Childs Nerv Syst (2011) 27:237–243
DOI 10.1007/s00381-010-1312-x

REVIEW PAPER

Cellular and paracellular transplants for spinal cord injury: a review of the literature

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Received: 23 September 2010 / Accepted: 11 October 2010 / Published online: 23 October 2010

Abstract
Background Experimental approaches to limit the spinal cord injury and to promote neurite outgrowth and improved function from a spinal cord injury have exploded in recent decades. Due to the cavitation resulting after a spinal cord injury, newer important treatment strategies have consisted of implanting scaffolds with or without cellular transplants. There are various scaffolds, as well as various different cellular transplants including stem cells at different levels of differentiation, Schwann cells and peripheral nerve implants, that have been reviewed. Also, attention has been given to different re-implantation techniques in avulsion injuries.
Methods Using standard search engines, this literature is reviewed.
Conclusion Cellular and paracellular transplantation for application to spinal cord injury offers promising results for those patients with spinal cord pathology.

Keywords Spinal cord · Injury · Paralysis · Treatment

Introduction

Filling the physical gap in a spinal cord injury (SCI) and replacing it with its lost elements has been one of the major fields of research toward finding a better treatment. Although the immediate posttraumatic ischemic and inflammatory reactions have been addressed with intensive research, and major progress has been made in both understanding and modulating the above, and a lot of new information is learned from understanding the differences between the mature and immature neural tissues in terms of their innate potential of regrowth, substituting the lost elements and filling the physical gap is one of the main pillars of spinal cord regeneration research. Here, we have gathered recent data on cell transplant, including stem cells, nonstem cells, different kinds and sources of stem cells, paracellular scaffolds such as peripheral nerve transplants, as well as noncellular scaffolding materials that are thought to offer a scaffold for the regenerating cells. Special attention is brought to the pathophysiology and actual results of reimplantation of avulsed roots to the spinal cord, as this may become a novel method for treatment of SCI in the future.

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Stem cell transplant

Replacing the lost cells and supporting tissue has been extensively studied. Different types of stem cells exist. Multipotent stem cells, i.e., neural precursor cells (NPC), can be differentiated toward both neural and non-neural lines. Neuronal restricted progenitors (NRP) can only be differentiated toward becoming neurons. The source can be from embryo and developing spinal cord [1] or from adult sources. Stem cells from adult sources have been used from the cerebral cortex [2, 3], subependymal zone of the lateral ventricles [4], and subependymal zone of the central canal of the mice spinal cord [5]. Amnionic multipotetent stem cells are among the fetal stem cells harvested after birth. Neuronal-restricted progenitors are more differentiated NPCs and are committed to neuronal line at the time of isolation. The dentate gyrus of the hippocampus has been a source of this cell type [6].As non-neuronal source of stem cells is the hematopoietic system with hematopoietic NPCs, being able to differentiate to neuronal and glial cell lines.

An obstacle of non-NRPs has been their nonuniformal differentiation to cell lines depending on the environment. An NPC being transplanted into a growing embryo differentiates differently if transplanted into an adult [7]. NPC being transplanted to a permissive neuronal environment of dentate gyrus [8, 9], or subventricular zone [10], differentiates to neurons while in the nonpermissive environment of the SCI, usually differentiates to a glial line [10]. Accordingly, in the traumatically injured spinal cord, differentiation of grafted NPCs is restricted toward the astrocytic lineage possibly due to the inflammatory environment. TNF-alpha, IL-1beta and IFN-gamma have been shown to play a major role in differentiation of NPCs in in vivo conditions [11].

In the mature central nervous system (CNS), NPCs usually have the tendency to differentiate toward the astroglial cell line rather than neuronal and oligodendroglial cell lines. The differentiation mechanism and factors affecting this have been studied extensively. Neuronal and myelin-producing oligodendrocyte differentiation in order to support neuro-axonal regeneration is the goal. Astroglial differentiation is mostly undesirable although a few aforementioned studies have shown some regeneration enhancing properties, together with growth factors or activated macrophages [12, 13].

