



Adult Stem Cell-Based Strategies for Peripheral Nerve Regeneration

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Abstract

Peripheral nerve injuries (PNI) occur as the result of sudden trauma and can lead to life-long disability, reduced quality of life, and heavy economic and social burdens. Although the peripheral nervous system (PNS) has the intrinsic capacity to regenerate and regrow axons to a certain extent, current treatments frequently show incomplete recovery with poor functional outcomes, particularly for large PNI. Many surgical procedures are available to halt the propagation of nerve damage,

and the choice of a procedure depends on the extent of the injury. In particular, recovery from large PNI gaps is difficult to achieve without any therapeutic intervention or some form of tissue/cell-based therapy. Autologous nerve grafting, considered the “gold standard” is often implemented for treatment of gap formation type PNI. Although these surgical procedures provide many benefits, there are still considerable limitations associated with such procedures as donor site morbidity, neuroma formation, fascicle mismatch, and scarring. To overcome such restrictions, researchers have explored various avenues to improve post-surgical outcomes. The most commonly studied methods include: cell transplantation, growth factor delivery to stimulate regenerating axons and implanting nerve guidance conduits containing replacement cells at the site of injury. Replacement cells which offer maximum benefits for the treatment of PNI, are Schwann cells (SCs), which are the peripheral glial cells and in part responsible for clearing out debris from the site of injury. Additionally, they release growth factors to stimulate myelination and axonal regeneration. Both primary SCs and genetically modified SCs enhance nerve regeneration in animal models; however, there is no good source for extracting SCs and the only method to obtain SCs is by sacrificing a healthy nerve. To overcome

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such challenges, various cell types have been investigated and reported to enhance nerve regeneration.

In this review, we have focused on cell-based strategies aimed to enhance peripheral nerve regeneration, in particular the use of mesenchymal stem cells (MSCs). Mesenchymal stem cells are preferred due to benefits such as autologous transplantation, routine isolation procedures, and paracrine and immunomodulatory properties. Mesenchymal stem cells have been transplanted at the site of injury either directly in their native form (undifferentiated) or in a SC-like form (transdifferentiated) and have been shown to significantly enhance nerve regeneration. In addition to transdifferentiated MSCs, some studies have also transplanted *ex-vivo* genetically modified MSCs that hypersecrete growth factors to improve neuroregeneration.

MSC	mesenchymal stem cell
NGF	nerve growth factor
NT-3	neurotrophin 3
NT-4/5	neurotrophins 4 and 5
PDGF	platelet-derived growth factor
PNI	peripheral nerve injury
PNS	peripheral nervous system
RGC	retinal ganglion cell
SC	Schwann cell
TDM	transdifferentiation media
TENG	tissue engineered nerve graft
Trk	tropomyosin receptor kinases
tMSC	transdifferentiated mesenchymal stem cell
uMSC	undifferentiated mesenchymal stem cell
VEGF	vascular endothelial growth factor

Keywords

Peripheral nerve regeneration · Neuroregeneration · Neuroprotection · Mesenchymal stem cells · Schwann cells · Genetic modification · Transplantation · Transdifferentiation · Brain-derived neurotrophic factor · Clinical trials

Abbreviations

AMD	age-related macular degeneration
BDNF	brain-derived neurotrophic factor
bFGF	basic fibroblast growth factor
BMMC	bone marrow mononuclear cell
CNTF	ciliary neurotrophic factor
CNV	choroidal neovascularization
CREB	cAMP-response-element-binding protein
DRG	dorsal root ganglia
ELISA	enzyme linked immunosorbent assay
GDNF	glial cell line-derived neurotrophic factor
GFP	green fluorescent protein
iPSC	induced pluripotent stem cell
MBP	myelin basic protein
MRI	magnetic resonance imaging

1 Introduction

Mesenchymal stem cells (MSCs) which have been altered to resemble and act like Schwann cells (SCs) have key beneficial properties within the context of peripheral nerve trauma such as enhancing neuron survival and improving return to function. The prevalence of peripheral nerve trauma remains surprisingly high and current treatment options such as nerve graft have several pitfalls. The traditional gold standard requires the sacrifice of a healthy nerve, thus alternative remedies, such as cell transplants, are in high demand. In particular, SCs are essential to Wallerian degeneration (Salzer and Bunge 1980; Stoll et al. 1989; Lee et al. 2006), and nerve regeneration (Hadlock et al. 2000; Mosahebi et al. 2001; Schlosshauer et al. 2003; Goto et al. 2010), making excellent transplant candidates (Hadlock et al. 2000; Mosahebi et al. 2001; Zhang et al. 2002; Goto et al. 2010). However, SCs are difficult to culture *in vitro* and require harvest from a healthy nerve in order to establish a mature cell line (Moreno-Flores et al. 2006). Studies within the last 20 years have instead searched for easily harvested cells such as MSCs that can be reprogrammed or transdifferentiated

into a SC-like phenotype. Transdifferentiated MSCs are capable of expressing SC marker proteins, promoting neural tissue survival, and improving return to function in peripheral nerve injuries (Cuevas et al. 2002; Ni et al. 2010; Dadon-Nachum et al. 2011; Oliveira et al. 2013; Thoma et al. 2014). In addition to mimicking SCs, MSCs have additional benefits, such as secreting neurotrophic factors and serving as targets for genetic modification (Keilhoff et al. 2006; Pereira Lopes et al. 2006; Ribeiro-Resende et al. 2009; Wang et al. 2009; Wyse et al. 2014). The following review will provide the reader with a more in-depth perspective of current treatment options and their pitfalls; the use of cell transplants, especially SCs and MSCs; and, finally, the use of transdifferentiation to create Schwann-like cells from MSCs and their benefits to peripheral nerve regeneration.

2 Peripheral Nerve Injuries- Causes & Prevalence

Peripheral nerve injuries (PNIs) may be caused by a variety of etiologies including trauma, metabolic disorders such as Diabetes mellitus, or iatrogenic surgical complications. The most common cause of PNI is trauma, during which nerves may suffer from traction, ischemia, crushing, or penetrating wounds (Campbell 2008). Other less common causes may include thermal, electric shock, radiation, or vibrational injuries (Robinson 2000, 2004). The majority of incidents are stretch-related injuries, especially in motor vehicle accidents (Stanec et al. 1997) 30% of injuries are due to lacerations by knife, glass, saw, or long bone fractures (Campbell 2008), and about 6% are related to sports injuries (Hirasawa and Sakakida 1983). In a retrospective study by Kouyoumdjian (2006), 456 cases of PNIs showed upper limb injuries to be the most common, with the ulnar nerve most often injured (Kouyoumdjian 2006). Again, these injuries were most often due to motor vehicle accidents, particularly motorcycle crashes. In addition to affecting civilians, PNIs can commonly occur in a combative setting, where nerve injuries are

commonly caused by shrapnel or blast injury from bombs or improvised explosive devices (Maricevic and Erceg 1997).

After suffering from a peripheral nerve injury, a patient's prognosis depends on the type of functional injury they have experienced. At the anatomic level, nerve injury can be divided into neurapraxia, axonotmesis, and neurotmesis (Seddon et al. 1943). In neurapraxia, the nerve remains intact but can no longer transmit impulses. Neurapraxia is typically due to segmental demyelination and is the mildest form of nerve injury. Distally, the nerve conducts normally but there is impaired conduction across the lesion due to the focal demyelination. Axons are typically anatomically intact but nonfunctional, which renders a body part paralyzed. There is sensory and motor loss due to demyelination but no Wallerian degeneration occurs. Clinically, muscle atrophy does not develop. Recovery time is typically rapid and ranges from hours to a few months. Full function is usually expected without any sort of intervention by approximately 12 weeks (Campbell 2008).

In axonotmesis, the axon is damaged but most of the surrounding connective tissue is intact. Wallerian degeneration does occur, a process which will be covered in Sect. 2. Axonotmesis is usually seen in stretch or crush injuries. Recovery and reinnervation depends upon the distance from nerve to muscle and the degree of internal axonal disorganization.

In neurotmesis, the nerve trunk is severed and most of the connective tissue is lost or distorted. Neurotmesis occurs with massive trauma, nerve avulsions, and sharp, cutting injury. There is loss of nerve trunk continuity and reinnervation typically does not occur. Without surgical intervention, the prognosis is poor. Recovery from this sort of trauma when there is significant axon loss and stromal disruption is usually prolonged and incomplete (Sunderland and Williams 1992).

When suffering from neurotmesis or axonotmesis, injuries can cause total or partial loss of motor, sensory, or even autonomic function. When left to repair itself, the peripheral nervous system can attempt one of three mechanisms: reinnervation by axonal

regeneration, reinnervation by collateral branching of uninjured surrounding axons, or remodeling of the nervous system circuitry; however, left to only these mechanisms, a full functional recovery is often not achieved (Sunderland and Williams 1992; Drake 1996; Lundborg 2000). Failure can be attributed to three problems: First, axons stop elongating and result in neuroma formation. Second, axon sprouts innervate more than one peripheral nerve branch and cause weak or contradicting muscle movements. Third, regeneration into the wrong nerve can occur if, for example, a sensory axon grows into a motor nerve or vice versa (Klimaschewski et al. 2013).

It is important to understand that while the peripheral nervous system retains the ability to reconstruct itself, only 60% of patients suffering from a PNI regain useful function (Klimaschewski et al. 2013). The occurrence of postparalytic syndromes such as paresis, synkinesis, and dysreflexia are common (Kerrebijn and Freeman 1998). Additionally, patients can experience chronic neuropathic pain, health care issues, and long periods of sick leave (Jaquet et al. 2001; Rosberg et al. 2005).

Due to the high incidence of unsatisfactory return of function, further improvements in peripheral nerve repair and regeneration have become an area of much interest. Today, PNIs have become the focus of new innovations which revolve around many different scientific disciplines. The following section will focus on the two most common areas of clinical treatment: surgery and transplantation. Other disciplines involved such as biomaterial sciences, physical therapy, and pharmacotherapy are outside of the realm of this review, though may be mentioned in the context of important interdisciplinary work.

2.1 Current Treatment Options for PNIs

The most common medical treatments rely largely on reconstructive microsurgery. Although nerve reconstruction has been attempted for centuries, techniques have improved drastically within the past few decades (Siemionow and

Brzezicki 2009). Procedural options include nerve autografts, neurolysis, nerve transfers, and direct suture (end to end neurorrhaphy) (Geuna et al. 2013). The nerve transfer method has seen widespread application in recent years and is used in severe nerve trauma, including brachial plexus avulsions (Tung and Mackinnon 2010; Zhang and Gu 2011).

Although advances in microsurgical techniques have plateaued, a few interesting technological advances have occurred within the past 10 years. For example, the use of glue rather than sutures has been tested in animal models, and results indicate that glue may be equal or even superior to epi- and perineural microsuturing (Whitlock et al. 2010, Sameem et al. 2011). Another area of advancement is robotics assisted surgery. Results from experimental studies are encouraging, and robot technologies may be favored by neurosurgeons in the future (Latif et al. 2008; Nectoux et al. 2009).

Microsurgical treatment alone has relatively low success rates, which is why transplantation is drawing the most interest in regenerative medicine (Geuna et al. 2013). The current “gold standard” includes transplantation of an autologous nerve segment which has been harvested from another healthy, less important nerve such as the sural nerve. The procedure was first developed by Millese (1981) and later deemed the standard of care (Siemionow and Brzezicki 2009). Although autografts are the “gold standard,” the harvesting of another healthy nerve represents obvious limitations, which is why veins are sometimes used as an alternative (Chiu and Strauch 1990). Although vein autografts may lead to satisfactory return of sensation, comparable to nerve grafting, they are only useful for short distances as longer veins tend to collapse (Chiu 1999).

In addition to nerve and vein grafts, skeletal muscle used as guiding fibers has also been tested with relative success. Various studies have shown that muscle conduits may potentially bridge peripheral nerve defects (Meek and Coert 2002) and that grafts may even gain some functional recovery (Pereira et al. 1991, 1996; Rath 2002).

Apart from tissue transplants, cell transplants are a large area of research. MSCs and glial cells,

specifically, SCs, are commonly studied for transplantation. The purpose for use and clinical studies of each cell type will be further discussed in Sects. 4, 5 and 6. The following section will explain the process of nerve breakdown and regeneration following a traumatic nerve injury and the essential role that SCs play.

3 Wallerian Degeneration

After damage to a peripheral nerve, a complex system of molecular and cellular events take place for nerve regeneration to begin. In 1850, August Waller first described Wallerian degeneration, a process characterized by degeneration in the distal nerve stump, with elongation and regeneration in the proximal nerve stump (Stoll et al. 1989, 2002).

Soon after a PNI, SCs in the distal nerve rapidly initiate detachment of their myelin sheaths (Guertin et al. 2005). The surrounding myelin and axonal tissue begin to break down. Within hours of injury, histological changes have occurred as neurotubules and neurofilaments become disarrayed (Burnett and Zager 2004). Within 24 h of injury, SCs are stimulated to proliferate by proteins released from the disintegrating axons (Karanth et al. 2006), and later, by macrophage-derived cytokines. The SCs exhibit an increased mitotic rate, nuclear and cytoplasmic enlargement, and rapid division to form daughter cells (Burnett and Zager 2004). These daughter cells produce cytokines and trophic factors which assist in degeneration and repair (Gaudet et al. 2011). During this time, local macrophages (Mast cells) interact with the SCs to remove degenerated axonal and myelin debris. SCs and macrophages work together to phagocytose and clear the site of injury. By 36–48 h, myelin disintegration is quite advanced (Burnett and Zager 2004). The elimination of myelin sheaths is important as it clears certain growth inhibitory factors such as myelin-associated glycoproteins (Huang et al. 2005). While the distal nerve is degenerating, the nerve cell body is undergoing a process known as

chromatolysis. Within 6 h of injury, the nucleus of the nerve cell body migrates to the periphery of the soma and the rough endoplasmic reticulum (Nissl bodies) breaks up and disperses (Lieberman 1971; Kreutzberg 1995). In this state, the neuron increases RNA synthesis and cellular protein content, and reduces DNA repression, in order to increase synthesis of growth-associated proteins and membrane structural components (Watson 1974).

Within 2 days, Schwann cell daughter cells have undergone rearrangement into a structure known as Bünger bands (Tetzlaff 1982). These bands act as a guidance skeleton for regenerating axon sprouts. Within a week, factors produced by SCs and injured axons leads to recruitment of hematogenous monocytes (Tofaris et al. 2002). The new macrophages continue to clear debris and produce factors which facilitate SC migration (Gaudet et al. 2011).

After weeks to months, axon sprouts begin to form, each with a specialized growth cone at the tip containing multiple filopodia. These filopodia adhere to the basal lamina of the Schwann cells within the Bünger bands, which serve as a guide toward potential new targets of innervation. Both physical and chemotactic guidance from the SCs are important in directing advancement of the growth cone (Gundersen and Barrett 1980; Dodd and Jessell 1988). Individual filopodia respond to environmental alterations in calcium (Lin and Forscher 1993) and different filopodia can react independently via local changes to actin metabolism (Kerrebijn and Freeman 1998). Once contacted by regenerative sprouts, SCs re-differentiate, express myelin mRNAs, and begin the process of remyelinating and ensheathing newly regenerated axons (Campbell 2008). If axonal sprouts are able to cross the injury site and contact a new peripheral target, then reinnervation may occur. The regeneration and repair phase may last for many months and is not always successful. Regenerating axons may enter surrounding tissue instead of the target organ or may enter the incorrect endoneurial tube, failing to reinnervate the correct target.

After nerve injury and repair, the conduction velocity of regenerated axons, their diameter, and their excitability remain below previous levels for a considerable period of time (Fields and Ellisman 1986).

In addition to the complex cellular response, PNIs induce the release of many neurotrophic factors and cytokines to create a favorable environment for axon regrowth. These polypeptides assure that the regenerating axons are growing towards the distal nerve stump and stimulate axonal sprouting. The following section will review the role of neuronal growth factors, particularly brain-derived neurotrophic factor, during Wallerian degeneration.

