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REVIEW PAPER

# Cellular Transplantation-Based Evolving Treatment Options in Spinal Cord Injury

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**Abstract** Spinal cord injury (SCI) often represents a condition of permanent neurologic deficit. It has been possible to understand and delineate the mechanisms contributing to loss of function following primary injury. The clinicians might hope to improve the outcome in SCI injury by designing treatment strategies that could target these secondary mechanisms of response to injury. However, the approaches like molecular targeting of the neurons or surgical interventions have yielded very limited success till date. In recent times, a great thrust is put on to the cellular transplantation mode of treatment strategies to combat SCI problems so as to gain maximum functional recovery. In this review, we discuss about the various cellular transplantation strategies that could be employed in the treatment of SCI. The success of such cellular approaches involving Schwann cells, olfactory ensheathing cells, peripheral nerve, embryonic CNS tissue and activated macrophage has been supported by a number of reports and has been detailed here. Many of these cell transplantation strategies have reached the clinical trial stages. Also, the evolving field of stem cell therapy has made it possible to contemplate the role of both embryonic stem cells and induced pluripotent stem cells to stimulate the differentiation of neurons when transplanted in SCI models. Moreover, the roles of tissue engineering techniques and synthetic biomaterials have also been explained with their beneficial and deleterious effects. Many of these cell-based therapeutic approaches have been able to cause only a little change in recovery and a combinatorial approach involving more than one strategy are now being tried out to successfully treat SCI and improve functional recovery.

**Keywords** Spinal cord injury (SCI) · Cellular transplantation · Neurologic deficit · Stem cell therapy

## Introduction

In the USA and other developed countries, it has been demonstrated that nearly 10–40 persons per annum per million populations are affected by SCI according to the epidemiologic research [1]. Many of the individuals sustaining SCI are young who are at the peak of their earning potential and personal lives or even older individuals getting injured by falls. The consequences of the neurologic injury is quite significant in either of the cases that has urged the scientific community to research for understanding the pathophysiological mechanisms associated with SCI so as to devise novel therapeutic strategies. A clear understanding about the root of secondary SCI mechanisms can form the basis of such strategies [2]. Neural element damage sustained during trauma is referred to as the primary SCI that generally causes irreversible injury and results from shear forces to blood vessels and axons. The body responses to the primary injury and is referred to as the secondary injury. A plethora of cellular events take place immediately following injury and exists for months to years [3]. These normal cellular events have

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been established to be an important part in inhibiting neurologic recovery and exacerbate underlying injury [4]. The researchers have now been able to demonstrate the significance of these cellular cascades and establish secondary SCI mitigating targets with patient outcome improving potential in SCI [5]. With such background, crucial advancements have been made in both operative and non-operative treatment modalities. Although there are a few reviews that discusses about the therapeutics in treating SCI, no such single review detailing the recent cellular transplantation-based treatment strategies in SCI could be found. In this review, we outline the various cellular transplantation strategies that could be effectively used in the treatment and control of SCI. A number of reports have supported the success of cellular approaches involving peripheral nerve, Schwann cells, embryonic CNS tissue, olfactory ensheathing cells and activated macrophages in SCI and have been detailed here. Subsequently, clinical trials are now underway with a number of these cell transplantation strategies. Also, stem cell therapy has come up as an evolving field in this regard and researchers have contemplated the role of both induced pluripotent stem cells and embryonic stem cells to induce the differentiation of neurons when transplanted in SCI models with no teratoma formation. In addition, the roles of synthetic biomaterials and tissue engineering techniques have been explained focusing on their both deleterious and beneficial aspects. The success achieved with these cell transplantation methods in treating SCI under in vitro conditions and in animal models augurs well for the future of SCI treatment that can only be verified with long-term clinical trials.

## Cellular Transplantation

There are a number of important issues to be solved by cellular transplantation in treating spinal cord injury that include creation of a favorable axon regeneration environment, dead cell replacement (e.g., providing new or myelinating neurons) and bridging of any existing cavities or cysts.