Bone morphogenic factor (BMF) is a promoter for astroglia differentiation and the BMF inhibitor, Noggin, prevents astroglial differentiation. Among promoters of neuronal and oligodendrocyte differentiation are helix loop helix factor (HLH-f), mammalian AS-C homolog (MASH), and Neurogenin [14–17]. One study transplanted predifferentiated embryonic NPCs to astrocytes into the lesioned spinal cord of the rat with resulting robust regeneration into and beyond the transplant as well as motor improvement [18]. Furthermore, human hematopoietic stem cells (HSCs) were transplanted into chicken embryo's spinal cord, differentiated to neurons [19, 20]. Several interesting aspects of this study are not only the HSCs ability to differentiate to neurons but also the permissive immature environment of the recipient embryo. A similar study was done with human stem cells transplanted into immunodeficient mice with differentiation toward neural and oligodendrocytic cell lines and locomotor improvement. This locomotor improvement could be abolished by human cell-specific diphtheria toxin, showing that these human stem cells were the source of the improvement [21]. In the same way, stem cells from one animal type have been transplanted to others. For instance, mouse stem cells have been transplanted into rats with differentiation into all three cell lines, as well as incorporation and growth into the recipient spinal cord [22].

Embryonic stem cells have also been used for myelination purposes, as they are directed toward differentiation to oligodendrocytes [23]. There is evidence suggesting the presence of progenitor cells committed to the oligodendrocyte lineage in the adult human CNS, but issues have been raised regarding the intrinsic capacity of these cells to contribute to the process of remyelination [24].

Various synthetic scaffolds have been used as vectors for stem cells, and stem cells have been shown to be able to continue their differentiation and growth when implanted in such vectors [25]. Even stimulating the NPCs toward neuronal and oligodendrocyte differentiation does not necessarily mean a functional durable regeneration. For a functional regeneration to occur, the new pathways need to be organized in a spatial manner, connecting the rostral pathways to the caudal ones in a normal anatomical order. In many studies, different implants together with neurotrophic factors have promoted regeneration not only into but also beyond the implants with some motor recovery, but whether this regrowth would have been more robust, or would have given better clinical recovery if the regenerating axons were spatially directed, is unclear.

The temporal aspect of the transplantation is also important. Early in the posttraumatic period, there is more intense inflammatory activity. Later on, there is less inflammatory activity but more scarring. Even in the early inflammatory phase, there are different subphases with different inflammatory characteristics. The study of Coumans et al. [26] compared acute and subacute regeneration after fetal cell and NT-3 implants and demonstrated that any approach may either be done after the acute phase or additional anti-inflammatory treatment can be used during the acute phase.

Amniotic stem cells

These cells are derived from the placenta and therefore solve the ethical aspect of harvesting. As the amniotic cells are immature and do not cause an autoimmune reaction, there is reason to believe that human amniotic cells can be used not only in human models but also in animal models to study efficacy prior to use in human models. A transection model in monkeys showed that human amniotic stem cells survived in the transplanted environment, supported growth of host axons, prevented the formation of glial scar at the cut ends and might prevent death in axotomized cells or attract the growth of new collateral sprouting [27]. Another study showed a positive functional outcome in rats [28].