4 The Importance of Neurotrophic Factors During Peripheral Nerve Regeneration

In response to a peripheral nerve injury, many neurotrophic factors are upregulated. These molecules may be classified either as neurotrophic factors or neurotrophic cytokines (Lewin and Barde 1996). This review will discuss neurotrophic factors and will focus primarily on the role of brain-derived neurotrophic factor (BDNF).

Neurotrophic factors are vital to healthy neuron function for the course of the cell's life. They are important for neurite outgrowth during embryonic development, maintenance of adult neurons, and regeneration following a PNI (Klimaschewski et al. 2013). The specific neurotrophins involved in regeneration include nerve growth factor (NGF), BDNF, and neurotrophins 3 (NT-3), 4, and 5 (NT-4/5). Several growth factors are also released, including glial cell line-derived neurotrophic factor (GDNF), fibroblast growth factors, insulin-like growth factors, neuregulins, and neuropeptides (galanin, vasoactive intestinal peptide, etc.) (Boyd and Gordon 2003; Gordon 2009). All neurotrophic factors are believed to be synthesized in target organs and then delivered via retrograde transport to the neuronal soma (Purves 1986; Oppenheim 1991). The

neurotrophin members (NGF, BDNF, NT-3/4/5) share a common low-affinity receptor p75 (Chao et al. 1986) to which they all bind equally. It is thought that p75 interacts with the tropomyosin receptor kinases (Trk) to assist in transport of neurotrophins within the neuronal terminals (Gargano et al. 1997). Each neurotrophin has a specific high affinity receptor: TrkA for NGF, TrkB is specific for BDNF, and NT-4/5, and NT-3 bind to TrkC (Terenghi 1999). Every Trk receptor is located in a discrete population of primary sensory neurons (McMahon et al. 1994; Wright and Snider 1995) and TrkB and C are also present in spinal motoneurons (Ernfors et al. 1993). The following section will focus on the TrkB receptor and the various roles that BDNF plays in neuronal regeneration.

4.1 Promotion of Neuron Survival

Activation of each neurotrophin is dependent on the type of neuronal damage (motor, sensory, or autonomic). BDNF, in particular, is upregulated in motor neurons, as is its receptor, TrkB, for 48 h following an axotomy lesion (Kobayashi et al. 1996). During this time, BDNF acts as a neuroprotectant. It has been shown to rescue motor neurons from natural cell death, as well as prevent their death following axotomy (Oppenheim et al. 1992; Yan et al. 1992, 1994). This ability of BDNF to rescue motor neurons is carried out through its TrkB receptor. Once BDNF binds to TrkB, several signal transduction cascades are activated. These include insulin receptor substrate-1, Ras, protein kinase C, and many other intermediate proteins. BDNF signaling pathways activate one or more transcription factors (cAMP-response-element-binding protein (CREB), and CREB-binding protein) which regulate the expression of genes encoding proteins that are involved in neural plasticity, stress resistance, and cell survival (Bonni et al. 1999; Brunet et al. 1999; Bathina and Das 2015).

Indeed, external application of BDNF following axotomy or ventral root avulsion reduces motoneuron death (Yan et al. 1992; Novikov et al. 1995) and continuous dose-dependent

administration of BDNF shows long-term survival effects on adult motoneurons after sciatic nerve avulsion (Kishino et al. 1997). Additionally, a few studies found that application of NGF, BDNF, and NT-3 can reverse detrimental changes induced by axotomy in adult and neonatal sensory neurons (Verge et al. 1992, 1995; Eriksson et al. 1994).

4.2 Remyelination

After Wallerian degeneration occurs, the next important step in peripheral nerve recovery is remyelination, in which BDNF plays an important role. Several studies have added exogenous BDNF to a peripheral nerve injury model and examined the effects on myelin protein synthesis and myelin sheath thickness. The first study to examine this phenomenon observed that when applied in combination with ciliary neurotrophic factor (CNTF), exogenous BDNF increases myelin thickness of regenerating sciatic nerves (Lewin et al. 1997). This work was continued by a study (Chan et al. 2001) that used a SC and dorsal root ganglion (DRG) cell co-culture model, as well as a sciatic nerve *in vivo* model, to test the effects of exogenous BDNF addition following an injury. Immediately following injury, BDNF caused an enhancement in the expression of myelin protein MAG and P0. This effect was seen in both the co-culture and sciatic nerve *in vivo* model. Furthermore, when endogenous BDNF levels were reduced in the co-culture via addition of the receptor scavenger TrkB, myelin protein synthesis was inhibited as was the formation of myelin, verifying that BDNF is indeed beneficial during remyelination.

Several studies found that BDNF increases myelination during peripheral nerve regeneration. With the use of electron microscopy, Chan et al. demonstrated that the addition of BDNF increased the number of myelinating axons and the thickness of the myelin sheath *in vivo* (Chan et al. 2001). A similar study (Zheng et al. 2016) created a mouse sciatic nerve injury model and administered exogenous BDNF injections to examine the effects on myelin sheaths in the distal

nerve stump. Their results showed that mice receiving BDNF administration had an increased number of myelinated fibers and that myelin sheaths were thicker when compared to control mice. Additionally, mice receiving BDNF blocking antibodies showed significant myelin deterioration in the distal sheath. Furthermore, a study by Zhang et al. 2000, demonstrated that treatment with BDNF antibody reduced the number and density of myelinated axons by 83%, and found that sensory reinnervation was impaired (Zheng and Kuffler 2000). Combined, these results demonstrate that BDNF is critical for preparing nerves for remyelination by increasing myelin proteins such as P0 and MAG, as well as protecting the distal nerve portion from atrophy by promoting remyelination.

4.3 Axonal Sprouting, Regeneration, and Functional Recovery

In addition to examining neuronal survival, regeneration, and re-myelination, several studies have looked at BDNF's role in axonal sprouting. It has been shown that following severe trauma such as ventral root avulsion, exogenous BDNF significantly increases axonal sprouting (Gordon 2009). In support of Gordon's findings, another study found that application of BDNF blocking antibodies on a transected facial nerve trunk significantly reduced axon sprouting up to 18% (Streppel et al. 2002). Axonal sprouting may increase in part, due to BDNF's role as a guidance molecule for the growth cone at the end of each axonal sprout. Studies in *Xenopus* spinal neuron models show that BDNF and NT-3 can attract or repulse growth cones based on concentration gradients (Song and Poo 1999; Zheng and Kuffler 2000).

Although BDNF may increase axonal sprouting, the data is controversial in regard to increased functional return upon application of BDNF. For example, using the sciatic function index (Bredesen and Rabizadeh 1997), gait analysis (Shirley et al. 1996), and force recovery, several studies failed to demonstrate a return to function with exogenous BDNF. One study even

showed that local long-term continuous infusion of low dose BDNF had no effect on tibial motoneurons after immediate microsurgical repair (Boyd and Gordon 2002).

On the other hand, a more recent study found that exogenous BDNF administration accelerates the recovery process in a mouse sciatic nerve injury model, while BDNF antibody treatment delayed it (Zheng et al. 2016). After the crush injury, control mice took 12 days to show initial improvement using the toe spreading score of gait analysis, and 24 days for a full recovery. Mice receiving the BDNF treatment required only 7 and 18 days, respectively. Conversely, BDNF antibody treatment delayed the processes to 17 and 30 days.

Another study created control and heterozygote BDNF knockout mice that received a left sciatic nerve crush (Takemura et al. 2012). Nerve function was evaluated using a rotarod test, sciatic function index, and motor nerve conduction velocity simultaneously with histological nerve analysis. Impaired nerve repair was observed in the BDNF heterozygote mice, which was consistent with attenuated function of BDNF. In contrast, the BDNF homozygote mice showed complete functional and histological recovery. These observations support the view that BDNF may play a pivotal role in functional return following a peripheral nerve injury.

Unlike other neurotrophic factors, BDNF is unique in that it regulates and maintains neuronal function, and when given exogenously, it counteracts degenerative changes in both sensory and motor axons. Unlike NGF, BDNF supports motoneuron survival *in vitro*, rescues from naturally-induced apoptosis, and prevents *in vivo* axotomy-induced cell death (Yin et al. 1998). While there are benefits of exogenous BDNF application to peripheral nerve lesion sites, its abilities to increase functional return are still controversial. Therefore, recent research has focused on the adjunct use of BDNF in combination with other therapies such as stem cell therapy, biomaterial conduits, pharmacotherapy, etc. A more in-depth discussion of BDNF therapy combined with stem cell use will be included in Sects. 6 and 8.

5 Cell-Based Therapy for Improving Nerve Regeneration

As discussed above, the gold standard of peripheral nerve repair continues to be the use of nerve grafting combined with direct nerve repair, and occasionally, the use of conduits to bridge larger nerve gaps. Recent research, however, has focused on cell therapy as a promising therapeutic approach for promoting nerve regeneration. Particularly, cell-based therapy has been widely studied as a delivery system for growth-promoting molecules and as a graft replacement. This section will focus briefly on the past use of glial cells such as SCs and then discuss the promising potential of bone marrow-derived MSCs.

5.1 Use of Schwann Cells

Schwann cells (SCs) play a key role in axonal regeneration, making them an attractive cell type to use for transplantation. During Wallerian degeneration, SCs remove necrotic tissue and myelin debris together with macrophages (Geuna et al. 2009). In the regeneration phase, SCs form the Bünger bands and increase synthesis of surface cell adhesion molecules and basement membrane proteins such as laminin and fibronectin to physically guide axons to distal innervation targets (Fu and Gordon 1997). SCs also produce neurotrophic factors, cytokines, and other compounds which promote neurite growth (Funakoshi et al. 1993; Hall 2001). Experimental evidence shows that addition of SCs to nerve conduits *in vitro* support axonal outgrowth (Schlosshauer et al. 2003), and improves the quality and rate of axon regeneration (Hadlock et al. 2000; Mosahebi et al. 2001; Goto et al. 2010). SCs combined with a vein conduit have even been used in bridging long nerve gaps (Strauch et al. 2001; Zhang et al. 2002). The Miami Project, a program for the investigation of brain and spinal cord injury has used SCs in a phase I clinical trial. Previous methods for SC culture were adapted for the manufacture of clinical

grade human SC products that meet FDA standards (Bunge et al. 2017), and the autologous transplant of SCs into individuals with spinal cord injury was deemed safe and feasible with no complications (Anderson et al. 2017).

Although SCs appear to be an ideal source of cells for regenerative therapy, there are several technical limitations. In order to obtain a source of autologous SCs, another healthy nerve must be sacrificed for harvesting, making donor site morbidity an additional concern. Use of SCs is thought by some to be impractical since the time requirement for expanding autologous cells in culture is lengthy (Moreno-Flores et al. 2006). There is also a risk of fibroblast contamination which would lead to unwanted scarring of the nerve (Mosahebi et al. 2001). SCs require stimulation by axons or specific growth factors that mimic axonal signals in order to proliferate, and do not proliferate in response to serum factors unlike other cell types. All of these limitations have led researchers to seek an alternative to SCs for cell transplantation, and stem cells have been posed as better candidates.

5.2 Mesenchymal Stem Cells

Stem cells are a distinct population of undifferentiated cells which are characterized by potency, the ability to differentiate into a wide variety of specialized cell types, and the ability to undergo numerous rounds of mitosis while remaining undifferentiated. There are embryonic, induced, fetal, and adult stem cells, of which this review will focus on adult stem cells.

Of these different categories, adult stem cells are thought to be the most limited in their potency and are generally considered multipotent in nature since their primary role is to repair damaged tissue in which they are found (Oliveira et al. 2013). Unlike fetal and embryonic stem cells, adult stem cells raise fewer ethical concerns as they do not require human embryo destruction. Additionally, adult stem cells have a lower risk of tissue rejection as auto-transplantation is a possibility, and the small risk of teratoma formation that pluripotent embryonic or induced stem cells

presents is almost null with adult cells (Bjorklund et al. 2002). Common sources of adult stem cells include mesenchymal, hematopoietic, or umbilical cord-derived. In particular, bone marrow-derived stem cells are known as mesenchymal stem cells and can differentiate into connective tissue types such as chondrocytes, adipocytes, myocytes, osteocytes, fibroblasts, and tenocytes (Muraglia et al. 2000). There is also extensive additional research to suggest that MSCs have the ability to transdifferentiate into ectodermal and endodermal lineages such as glial cells, neurons, hepatocytes, etc (Fig. 1) (Woodbury et al. 2000; Dezawa et al. 2001; Kim et al. 2002).

In addition to being a source for many cell types, MSCs are easily accessible and have the ability to rapidly divide under culture, allowing them to meet the requirements of an *in vitro* cell system. Additionally, MSCs are excellent candidates for allogeneic transplantation as they are immune privileged cells and often do not require the use of immune suppressive drugs (Oliveira et al. 2013). Besides their high safety and efficacy, MSCs release paracrine factors, survive and integrate into host tissue, and concentrate in injured tissues. (Keilhoff and Fansa 2011).

6 Mechanisms Behind Nerve Regeneration Potential of MSCs

Although MSCs are highly regarded for their plasticity and ability to differentiate into many cell types, there are other mechanisms by which MSCs are thought to promote and support nerve regeneration. Such mechanisms include immunomodulation, transdifferentiation into SCs, paracrine activity, genetic manipulations, and mitochondrial transfer/cellular fusion (Fig. 2).

6.1 Secretion of Neurotrophins

As discussed already, neurotrophins promote neuronal survival, reverse the negative effects of PNIs, and lead to SC proliferation and

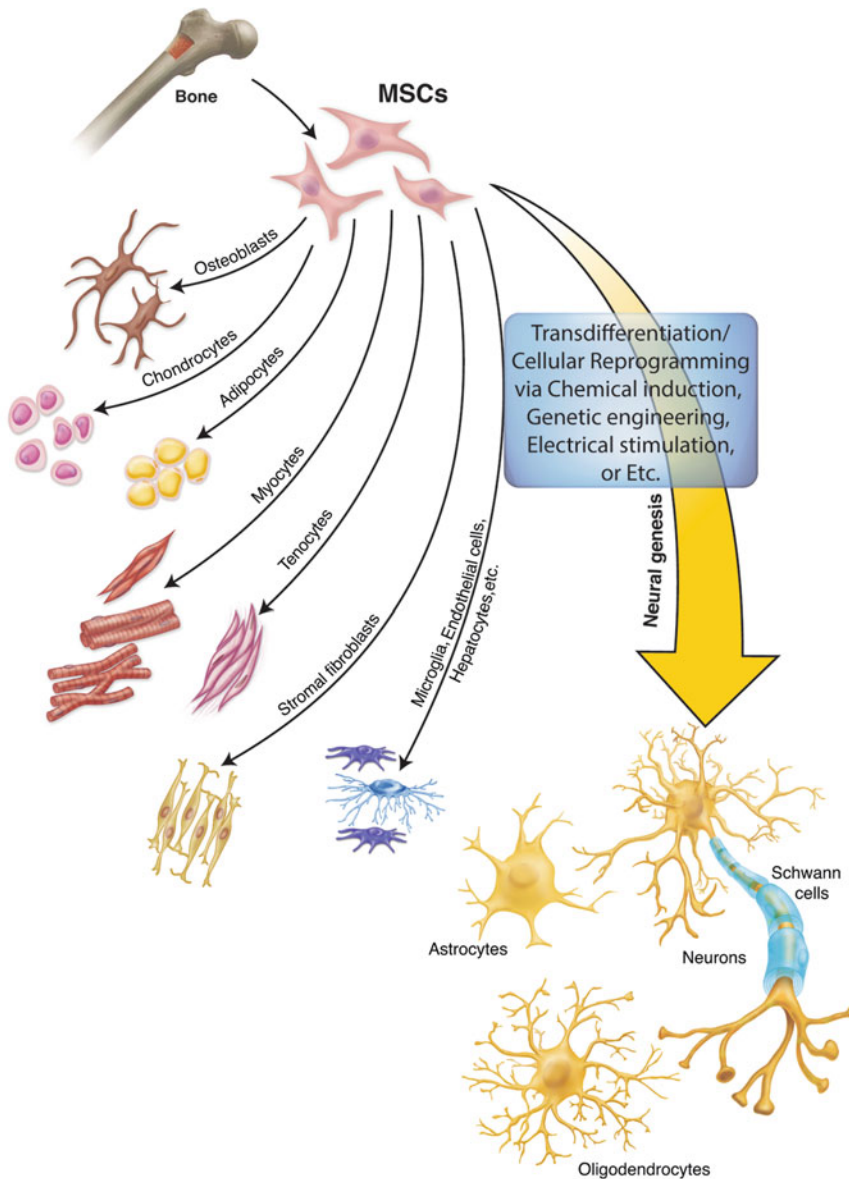


Fig. 1 Differentiation potential of multipotent bone marrow-derived mesenchymal stem cells (MSCs). Mesenchymal stem cells are capable of differentiating into mesodermal lineages including osteoblasts, chondrocytes, adipocytes, myocytes, tenocytes and stromal fibroblasts. A number of studies have also demonstrated that MSCs can

be transdifferentiated or reprogrammed into endodermal and ectodermal lineages including, microglia, endothelial cells, hepatocytes and neural cells (neural genesis into: Schwann cells, neurons, astrocytes and oligodendrocytes). (Illustration modified from Sandquist et al. 2016)

differentiation. MSCs can produce neurotrophic substances for paracrine signaling, which is likely one of the key ways that MSCs are thought to help in regeneration. Gu et al. investigated DRG

explants and neurons co-cultured with MSCs and showed enhanced neurite outgrowth and neuronal cell survival due to the production of NGF, CNTF, BDNF, and basic fibroblast growth factor

