat model of long-segment spinal cord injury allowed the researchers to assess the cells' ability to regenerate damaged nerves and how well they work together. Regeneration of nerve fibers and considerable recovery of motor function both followed successful implantation. Because of their involvement in antioxidant microenvironment mitigation and the resultant protection for the implanted MSC, MnO2 NPs likely improved the HA hydrogel's capacity to regenerate nerve tissue

Chapter 6

Clinical trials

The effectiveness of cell treatment in spinal cord injury patients has been the subject of several clinical investigations. Clinical trials that have been recently registered at ClinicalTrials.gov as of 2016 are summarized in table 2. Out of all the finished trials, only one was a phase III clinical study; the others were all conducted in earlier stages. Current stem cell therapy clinical studies for SCI are shown in Table 2.

Table 2: Current clinical trials on stem cell therapy for SCI treatment. Adapted from (Saremi et al., 2022).

ClinicalTrials.gov identifier	Stem cells	Country; sponsor	Phase; Asia scale	No. of the patients	Timing of transplantation After injury	Route of cell delivery
NCT03308565	ASCs	USA; Allan Dietz Mayo Clinic	I; ASIA A-B	10 (18 Years and older)	1–24 months	Intrathecal
NCT04520373	Autologous AD-MSCs	USA; Allan Dietz Mayo Clinic	II; ASIA A-B	40 (18 Years and older)	1–24 months	Intrathecal

NCT03979742	UCBCs	USA; StemCyte, Inc.	II; ASIA A	27 (18–60 Years)	2–48 weeks	Site of injury
NCT03225625	Autologous BMSCs	USA; United Arab Emirates, MD Stem Cells	Not indicated	40 (18 Years and older)	1–12 months	Paraspinal, Intravenous, Intranasal
NCT04288934	Auto BM-MSCs, WJ-MSCs	Jordan; University of Jordan	I; ASIA A-C	20 (18–70 Years)	3–12 months	Into the spinal medulla
NCT02981576	BM-MSC, AT-MSCs	Jordan; University of Jordan	I/II; ASIA A-C	14 (18–70 Years)	12 months	Intrathecal
NCT02687672	Autologous BM, leukapheresis-derived SCs	Jordan; Stem Cells Arabia	II; ASIA scale not indicated	50 (5–50 Years)	6–60 months	Not indicated
NCT02481440	UC-MSCs	China; Sun Yat-Sen University	I/II; ASIA A-D	102 (18–65 Years)	1–12 months	Intrathecal

NCT03505034	UC-MSCs	China; Sun Yat-Sen University	II; ASIA A-D	43 (18–65 Years)	1–12 months	Intrathecal
NCT03521336	UC-MSCs	China; Sun Yat-Sen University	II; ASIA A-D	84 (18–65 Years)	1–12 months	Intrathecal
NCT02574572	Autologous MSCs	Brazil; Hospital Sao Rafael	I; ASIA A	10 (18–65 Years)	12 months	Site of injury
NCT02574585	Autologous MSCs	Brazil; Hospital Sao Rafael	II; ASIA A	40 (18–65 Years)	12 months	Not indicated
NCT03003364	WJ-MSCs	Spain; Banc de Sang i Teixits,	I/II; ASIA A	10 (18–65 Years)	1–12 months	Intrathecal

ASCs: adipose stem cells; **AD-MSCs**: adipose-derived mesenchymal stem cells; **UCBCs**: umbilical cord blood cells; **AT-MSCs**: adipose tissue-derived MSCs; **BM-MCs**: bone marrow-derived mononuclear cells; **BM**: bone marrow; **BM-MSCs**: bone marrow-derived mesenchymal stem cells; **BMSCs**: bone marrow-derived stem cells; **hUCB-MSCs**: human blood umbilical cord-derived mesenchymal stem cells; **MSCs**: mesenchymal stem cells; **SCs**: stem cells; **WJ-MSCs**: Wharton's jelly mesenchymal stem cells; **UC-MSCs**: umbilical cord-derived mesenchymal stem cells.

On another approach, a large number of trials have been carried out on tissue engeenering. The following list includes current clinical trials (from 2016) by researchers conducted at ClinicalTrials.gov (Table 3).

Table 3: Current clinical trials on scaffolds with and without cells for SCI treatment. Adapted from (Saremi et al., 2022).