Nonstem cell biological transplants

Olfactory ensheathing cells (OECs), being glial cells, ensheathing the axons of the olfactory receptor neurons

having the properties of both Schwann cells and astrocytes, have shown both regeneration and functional recovery in spinal cord contusion, hemisection, and transection [29–35]. Genetically modified OECs secrete glial cell line-derived neurotrophic factor (GDNF) and, using a retroviral-based system, have been transplanted into a complete spinal transection and demonstrated the capability of producing in vivo GDNF significant recovery [36]. OEC transplants together with methylprednisolone have also shown promising results [37]. Schwann cells have extensively been used [38], but one interesting study raised some questions about OEC's potential when Schwann cell transplants were compared to OEC and Schwann cell–OEC transplants together, showing that all had diminished cavitation 12 weeks postinjury, but Schwann cell grafts contained more myelinated axons than OEC and the combined group. Retrograde tracing demonstrated longer axonal sprouting in certain tracts (propriospinal and brainstem axons) in the groups including Schwann cells but not OECs. On the other hand, glial fibrillary acidic protein and chondroitin sulfate staining in the Schwann cell-containing groups were greater [39]. Together with the fact that Schwann cell groups had better regeneration, this puts other studies showing inhibitory effects of chondroitine sulfates on axonal regeneration under question, or it means that their role is not as important as other factors in preventing regeneration.

Another widely used transplant is the peripheral nerve. Intercostal nerves together with local application of aFGF have facilitated regrowth of axons [40]. Intercostal nerves transduced with an adenoviral vector-encoding NT-3 have shown regrowth, whereas no regrowth has been demonstrated in intercostal nerve-only group [41]. Similarly, the use of nerve graft together with chondroitinase ABC has shown significantly robust regeneration compared to nerve graft-only and controls [42]. Stressed sciatic nerve graft has been used with some positive clinical results with some regeneration into the graft but not beyond. The motor recovery has been postulated to be secondary to recruitment of surviving nerves as long as there has been no observed regeneration that passed the damaged area [43].

Fibroblasts, genetically engineered to express nerve growth factor (NGF), have been grafted in the striatum, causing cholinergic axons to arise from the nucleus basalis toward and into the grafts within 8 weeks. They also showed unmyelinated axons growing into the grafts of NGF-producing cells only on reactive astrocytic processes that contribute to a surrounding glial border [44]. This has therefore raised two questions. Are astrocytes really inhibitory for regeneration and what happens when fibroblasts are transplanted into the spinal cord? To answer this question, fibroblasts derived from the meninges overlying cerebral cortex were implanted into the injured adult rat spinal cord, expecting to demonstrate posttraumatic adhesive scar. One month past trauma, surprisingly, there was not only a lack of the anticipated adhesive scar but also regeneration of peptidergic axons originating from dorsal root afferents and, to a lesser extent, of supraspinal serotonergic fibers at the periphery of the grafts. mRNA for b-NGF, NT-3, aFGF, and bFGF were demonstrated in these cultures, suggesting that the nerve-promoting effect of these fibroblasts may be due to their capacity to synthesize neurotrophic factors [45].

Bridging transplants

Autologous segments of the sciatic nerve have been used to make an extraspinal subcutaneous bridge between the medulla oblongata and the lower cervical or upper thoracic spinal cord in both mice and rats, with the spinal cord between these two levels being intact. They demonstrated that the sciatic nerve bridges contained axons from spinal cord and brain stem neurons and that they had a maximum growth of 1.5 cm in mice and 3 cm in rats [46]. Peroneal nerve autograft have been used for anastomosis of lumbar roots to spinal cord rostral to the SCI with both regeneration and positive clinical response [37, 47].

Autoimmune reaction to allogenic transplants

Several immunosuppressing agents have been studied in conjunction with transplants in order to decrease the autoimmune reactions. A study comparing cyclosporin and FK506 together with transplated fibroblasts showed no difference as both treatments improved fibroblast survival, diminished immune cell invasion, and promoted axonal growth [48].

Topographical bioengineering

An interesting study replaced a thoracic segment of neonatal rat cord into an embryonal spinal cord segment. The importance of the positioning of the implanted spinal cord segment was also studied. It demonstrated that the majority of the isotopic implants grew so well with regeneration rostral and caudal to the implant that it was difficult to differentiate them from the recipient spinal cord. The minority that did not follow the gray-white matter boundaries became primarily white matter. In the experimental group with inverted embryologic spinal cord implants, there was no growth. This study demonstrated the capabilities of immature embryonic graft and neonate recipient and the importance of correct spatial positioning on implants [49]. Several groups have performed scaffolding experiments including exogenous channels to direct the growing axons with moderate success [50].

