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Peripheral nerve injury and myelination: potential therapeutic strategies

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Abstract

Traumatic peripheral nerve injury represents a major clinical and public health problem that often leads to significant functional impairment and permanent disability. Despite modern diagnostic procedures and advanced microsurgical techniques, functional recovery after peripheral nerve repair is often unsatisfactory. Therefore, there is an unmet need for new therapeutic or adjunctive strategies to promote the functional recovery in nerve injury patients. In contrast to the central nervous system, Schwann cells in the peripheral nervous system play a pivotal role in several aspects of nerve repair such as degeneration, remyelination, and axonal growth. Several non-surgical approaches, including pharmacological, electrical, cell-based, and laser therapies, have been employed to promote myelination and enhance functional recovery after peripheral nerve injury. This review will succinctly discuss the potential therapeutic strategies in the context of myelination following peripheral neurotrauma.

Keywords

peripheral nerve injury; therapeutic strategies; physical therapy; pharmacotherapy; myelination; epigenetics; limitations

Traumatic peripheral nerve injury (TPNI) represents a major clinical and public health problem that often leads to significant functional impairment and permanent disability.[1] It is estimated that roughly 3% of all trauma patients have peripheral nerve injuries[2] and more

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than 50,000 peripheral nerve repair procedures are performed annually in the United States alone.[3] TPNIs are generally associated with motor vehicle collisions, penetrating injuries, lacerations, gun-shots, falls, burns, fractures, ischemia, traction and crush injuries,[4] and have been studied in both civilian[2,5–7] and military populations.[8] TPNIs are also more common in children than previously identified and can result in major social and economic burdens.[9] Despite progress in understanding the pathophysiology and the biological factors involved in of nerve injury and recovery, as well as the availability of modern diagnostic procedures and advanced microsurgical techniques, few treatment options exist to alter the course of recovery after TPNI.[4,10] This review will discuss the current knowledge of TPNI and recovery in terms of pathophysiology of nerve injury, classification, regeneration, diagnostic consideration and therapeutic strategies. The main focus is to provide a better understanding of potential non-surgical therapeutic approaches in promoting myelination following peripheral neurotrauma.

Pathophysiology and classification of nerve injury

At the cellular level, TPNIs exist in varying severities, which relate directly to the classification of these injuries. The severity of injury is related to the extent of anatomic disruption to the constituents of the nerve. On an anatomic level, the nerves grossly consist of axons, myelin (in myelinated fibers), Schwann cells, blood vessels, fat and connective tissue.[10–12] The outer covering of the nerve is a connective tissue epineurium, which is the site of most microsurgical techniques for repair. Within the nerve there are fascicles embedded in an internal connective tissue epineurium. Each individual fascicle is surrounded by perineurial connective tissue, and each fascicle contains many nerve fibers, which in turn, are encapsulated by longitudinally oriented endoneurial tubes. Nerves with fewer and larger fascicles may be more vulnerable to injury as a result of their inherent deficiency in connective epineurial tissue.[11,12] Some fibers within any given fascicle contain myelin which is compact in distribution around internodes, in contrast to the less myelin-dense paranodal and myelin-deficient nodes of Ranvier, where sodium channels are concentrated allowing propagation of action potentials (saltatory conduction). In practice, injuries to connective tissue, myelin, and axons all comingle in most TPNI.