## Schwann Cells

Peripheral nerve derived Schwann cells have been transplanted into the SCI rat models, implanted into the extracellular matrix containing channels post-complete transection or lateral hemisection or post-contusion injury and injected as suspensions [6, 7]. After the completion of Schwann cell implantation and transection, these bridge gaps are traversed by the extension of the cell body consisting spinal and sensory axons. Subsequently, myelinating and electrophysiological activities are detected while the regenerated axons do not reinnervate the host by leaving the grafts distally. Following Schwann cell implantation and contusion, extension of spinal and sensory axons into the grafts takes place with myelination and a concomitant reduction in cavitation [6]. Although some of the studies reported for hindlimb function recovery, the fact was not confirmed by others [8]. As a result of such observations, combinatorial therapeutic approaches have been evaluated. Beyond bridges, increased CNS axon regeneration has been demonstrated with thoracic transection in response to Schwann cell transplantation along with olfactory ensheathing glia, steroid (methylprednisolone sodium succinate) or neurotrophin delivery [9, 10]. SCI rat models with attenuated immune system have also been transplanted with human Schwann cells where spinal axons extended distal to grafts while the brainstem axons resulted in graft regeneration. There are reports of functional improvements with limited cases of weight-supported stepping [11]. So, the determination of the most effective and efficacious Schwann cell involved combinatorial therapy that is safe and reproducible remains the key aspect of the cellular transplantation. The safety and effectiveness of these methods can be analyzed from the transplantation of the autologous Schwann cells into contusive SCI non-human primates which could culminate into human clinical trials [12]. There is yet no report on the human clinical trials that involve Schwann cell transplantation following SCI.

## Olfactory Ensheathing Cells

Under laboratory conditions, olfactory ensheathing cells (OECs) have been reportedly isolated from Fischer rat pulps or Sprague–Dawley olfactory bulbs with the help of marker antibodies and fluorescence-activated cell sorting (FACS) [13]. The maintenance and growth of purified OECs in large numbers can be obtained from mitogen mixture of heregulin β1, forskolin, FGF2 and confluent cortical astrocyte culture-conditioned serum-free medium [14]. Upon immediate culture of the OECS purified with FACS, inhibition of apoptosis is possible, proliferating for 12 days and then differentiating into two subtypes [15]. Different morphological and antigenic characteristics are exhibited by these subtypes, with one resembling the astrocytes and the other Schwann cells [16]. Astrocyte-like OECs show higher expression of glial fibrillary acidic protein immunoreactivity (GFAP-IR) and embryonic form of neural cell adhesion molecule (ENCAM) with a flat morphology. However, the cells express no or little p75NTR, the non-myelin Schwann cell marker [17]. The NCAM polysialylated isoform, ENCAM, has been detected in those adult cells that seem to retain embryonic features, e.g., hypothalamoneurohypophysial system astrocytes [18].

The expression of polysialyic acid by NCAM marks for reduced adhesive properties that is associated with the required cellular rearrangements during plasticity and neurohistogenesis [19]. On the other hand, the OECs with Schwann cell-like features exhibit higher p75NTR levels with little or negligible ENCAM and diffused levels of GFAP-IR with the cells showing spindle-shaped morphology [17]. It is highly probable that other OEC subtypes are present in culture that can show dramatic ability of changing size and shape while exhibiting considerable cellular plasticity [16].

### Peripheral Nerve Transplantation

Autologous peripheral transplantation in SCI adult rats resulted in supporting various types of axonal growth but not the supraspinal axon. Thirty-five peripheral nerve grafts along with a host of other therapies (e.g., vertebral wiring, anti-inflammatory drugs, acidic fibroblast growth factor and fibrin glue) aid in promoting recovery besides facilitating supraspinal axon regeneration through and beyond grafts [20, 21]. Similar kind of approach has been taken following lateral spinal hemisection in non-human primates [22]. Although hardly any functional difference could be detected, after 4 months SCI regeneration of some of the spinal axons was found. The method has been applied in treating incomplete, chronic human SCI with a report for restricted functional recovery. This observation was demonstrated in one patient, but the study did not include any control patient [23]. However, the procedure was found to be unsuccessful in complete SCI patients. Therefore, in order to define the role of the peripheral nerve bridge grafting, a lot of work is to be done so as to effectively cause improvements in outcome in SCI patients.