Synthetic scaffolds

Despite identifying all the factors above, there is a physical gap in the damaged spinal cord, with scar tissue and lack of cells and tracts to connect the two ends of the damage, the so-called cavitation. Polylactic glycolic acid has been used together with neural stem cells with some corticospinal tract fibers passing through the injury to the caudal cord with recovery of some motor function [51]. Collagen has commercially been used for peripheral nerve grafting, but there is evidence of its inhibitory effect on in the spinal cord [52]. Alginate, derived from brown seaweed, has been found to have some regenerative properties [53–55]. Synthetic gels such as poly hydroxypropyl methacrylamide have also shown some promising results with both regeneration and positive functional outcomes. One major advantage of these products has been that they are nonbiodegradable, not exposing the tissue to metabolites. These metabolites may have harmful effects on the tissue and may also inhibit the regenerative cascade [56]. Polyethylene glycol has also been used in guinea pig with only functional results, as prolonged application can induce conduction block [57, 58]. In vitro, fibrin has shown inhibitory effects on the nerve growth. In vivo application with different growth factors has shown some regenerative results probably due to the growth factors rather than the fibrin itself [59]. Matrigel being extracted from the Engelbreth–Holm–Swarm sarcoma, containing laminin, fibronectin, and proteoglycans has demonstrated some regenerative potential in vitro but has not shown any regenerative characteristics in vivo [60]. Fibronectin has also demonstrated some ability to carry many cell lines but not promoting regeneration [61]. Agarose, a seawood polysaccharide, implanted with brain-derived neurotrophic factor has been used in SCI with some axonal growth into scaffolds [62]. Many of the scaffolds above have been used alone or together with NPC/growth factors [51, 63, 64].

Artificially made scaffolds also have the risk of causing autoimmune reactions. Hyaluronic acid (Hyaluronan, HA) is a proteoaminoglycan inherent in the extracellular matrix and has been studied extensively, industrially manufactured, and commercially used in subcutaneous, intra-articular, and intra-abdominal contexts with good results [65]. An HA-receptor in early cellular events in the brain was first suggested in 1991 [66]. HA's role on astrocytes was further demonstrated showing that HA, together with its receptor CD44, induced cytoskeleton activation and astrocyte migration [67]. High molecular weight HA, however, has shown properties that maintain astrocytes in a state of quiescence, and its degradation with hyaluronidase has resulted in some astrocyte proliferation [68]. Separate groups have also confirmed the potential utility of HA–DTPH–poly (ethylene glycol) diacrylate hydrogel as an in situ crosslinkable injectable material for tissue engineering [69, 70]. In vitro controlled studies have shown very promising results with extensive neurite growth, but in vivo application in spinal cord transection has not shown any signs of regeneration [70]. However, another in vivo application hemi-transection study showed signs of regeneration [71].

Reimplantation

The peripheral–central transitional zone is that length of rootlet containing both central and peripheral nervous tissues. The two tissues are separated by a very irregular but clearly defined interface, consisting of the surface of the astrocytic tissue comprising the central component of the transitional zone (TZ). Central to this, myelin sheaths are formed by oligodendrocytes and the supporting tissue is astrocytic. Peripheral to it, sheaths are formed by Schwann cells. Regeneration through this area can happen if this segment is absent, or undeveloped as in immature tissue. Thus, the experimental strategy has been to delete the TZ in mature tissue [72–75]. With this background, reinsertion of the dorsal roots into the peripheral–central TZ has been attempted both in dorsal roots beyond the TZ [74–76] and into the gray matter [76] with promising results. Similar attempts with reimplantation of the ventral roots have given recovery of some function [77, 78]. For example, reinsertion of the ventral roots past the dorsal TZ has been attempted with electron microscopic evidence of growth from the dorsal spinal cord neuron axons into the reinserted ventral root [79].