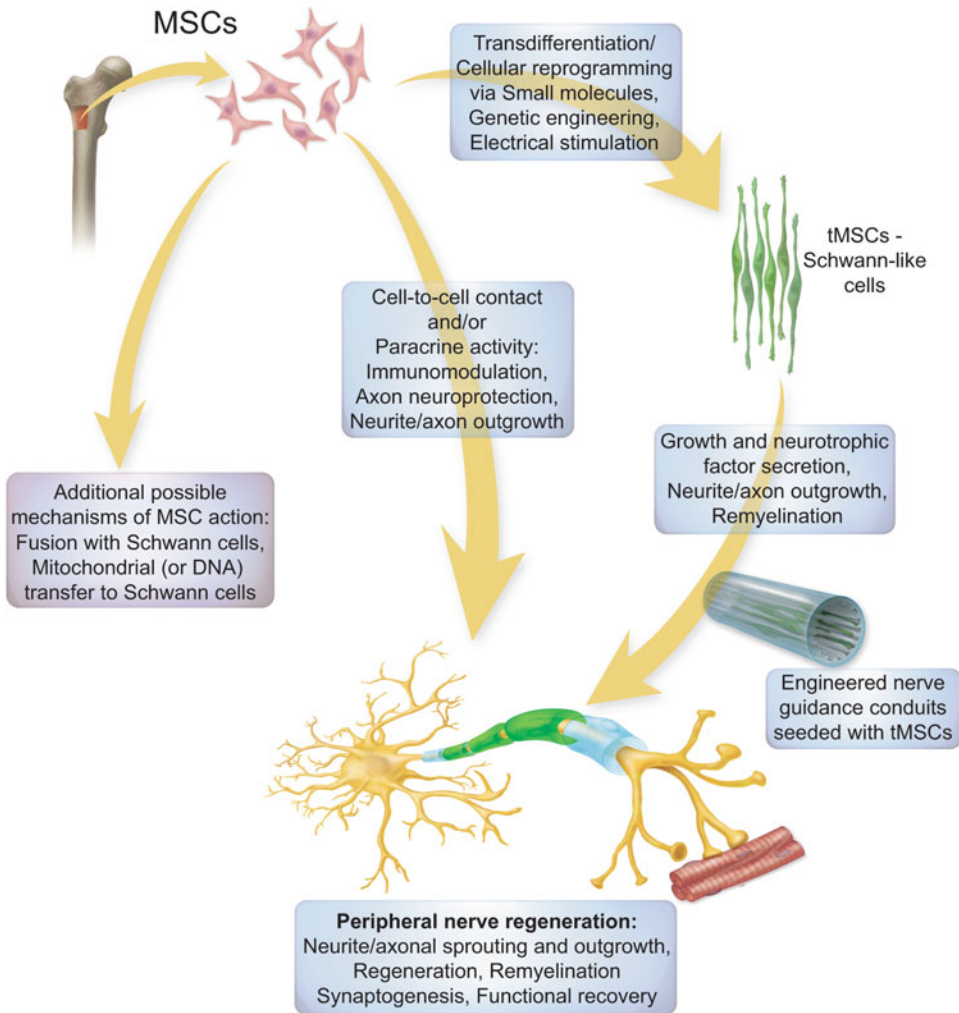


Fig. 2 Mechanisms and strategies using MSCs to promote and support nerve regeneration. Abbreviations: MSCs, mesenchymal stem cells; tMSCs, transdifferentiated MSCs; DNA, deoxyribonucleic acid. (Illustration

prepared by S. Mientka, K. Moss, D. S. Sakaguchi, Biological Pre/Medical Illustration (BPMI) program, Iowa State University)

(bFGF) by MSCs (Gu et al. 2010). In the culture system, there was no direct contact between the neurons/explants and MSCs, leading researchers to believe that positive effects were due to the release of soluble growth factors. Other studies have found that DRG explants or adult neural progenitor cells treated with MSC-conditioned media also showed increased neurite outgrowth, presumably due to the presence of released growth factors in the media (Ribeiro-Resende et al. 2009; Ye et al. 2016). In addition to their

direct paracrine effects, MSCs can induce SCs to produce neurotrophic mediators as well. In co-culture studies of rat SCs and MSCs, increased survival and proliferation rates of SCs was noted as well as high expression of mRNA and protein levels for NGF, BDNF, and Trk/p75^{NTR} receptors (Wang et al. 2009). The same group also examined the effect of MSCs on SCs in a rat peripheral nerve repair model, and showed that MSCs increased the generation of SCs and promoted SC-mediated neurotrophic functions. Thus,

MSCs are considered beneficial for regeneration due to their production and secretion of neurotrophic factors.

After *in vitro* co-culture studies, the next step was to determine whether MSCs continued to produce growth factors *in vivo* after transplantation, and whether these factors were biologically active. Fortunately, this inquiry returned positive results. Several studies were able to document expression of GDNF, CNTF, FGF, and even BDNF by MSCs *in vivo*, allowing for survival and elongation of neuronal growth cones (Pereira Lopes et al. 2006; Chen et al. 2007; Pan et al. 2007; Yang et al. 2011). A similar study (Ribeiro-Resende et al. 2009) transplanted MSCs at a rat sciatic nerve lesion and the results demonstrated improved regeneration of motor and sensory axons due to the production of growth factors. Other studies incorporated conduits filled with MSCs to test models of long sciatic nerve gaps (Wang et al. 2011; Yang et al. 2011; Hu et al. 2013). For example, one group implanted a collagen conduit filled with MSCs at a mouse sciatic nerve transection lesion and saw enhanced axon regeneration and remyelination (Pereira Lopes et al. 2006). Additionally, high levels of NGF and BDNF were detected, suggesting that MSCs were expressing these neurotrophins *in vivo*.

Combined, these results demonstrate that MSC-based therapy improves peripheral nerve regeneration through direct secretion of neurotrophic factors which may act locally as well as on glial cells further away.

6.2 Immuno-modulatory Effects

One of the most interesting features of MSCs is their ability to modulate the immune system making it a popular candidate for transplant therapy. When transplanted into tissues, MSCs decrease tissue inflammation and can have immunosuppressive effects by restraining T-cell proliferation and inhibiting natural killer T cell signaling (Di Nicola et al. 2002).

Additionally, MSCs promote anti-inflammatory T helper 2 cells (Aggarwal and Pittenger 2005). MSCs also suppress monocyte

differentiation into dendritic cells, thus decreasing the amount of antigen presentation to T cells (Jiang et al. 2005). In a spinal cord injury model, MSC transplantation favored the development of M2 macrophages and suppressed M1 activation (Nakajima et al. 2012). M2 macrophages have anti-inflammatory activity while the classic M1 phenotype has pro-inflammatory effects in damaged tissue (Nakajima et al. 2012). The complex mechanisms behind MSCs immunomodulatory properties are still being uncovered, but they are thought to be facilitated by cell-cell contact and the secretion of soluble factors such as indoleamine-pyrrole 2,3-dioxygenase (IDO) and nitric oxide that are known to inhibit T-Cell proliferation (Mazzoni et al. 2002; Terness et al. 2002; Wang et al. 2014). A recent review by Gao et al. further details immunomodulatory properties of MSCs (Gao et al. 2017). Though exact mechanisms of MSC immunomodulation are not fully understood, their ability to decrease inflammation has been widely described, supporting the therapeutic merits of stem cells.

6.3 Cellular Fusion

In addition to the various nerve regeneration mechanisms discussed, a few studies have documented the spontaneous transfer of mitochondria from MSCs with a variety of other cell types. MSCs can form tunneling nanotubes through which mitochondria and nuclear DNA can be transferred. Several studies have utilized MSCs in acute pulmonary damage models to demonstrate mitochondrial transfer from MSCs to alveolar cells and airway epithelial cells (Spees et al. 2006; Islam et al. 2012; Li et al. 2014). Mitochondrial transfer has also been demonstrated between MSCs and cardiomyocytes, causing increased proliferation and, in Acquistapace's study, reprogramming towards a progenitor-like state (Plotnikov et al. 2008; Acquistapace et al. 2011; Vallabhaneni et al. 2012). The majority of these studies involved use of epithelial or muscle cells; however, one study found that bone marrow-derived MSCs were able to fuse with neuronal cell types,

including Purkinje cells (Weimann et al. 2003). To date, there is no evidence of mitochondrial transfer or MSC fusion with SCs, but this could represent an alternative mechanism by which MSCs support SC activity and regeneration.

7 Clinical Trials with MSCs for Neurological Disorders

Autologous cell transplantation has been investigated extensively as a therapeutic strategy for neurological disorders. Extensive *in vitro* and *in vivo* data suggest that MSCs secrete several trophic factors, support neurogenesis and neurite growth, and promote survival and elongation of damaged peripheral nerves. An even larger body of work exists demonstrating the benefits of MSCs within the context of central nervous system disorders and spinal cord trauma. Combined, the data has proven the safety and efficacy of MSCs and allowed their use in human clinical trials – a key stepping stone to their common use as a clinical therapy.

A large number of studies have reported the use of MSCs in treatment of neurological disease and trauma (Harrop et al. 2012). Clinical trials include treatment of Multiple Sclerosis (23%), Amyotrophic Lateral Sclerosis (14%), Alzheimer's disease (5%), Duchene muscular dystrophy (3%), Parkinson's Disease (3%) to treatment of traumatic injury, with spinal cord injury models having the largest number of trials (29%) (Squillaro et al. 2016).

Fewer clinical trials have utilized MSCs within a peripheral nerve context. One retrospective study reports the use of bone marrow mononuclear cells (BMMCs) as a source of MSCs to treat patients with a median or ulnar nerve severed by knife or glass. Cells were collected from the patient's iliac crest and injected into a silicone conduit used to bridge the nerve gap. Patients implanted with the BMMC-filled conduits scored higher for motor function, sensation, and effect of pain on function than those who received empty conduits (Braga-Silva et al. 2008). Though these results are promising, the two groups of patients (with and without BMMCs) were studied decades

apart; furthermore, it is unclear whether the improvements were mediated specifically by MSCs within the BMMC fraction. Nonetheless, this study provides a basis for future clinical trials.

Most clinical trials related to peripheral nerve repair with MSCs focus on diabetic peripheral neuropathy patients. For diabetic patients, MSCs are an effective therapeutic agent due to secretion of bFGF and vascular endothelial growth factor (VEGF) (Shibata et al. 2008). Evidence suggests that the effects are not mediated through differentiation into neural cell types, but rather through the secretion of these beneficial factors. (Siniscalco et al. 2011). One clinical trial was conducted using MSCs to treat patients with diabetic foot disease – a complication in which hyperglycemia induces peripheral nerve damage. Human umbilical cord blood-MSCs were injected into the patient's impaired limb resulting in obvious improvement 12 weeks after treatment. This result was attributed to the MSC's bFGF and VEGF production and also to their immune cell modulatory effects (Li et al. 2013). Current clinical trials are in stage II and III and revolve around change of nerve conduction velocities before and after stem cell intravenous transfusion (clinicaltrials.gov; NCT02387749). Results indicate that autologous bone marrow stem cells have increased bFGF and epidermal growth factor (EGF) levels at time of transfusion. Patients with MSC transfusion had greater sural nerve conduction velocities 90 days after treatment.

Other conditions with MSC-based clinical trials involving the peripheral nervous system include hemifacial spasm and burn wound healing (clinicaltrials.gov; NCT02394873, NCT03183622). One clinical trial in early phase I aims to inject autologous adipose-derived MSCs into patients with hemifacial spasm in hopes to increase facial nerve function and evaluate changes in facial nerve electrophysiology (clinicaltrials.gov; NCT02853942). While peripheral nerve regeneration is not the primary focus of some of these studies, transplanted MSCs and their secreted factors are likely to facilitate the overall repair of damaged nerves.

Unfortunately, there are no current clinical trials examining the use of MSCs in traumatic peripheral nerve damage, but important pre-clinical studies are underway. Xue et al. transplanted autologous bone marrow-MSCs into a 60 mm-long canine sciatic nerve gap using a tissue-engineered nerve graft (TENG) with improved repair and regeneration (Xue et al. 2012). The same group then moved on to implanting the MSC-TENGs into a rhesus monkey median nerve gap. Animals with MSC-TENG implants recovered motor function comparable to autologous nerve grafted animals and with greater recovery than the animals with tissue-engineered scaffold alone. Transplanted MSCs were found to express SC marker S100 and neurotrophic factors BDNF, CNTF and bFGF. Thus, it is likely that the autologous MSCs contribute to the peripheral nerve regeneration via cell replacement and secretion of beneficial factors. Lastly, after extensive safety evaluations, it was concluded that autologous MSCs could safely be used in a primate (Hu et al. 2013). Altogether, these studies provide strong support for the future clinical use of MSCs for traumatic peripheral nerve damage.

The data obtained from clinical trials, as well as *in vitro* and *in vivo* studies shows that unaltered MSCs offer many benefits for nerve regeneration, mainly by secretion of neurotrophic factors, as well as by support of SCs. However, MSCs may hold even greater potential when transdifferentiated into another cell type, such as Schwann cells. The various benefits and methods of transdifferentiated MSCs will be discussed below.

8 Transdifferentiation

Bone mesenchymal stem cells were once thought to be fairly restricted in their differentiation patterns but more studies are demonstrating that they are capable of versatility and greater plasticity. In response to a variety of culture conditions, specialized *in vivo* microenvironments and genetic manipulations, MSCs can turn into different phenotypes such as glial cells (Fig. 3). In

particular, turning MSCs into a SC-like phenotype is of high interest due to the beneficial effects on nerve regeneration. MSCs can be transdifferentiated with a variety of methods, including the use of transplantation, co-cultures, small molecule cues, genetic manipulation, or as most recently described, through electric stimulation. Each method will be discussed in greater detail below.