ClinicalTrials.gov identifier	**Stem cells**	**Biomaterials**	**Country; Sponsor**	**Phase; ASIA scale**	**Timing of transplantation after injury**	**Route of scaffold transplantation**
NCT02138110	Not used	PLGA-bP (Lys)	USA: In Vivo Therapeutics	Not Indicated; ASIA A	3–12 months	Epicenter of the post-irrigation contusion cavity, OSS, T2-T12
NCT03762655	Not used	Neuro-Spinal Scaffold™	USA; In Vivo Therapeutics	Not Indicated; ASIA A	1–24 months	Epicenter of the contusion cavity, OSS, T2 - T12

NCT03933072	Autologous OECs, ONFs	Peripheral nerve grafts	Poland; Nicholls Spinal Injury Foundation	I/II; ASIA A	2–3 years	Bridging of the spinal cord gap, C5-Th10
NCT02688049	MSCs	NeuroRegen Scaffold	China; Chinese Academy of Sciences	I/II; ASIA A	1–24 months	Site of injury, after scars removal, C5-T12
NCT02688062	BMMCs	NeuroRegen Scaffold	China; Chinese Academy of Sciences	I/II; ASIA A	1–24 months	Site of injury, after scars removal, C5-T12
NCT02688049	MSCs, NSCs	NeuroRegen Scaffold	China; Chinese Academy of Sciences	I/II; ASIA A	1–24 months	Site of injury, after scars removal, C5-T12
NCT02510365	Not used	Collagen	China;	I; ASIA A	12 months	C4-T12

			Chinese Academy of Sciences			
NCT03966794	Not used	Collagen	China; Chinese Academy of Science	I/II; ASIA A	1–24 months	C4- T12/L1

ASIA: American Spinal Injury Association; BMMCs: bone marrow mononuclear cells; MSCs: Mesenchymal stem cells; NSCs: Neural stem cells; OECs: Olfactory ensheathing cells; ONFs: olfactory nerve fibroblasts; PLGA-bP (Lys): poly (lactic-co-glycolic acid)-b-poly (L-lysine).

Chapter 7

Challenges and drawback

There are still several challenges that pose limitation to find an effective treatment for SCI which need to be solved for accelerating the research.

Figure 5: Challenges and drawbacks in the field of SCI research. Inspired by (Tator, 2006)

Several obstacles remain that must be addressed in order to speed up research towards viable therapies for SCI. Figure 5 displays the main challenges of regenerative medicine's translation of sophisticated therapeutic ideas into effective treatment. However, because there is such a wide range of people who can incur a SCI, as well as different degrees of injury, different methods of harm, and different causes of injury, studies should only include people who have sustained those particular types or severity levels of injury(Tator, 2006). Another major obstacle to transplantation

is overcoming immunogenicity, and traditional immunosuppressants come with serious side effects that must be managed in the host (Martin-Lopez et al., 2021). The lack of information gained from pre-clinical and clinical studies is also a barrier to developing a reliable regenerative treatment. Examples include the difficulties inherent in preclinical processing and clinical administration of cell-based treatments (Ashammakhi et al., 2019). High costs and seldom encountered difficulties in research characterize many of the issues inherent to GMP-grade production (Ahuja et al., 2020). High-throughput whole population screening methods and environment-controlled cell culture robots are two examples of the cutting-edge technologies being developed to overcome these obstacles. However, regulatory frameworks for the clinical use of stem cell products and other regenerative goods are still in flux in many nations. In addition, there is a need for a robust clinical and regulatory infrastructure in order to address the lack of globally binding standards and a unified regulatory framework for clinical treatments, which now exist in many different forms across different nations (Rosemann, 2014).). In addition, there are escalating expenses associated with meeting ever-growing regulatory demands. Public money, on the other hand, is extremely scarce. Therefore, this limited budget, together with the imposition of higher regulatory obligations on investigators and corporations, presents additional obstacles in attracting financing.