The two commonly used classifications of nerve injury are the Seddon and Sunderland Classifications.[11,13] Seddon’s scheme, first published in 1943, divides nerve injuries into neurapraxia, axonotmesis, and neurotmesis in order of increasing severity. Neurapraxia, the mildest class of nerve injury, is characterized as a conduction block from a seemingly isolated injury to myelin, usually along a particular section of the nerve (segmental demyelination). In practice, myelin is not likely the only tissue type to be disrupted in these injuries, as nerve dysfunction from focal ischemia and subtle injuries to other tissue types may also play a role. Nonetheless, neuropraxic injuries are believed to spare most, if not all axons and these injuries often result from compression or traction on the nerve. In axonotmesis, the hallmark is axonal disruption and resulting Wallerian degeneration, but some connective tissue structures are preserved. Axonotmesis is typical after peripheral nerve crush and stretch injuries. Neurotmesis, where the nerve is severed, is the most severe class of nerve injury and is often caused by substantial trauma to the nerve and adjoining structures.[11–13]

Sunderland's classification breaks down nerve injury into five categories:[12] the first degree of nerve injury corresponds to neurapraxia, here with the distinction that the distal segment of the nerve, which is outside of the zone of injury, can conduct normally. Here too, the axonal structure is believed to be preserved. The second degree of nerve injury consists of axon degeneration distally with preserved endoneurial tubes with a good chance of near normal recovery if the injuries are not too distant from the targets. The third degree of nerve injury consists of the destruction of endoneurial tubes and nerve fibers with preserved fascicles and perineurium. Intrafascicular structures are disorganized but fascicular continuity is preserved. The fourth degree of nerve injury is associated with fascicular and perineurial destruction with only epineurium spared. The fifth degree of nerve injury is of complete loss of nerve continuity and complete destruction of axon and surrounding connective tissue, which corresponds to neurotmesis in Seddon's classification. In neurotmesis the prognosis of spontaneous recovery is poor, and nerve continuity is believed unlikely or even impossible without surgical intervention.

Regeneration

Recovery of nerve function after injury depends on several factors including the degree and location of the nerve injury and patient related factors. The degree of nerve injury itself depends on number and size of fascicles within the injured nerve (e.g. partial vs. complete severance of fascicles). Not all nerves have the same capacity for recovery, and therefore the type and location of nerve injured (e.g. proximal vs distal nerve segment, mixed nerve, etc.), are critical prognostic factors for regeneration.[4,12,14,15] Finally, advanced patient age and the chronicity of the injury each negatively affect the capacity for regeneration with older patients and those with delayed presentations or intervention often faring most poorly. Regeneration and repair processes of injured nerve occur at multiple levels including the nerve cell body, the segment between the neuron and the injury site (proximal stump), the injury site itself, the segment between the injury site and the end organ (distal stump), and the end organ.[4] One critical factor in regeneration is the process of Wallerian degeneration, which affects the segment of nerve distal to transected axons. This process starts within 48 hours and is usually complete by day 7–9 for motor axons and by day 11 for sensory nerves, with the difference in times ascribed to special consequences to dysfunction and early failure in neuromuscular transmission in motor fibers. Wallerian degeneration itself is an early means to distinguish neurapraxic from axonal injuries provided electrodiagnostic studies are performed after this period.

There are generally accepted three mechanisms of repair: remyelination, collateral sprouting, and axon regrowth.[4,16] In neurapraxic lesions, Schwann cells must de-differentiate to a stage where cell division is possible to proliferate and make new myelin, and this stage may take three months. Beyond this time period, persistent deficits imply axonal damage. The partial axon lesions recover through the mode of collateral sprouting that can take up to six months. Terminal sprouts are chemotrophic outgrowths from the intact nerve endings which grow from the conical growth-cone which emanates often from myelin-free nodes of Ranvier within the intramuscular nerves.[1] This process sacrifices single fiber motor-specificity by placing more muscle fibers under the control of remaining axons. Therefore, the motor unit size (the number of muscle fibers dependent on one nerve fiber),

can increase fivefold through the process of collateral sprouting.[14] In complete lesions, recovery depends on an axon regrowth rate of approximately 1 mm/day, with some variation in this rate related to patient factor (age) and injury factors (injury site, mechanism of injury, proximity of the injury to the nerve cell body, time and type of repair),[14,15] with the fastest reported regeneration rates associated with proximal limb and younger patients.[12,14]