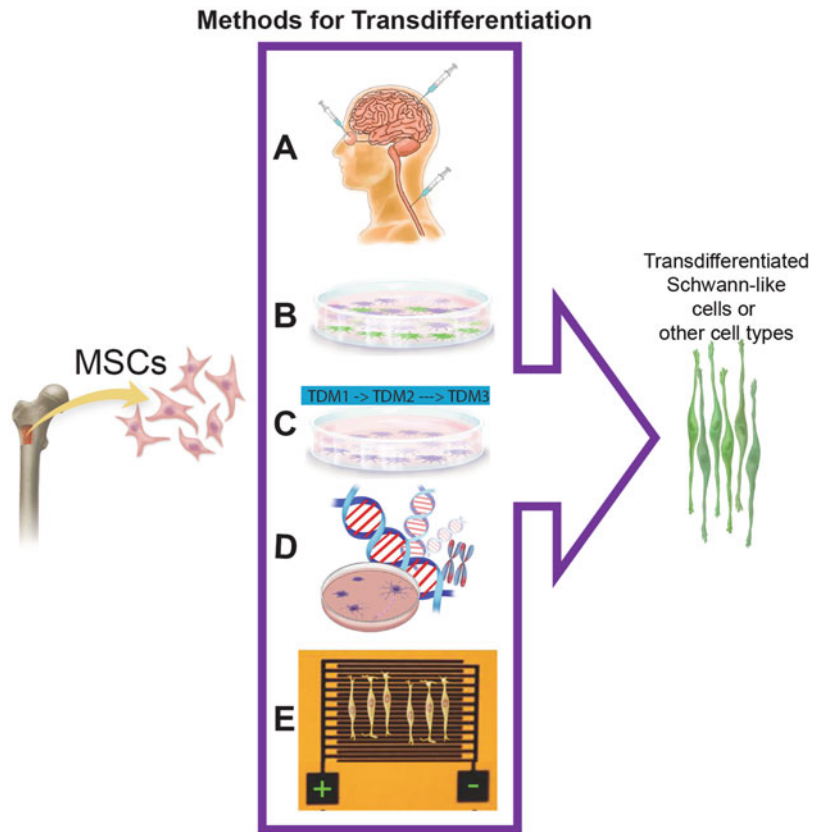
8.1 Transdifferentiation via Transplantation

During Wallerian degeneration and nerve regeneration, a wide variety of cytokines and growth factors are released, creating a specialized microenvironment with the capacity to greatly influence cell differentiation patterns. Although controversial, these environmental signals have been utilized to transdifferentiate MSCs in response to injury or inflammation. Bone marrow-derived MSCs injected at the site of a rat sciatic nerve transection were capable of surviving and migrating, as well as differentiating into an SC-like phenotype, based on S100 immunoreactivity patterns (Cuevas et al. 2002). In this study, it was presumed that MSC transdifferentiation occurred in response to physiological environmental cues, as no MSC medium changes were made. Although transdifferentiation may have occurred, the percentage of cells positive for S100 was so low that this may not be a very efficacious method. Another 2010 study demonstrated similar results, with few numbers of transplanted MSCs at an injury site converting to an SC-like phenotype (Oliveira et al. 2010).

8.2 Transdifferentiation via Co-culture

A more simplistic approach to changing a cell's microenvironment is to adjust its neighboring interactions using co-culture methods. One study showed that direct contact co-cultures of DRG neurons and MSCs could cause a phenotypic and morphological change in MSCs to resemble

Fig. 3 Methods for MSC transdifferentiation. MSCs can be transdifferentiated with a variety of methods, including the use of transplantation (a), co-cultures (b), small molecule cues (c), genetic manipulation (d), or through electric stimulation (e). (Illustration prepared by S. Mientka, K. Moss, D. S. Sakaguchi, Biological Pre/Medical Illustration (BPMI) program, Iowa State University; ((a) adapted from Sakaguchi 2017, (e) adapted from Das et al. 2017)



SCs (Yang et al. 2008). Researchers suggested that the release of cytokines and other neuronal molecules on the axonal surface may play a role in the transdifferentiation process. However, this method alone did not allow transdifferentiated MSCs (tMSCs) to form compact myelin, suggesting that further molecular cues are necessary for a complete transdifferentiation process. Another study looked at co-culture of MSCs with olfactory ensheathing cells and saw a dramatic increase in the number of MSCs resembling a neural morphology which were immunoreactive to various neural markers such as GFAP, p75^{NTR}, and MAP 2 (Ni et al. 2010). These studies demonstrated that co-culturing methods may be sufficient to begin the transdifferentiation protocol, but additional small molecules may be needed to affect a functional change in tMSCs.

8.3 Use of Small Molecules in Media

Although transdifferentiation via transplantation and co-culture has shown some success, this method is not as successful or efficient as the addition of small molecules to culture medium. These specific molecules can trigger cell-signaling pathways and rapidly modulate cell phenotype. In 2001, Dezawa et al., discovered a cell medium protocol for transdifferentiation of MSCs into an SC-like morphology requiring incubation with beta-mercaptoethanol, then retinoic acid for 3 days, followed by forskolin, bFGF, platelet-derived growth factor (PDGF) and heregulin (Dezawa et al. 2001). After induction, these cells physically resembled SCs and expressed several SC markers including p75, S100, GFAP and O4. Bierlein De la Rosa et al.

used Dezawa et al.'s protocol to transdifferentiate MSCs which had been genetically modified to hypersecrete BDNF. The cells were morphologically similar to SCs and expressed SC markers S100 and p75 even after 20 days in transdifferentiation media. Additionally, secreted BDNF levels increased after 20 days and the BDNF secreting MSCs actually showed more SC markers after 8 days compared to the control, green fluorescent protein (GFP) expressing MSCs, indicating the BDNF itself may facilitate faster conversion to SC phenotype (De la Rosa et al. 2017).

Biomaterial-based scaffolds are also being investigated as a means to augment the transdifferentiation of MSCs into Schwann-like cells. For example, gelatin-based 3D conduits with different microstructures (nanofibrous, macroporous and ladder-like) have been fabricated for peripheral nerve regeneration applications (Uz et al. 2017). Their results indicated that 3D macroporous and ladder-like 3D microstructures enhanced MSC attachment, proliferation and spreading, creating interconnected cellular networks. This type of approach investigates the effects of 3D conduit microstructure and mechanical properties and may provide a better understanding of how material-cell interfaces can influence the transdifferentiation process.

Recently, the first comparative proteomic evaluation of MSC transdifferentiation was undertaken to uncover the protein contents that affects SC formulation (Sharma et al. 2017). This study identified a number of MSC proteins that were significantly regulated during SC transdifferentiation. Many of these proteins support axonal guidance, myelination, neural development and differentiation. These results provide clues to unraveling the molecular events that underlie the transdifferentiation process and may ultimately serve to facilitate nerve regeneration and repair.

Other studies have utilized compounds such as valproic acid and histone deacetylase inhibitors, along with neural inducing signaling molecules to create mature neural cells (Sandquist et al. 2016). A 2014 study used a two-step method to first create neural precursor cells, and then induced

SCs from human foreskin fibroblasts (Thoma et al. 2014). These cells may potentially be used to treat peripheral nerve injuries in the future.

8.4 Genetic Modification for Transdifferentiation

A newer transdifferentiation method can now convert adult differentiated cells to a specific terminal cell type without going through pluripotency. This methodology is based on the idea of 'master control genes' in somatic cells which can be overexpressed to induce a cascade of cell fate changes (Lewis 1992; Nizzardo et al. 2013; Prasad et al. 2016). The earliest evidence of this possibility was provided by Weintraub et al, who confirmed conversion of fibroblasts to myogenic lineage by transfection of a master regulatory gene (*MyoD*) (Weintraub et al. 1991). Later, *Pax6* was recognized as a master gene responsible for neuronal differentiation. Vierbuchen et al. identified the combination of *Asc11*, *Brn2* and *Myt11* as able to convert mouse embryonic fibroblasts into mature neurons (Vierbuchen et al. 2010). Cells transdifferentiated in this manner exhibited similar functionality to cells differentiated from induced pluripotent stem cells (iPSCs) or wild-type analogs and show no tumorigenicity when transplanted *in vivo* (López-León et al. 2017). Unfortunately, this method of generating target cells through transdifferentiation relies on viral expression of exogenous transcription factors which makes demonstration of safety for clinical trials difficult; however, the method holds promise for direct cellular conversion.

8.5 Electrical Transdifferentiation

A recent study by Das et al. 2017 described a novel procedure for transdifferentiation of MSCs through the application of electrical stimuli via graphene-based electrodes (Fig. 4) (Das et al. 2017; Uz et al. 2018). Rat MSCs were seeded on a graphene interdigital electrode and subjected to either electrical or chemical

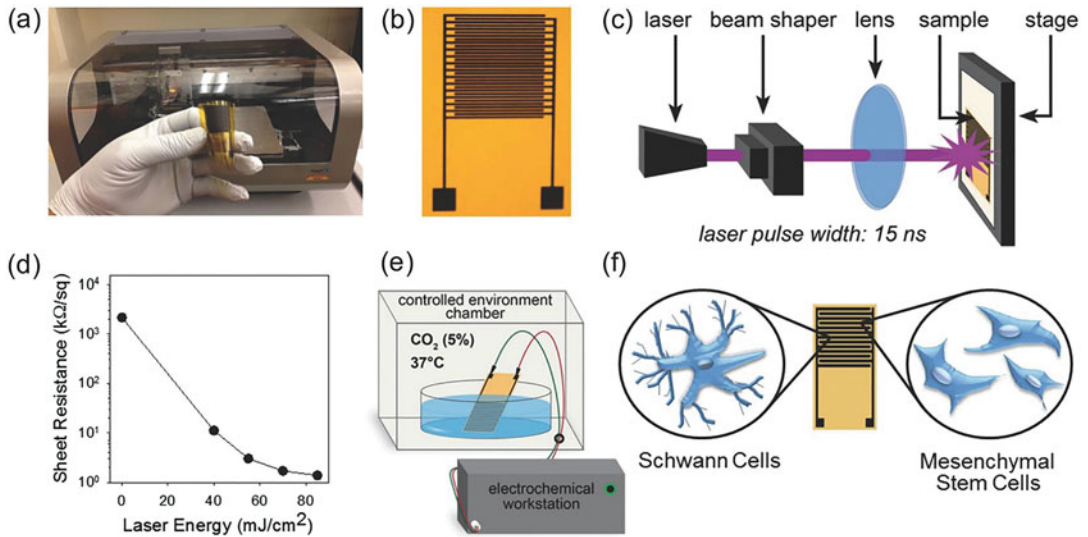


Fig. 4 Fabrication and MSC differentiation protocol on graphene interdigitated electrodes (IDEs). (a) The inkjet printing of the graphene IDE on a flexible and bendable polyimide substrate (Fujifilm Dimatix Materials Printer is shown in the background). (b) An optical image of the graphene IDE circuit with 400 μm finger width, pitch of 250 μm , and printed graphene thickness of 5–7 μm . (c) Schematic diagram of the pulsed-laser processing setup

used for postprint annealing. (d) Electrical sheet resistance measurements of the printed graphene IDE irradiated with distinct laser energies. (e) Schematic diagrams displaying the cell culture medium and application of sole-electrical stimulation to the IDE circuit while (f) displays schematic magnified views of MSCs and postelectrical stimulated differentiated SCs. (Adapted from Das et al. 2017)

transdifferentiation, then expression of cell surface markers such as p75, S100, and S100 β were analyzed with immunocytochemistry after 15 days. The results for electrical tMSCs were compelling, showing the highest degree of preferential immunolabeling, with more than 85% of cells demonstrating labeling for SC markers vs. 75% for chemically transdifferentiated MSCs. Additionally, electrically stimulated cells secreted significantly higher levels of NGF as compared to their chemically transdifferentiated counter-parts. Although not statistically significant, higher levels of BDNF and GDNF were also noted. While other reports have shown that electrical stimuli can increase growth factor level production in SCs (Huang et al. 2010; Koppes et al. 2011, 2014), this paper is the first to describe such effects in transdifferentiated MSCs. Furthermore, Das et al., demonstrated that electrical stimuli alone can transdifferentiate MSCs to a SC-like phenotype without the need for chemical growth

factors, thus saving researchers time, labor, and money, while providing a novel platform for an artificial neural network circuit.

8.6 Beneficial Properties of tMSCs

Once methods of transdifferentiation had been discovered, scientists moved on to *in vivo* studies to determine the effect of tMSCs on models of peripheral nerve injury. After Dezawa et al. performed their *in vitro* cell characterization, tMSCs were transplanted into the cut end of a rat sciatic nerve. Results showed that the transplanted cells remained in a Schwann-cell like state and were capable of forming myelin sheaths, as well as supporting nerve fiber regrowth (Keilhoff and Fansa 2011). Additionally, Dezawa and collaborators also showed that tMSCs co-localized with the myelin-associated glycoprotein antibody signal, suggesting that MSCs may be able to differentiate into

myelinating cells. After this initial trial, many labs followed suite by implanting transdifferentiated MSCs into a variety of peripheral nerve and spinal cord injury models. In 2004, Mimura et al. supported Dezawa's work by showing that human and rodent MSC-derived SCs expressed myelin-related markers and contributed to re-myelination when transplanted into a rat sciatic nerve injury (Mimura et al. 2004). Using a similar transdifferentiation protocol, Keilhoff et al. (2006) also demonstrated that transplanted tMSCs within a muscle conduit promoted remyelination, and electron microscopy showed that single tMSCs were even capable of wrapping more than one axon, similar to an oligodendrocyte (Keilhoff et al. 2006).

In addition to providing functional support, transdifferentiated MSCs are capable of producing trophic factors at even higher levels than SCs. When transdifferentiated MSCs were placed in a DRG co-culture system without direct contact, tMSCs showed upregulation of BDNF and NGF. Additionally, neurite outgrowth was observed even in the presence of NGF and BDNF blocking antibodies, suggesting that other trophic cytokines or factors may be produced by tMSCs (Mahay et al. 2008). Another interesting study used a combination of two different mediums to transdifferentiate MSCs, facilitating production of large amounts of BDNF and GDNF. Interestingly, cells resembled an astrocyte morphology and expressed certain astrocyte markers. When transplanted, the cells improved muscle reinnervation and restored motor function in a rat sciatic nerve crush model (Dadon-Nachum et al. 2011). Combined, these results support the idea that MSCs display functional characteristics similar to SCs by secreting bioactive neurotrophic factors.

Soon after the introduction of tMSC transplants, scientists began to question the duration of a SC-like state once cells were placed in an *in vivo* environment. Shimizu et al. transplanted MSC Schwann-like cells within a transpermeable tube into a rat sciatic nerve gap (Fig. 5) (Shimizu et al. 2007). After 3 weeks, tMSCs continued to express SC markers such as p75, GFAP and increased S100 expression. Most importantly,

the MSCs expressed myelin-associated markers such as MAG and myelin basic protein (MBP) even after 3 weeks *in vivo*, which the authors contend supports the premise that MSCs may retain SC-like characteristics even after transplantation. It is important to note however, that remyelination was not seen via immunohistochemistry or electron microscopy, as in other studies. A different study by Ishikawa et al. 2009 transplanted MSC-derived SCs within chitosan gel sponges and found that cells were able to form myelin sheaths 1 month after transplant (Ishikawa et al. 2009). The mean diameter of myelinated fibers increased continuously, even out to 4 months post-transplant. This study, along with the work by Dezawa et al. 2001, demonstrates that rat tMSCs may contribute to remyelination after transplantation into an injured PNS model. Similar results have been found in spinal cord injury models (Someya et al. 2008; Wakao et al. 2010; Kamada et al. 2011), indicating that MSC-derived SCs are effective for both PNS and CNS regeneration. Thus, MSCs are capable of expressing SC biomarkers, may express myelin markers, and even physically form myelin sheaths. Moreover, these effects may last well past the time that MSCs were last exposed to transdifferentiation media, suggesting that the acquired SC-like state is at least semi-permanent and allows cells to persist well into the acute phase of Wallerian degeneration.

Unfortunately, there have never been clinical trials involving the use of chemically transdifferentiated MSCs for the nervous system. However, a non-human primate study has been completed as an important pre-clinical step. Wakao et al. 2010, used a monkey model and followed subjects out to a year post transplant (Wakao et al. 2010). MSCs were chemically induced to resemble SCs and cell marker expression patterns were confirmed with both immunocytochemistry and reverse transcription-PCR. Cells were transplanted for 1 year in a median nerve transection model. During this year, no major health abnormalities were observed in the monkeys. Immunohistochemistry with Ki-67 revealed no signs of massive proliferation and the ^{18}F -FDG-PET scan, which detects neoplastic cells, demonstrated no abnormalities.