### Embryonic CNS Tissue

Following fetal spinal cord transplantation into the site of lesion and spinal cord transection in animal models, the number of host axon regeneration into the transplant is small and termination is observed near the transplant border of the host [10]. However, although small but significant recovery in function has been observed in cats and rats [24]. The recovery is not dependent on long distance growth through, beyond and into the grafts. Some of the researchers are of the opinion that in fact it is brought about by the relaying activities of the transplants that makes the neuronal signal transmission affordable in turn innervated by host proximal neurons and projecting to host distal neurons. The grafts might also be useful in improving conduction and providing growth factors to the spared neurons [10]. In adult rats, upon fetal spinal cord transplantation in combination with the delivery of neurotrophin factors following complete spinal cord transection, functional recovery has been observed [25] where the growth of caudal spinal cord has been found from some propriospinal and supraspinal axons. In a clinical trial with syringomyelia patients, fetal spinal cord intraspinal transplantation has been tested with cyst obliteration in all the patients and no associated complications [26, 27]. Although these studies provided promising insights into the SCI treatment modalities, they have not been able to provide a marked improvement in the SCI or syringomyelia treatment standards, perhaps due to the problems faced in procuring fetal tissues for transplantation.

### Activated Macrophages

It has now been known that the macrophages play an important role in spinal cord repair, and the failure in SCI repair can be largely associated with the kind of macrophage response achieved [28]. Following transection and activated macrophage transplantation (incubated with skin tissue or PNS), hindlimb function recovery in rats has been achieved. The extension of the fibers through the lesions took place while spinal cord re-transection led to abolishment of previous functional recovery [29]. But the recovery rate was comparable to that achieved with other cell-type transplantations [30]. On the other hand, the intrinsic macrophage activation via a proinflammatory agent injection at the spinal contusion site exerts negative effects on tissue survival and functional recovery of hindlimb. On the contrary, macrophage depletion has led to extensive white matter sparing, better hindlimb usage during overground locomotion and reduced tissue cavitation [31]. Therefore, it can be concluded that the macrophages can be both beneficial as well deleterious in providing recovery of function which makes it necessary for more in-depth studies with the macrophages directed at SCI treatment. Till now, no peer reviewed report involving non-human primates for activated macrophage transplantation has been available. In Israel and Belgium, Proneuron has sponsored the clinical trials (Phase I; from 2000 to 2003) of activated macrophage transplantation in humans. Blood-derived monocytes that have been activated with biopsied skin were used for transplantation in SCI patients who showed no irresolvable adverse effects [32]. Currently, a randomized controlled, multicentre, clinical trial (Phase II) is underway at USA and Israel hospitals.

### Stem Cell Transplantation (18)

The spinal cord and the brain, the components of the central nervous system (CNS), have long been considered

as examples of organs where the regeneration has been found to be difficult. However, this notion has been disproved by large with the recent advancements made in the field of stem cell biology. First identified in 1992 by Reynolds and Weiss [33], the culturing methods for neural stem/progenitor cells (NS/PCs) from mammals including humans have now been well established. Similarly, an increased interest has been evoked in induced pluripotent stem cells following the success of Sir John Gurdon and Shinya Yamanaka [34] who received the Nobel Prize for Physiology or Medicine in 2012. In the repair and regeneration in spinal cord injury cases, the transplantation of both the embryonic and induced pluripotent stem cells has been contemplated.