During the regeneration process, the gap caused by the retracted stumps of a fully transected nerve is bridged by new tissue termed as “nerve bridge” and the regrowing axons from the proximal stump follow blood vessels through newly formed nerve bridge to reach the distal stump and their target organs.[17,18] However, there can be scar formation within the nerve bridge that can lead to misdirection and aberrant regeneration of the sprouting axons.[4] Therefore proximal lesions and those that must regenerate through scar hold the worst prognosis owing to the time lost during which reversible target muscle denervation atrophy becomes irreversible (12–18 months) in axotomy state.[4,19] In the most severe forms of axonotmeses and neurotmeses (Sunderland class 3–5), recovery is encumbered by increasing disorganization of fascicular structure and scar formation inside the nerve. This is perhaps related to the random and disorganized growth of regenerating axons towards the end-organ.[1,12] This may predispose more severe injuries to incomplete recovery of muscle function. Endoneurial tubes, Schwann cells and muscle fibers may indeed be viable for 12–18 months after prolonged axotomy;[1,4,19] however, motor recovery is rarely possible after this time period even with surgical repair because of reduced capacity of motor axons to regenerate.[16] Sensory fibers, without their inherent dependency on a viable muscle end-organ, may enjoy a slightly longer period of viable recovery.[19]

Electrodiagnostic evaluation of TPNI

The nature of the cellular and structural injury in the nerve defies imaging in most scenarios, and electrodiagnostic testing (EDX) is currently the most sensitive and specific method to evaluate TPNI. However, EDXs such as nerve conduction study (NCS) and electromyography (EMG) depend critically on the time-dependent post injury sequelae to the nerve and end-organ denervation, for a sensitivity that evolves over time after the injury. NCS determines nerve conduction velocities to help evaluate axonal degeneration from demyelinating disorders and EMG evaluates the motor unit action potential (MUAP) of a motor unit. Unlike surgical intervention, which can give some immediate information about the nature of the nerve injury, EDX has the key benefit of offering information about the functional status of the nerve and distally innervated units and carries far less risk of damage to the nerve or perioperative complications. It is for these reasons EDX plays a crucial ongoing role in the evaluation of these patients. The main aim of EDX in TPNI is to classify the pathophysiology of nerve injury as to axonal or demyelinating in nature, as well as to identify the location, grade and prognosis of the injury. This information is crucial in deciding the plans for an intervention. Despite the importance of these studies, it is also crucial to recognize the limitations and time dependency of EDX data, which typically include information from distinct NCSs and EMG studies obtained in a single session.

Neurapraxia and axonotmesis are indistinguishable using EDX testing for the first few days after nerve injury even when information is combined for sensory and motor nerves. The

distal segment of an injured nerve remains excitable during this time, and therefore EDX may not provide sufficient information to inform prognosis for approximately 11 days after injury. Testing, during this period can nonetheless aid in localization of the injury and grading severity with the severest lesions (whether neurapraxic or axonotmetic) failing to evidence MUAP recruitment. In neurapraxia, the distal compound muscle action potential (CMAP) is normal, but stimulation proximal to the lesion will not result in responses in the case of complete conduction block. EMG in these cases will show no MUAP (indicating a complete neurapraxic lesion) or reduced recruitment (indicating a partial neurapraxic lesion) – all without abnormal spontaneous activity. These changes usually resolve by 12 weeks, but delayed conduction may persist owing to thinner and shorter internodes associated with remyelination.[4,20] In axonotmesis, distal CMAP may be decreased or absent, but only after 7–9 days for motor and 11 days for sensory fiber injury. A decrease in amplitude of CMAP is proportional to the degree of axonal loss. EMG examination will show reduced recruitment or absent MUAP in the case of neurotmesis. MUAP recruitment can also serve to indicate axonal continuity after Wallerian degeneration is complete. Abnormal spontaneous activity will begin approximately 2–3 weeks after injury however; the extent of this activity may not correlate with severity.[20] There are some notable shortcomings of commonly used EDX techniques. EDX does not allow the critical distinction of subtypes of intermediate (Sunderland class 3–5) injuries.[1] The absent MUAP recruitment can be erroneously ascribed to loss of axonal continuity in mixed neurapraxic and axonotmetic lesions when NCS-EMG is performed before remyelination takes place.