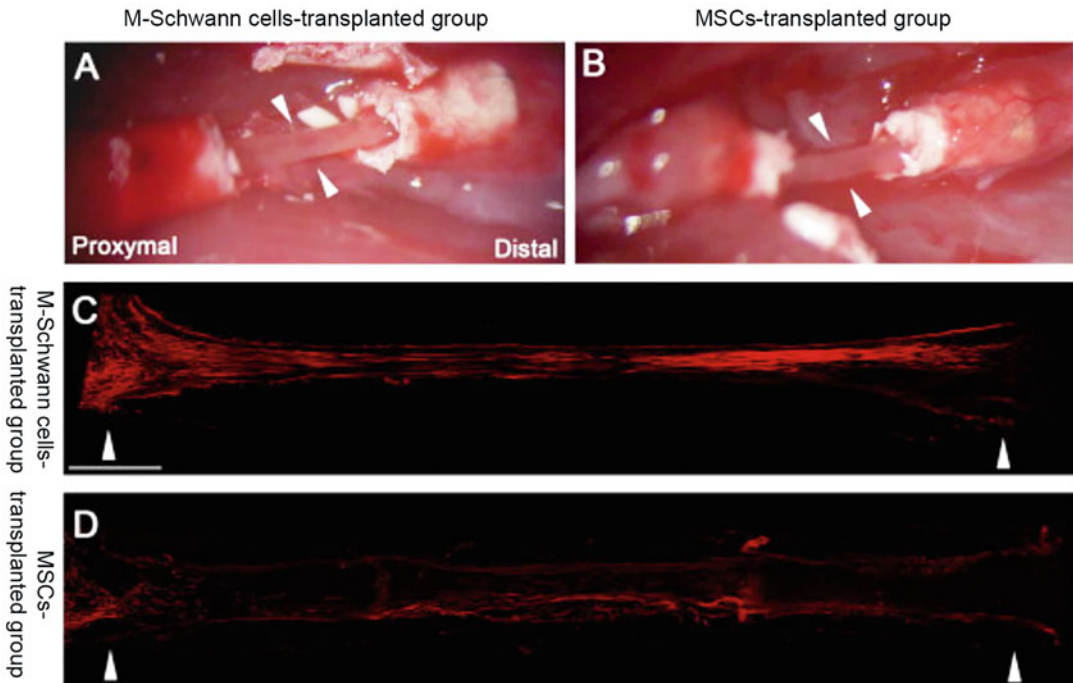


Fig. 5 Transdifferentiated MSCs bridge 10 mm nerve gap. Transected rat sciatic nerve gap bridged with graft containing MSCs transdifferentiated into SCs (M-Schwann cells, (a) and undifferentiated MSCs (b) 3 weeks after transplantation. Newly formed tissue

indicated by arrowheads. Nerve fibers stained with neurofilament (red) for M-Schwann cell (c) and MSC (d) groups. Scale bar = 100 μ m. (Figure adapted from Shimizu et al. 2007)

Furthermore, monkeys regained function, and electrophysiology with histology revealed restoration of the severed nerve. This study is particularly important because it demonstrated not only the efficacy of transdifferentiation, but also the safety of long-term implantation of tMSCs in nonhuman primates.

9 Genetically Modified MSCs

The literature has aptly demonstrated that undifferentiated MSCs can produce neurotrophic factors *in vitro* as well as *in vivo* and that the process of transdifferentiation can even further increase production levels. Only in recent years have researchers begun to investigate the continuous production of these proteins via functional gene insertion. As one of their novel features,

MSCs are suitable for transduction and expression of exogenous genes, making them a good candidate system for genetic engineering. The most widely used systems are now either lentivirus or retrovirus-based. In regards to nervous system disorders, MSC lines have been created to over express a wide variety of neurotrophic factors such as GDNF, NGF, and BDNF (Wyse et al. 2014), as well as other growth factors. Pre-clinical studies by Sharma et al. 2015, demonstrated that MSCs genetically modified for production and secretion of BDNF, GDNF and even a hybrid MSC line hyper-secreting both BDNF and GDNF, had similar viability and proliferation rates when compared to the non-genetically modified original MSCs (Sharma et al. 2015). One 2008 study by Bauer et al., went so far as to develop an in depth biosafety model to specifically assess the risk of retro- and lentiviral

vectors (Bauer et al. 2008). Human hematopoietic stem cells and MSCs were transduced with Moloney murine leukemia virus and transplanted into 481 immunodeficient mice. There was no detectable evidence of insertional mutagenesis leading to human leukemias or solid tumors during the 18 months animals were studied. Additionally, no vector-associated adverse events were observed and in 117 serum samples analyzed, there was no detectable viral DNA. These findings indicate that virally transduced MSCs are stable and may behave biologically like the wild type MSC population, making them suitable for *in vivo* study and use in a variety of disease and injury models. Genetically modified MSCs have been used in studies ranging from the treatment of neurodegenerative disorders, including ischemic injury, retinal degeneration, spinal cord injuries and peripheral nerve transections. Studies in each of these areas will be discussed below.

9.1 Use of Genetically Modified MSCs in Neurodegenerative Disorders

9.1.1 Parkinson's Disease

Use of GDNF was first described in 1993 as a potential treatment for Parkinson's disease because of its ability to increase dopamine uptake and aid in the survival of embryonic midbrain dopaminergic neurons (Lin et al. 1993). With the challenge of administering GDNF infusions, cell-based strategies to deliver GDNF have been receiving attention. In one study, MSCs transduced with a GDNF retrovirus vector increased dopaminergic neuron sprouting (Moloney et al. 2010). A similar study found that injections of GDNF MSCs given 1 week before a lactacystin lesion of the medial forebrain also significantly increased dopamine levels (Wu et al. 2010). Furthermore, Ren et al. (2013) transplanted GDNF MSCs into the brain of non-human primates and saw increased dopamine levels and improved contralateral limb function (Ren et al. 2013). Pre-clinical studies provide evidence that GDNF MSCs produce high levels of a functional trophic factor, which, with further safety and efficacy

data, could be used in clinical trials as adjunct treatment for Parkinson's disease.

9.1.2 Alzheimer's Disease

Treatment options for Alzheimer's disease are limited and focus on symptoms related to neurotransmitter systems, rather than targeting the underlying pathologies. Given the prevalence of the disease and lack of treatments, new strategies are being developed which focus around the use of NGF. Autologous fibroblasts engineered to express NGF were transplanted in eight patients with Alzheimer's. Patients saw an improvement of Mini-Mental Status Examination scores and a reduced decline in cognitive scores (Ren et al. 2013). A phase II clinical trial is still open for this method (Wyse et al. 2014). MSCs have not directly been used in human clinical trials, however, promising work by Li et al. (2008) demonstrated reduced memory deficits in the Morris-water-maze task in a rat model when NGF MSCs were transplanted to the hippocampus (Li et al. 2008). Future studies will likely include further *in vivo* transplantation trials with NGF MSCs in both rodent and non-human primate models, and in human trials.

9.1.3 Huntington's Disease

Compared to the other neurodegenerative diseases discussed, Huntington's disease is unique in that clinical signs may be directly correlated to reduced levels of neurotrophic factor BDNF. Low BDNF levels in the striatum are due to loss of function of the wild-type huntingtin protein. This protein modulates BDNF transcription and plays a role in BDNF transport and secretion (Zuccato et al. 2001). The Dunbar laboratory first demonstrated that murine MSCs engineered to overexpress BDNF improved disease progression on a transgenic mouse model of Huntington's (Dey et al. 2010). Important pre-clinical trials by Pollock et al. 2016 utilized a double-blind study to examine the effects of transplanted human BDNF MSCs on disease progression in a mouse Huntington's disease model (Pollock et al. 2016). Treatment with MSCs decreased striatal atrophy and significantly reduced anxiety. BDNF MSC treatment also

increased the mean lifespan of mice. This study demonstrated the efficacy of BDNF hypersecreting MSCs as a medical therapy for Huntington's disease and set the groundwork for future clinical trials.

9.2 Ischemic Brain Injury

Ischemic brain injury causes the death of various important cell types such as neurons, glial, and endothelial cells. Regain of function and brain tissue repair necessitates cell replacement and formation of a new network (van Velthoven et al. 2009). When transplanted into ischemic regions of the rat brain, MSCs reduced functional deficits after 14 days, scar thickness was decreased, and the number of proliferating cells in the subventricular zone was increased (Chen et al. 2001; Li et al. 2001; Lu et al. 2001). Improvement by MSC treatment has been attributed to decreased apoptosis, MSC differentiation into neuronal cells, and promotion of neurogenesis, angiogenesis, and synaptogenesis (Chen et al. 2003; Iihoshi et al. 2004; Mimura et al. 2004; Mimura et al. 2005; Li et al. 2006). Several groups have used genetically modified stem cells that overexpress growth factors known to enhance neuronal survival including BDNF and GDNF. When BDNF overexpressing MSCs were delivered to an ischemic brain model via injection, infarct volume was reduced and functional outcome was improved (Kurozumi et al. 2004; Nomura et al. 2005; Horita et al. 2006). Furthermore, BDNF expressing MSCs can significantly improve behavioral test results and reduce ischemic damage as indicated via magnetic resonance imaging (MRI) analysis after 7 and 14 days (Kurozumi et al. 2005; Nomura et al. 2005).

9.3 Retinal Degenerative Disease – Glaucoma

Glaucoma is a leading cause of progressive blindness and is estimated to affect over 2 million Americans (Friedman et al. 2004). Glaucoma is

an optic neuropathy resulting in progressive loss of visual function due to loss of retinal ganglion cells (RGC) whose axons project through the optic nerve and terminate in visual centers. To prevent this loss of retinal cells, several research groups have investigated the neuroprotective effects of MSCs which have been genetically modified (Hou et al. 2010; Harper et al. 2011; Park et al. 2012; Machalińska et al. 2013) or chemically stimulated (Levkovitch-Verbin et al. 2010) to augment secretion of neurotrophic factors as a strategy for retinal neuroprotection. In these studies, modified MSCs were successfully transplanted whether intravitreally or subretinally, though subretinal transplant appears to yield greater neurotrophic factor mRNA and protein levels in the rat retina (Park et al. 2012).

Harper et al found that intravitreal transplant of BDNF-expressing MSCs preserved RGCs to a greater degree than unmodified MSCs and allowed for greater protection of retina and optic nerve function in a rat glaucoma model (Harper et al. 2011). Rat and human MSCs chemically stimulated to secrete BDNF and GDNF were also neuroprotective after intravitreal transplant in rats with optic nerve transection (Levkovitch-Verbin et al. 2010). Hou et al. used MSCs genetically modified to secrete an anti-angiogenic factor called pigment epithelial-derived factor (PEDF) as a strategy to inhibit choroidal neovascularization (CNV) – the underlying cause of wet AMD. The results indicate a recruitment of MSCs to sites of CNV, a reduction in the CNV proliferation and an increase in retinal pigment epithelial cells that protect photoreceptor cells (Hou et al. 2010). These studies provide a promising basis to the use of modified MSCs as a therapy for retinal degenerative diseases such as glaucoma and AMD via neuroprotection of cells vulnerable to these diseases.

9.4 Spinal Cord Injuries

In addition to various therapies within the brain and retina, modified MSCs have been used with variable success in the spinal cord. In a 2005 study by Lu, Jones, and Tuszynski, BDNF

MSCs were injected into a crushed rat spinal cord injury and the extent and diversity of axonal growth was increased (Lu et al. 2005). Additionally, SCs preferentially migrated to the BDNF secreting grafts. Unfortunately, functional recovery was not achieved for any of the studied rats. Another study was performed by Sasaki et al. 2009, in which BDNF secreting human MSCs were implanted at a T9 spinal cord lesion (Sasaki et al. 2009). After 5 weeks, locomotor improvement was observed for the BDNF group and there was increased axonal sprouting. Specifically, an increased number of serotonergic fibers were observed in the ventral horn grey matter, an area important for motor controlled movement. Unlike the 2005 Lu study, Sasaki's group demonstrated that BDNF MSCs are associated with improved functional outcome following a spinal cord injury. Due to the conflicting data reports, additional studies are necessary before the full benefits of BDNF delivery via engineered MSCs can be determined for the treatment of spinal cord damage.

9.5 Peripheral Nerve Injury

Of all the disease models discussed so far, peripheral nerve injuries have the fewest published studies involving transplantation of genetically modified MSCs. This may be because researchers are now utilizing a multi-disciplinary approach and studies often involve the use of engineered conduits, cell transplants, and now even gene therapy. One of the first studies to use a MSC gene delivery system transplanted MSC spheroids transfected with the BDNF gene (Tseng and Hsu 2014). Spheroids were combined with a polymeric nerve conduit to bridge a 10 mm rat sciatic nerve transection gap. MRI was used to track the transplanted cells. Animals receiving the BDNF MSC spheroids had the shortest gap bridging time, the largest regenerated nerve, and the thickest myelin sheath at 31 days. Furthermore, BDNF MSC spheroids significantly promoted functional recovery. A more recent study (Gao et al. 2016) combined multi-channel agarose scaffolds with BDNF MSCs to bridge a 15 mm

sciatic nerve transection gap. Additionally, the distal sciatic nerve segment was injected with a BDNF lentiviral vector. Twelve weeks after injury, BDNF secreting cells significantly increased axonal regeneration and injection of the lentiviral vector at the distal segment enhanced axonal regeneration beyond the lesion. A recent study investigated the efficacy of BDNF *ex vivo* gene transfer to umbilical cord blood-derived MSCs in a rat sciatic nerve crush injury model (Hei et al. 2017). Four weeks post-surgery, the BDNF expressing MSCs exhibited more peripheral nerve regeneration than the controls. Additionally, sciatic function index, axon counts, and axon density were significantly increased for both the BDNF MSC and regular MSC groups. The results from these studies are promising and indicate that BDNF hypersecreting MSCs in particular can improve sciatic nerve regeneration. Unlike other areas of research, no pre-clinical characterization studies looking at safety and appropriate dosage ranges have been published. This would be a necessary step before testing BDNF MSCs outside of a rat model.

10 Conclusions and Future Directions

Peripheral nerve injury limits mobility and sensation in up to 2.8% of all trauma patients and often results in unsatisfactory return to function (Noble et al. 1998). Although the gold standard of microsurgery with autograft has seen advances in the last decade, there are significant drawbacks associated with the procedure. For this reason, scientists have proposed the use of an alternative transplant type, in the form of autologous stem cells. Specifically, research is directed at the conversion of mesenchymal stem cells towards a SC-like fate to aid in Wallerian degeneration, neuronal regeneration, and possibly even remyelination. Additionally, MSCs have their own unique benefits such as immunomodulatory properties, secretion of neurotrophic factors, possible mitochondrial transfer, and the ability to be easily genetically modified. In order to resemble a SC, MSCs must undergo transdifferentiation

which can be achieved through a variety of methods including incorporating specific factors into the growth media, co-culture method, *in vivo* transdifferentiation, and others. Although these older techniques have their benefits, methods of transdifferentiation have changed drastically within the last 10 years and now include master gene modification as well as the use of specific cell signaling molecules combined with histone deacetylase inhibitors.

As demonstrated by the more recent body of literature, scientists are beginning to investigate other somatic cell types in addition to bone marrow MSCs including but not limited to fibroblasts, adipocytes, and even hair follicle stem cells (Amoh et al. 2005; Kingham et al. 2007; Thoma et al. 2014). These studies rely largely on immunocytochemical staining, co-culture neurite outgrowth, and gene expression patterns to support transdifferentiation of cells into SCs. Unfortunately, none of these studies have measured growth factor secretion levels from transdifferentiated cells, and only Thoma's study looked at the ability of these cells to create myelin. In order to truly assess whether these transdifferentiated cells are SCs, future work should test growth factor secretion, perform patch-clamp recordings, transplant cells into rat sciatic nerve models, and examine myelin formation via electron microscopy.