In another trial, ventral roots were reimplanted and combined with application of either glioma-derived growth factor (GDGF) or brain-derived growth factor (BDGF). This showed formation of a neuronal network at the ventral horn, but failure of the motor neuron axons to extend into the reinserted root, suggesting that neurotrophic factors in the ventral horn promote sprouting, but prevent directional growth of axons of surviving motor neurons into the implanted root [80]. Due to these promising animal results, trials, although in relatively small numbers, have been performed with reinsertion of the roots of brachial plexus injury [81, 82] and later also of the lumbosacral plexus roots [83]. In this approach, the avulsed root/roots are reinserted into their normal anatomic position beyond the TZ. One major difference of the reimplantation situation compared to SCI is that, in avulsion injuries, the resulting posttraumatic ischemic/inflammatory cascade is far less than with hemi-transection of the spinal cord.

Discussion

SCI is the product of the immediate mechanical trauma and a very complex ischemic and inflammatory cascade

secondary to the initial trauma. Months after trauma, there is cell loss, loss of tracts, and appearance of scar tissue and cavitation that act as barriers toward regeneration. Apparently, prevention of the initial mechanical trauma is beyond the scope of this paper. The last two decade's research on the pathophysiology of the post-traumatic ischemic and inflammatory cascade has shed light onto possible avenues of action in order to limit this cascade [84, 85]. Numerous experimental works have been done with variable results. Among these are trials focusing on optimizing the circulation and perfusion to the spinal cord, limiting the inflammation, modifying the neurite outgrowth inhibitory substances influence, and altering the metabolism by changing the temperature. Here, we specifically looked at cellular and noncellular scaffolding means that either, by themselves, promote regeneration, as stem cells, or can work as reliable vectors for stem cells, or can function as matrix for regenerating neuritis. The temporal aspect of the treatment is obviously important. We observed that the immediate posttraumatic environment is not the most optimal for implantation of these cells or scaffolds unless some anti-inflammatory additive is to be used.

Another important aspect is the possibility of neuronal cell growth together with myelination. Many neuronal cell lines survive in vivo, but function will not be optimal due to a lack of myelination. Currently, we know of multiple sources of cell lines at different stages of differentiation, promoters and inhibitors of differentiation toward different cell lines. The more immature the graft and the recipient are, the better the outcome will be.

In summary, at the cell level, both pluripotential and neuronal-restricted stem cells have been used with varying results. Olfactory ensheating cells aimed at myelinating neurons have shown promising results as have Schwann cells. They potentially produce several growth factors that stimulate their surroundings. Different peripheral nerve grafts have been used such as the combination of being rich in Schwann cells as well as functioning as a scaffold that promote neurite outgrowth. Noncellular scaffolds have followed the same principal as peripheral nerve scaffolds, with the difference that the latter produces growth factors through its richness in Schwann cells.

If we look beyond micromanagement, we can see promising results with transplantation of segments of the spinal cord as well as the importance of the topography of the transplant. To date, all successful trials have been done in fetal recipients. It is apparent that these methods will not have an application in the immediate future of the majority of the patients with SCI. However, as the science develops, future intrauterine treatment of spinal cord pathology in fetuses may be considered.

Conclusion

Numerous cells and substances have been used to substitute for neuronal damage, to sustain the surviving neurons as well as promote their neurite outgrowth, and to promote myelinization. Moreover, multiple cellular and noncellular scaffolds have been suggested and studied to guide neurite outgrowth. No one single-treatment strategy has so far given a significantly positive outcome. It appears that the ideal treatment in the future will be a combination of several cell groups and scaffolds, at an optimal time window and in a correct topographical fashion.

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