## ESC-Derived NS/PCs (18)

Neural stem/progenitor cells (NS/PCs) are characterized as cells with multipotentiality and self-renewal capacity. But the rate of proliferation and differentiation of the cells is strictly controlled depending upon the localization and exact stage of their production. It has been observed that the characteristics of the NS/PCs vary to a large extent. Generally, the NS/PCs exist around the 5th day of embryogenesis and leukemia inhibitory factor (LIF) can be used to culture the NS/PCs from this stage [35]. However, if the NS/PCs are procured on the 8th day of embryogenesis, they can be cultured with fibroblast growth factor-2 (FGF-2) in vitro. The ventricle surrounding radial glial cells from this stage up to the stages of late embryogenesis can be the NS/PC source, and these cells can give rise to neuronal cells by asymmetric divisions and self-renew by symmetric divisions [36, 37]. In the adult brain, the NS/PCs exist majorly around the ventricles up to the neonatal stage from the late developmental stage and differentiate into glial cells (oligodendrocytes and astrocytes) besides differentiating into neuronal cells [38]. Both FGF-2 and epidermal growth factor (EGF) stimulate NS/PC proliferation in the late embryonic stages. The NS/PCs appearing after the late embryonic stages are unable to give rise to early-born neuronal projections like motor neurons, dopaminergic neurons and forebrain cholinergic neurons. Highly plastic NS/PC development could be induced by Okada et al. [39] using the ESCs from the mouse inner cell mass from the early developmental stages. They succeeded in establishing a culture system that mimicked spatial and temporal developmental specificity. In this system of culture, first of all LIF (required in the maintenance of undifferentiated state) is removed and the ESCs are maintained in suspension culture so as to form embryoid bodies (EBs) constituted by the three germ layer cells. The relatively early stage NS/PCs present in these EBs can be maintained as neurospheres selectively in suspension cultures in the presence of FGF-2 in serum-free medium. Besides, during EB formation, addition of Noggin that plays a critical role in the formation of retinoic acid (a known player in the development of anterior spinal cord and hindbrain as well as in neural induction) or forebrain by preventing BMP-mediated differentiation promotion into neuroepithelium at low concentration increases EB constituting NS/PCs, thereby enhancing the neurosphere forming efficiency. Secondary and tertiary neurospheres can be generated from the passaging of primary neurospheres. It is noteworthy to mention that while the induction of tertiary and secondary neurospheres give rise to glial cells like oligodendrocytes besides the neurons, the primary neurospheres can generate only neurons upon induction. These neurospheres derived form mouse ESCs show increased multipotentiality and self-renewal capacity as their repeated passage results in the generation of astrocytes, oligodendrocytes and neurons. Moreover, CNS early developmental stages are well reflected with the change observed in the capacity of differentiation depending on the passaging of the cultures wherein earlier passaged neurospheres give rise to only neurons while glial cells are developed after the mid-gestation period for the first time. During EB formation, retinoic acid and Noggin concentration modulation can control the induced NS/PC regional specificity along the anteroposterior axis. While during primary neurosphere formation, dorsalizing factors such as Wnt3a and BMP4 and ventralizing factors like Sonic Hedgehog can be successfully used in the regulation of regional specificity along the dorsoventral axis [39]. These results go on to indicate that NS/PC regional specificity can be controlled by appropriate factor addition during specific stages of differentiation induction.

## Neurospheres Derived from iPSC

The establishment of iPSC by Yamanaka et al. has paved the path for the development of novel therapeutic strategies in the treatment of many diseases. Induced pluripotent stem cells are generated by the reprogramming of somatic cells following the introduction of Klf4, Oct4, Sox2 and c-Myc genes into human/mouse fibroblasts [34]. The resultant cells show differentiation and proliferative ability almost similar to that of the ESCs. Moreover, iPSCs can be an answer to problems like immunological rejection and ethical issues since these cells could easily be established from patient somatic cells. However, greater challenges like tumorigenesis might be associated with iPSCs as compared to ESCs due to (i) the introduction of foreign genes into chromosomes, (ii) that reprogramming may be incomplete. The safety of the mouse iPSCs following transplantation