In addition to testing new cell types, researchers are trying new methods of transdifferentiation and emphasizing the use of genetic control and epigenetic cues. Future research may focus on SC de-differentiation or multi-step transdifferentiation in which a less-differentiated intermediate is first created, and then the fully transdifferentiated cell type is achieved, such as in Thoma et al.'s work with fibroblasts. While these cell fate reprogramming methods are promising, they can often be time consuming, difficult to consistently reproduce, and cost prohibitive. Additionally, rigorous studies have yet to be performed which examine the tumorigenic capacity of these cells and their long term genetic stability. While the field of transdifferentiation still has many challenges to overcome, it is a promising focus in the study of regenerative

medicine and offers new insight into cell fate plasticity.

Specifically, in regards to the peripheral nervous system, researchers have shown that a variety of regenerative cell types may act like SCs by secreting trophic factors, supporting re-myelination, and decreasing time to functional return of severed nerves. When transdifferentiated cells are combined with multiple neuro-regenerative strategies such as *ex vivo* gene delivery, and biomaterial conduits, they may become powerful alternatives to traditional peripheral nerve regeneration therapies.

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Conflict of Interest The authors declare no conflict of interest.

References

- Acquistapace A, Bru T, Lesault PF, Figeac F, Coudert AE, le Coz O, Christov C, Baudin X, Auber F, Yiou R, Dubois-Rande JL, Rodriguez AM (2011) Human mesenchymal stem cells reprogram adult cardiomyocytes toward a progenitor-like state through partial cell fusion and mitochondria transfer. *Stem Cells* 29 (5):812–824
- Aggarwal S, Pittenger MF (2005) Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 105(4):1815–1822
- Amoh Y, Li L, Campillo R, Kawahara K, Katsuoka K, Penman S, Hoffman RM (2005) Implanted hair follicle stem cells form Schwann cells that support repair of severed peripheral nerves. *Proc Natl Acad Sci U S A* 102(49):17734–17738
- Anderson KD, Guest JD, Dietrich WD, Bunge MB, Curiel R, Dididze M, Green BA, Khan A, Pearse DD, Saraf-Lavi E (2017) Safety of autologous human schwann cell transplantation in subacute thoracic spinal cord injury. *J Neurotrauma* 34:2950–2963
- Bathina S, Das UN (2015) Brain-derived neurotrophic factor and its clinical implications. *Arch Med Sci* 11 (6):1164–1178
- Bauer G, Dao MA, Case SS, Meyerrose T, Wirthlin L, Zhou P, Wang X, Herrbrich P, Arevalo J, Csik S, Skelton DC, Walker J, Pepper K, Kohn DB, Nolte JA (2008) In vivo biosafety model to assess the risk of adverse events from retroviral and lentiviral vectors. *Mol Ther* 16(7):1308–1315
- Bjorklund LM, Sanchez-Pernaute R, Chung S, Andersson T, Chen IY, McNaught KS, Brownell AL,

- Jenkins BG, Wahlestedt C, Kim KS, Isacson O (2002) Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model. *Proc Natl Acad Sci U S A* 99(4):2344–2349
- Bonni A, Brunet A, West AE, Datta SR, Takasu MA, Greenberg ME (1999) Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. *Science* 286(5443):1358–1362
- Boyd JG, Gordon T (2002) A dose-dependent facilitation and inhibition of peripheral nerve regeneration by brain-derived neurotrophic factor. *Eur J Neurosci* 15(4):613–626
- Boyd JG, Gordon T (2003) Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. *Mol Neurobiol* 27(3):277–324
- Braga-Silva J, Gehlen D, Padoin A, Machado D, Garicochea B, Costa da Costa J (2008) Can local supply of bone marrow mononuclear cells improve the outcome from late tubular repair of human median and ulnar nerves? *J Hand Surg Eur Vol* 33(4):488–493
- Bredesen DE, Rabizadeh S (1997) p75NTR and apoptosis: Trk-dependent and Trk-independent effects. *Trends Neurosci* 20(7):287–290
- Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME (1999) Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* 96(6):857–868
- Bunge MB, Monje PV, Khan A, Wood PM (2017) From transplanting Schwann cells in experimental rat spinal cord injury to their transplantation into human injured spinal cord in clinical trials. *Prog Brain Res* 231:107–133
- Burnett MG, Zager EL (2004) Pathophysiology of peripheral nerve injury: a brief review. *Neurosurg Focus* 16(5):E1
- Campbell WW (2008) Evaluation and management of peripheral nerve injury. *Clin Neurophysiol* 119(9):1951–1965
- Chan JR, Cosgaya JM, Wu YJ, Shooter EM (2001) Neurotrophins are key mediators of the myelination program in the peripheral nervous system. *Proc Natl Acad Sci U S A* 98(25):14661–14668
- Chao MV, Bothwell MA, Ross AH, Koprowski H, Lanahan AA, Buck CR, Sehgal A (1986) Gene transfer and molecular cloning of the human NGF receptor. *Science* 232(4749):518–521
- Chen J, Li Y, Wang L, Lu M, Zhang X, Chopp M (2001) Therapeutic benefit of intracerebral transplantation of bone marrow stromal cells after cerebral ischemia in rats. *J Neurol Sci* 189(1-2):49–57
- Chen J, Zhang ZG, Li Y, Wang L, Xu YX, Gautam SC, Lu M, Zhu Z, Chopp M (2003) Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats. *Circ Res* 92(6):692–699
- Chen CJ, Ou YC, Liao SL, Chen WY, Chen SY, Wu CW, Wang CC, Wang WY, Huang YS, Hsu SH (2007) Transplantation of bone marrow stromal cells for peripheral nerve repair. *Exp Neurol* 204(1):443–453
- Chiu DT (1999) Autogenous venous nerve conduits. A review. *Hand Clin* 15(4):667–671 ix
- Chiu DT, Strauch B (1990) A prospective clinical evaluation of autogenous vein grafts used as a nerve conduit for distal sensory nerve defects of 3 cm or less. *Plast Reconstr Surg* 86(5):928–934
- Cuevas P, Carceller F, Dujovny M, Garcia-Gomez I, Cuevas B, Gonzalez-Corrochano R, Diaz-Gonzalez D, Reimers D (2002) Peripheral nerve regeneration by bone marrow stromal cells. *Neurol Res* 24(7):634–638
- Dadon-Nachum M, Sadan O, Srugo I, Melamed E, Offen D (2011) Differentiated mesenchymal stem cells for sciatic nerve injury. *Stem Cell Rev* 7(3):664–671
- Das SR, Uz M, Ding S, Lentner MT, Hondred JA, Cargill AA, Sakaguchi DS, Mallapragada S, Claussen JC (2017) Electrical differentiation of mesenchymal stem cells into Schwann-cell-like phenotypes using inkjet-printed graphene circuits. *Adv Healthc Mater* 6(7)
- De la Rosa MB, Sharma AD, Mallapragada SK, Sakaguchi DS (2017) Transdifferentiation of brain-derived neurotrophic factor (BDNF)-secreting mesenchymal stem cells significantly enhance BDNF secretion and Schwann cell marker proteins. *J Biosci Bioeng* 124(5):572–582
- Dey ND, Bombard MC, Roland BP, Davidson S, Lu M, Rossignol J, Sandstrom MI, Skeel RL, Lescaudron L, Dunbar GL (2010) Genetically engineered mesenchymal stem cells reduce behavioral deficits in the YAC 128 mouse model of Huntington's disease. *Behav Brain Res* 214(2):193–200
- Dezawa M, Takahashi I, Esaki M, Takano M, Sawada H (2001) Sciatic nerve regeneration in rats induced by transplantation of in vitro differentiated bone-marrow stromal cells. *Eur J Neurosci* 14(11):1771–1776
- Di Nicola M, Carlo-Stella C, Magni M, Milanese M, Longoni PD, Matteucci P, Grisanti S, Gianni AM (2002) Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or non-specific mitogenic stimuli. *Blood* 99(10):3838–3843
- Dodd J, Jessell TM (1988) Axon guidance and the patterning of neuronal projections in vertebrates. *Science* 242(4879):692–699
- Drake DB (1996) Nerve injuries: operative results for major nerve injuries, entrapments and tumors. *Plast Reconstr Surg* 98(4):749
- Eriksson NP, Lindsay RM, Aldskogius H (1994) BDNF and NT-3 rescue sensory but not motoneurons following axotomy in the neonate. *Neuroreport* 5(12):1445–1448
- Ernfors P, Rosario CM, Merlio JP, Grant G, Aldskogius H, Persson H (1993) Expression of mRNAs for neurotrophin receptors in the dorsal root ganglion and spinal cord during development and following peripheral or central axotomy. *Brain Res Mol Brain Res* 17(3-4):217–226

- Fields RD, Ellisman MH (1986) Axons regenerated through silicone tube splices. I. Conduction properties. *Exp Neurol* 92(1):48–60
- Friedman DS, Wolfs R, O'Colmain BJ, Klein BE, Taylor HR, West S, Leske MC, Mitchell P, Congdon N, Kempen J (2004) Prevalence of open-angle glaucoma among adults in the United States. *Arch Ophthalmol* 122:532–538
- Fu SY, Gordon T (1997) The cellular and molecular basis of peripheral nerve regeneration. *Mol Neurobiol* 14 (1-2):67–116
- Funakoshi H, Frisen J, Barbany G, Timmusk T, Zachrisson O, Verge VM, Persson H (1993) Differential expression of mRNAs for neurotrophins and their receptors after axotomy of the sciatic nerve. *J Cell Biol* 123(2):455–465
- Gao M, Lu P, Lynam D, Bednark B, Campana WM, Sakamoto J, Tuszynski M (2016) BDNF gene delivery within and beyond templated agarose multi-channel guidance scaffolds enhances peripheral nerve regeneration. *J Neural Eng* 13(6):066011
- Gao F, Chiu S, Motan D, Zhang Z, Chen L, Ji H, Tse H, Fu Q-L, Lian Q (2017) Mesenchymal stem cells and immunomodulation: current status and future prospects. *Cell Death Dis* 7(1):e2062
- Gargano N, Levi A, Alema S (1997) Modulation of nerve growth factor internalization by direct interaction between p75 and TrkA receptors. *J Neurosci Res* 50 (1):1–12
- Gaudet AD, Popovich PG, Ramer MS (2011) Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury. *J Neuroinflammation* 8:110
- Geuna S, Raimondo S, Ronchi G, Di Scipio F, Tos P, Czaja K, Fornaro M (2009) Chapter 3: histology of the peripheral nerve and changes occurring during nerve regeneration. *Int Rev Neurobiol* 87:27–46
- Geuna S, Gnani S, Perroteau I, Tos P, Battiston B (2013) Tissue engineering and peripheral nerve reconstruction: an overview. *Int Rev Neurobiol* 108:35–57
- Gordon T (2009) The role of neurotrophic factors in nerve regeneration. *Neurosurg Focus* 26(2):E3
- Goto E, Mukozawa M, Mori H, Hara M (2010) A rolled sheet of collagen gel with cultured Schwann cells: model of nerve conduit to enhance neurite growth. *J Biosci Bioeng* 109(5):512–518
- Gu Y, Wang J, Ding F, Hu N, Wang Y, Gu X (2010) Neurotrophic actions of bone marrow stromal cells on primary culture of dorsal root ganglion tissues and neurons. *J Mol Neurosci* 40(3):332–341
- Guertin AD, Zhang DP, Mak KS, Alberta JA, Kim HA (2005) Microanatomy of axon/glia signaling during Wallerian degeneration. *J Neurosci* 25(13):3478–3487
- Gundersen RW, Barrett JN (1980) Characterization of the turning response of dorsal root neurites toward nerve growth factor. *J Cell Biol* 87(3 Pt 1):546–554
- Hadlock T, Sundback C, Hunter D, Cheney M, Vacanti JP (2000) A polymer foam conduit seeded with Schwann cells promotes guided peripheral nerve regeneration. *Tissue Eng* 6(2):119–127
- Hall S (2001) Nerve repair: a neurobiologist's view. *J Hand Surg Br* 26(2):129–136
- Harper MM, Grozdanic SD, Blits B, Kuehn MH, Zamzow D, Buss JE, Kardon RH, Sakaguchi DS (2011) Transplantation of BDNF-secreting mesenchymal stem cells provides neuroprotection in chronically hypertensive rat eyes. *Invest Ophthalmol Vis Sci* 52 (7):4506–4515
- Harrop JS, Hashimoto R, Norvell D, Raich A, Aarabi B, Grossman RG, Guest JD, Tator CH, Chapman J, Fehlings MG (2012) Evaluation of clinical experience using cell-based therapies in patients with spinal cord injury: a systematic review. *J Neurosurg Spine* 17 (1 Suppl):230–246
- Hei WH, Almansoori AA, Sung MA, Ju KW, Seo N, Lee SH, Kim BJ, Kim SM, Jahng JW, He H, Lee JH (2017) Adenovirus vector-mediated ex vivo gene transfer of brain-derived neurotrophic factor (BDNF) to human umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs) promotes crush-injured rat sciatic nerve regeneration. *Neurosci Lett* 643:111–120
- Hirasawa Y, Sakakida K (1983) Sports and peripheral nerve injury. *Am J Sports Med* 11(6):420–426
- Horita Y, Honmou O, Harada K, Houkin K, Hamada H, Kocsis JD (2006) Intravenous administration of glial cell line-derived neurotrophic factor gene-modified human mesenchymal stem cells protects against injury in a cerebral ischemia model in the adult rat. *J Neurosci Res* 84(7):1495–1504
- Hou H-Y, Liang H-L, Wang Y-S, Zhang Z-X, Wang B-R, Shi Y-Y, Dong X, Cai Y (2010) A therapeutic strategy for choroidal neovascularization based on recruitment of mesenchymal stem cells to the sites of lesions. *Mol Ther* 18(10):1837–1845
- Hu N, Wu H, Xue C, Gong Y, Wu J, Xiao Z, Yang Y, Ding F, Gu X (2013) Long-term outcome of the repair of 50 mm long median nerve defects in rhesus monkeys with marrow mesenchymal stem cells-containing, chitosan-based tissue engineered nerve grafts. *Biomaterials* 34(1):100–111
- Huang JK, Phillips GR, Roth AD, Pedraza L, Shan W, Belkaid W, Mi S, Fex-Svenningsen A, Florens L, Yates JR 3rd, Colman DR (2005) Glial membranes at the node of Ranvier prevent neurite outgrowth. *Science* 310(5755):1813–1817
- Huang J, Ye Z, Hu X, Lu L, Luo Z (2010) Electrical stimulation induces calcium-dependent release of NGF from cultured Schwann cells. *Glia* 58(5):622–631
- Iihoshi S, Honmou O, Houkin K, Hashi K, Kocsis JD (2004) A therapeutic window for intravenous administration of autologous bone marrow after cerebral ischemia in adult rats. *Brain Res* 1007(1-2):1–9
- Ishikawa N, Suzuki Y, Dezawa M, Kataoka K, Ohta M, Cho H, Ide C (2009) Peripheral nerve regeneration by transplantation of BMSC-derived Schwann cells as chitosan gel sponge scaffolds. *J Biomed Mater Res A* 89(4):1118–1124