Chapter 8

Conclusion and future direction

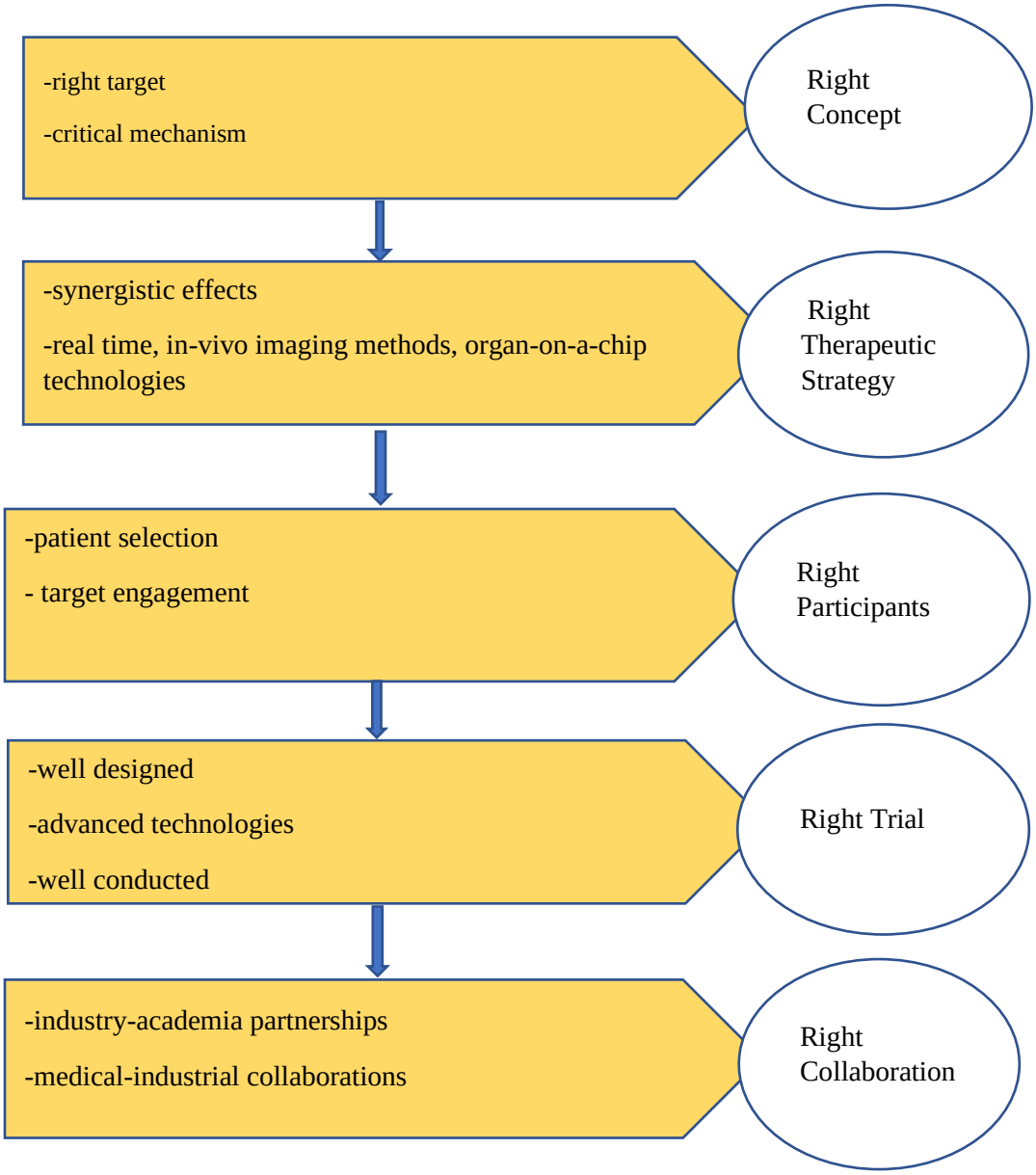

Figure 6: 5 "Rights" of future direction for SCI research. Inspired by (Ashammakhi et al., 2019)

First, the use of novel biomarkers and the targeting of more specific SCI diseases in trial design hold promise for better evaluation of results. Second, researchers may use technologies like real-time, in-vivo imaging approaches, and organ-on-a-chip technologies to meet the pressing demand

for effective treatment options. Additionally, scientists need to zero down on a combinatorial strategy that maximizes synergistic effects (Ashammakhi et al., 2019). In addition, it is difficult to identify the optimal patient group for transplantation regenerative treatments, particularly cell transplants, in SCI. The mechanism of treatment plan, stages, and severity of SCI must be accurately identified before selecting participants. In addition, proper trials need meticulous planning and execution of both preclinical and clinical phases. Clinical studies involving SCI must be effectively conceived and carried out in a safe manner in order to provide the evidence required for subsequent treatments(Tator, 2006). Last but not least, it appears that increased funding is necessary for the delivery of even more potent and well-designed phase I and II clinical trials, which will pave the way for the first phase III studies in the coming years, and this can be achieved through the right collaboration among academia, industry, medical centers, research institutes, and funding organization at the national or international level.

To conclude, promising outcomes have been attained by evaluating a variety of treatment strategies for achieving spinal cord regeneration and repair. yet further research is required concerning their efficacy and safety in human use before these strategies can be implemented in clinical practice. The key to successful neuroregeneration after SCI may lie in the development of sophisticated innovative treatment techniques that combine or include cell-based treatments, growth factors, biomaterials, and nanocarriers together.

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