and their neural differentiation depend greatly on the somatic cells from which the iPSCs have been derived [40]. In this study, thirty-six mouse iPSCs were established and transplanted into the NOD/SCID mice as neurospheres after being differentiated into neural lineages so as to detect their safety post-transplantation and differentiation capacity in vivo. Neurospheres so derived from the iPSCs were proceeded for FACS analysis which showed great degree of variation in the percentage of undifferentiated Nanog-EGFP + cells and depended on the iPSC generating source somatic cells [40]. However, the neurospheres generated from MEF-derived iPSCs exhibited the presence of no undifferentiated cells with differentiation potential almost equivalent to that of the ESCs upon induction. Also, the rate of teratoma formation in the mouse group transplanted with MEF-iPSC neurospheres was similar to that in the ESC-derived neurosphere transplanted group. Gastric epithelial cell-derived iPSCs showed no teratoma formation when transplanted as neurospheres into the mice. However, the iPSCs derived form adult tail tips fibroblast (TTF) exhibited the presence of significant number of undifferentiated cells in the neurospheres with resistance to differentiation upon induction. Subsequently, such neurospheres when transplanted into mice died becoming weaker within a short period of time due to the high rate of teratoma formation. Intermediate response (between TTF-iPSC and MEF-iPSC) to teratoma formation and differentiation were observed with the iPSCs derived form adult hepatocytes. Such differences in iPSC differentiation capacity based on the source of somatic cells from where they are derived may be attributed to the epigenetic memory, i.e., the genetic profile of the genes remained to be expressed that requires further detailed analysis of these cells.

## Tissue Engineering

Tissue engineering comes as a highly promising and exciting procedure in the treatment of SCI. It generally consists of three factors: seed cell, growth factor and the scaffold. For achieving a successful repair, the appropriate selection of growth factor, scaffold and seed cell is very important. While selecting the seed cell, it has been observed that the adipose tissue-derived adult stem cells are more appropriate as compared to embryonic stem cells, neuronal stem cells and fibroblasts. Similarly, natural component-derived scaffolds are found to be more effective as compared to those formed of synthetic and artificial materials [41]. It has been established that the use of blending materials composed of synthetic and natural materials can decrease the problems associated with the use of natural or synthetic materials alone. Similarly, the importance of the growth factor lies in its repairing action, specifically as the virus carrier-mediated transfection of the stem cells leads to consistent growth factor gene expression [42]. It has been hypothesized accordingly that a combinatorial approach using more than one growth factor is useful in this regard. Therefore, the study of virus constructs carrying several genes together requires further attention. Although the technique of tissue engineering can be regarded highly promising in SCI treatment, further extensive works are required in order to achieve successful SCI treatment with the method.

## Synthetic Biomaterials

One of the major factors that contribute to the SCI continuance is the problem with regeneration of the damaged axons interfering with the axonal circuit re-establishment involved in function. Different research groups are working toward addressing the issue of lack of regenerating axons in SCI. The latest development in this field have been the emergence of biomaterials as regenerating materials that calls for increased level of collaborative work between the clinicians, basic and translational researchers for effective therapy development in SCI. A number of available biomaterials have been tested in the SCI models [43, 44]. The biomaterials may provide their effects by acting as a structural support as well as provide factors for the promotion of axonal growth by preventing growth inhibition. The specific needs of the damaged CNS can be addressed by appropriate designing of the materials with the current updated technology. Although, a number of biomaterial is available with the potential to be used in SCI repair, they come with their own sets of pros and cons upon incorporation into the site of injury. The most important thing to consider is that the specific needs of the injury should be the ultimate determining factor while selecting a biomaterial for the repair of spinal cord. In humans, contusion which is found associated with more than 75 percent of all SCI cases is regarded as the most clinically relevant injury. It has been found subsequently that in situ injectable gelling materials possess potential to be applied in clinical treatment procedures [43]. The materials can be injected at either the adjacent or epicenter of the site of lesion directly without causing very little additional damage. However, while using the gelling materials, it should be kept in mind that the material should have very low immunogenicity along with reliable sterilization techniques. Although the synthetic polymers could be sterilized quite safely, there is some concern associated with the reliability of the natural polymer sterilization. In addition, the material should be slowly biodegraded with time as naturally regenerated environment replaces it. It has been demonstrated that PEG-based hydrogels can fulfill most of the

aforementioned criteria [45]. With its innate reactive oxygen species sequestering ability, it is highly probable that this group of in situ gelling materials can make the required progress so as to provide important contribution in SCI therapy development.