- Islam MN, Das SR, Emin MT, Wei M, Sun L, Westphalen K, Rowlands DJ, Quadri SK, Bhattacharya S, Bhattacharya J (2012) Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nat Med* 18(5):759–765
- Jaquet JB, Luijsterburg AJ, Kalmijn S, Kuypers PD, Hofman A, Hovius SE (2001) Median, ulnar, and combined median-ulnar nerve injuries: functional outcome and return to productivity. *J Trauma* 51(4):687–692
- Jiang XX, Zhang Y, Liu B, Zhang SX, Wu Y, Yu XD, Mao N (2005) Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. *Blood* 105(10):4120–4126
- Kamada T, Koda M, Dezawa M, Anahara R, Toyama Y, Yoshinaga K, Hashimoto M, Koshizuka S, Nishio Y, Mannoji C, Okawa A, Yamazaki M (2011) Transplantation of human bone marrow stromal cell-derived Schwann cells reduces cystic cavity and promotes functional recovery after contusion injury of adult rat spinal cord. *Neuropathology* 31(1):48–58
- Karanth S, Yang G, Yeh J, Richardson PM (2006) Nature of signals that initiate the immune response during Wallerian degeneration of peripheral nerves. *Exp Neurol* 202(1):161–166
- Keilhoff G, Fansa H (2011) Mesenchymal stem cells for peripheral nerve regeneration—a real hope or just an empty promise? *Exp Neurol* 232(2):110–113
- Keilhoff G, Goihl A, Langnase K, Fansa H, Wolf G (2006) Transdifferentiation of mesenchymal stem cells into Schwann cell-like myelinating cells. *Eur J Cell Biol* 85(1):11–24
- Kerrebijn JD, Freeman JL (1998) Facial nerve reconstruction: outcome and failures. *J Otolaryngol* 27(4):183–186
- Kim BJ, Seo JH, Bubien JK, Oh YS (2002) Differentiation of adult bone marrow stem cells into neuroprogenitor cells in vitro. *Neuroreport* 13(9):1185–1188
- Kingham PJ, Kalbermatten DF, Mahay D, Armstrong SJ, Wiberg M, Terenghi G (2007) Adipose-derived stem cells differentiate into a Schwann cell phenotype and promote neurite outgrowth in vitro. *Exp Neurol* 207(2):267–274
- Kishino A, Ishige Y, Tatsuno T, Nakayama C, Noguchi H (1997) BDNF prevents and reverses adult rat motor neuron degeneration and induces axonal outgrowth. *Exp Neurol* 144(2):273–286
- Klimaschewski L, Hausott B, Angelov DN (2013) The pros and cons of growth factors and cytokines in peripheral axon regeneration. *Int Rev Neurobiol* 108:137–171
- Kobayashi NR, Bedard AM, Hincke MT, Tetzlaff W (1996) Increased expression of BDNF and *trkB* mRNA in rat facial motoneurons after axotomy. *Eur J Neurosci* 8(5):1018–1029
- Koppes AN, Seggio AM, Thompson DM (2011) Neurite outgrowth is significantly increased by the simultaneous presentation of Schwann cells and moderate exogenous electric fields. *J Neural Eng* 8(4):046023
- Koppes AN, Nordberg AL, Paolillo GM, Goodsell NM, Darwish HA, Zhang L, Thompson DM (2014) Electrical stimulation of schwann cells promotes sustained increases in neurite outgrowth. *Tissue Eng Part A* 20(3-4):494–506
- Kouyoumdjian JA (2006) Peripheral nerve injuries: a retrospective survey of 456 cases. *Muscle Nerve* 34(6):785–788
- Kreutzberg GW (1995) Reaction of the neuronal cell body to axonal damage. In: Waxman SG, Kocsis JD, Stys PK (eds) *The axon: structure, function and pathophysiology*. Oxford University Press, Oxford, pp 355–374
- Kurozumi K, Nakamura K, Tamiya T, Kawano Y, Kobune M, Hirai S, Uchida H, Sasaki K, Ito Y, Kato K, Honmou O, Houkin K, Date I, Hamada H (2004) BDNF gene-modified mesenchymal stem cells promote functional recovery and reduce infarct size in the rat middle cerebral artery occlusion model. *Mol Ther* 9(2):189–197
- Kurozumi K, Nakamura K, Tamiya T, Kawano Y, Ishii K, Kobune M, Hirai S, Uchida H, Sasaki K, Ito Y, Kato K, Honmou O, Houkin K, Date I, Hamada H (2005) Mesenchymal stem cells that produce neurotrophic factors reduce ischemic damage in the rat middle cerebral artery occlusion model. *Mol Ther* 11(1):96–104
- Latif MJ, Afithinos JN, Connery CP, Perin N, Bhora FY, Chwajol M, Todd GJ, Belsley SJ (2008) Robotic intercostal nerve graft for reversal of thoracic sympathectomy: a large animal feasibility model. *Int J Med Robot* 4(3):258–262
- Lee H, Jo EK, Choi SY, Oh SB, Park K, Kim JS, Lee SJ (2006) Necrotic neuronal cells induce inflammatory Schwann cell activation via TLR2 and TLR3: implication in Wallerian degeneration. *Biochem Biophys Res Commun* 350(3):742–747
- Levkovitch-Verbin H, Sadan O, Vander S, Rosner M, Barhum Y, Melamed E, Offen D, Melamed S (2010) Intravitreal injections of neurotrophic factors secreting mesenchymal stem cells are neuroprotective in rat eyes following optic nerve transection. *Invest Ophthalmol Vis Sci* 51(12):6394–6400
- Lewin GR, Barde YA (1996) Physiology of the neurotrophins. *Annu Rev Neurosci* 19:289–317
- Lewin SL, Utley DS, Cheng ET, Verity AN, Terris DJ (1997) Simultaneous treatment with BDNF and CNTF after peripheral nerve transection and repair enhances rate of functional recovery compared with BDNF treatment alone. *Laryngoscope* 107(7):992–999
- Lewis EB (1992) The 1991 Albert Lasker Medical Awards. Clusters of master control genes regulate the development of higher organisms. *JAMA* 267(11):1524–1531
- Li Y, Chen J, Wang L, Lu M, Chopp M (2001) Treatment of stroke in rat with intracarotid administration of marrow stromal cells. *Neurology* 56(12):1666–1672

- Li Y, McIntosh K, Chen J, Zhang C, Gao Q, Borneman J, Raginski K, Mitchell J, Shen L, Zhang J, Lu D, Chopp M (2006) Allogeneic bone marrow stromal cells promote glial-axonal remodeling without immunologic sensitization after stroke in rats. *Exp Neurol* 198 (2):313–325
- Li LY, Li JT, Wu QY, Li J, Feng ZT, Liu S, Wang TH (2008) Transplantation of NGF-gene-modified bone marrow stromal cells into a rat model of Alzheimer' disease. *J Mol Neurosci* 34(2):157–163
- Li X-Y, Zheng Z-H, Li X-Y, Guo J, Zhang Y, Li H, Wang Y-W, Ren J, Wu Z-B (2013) Treatment of foot disease in patients with type 2 diabetes mellitus using human umbilical cord blood mesenchymal stem cells: response and correction of immunological anomalies. *Curr Pharm Des* 19(27):4893–4899
- Li X, Zhang Y, Yeung SC, Liang Y, Liang X, Ding Y, Ip MS, Tse HF, Mak JC, Lian Q (2014) Mitochondrial transfer of induced pluripotent stem cell-derived mesenchymal stem cells to airway epithelial cells attenuates cigarette smoke-induced damage. *Am J Respir Cell Mol Biol* 51(3):455–465
- Lieberman AR (1971) The axon reaction: a review of the principal features of perikaryal responses to axon injury. *Int Rev Neurobiol* 14:49–124
- Lin CH, Forscher P (1993) Cytoskeletal remodeling during growth cone-target interactions. *J Cell Biol* 121 (6):1369–1383
- Lin LF, Doherty DH, Lile JD, Bektesh S, Collins F (1993) GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science* 260 (5111):1130–1132
- López-León M, Outeiro TF, Goya RG (2017) Cell reprogramming: therapeutic potential and the promise of rejuvenation for the aging brain. *Ageing Res Rev* 40:168–181
- Lu D, Mahmood A, Wang L, Li Y, Lu M, Chopp M (2001) Adult bone marrow stromal cells administered intravenously to rats after traumatic brain injury migrate into brain and improve neurological outcome. *Neuroreport* 12(3):559–563
- Lu P, Jones LL, Tuszynski MH (2005) BDNF-expressing marrow stromal cells support extensive axonal growth at sites of spinal cord injury. *Exp Neurol* 191 (2):344–360
- Lundborg G (2000) A 25-year perspective of peripheral nerve surgery: evolving neuroscientific concepts and clinical significance. *J Hand Surg* 25(3):391–414
- Machalińska A, Kawa M, Pius-Sadowska E, Stępniewski J, Nowak W, Rogińska D, Kaczyńska K, Baumert B, Wiszniewska B, Józkowicz A (2013) Long-Term Neuroprotective Effects of NT-4–Engineered Mesenchymal Stem Cells Injected Intravitreally in a Mouse Model of Acute Retinal Injury. *Invest Ophthalmol Vis Sci* 54(13):8292–8305
- Mahay D, Terenghi G, Shawcross SG (2008) Schwann cell mediated trophic effects by differentiated mesenchymal stem cells. *Exp Cell Res* 314(14):2692–2701
- Maricevic A, Erceg M (1997) War injuries to the extremities. *Mil Med* 162(12):808–811
- Mazzoni A, Bronte V, Visintin A, Spitzer JH, Apolloni E, Serafini P, Zanollo P, Segal DM (2002) Myeloid suppressor lines inhibit T cell responses by an NO-dependent mechanism. *J Immunol* 168 (2):689–695
- McMahon SB, Armanini MP, Ling LH, Phillips HS (1994) Expression and coexpression of Trk receptors in subpopulations of adult primary sensory neurons projecting to identified peripheral targets. *Neuron* 12 (5):1161–1171
- Meek MF, Coert JH (2002) Clinical use of nerve conduits in peripheral-nerve repair: review of the literature. *J Reconstr Microsurg* 18(2):97–109
- Millesi H (1981) Interfascicular nerve grafting. *Orthop Clin North Am* 12(2): 287–301
- Mimura T, Dezawa M, Kanno H, Sawada H, Yamamoto I (2004) Peripheral nerve regeneration by transplantation of bone marrow stromal cell-derived Schwann cells in adult rats. *J Neurosurg* 101(5):806–812
- Mimura T, Dezawa M, Kanno H, Yamamoto I (2005) Behavioral and histological evaluation of a focal cerebral infarction rat model transplanted with neurons induced from bone marrow stromal cells. *J Neuropathol Exp Neurol* 64(12):1108–1117
- Moloney TC, Rooney GE, Barry FP, Howard L, Dowd E (2010) Potential of rat bone marrow-derived mesenchymal stem cells as vehicles for delivery of neurotrophins to the Parkinsonian rat brain. *Brain Res* 1359:33–43
- Moreno-Flores MT, Bradbury EJ, Martin-Bermejo MJ, Agudo M, Lim F, Pastrana E, Avila J, Diaz-Nido J, McMahon SB, Wandosell F (2006) A clonal cell line from immortalized olfactory ensheathing glia promotes functional recovery in the injured spinal cord. *Mol Ther* 13(3):598–608
- Mosahebi A, Woodward B, Wiberg M, Martin R, Terenghi G (2001) Retroviral labeling of Schwann cells: in vitro characterization and in vivo transplantation to improve peripheral nerve regeneration. *Glia* 34 (1):8–17
- Muraglia A, Cancedda R, Quarto R (2000) Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model. *J Cell Sci* 113. (Pt 7:1161–1166
- Nakajima H, Uchida K, Guerrero AR, Watanabe S, Sugita D, Takeura N, Yoshida A, Long G, Wright KT, Johnson WE, Baba H (2012) Transplantation of mesenchymal stem cells promotes an alternative pathway of macrophage activation and functional recovery after spinal cord injury. *J Neurotrauma* 29 (8):1614–1625
- Nectoux E, Taleb C, Liverneaux P (2009) Nerve repair in telemicrosurgery: an experimental study. *J Reconstr Microsurg* 25(4):261–265
- Ni WF, Yin LH, Lu J, Xu HZ, Chi YL, Wu JB, Zhang N (2010) In vitro neural differentiation of bone marrow

- stromal cells induced by cocultured olfactory ensheathing cells. *Neurosci Lett* 475(2):99–103
- Nizzardo M, Simone C, Falcone M, Riboldi G, Comi GP, Bresolin N, Corti S (2013) Direct reprogramming of adult somatic cells into other lineages: past evidence and future perspectives. *Cell Transplant* 22(6):921–944
- Noble J, Munro CA, Prasad VS, Midha R (1998) Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries. *J Trauma* 45(1):116–122
- Nomura T, Honmou O, Harada K, Houkin K, Hamada H, Kocsis JD (2005) I.V. infusion of brain-derived neurotrophic factor gene-modified human mesenchymal stem cells protects against injury in a cerebral ischemia model in adult rat. *Neuroscience* 136(1):161–169
- Novikov L, Novikova L, Kellerth JO (1995) Brain-derived neurotrophic factor promotes survival and blocks nitric oxide synthase expression in adult rat spinal motoneurons after ventral root avulsion. *Neurosci Lett* 200(1):45–48
- Oliveira JT, Almeida FM, Biancalana A, Baptista AF, Tomaz MA, Melo PA, Martinez AM (2010) Mesenchymal stem cells in a polycaprolactone conduit enhance median-nerve regeneration, prevent decrease of creatine phosphokinase levels in muscle, and improve functional recovery in mice. *Neuroscience* 170(4):1295–1303
- Oliveira JT, Mostacada K, de Lima S, Martinez AM (2013) Bone marrow mesenchymal stem cell transplantation for improving nerve regeneration. *Int Rev Neurobiol* 108:59–77
- Oppenheim RW (1991) Cell death during development of the nervous system. *Annu Rev Neurosci* 14:453–501
- Oppenheim RW, Yin QW, Prevette D, Yan Q (1992) Brain-derived neurotrophic factor rescues developing avian motoneurons from cell death. *Nature* 360(6406):755–757
- Pan HC, Cheng FC, Chen CJ, Lai SZ, Lee CW, Yang DY, Chang MH, Ho SP (2007) Post-injury regeneration in rat sciatic nerve facilitated by neurotrophic factors secreted by amniotic fluid mesenchymal stem cells. *J Clin Neurosci* 14(11):1089–1098
- Park H-YL, Kim JH, Kim HS, Park CK (2012) Stem cell-based delivery of brain-derived neurotrophic factor gene in the rat retina. *Brain Res* 1469:10–23
- Pereira Lopes FR, Camargo de Moura Campos L, Dias Correa J Jr, Balduino A, Lora S, Langone F, Borojevic R, Blanco Martinez AM (2006) Bone marrow stromal cells and resorbable collagen guidance tubes enhance sciatic nerve regeneration in mice. *Exp Neurol* 198(2):457–468
- Pereira JH, Bowden RE, Gattuso JM, Norris RW (1991) Comparison of results of repair of digital nerves by denatured muscle grafts and end-to-end sutures. *J Hand Surg Br* 16(5):519–523
- Pereira JH, Bowden RE, Narayanakumar TS, Gschmeissner SE (1996) Peripheral nerve reconstruction using denatured muscle autografts for restoring protective sensation in hands and feet of leprosy patients. *Indian J Lepr* 68(1):83–91
- Plotnikov EY, Khryapenkova TG, Vasileva AK, Marey MV, Galkina SI, Isaev NK, Sheval EV, Polyakov VY, Sukhikh GT, Zorov DB (2008) Cell-to-cell cross-talk between mesenchymal stem cells and cardiomyocytes in co-culture. *J Cell Mol Med* 12(5A):1622–1631
- Pollock K, Dahlenburg H, Nelson H, Fink KD, Cary W, Hendrix K, Annett G, Torrest A, Deng P, Gutierrez J, Nacey C, Pepper K, Kalomoiris S, J DA, McGee J, Gruenloh W, Fury B, Bauer G, Duffy A, Tempkin T, Wheelock V, Nolta JA (2016) Human Mesenchymal Stem Cells Genetically Engineered to Overexpress Brain-derived Neurotrophic Factor Improve Outcomes in Huntington's Disease Mouse Models. *Mol Ther* 24(5):965–977
- Prasad A, Manivannan J, Loong DT, Chua SM, Gharibani PM, All AH (2016) A review of induced pluripotent stem cell, direct conversion by trans-differentiation, direct reprogramming and oligodendrocyte differentiation. *Regen Med* 11(2):181–191
- Purves D (1986) The trophic theory of neural concentrations. *Trends Neurosci* 9:486–489
- Rath EM (2002) Skeletal muscle autograft for repair of the human inferior alveolar nerve: a case report. *J Oral Maxillofac Surg* 60(3):330–334
- Ren Z, Wang J, Wang S, Zou C, Li X, Guan Y, Chen Z, Zhang YA (2013) Autologous transplantation of GDNF-expressing mesenchymal stem cells protects against MPTP-induced damage in cynomolgus monkeys. *Sci Rep* 3:2786
- Ribeiro-Resende VT, Pimentel-Coelho PM, Mesentier-Louro LA, Mendez RM, Mello-Silva JP, Cabral-da-Silva MC, de Mello FG, de Melo Reis RA, Mendez-Otero R (2009) Trophic activity derived from bone marrow mononuclear cells increases peripheral nerve regeneration by acting on both neuronal and glial cell populations. *Neuroscience* 159(2):540–549
- Robinson LR (2000) Traumatic injury to peripheral nerves. *Muscle Nerve* 23(6):863–873
- Robinson LR (2004) traumatic injury to peripheral nerves. *Suppl Clin Neurophysiol* 57:173–186
- Rosberg HE, Carlsson KS, Dahlin LB (2005) Prospective study of patients with injuries to the hand and forearm: costs, function, and general health. *Scand J Plast Reconstr Surg Hand Surg* 39(6):360–369
- Sakaguchi DS (2017) Regenerative and repair strategies for the central nervous system. In: *Neuroimmune pharmacology*. Springer, Cham, pp 799–818
- Salzer JL, Bunge RP (1980) Studies of Schwann cell proliferation. I. An analysis in tissue culture of proliferation during development, Wallerian degeneration, and direct injury. *J Cell Biol* 84(3):739–752
- Sameem M, Wood TJ, Bain JR (2011) A systematic review on the use of fibrin glue for peripheral nerve repair. *Plast Reconstr Surg* 127(6):2381–2390
- Sandquist EJ, Uz M, Sharma AD, Patel BB, Mallapragada SK, Sakaguchi DS (2016) Stem cells, bioengineering