## Neurotrophic Growth Factor (10)

Since, the neurotrophic factors promote axon regeneration and protect neuronal cells, they have an important role to play in the functional recovery after SCI [46]. The neurotrophic factors are subdivided into growth factors, neurotrophins, cytokines, neurotrophic factor derived from glial cell lines and ciliary neurotrophic factor [47]. The most commonly used neurotrophic factors include BDNF, NT3 and NGF. Discovered in the 1950s, NGF has been found to be a core factor in the peripheral innervation regulation with an impact on CNS as well [48]. It was demonstrated by Allen et al. that NGF can serve as an effective option of therapy in neurodegeneration [49]. Simultaneously, other works showed that porcine brain extracted BDNF can elicit a broad spectrum effect on the central and peripheral neurons. However, such effects could not be found in the hippocampal neurons, sensory neurons, motor neurons, ciliary ganglion neurons, midbrain dopaminergic neurons, basal forebrain cholinergic neurons and cerebellar neurons [50]. The biological effects of BDNF are mainly exerted by its binding and activation of TrkB [51]. It was also found that agarose scaffold incorporated with BDNF when implanted in SCI rat model could cause the regeneration of axons through the scaffold in a linear fashion [52]. NT-3 on the other hand, besides maintaining the sympathetic neurons, motor neurons and differentiation of dopaminergic neurons, promotes the in vitro nerve outgrowth and maintains the survival of sensory and sympathetic neurons [53]. The only gene to be regarded to play a role in the promotion of cortico-spinal tract growth after SCI is NT-3. NT-3 exerts its biological effects mainly by its activation and binding of TrkC. It also shows some effects with TrkA and TrkB which are apparently weaker as compared to its role with TrkC [54]. The application of neurotrophic factors may be brought about by the three following methods: gene modification, cerebrospinal fluid injection and local injection [55]. However, gene modification seems to be the most important among these methods. There are studies that have shown marked improvements in functional recovery upon the neurotrophic growth factor administration as compared to the control sets where no such growth factor administration was done. But the treatment method could not fully produce the desired effects of nerve generation owing to a number of limiting factors like short half-life, time limitation and concentration [56]. Similarly, the injection of cerebrospinal fluid poses some disadvantages since it is not possible to limit the neurotrophic factor to only the site of injury as also a reduced recovery rate is associated with the method as compared to the other procedures. So, increased attention is paid toward the development and upgradation of gene modification [57]. Gene therapy in SCI cases can be employed by two methods: cell-mediated gene therapy and transfer of the gene directly to human body target cell. Cell-mediated gene therapy requires transferring of the target gene into a proper transplant cell. The cell selection is based on the high gene expression levels and the cell transplantation into the target tissue. Accordingly, the researchers have tried to modify Schwann cells, muscle cells and fibroblast cells for the transplantation into the injured region. Such cells which have been modified genetically may promote nerve regeneration by the continued expression of nutritional factors. The vectors used for the transfer of gene can be non-viral or viral [58]. Viral vectors involve chronic viruses, retroviruses and adenoviruses among which chronic viruses and retroviruses are of major interest since their application in the transfer of genes into the host genome results in long-term expression. The detection of the effect of a chronic virus-mediated exogenous gene transfer into adipose stem cells is possible if the cells undergo adipogenic and osteogenic differentiation. Hence, the efficiency of chronic virus carrier and stem cell combinations is currently under investigation [59]. However, the application of the method clinically can be made simpler if the growth factors are made to be released from scaffolds slowly with the aid of biomaterial-based protracted release of the loaded proteins as compared to the ones delivered by gene transfer.

## Conclusion

There has been very limited cure in the devastating condition of SCI. Molecular and rehabilitating therapies have been tried out with very little success in the recovery in SCI. Recently, cellular transplantations have been used either singly or in combination with molecular therapeutic agents that have produced significant improvements in SCI cases. Many such therapeutic approaches are now moving toward the clinical trial stages. Upon completion of these clinical trials, it can be ascertained whether these cell transplantation methods can be used in the functional recovery in SCI. Nonetheless, the laboratory success coupled with the results obtained in animal models of SCI, the cell transplantation creates a great deal of interest in improving the outcome in SCI. Now, it is to be seen whether the initial success achieved gets translated under clinical conditions as well.

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