- and 3-D scaffolds for nervous system repair and regeneration. In: Zhang LG, Kaplan D (eds) *Neural engineering: from advanced biomaterials to 3D fabrication techniques*. Springer, New York
- Sasaki M, Radtke C, Tan AM, Zhao P, Hamada H, Houkin K, Honmou O, Kocsis JD (2009) BDNF-hypersecreting human mesenchymal stem cells promote functional recovery, axonal sprouting, and protection of corticospinal neurons after spinal cord injury. *J Neurosci* 29(47):14932–14941
- Schlosshauer B, Muller E, Schroder B, Planck H, Muller HW (2003) Rat Schwann cells in bioresorbable nerve guides to promote and accelerate axonal regeneration. *Brain Res* 963(1-2):321–326
- Seddon HJ, Medawar PB, Smith H (1943) Rate of regeneration of peripheral nerves in man. *J Physiol* 102(2):191–215
- Sharma AD, Brodskiy PA, Petersen EM, Dagdeviren M, Ye EA, Mallapragada SK, Sakaguchi DS (2015) High throughput characterization of adult stem cells engineered for delivery of therapeutic factors for neuroprotective strategies. *J Vis Exp* (95)
- Sharma AD, Wiederin J, Uz M, Ciborowski P, Mallapragada SK, Gendelman HE, Sakaguchi DS (2017) Proteomic analysis of mesenchymal to Schwann cell transdifferentiation. *J Proteomics* 165:93–101
- Shibata T, Naruse K, Kamiya H, Kozakae M, Kondo M, Yasuda Y, Nakamura N, Ota K, Tosaki T, Matsuki T (2008) Transplantation of bone marrow-derived mesenchymal stem cells improves diabetic polyneuropathy in rats. *Diabetes* 57(11):3099–3107
- Shimizu S, Kitada M, Ishikawa H, Itokazu Y, Wakao S, Dezawa M (2007) Peripheral nerve regeneration by the in vitro differentiated-human bone marrow stromal cells with Schwann cell property. *Biochem Biophys Res Commun* 359(4):915–920
- Shirley DM, Williams SA, Santos PM (1996) Brain-derived neurotrophic factor and peripheral nerve regeneration: a functional evaluation. *Laryngoscope* 106(5 Pt 1):629–632
- Siemionow M, Brzezicki G (2009) Chapter 8: Current techniques and concepts in peripheral nerve repair. *Int Rev Neurobiol* 87:141–172
- Siniscalco D, Giordano C, Galderisi U, Luongo L, de Novellis V, Rossi F, Maione S (2011) Long-lasting effects of human mesenchymal stem cell systemic administration on pain-like behaviors, cellular, and biomolecular modifications in neuropathic mice. *Front Integr Neurosci* 5:79
- Someya Y, Koda M, Dezawa M, Kadota T, Hashimoto M, Kamada T, Nishio Y, Kadota R, Mannoji C, Miyashita T, Okawa A, Yoshinaga K, Yamazaki M (2008) Reduction of cystic cavity, promotion of axonal regeneration and sparing, and functional recovery with transplanted bone marrow stromal cell-derived Schwann cells after contusion injury to the adult rat spinal cord. *J Neurosurg Spine* 9(6):600–610
- Song HJ, Poo MM (1999) Signal transduction underlying growth cone guidance by diffusible factors. *Curr Opin Neurobiol* 9(3):355–363
- Spees JL, Olson SD, Whitney MJ, Prockop DJ (2006) Mitochondrial transfer between cells can rescue aerobic respiration. *Proc Natl Acad Sci U S A* 103(5):1283–1288
- Squillaro T, Peluso G, Galderisi U (2016) Clinical trials with mesenchymal stem cells: an update. *Cell Transplant* 25(5):829–848
- Stanec S, Tonkovic I, Stanec Z, Tonkovic D, Dzepina I (1997) Treatment of upper limb nerve war injuries associated with vascular trauma. *Injury* 28(7):463–468
- Stoll G, Griffin JW, Li CY, Trapp BD (1989) Wallerian degeneration in the peripheral nervous system: participation of both Schwann cells and macrophages in myelin degradation. *J Neurocytol* 18(5):671–683
- Stoll G, Jander S, Myers RR (2002) Degeneration and regeneration of the peripheral nervous system: from Augustus Waller's observations to neuroinflammation. *J Peripher Nerv Syst* 7(1):13–27
- Strauch B, Rodriguez DM, Diaz J, Yu HL, Kaplan G, Weinstein DE (2001) Autologous Schwann cells drive regeneration through a 6-cm autogenous venous nerve conduit. *J Reconstr Microsurg* 17(8):589–595 discussion 596–587
- Streppel M, Azzolin N, Dohm S, Guntinas-Lichius O, Haas C, Grothe C, Wevers A, Neiss WF, Angelov DN (2002) Focal application of neutralizing antibodies to soluble neurotrophic factors reduces collateral axonal branching after peripheral nerve lesion. *Eur J Neurosci* 15(8):1327–1342
- Sunderland S, Williams HB (1992) Nerve injuries and their repair: a critical appraisal. LWW
- Takemura Y, Imai S, Kojima H, Katagi M, Yamakawa I, Kasahara T, Urabe H, Terashima T, Yasuda H, Chan L, Kimura H, Matsusue Y (2012) Brain-derived neurotrophic factor from bone marrow-derived cells promotes post-injury repair of peripheral nerve. *PLoS One* 7(9):e44592
- Terenghi G (1999) Peripheral nerve regeneration and neurotrophic factors. *J Anat* 194. (Pt 1):1–14
- Terness P, Bauer TM, Röse L, Dufter C, Watzlik A, Simon H, Opelz G (2002) Inhibition of allogeneic T cell proliferation by indoleamine 2, 3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites. *J Exp Med* 196(4):447–457
- Tetzlaff W (1982) Tight junction contact events and temporary gap junctions in the sciatic nerve fibres of the chicken during Wallerian degeneration and subsequent regeneration. *J Neurocytol* 11(5):839–858
- Thoma EC, Merkl C, Heckel T, Haab R, Knoflach F, Nowaczyk C, Flint N, Jagasia R, Jensen Zoffmann S, Truong HH, Petitjean P, Jessberger S, Graf M, Iacone R (2014) Chemical conversion of human fibroblasts

- into functional Schwann cells. *Stem Cell Rep* 3 (4):539–547
- Tofaris GK, Patterson PH, Jessen KR, Mirsky R (2002) Denervated Schwann cells attract macrophages by secretion of leukemia inhibitory factor (LIF) and monocyte chemoattractant protein-1 in a process regulated by interleukin-6 and LIF. *J Neurosci* 22(15):6696–6703
- Tseng TC, Hsu SH (2014) Substrate-mediated nanoparticle/gene delivery to MSC spheroids and their applications in peripheral nerve regeneration. *Biomaterials* 35(9):2630–2641
- Tung TH, Mackinnon SE (2010) Nerve transfers: indications, techniques, and outcomes. *J Hand Surg [Am]* 35(2):332–341
- Uz M, Büyüköz M, Sharma AD, Sakaguchi DS, Altinkaya SA, Mallapragada SK (2017) Gelatin-based 3D conduits for transdifferentiation of mesenchymal stem cells into Schwann cell-like phenotypes. *Acta Biomater* 53:293–306
- Uz M, Das SR, Ding S, Sakaguchi DS, Claussen JC, Mallapragada SK (2018) Advances in controlling differentiation of adult stem cells for peripheral nerve regeneration. *Adv Healthc Mater*:1701046
- Vallabhaneni KC, Haller H, Dumler I (2012) Vascular smooth muscle cells initiate proliferation of mesenchymal stem cells by mitochondrial transfer via tunneling nanotubes. *Stem Cells Dev* 21(17):3104–3113
- van Velthoven CT, Kavelaars A, van Bel F, Heijnen CJ (2009) Regeneration of the ischemic brain by engineered stem cells: fuelling endogenous repair processes. *Brain Res Rev* 61(1):1–13
- Verge VM, Merlio JP, Grondin J, Ernfors P, Persson H, Riopelle RJ, Hokfelt T, Richardson PM (1992) Colocalization of NGF binding sites, trk mRNA, and low-affinity NGF receptor mRNA in primary sensory neurons: responses to injury and infusion of NGF. *J Neurosci* 12(10):4011–4022
- Verge VM, Richardson PM, Wiesenfeld-Hallin Z, Hokfelt T (1995) Differential influence of nerve growth factor on neuropeptide expression in vivo: a novel role in peptide suppression in adult sensory neurons. *J Neurosci* 15(3 Pt 1):2081–2096
- Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Sudhof TC, Wernig M (2010) Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* 463(7284):1035–1041
- Wakao S, Hayashi T, Kitada M, Kohama M, Matsue D, Teramoto N, Ose T, Itokazu Y, Koshino K, Watabe H, Iida H, Takamoto T, Tabata Y, Dezawa M (2010) Long-term observation of auto-cell transplantation in non-human primate reveals safety and efficiency of bone marrow stromal cell-derived Schwann cells in peripheral nerve regeneration. *Exp Neurol* 223(2):537–547
- Wang J, Ding F, Gu Y, Liu J, Gu X (2009) Bone marrow mesenchymal stem cells promote cell proliferation and neurotrophic function of Schwann cells in vitro and in vivo. *Brain Res* 1262:7–15
- Wang X, Luo E, Li Y, Hu J (2011) Schwann-like mesenchymal stem cells within vein graft facilitate facial nerve regeneration and remyelination. *Brain Res* 1383:71–80
- Wang Y, Chen X, Cao W, Shi Y (2014) Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. *Nat Immunol* 15(11):1009
- Watson WE (1974) The binding of actinomycin D to the nuclei of axotomized neurones. *Brain Res* 65(2):317–322
- Weimann JM, Johansson CB, Trejo A, Blau HM (2003) Stable reprogrammed heterokaryons form spontaneously in Purkinje neurons after bone marrow transplant. *Nat Cell Biol* 5(11):959–966
- Weintraub H, Davis R, Tapscott S, Thayer M, Krause M, Benzeval R, Blackwell TK, Turner D, Rupp R, Hollenberg S et al (1991) The myoD gene family: nodal point during specification of the muscle cell lineage. *Science* 251(4995):761–766
- Whitlock EL, Kasurkuri R, Yan Y, Tung TH, Hunter DA, Mackinnon SE (2010) Fibrin glue mitigates the learning curve of microneurosurgical repair. *Microsurgery* 30(3):218–222
- Woodbury D, Schwarz EJ, Prockop DJ, Black IB (2000) Adult rat and human bone marrow stromal cells differentiate into neurons. *J Neurosci Res* 61(4):364–370
- Wright DE, Snider WD (1995) Neurotrophin receptor mRNA expression defines distinct populations of neurons in rat dorsal root ganglia. *J Comp Neurol* 351(3):329–338
- Wu J, Yu W, Chen Y, Su Y, Ding Z, Ren H, Jiang Y, Wang J (2010) Intrastratial transplantation of GDNF-engineered BMSCs and its neuroprotection in lactacystin-induced Parkinsonian rat model. *Neurochem Res* 35(3):495–502
- Wyse RD, Dunbar GL, Rossignol J (2014) Use of genetically modified mesenchymal stem cells to treat neurodegenerative diseases. *Int J Mol Sci* 15(2):1719–1745
- Xue C, Hu N, Gu Y, Yang Y, Liu Y, Liu J, Ding F, Gu X (2012) Joint use of a chitosan/PLGA scaffold and MSCs to bridge an extra large gap in dog sciatic nerve. *Neurorehabil Neural Repair* 26(1):96–106
- Yan Q, Elliott J, Snider WD (1992) Brain-derived neurotrophic factor rescues spinal motor neurons from axotomy-induced cell death. *Nature* 360(6406):753–755
- Yan Q, Matheson C, Lopez OT, Miller JA (1994) The biological responses of axotomized adult motoneurons to brain-derived neurotrophic factor. *J Neurosci* 14(9):5281–5291
- Yang J, Lou Q, Huang R, Shen L, Chen Z (2008) Dorsal root ganglion neurons induce transdifferentiation of mesenchymal stem cells along a Schwann cell lineage. *Neurosci Lett* 445(3):246–251
- Yang Y, Yuan X, Ding F, Yao D, Gu Y, Liu J, Gu X (2011) Repair of rat sciatic nerve gap by a silk fibroin-based scaffold added with bone marrow mesenchymal stem cells. *Tissue Eng Part A* 17(17-18):2231–2244
- Ye E-A, Chawla SS, Khan MZ, Sakaguchi DS (2016) Bone marrow-derived mesenchymal stem cells (MSCs) stimulate neurite outgrowth from

- differentiating adult hippocampal progenitor cells. *Stem Cell Biol Res* 3(1):3
- Yin Q, Kemp GJ, Frostick SP (1998) Neurotrophins, neurones and peripheral nerve regeneration. *J Hand Surg Br* 23(4):433–437
- Zhang CG, Gu YD (2011) Contralateral C7 nerve transfer – our experiences over past 25 years. *J Brachial Plex Peripher Nerve Inj* 6(1):10
- Zhang JY, Luo XG, Xian CJ, Liu ZH, Zhou XF (2000) Endogenous BDNF is required for myelination and regeneration of injured sciatic nerve in rodents. *Eur J Neurosci* 12(12):4171–4180
- Zhang F, Blain B, Beck J, Zhang J, Chen Z, Chen ZW, Lineaweaver WC (2002) Autogenous venous graft with one-stage prepared Schwann cells as a conduit for repair of long segmental nerve defects. *J Reconstr Microsurg* 18(4):295–300
- Zheng M, Kuffler DP (2000) Guidance of regenerating motor axons in vivo by gradients of diffusible peripheral nerve-derived factors. *J Neurobiol* 42(2):212–219
- Zheng J, Sun J, Lu X, Zhao P, Li K, Li L (2016) BDNF promotes the axonal regrowth after sciatic nerve crush through intrinsic neuronal capability upregulation and distal portion protection. *Neurosci Lett* 621:1–8
- Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L, MacDonald ME, Friedlander RM, Silani V, Hayden MR (2001) Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science* 293(5529):493–498