Reinnervation changes first appear 2–3 months after axonotmesis and are typified by nominal increases in CMAP amplitude, however are probably better predicted based on MUAP configuration. Collateral sprouting results in longer duration polyphasic complexes and unstable motor units in the subacute phase followed by large amplitude and long duration motor unit potentials with persistently reduced recruitment. The very first sign of axon regrowth can be nascent units, which are small, polyphasic motor unit potentials.[20]

Taken together, the nature of EDX combined with the nature of nerve recovery allows the assessment of valuable clinical information, albeit with constraints on timing. The more detailed information is obtained once denervation has taken place with EDX yielding more definitive answers over time after TPNI.[4] Studies performed within a week of injury allow localization of the injured segment in addition to some information about the grade or completeness of the injury. After one week, studies that focus on motor nerves can distinguish between axonotmesis and neurapraxia, in addition to the information about completeness of the injury. This same distinction is possible for sensory fibers at 11 days post-injury. Studies performed at 3–4 weeks allow all of the above distinctions in addition to assessment of abnormal spontaneous activity. Studies performed at 3–4 months allow the documentation of reinnervation.

Therapeutic strategies

Currently the treatment of choice for peripheral nerve injury that results in laceration is advanced microsurgical end-to-end repair with tensionless epineurial sutures or autologous nerve grafting where end-to-end anastomosis is not possible (Table 1).[21–24] However,

functional recovery after peripheral nerve repair is often unsatisfactory and it is apparent that microsurgical approaches fail to address the complex cellular and molecular events associated with TPNI. Therefore, there is an unmet need for new therapeutic or adjunctive strategies to promote functional recovery in TPNI patients. In contrast to the central nervous system (CNS), Schwann cells in the peripheral nervous system (PNS) produce a growth-permissive environment for the injured nerve and play a pivotal role in several aspects of nerve repair such as degeneration, remyelination, and axonal growth.[7,16] Several treatment strategies have been employed to enhance the recovery process after TPNI, including pharmacological, electrical and cell based therapies.[7,25–27] While each has shown some promise in treating patients, none has provided a single universally applicable cure for the consequences of TPNI and they are also not without significant pitfalls or potent side-effect profiles. Surgical interventions have been extensively reviewed by several authors;[4,24,28,29] this section will highlight the usefulness of novel non-surgical therapeutic approaches in promoting myelination following peripheral neurotrauma (Table 1).

Pharmacotherapy

At present, there is no clinically approved pharmacological agent available for the treatment of TPNI. However, several agents have reported to be potential candidates to improve nerve regeneration by promoting myelination in the peripheral nerves.

Steroid Hormones

Steroid hormones, such as estrogen and progesterone, have been investigated for use in TPNI,[30–32] with both *in vivo* and *in vitro* evidence demonstrating the receptors for these hormones in the constituent cells of the peripheral nerve such as Schwann cells, DRG neurons, sensory and autonomic neurons.[27] Both hormones are known to be neuroprotective and promote myelination, with estrogen (17β-Estradiol) promotes nerve recovery in both the central and peripheral nervous systems.[33,34] In the PNS, estrogen exerts its effects through the modulation of steroid nuclear receptors, which in turn upregulate the PI3K/AKT/mTOR signaling pathway and thereby promote myelination.[32] It is reported that PNS dorsal root ganglion neurons activate mTOR following a sciatic nerve injury and this activity enhances axonal growth capacity with increased expression of growth-associated protein, GAP-43.[35] Activation of mTOR can also lead to downstream phosphorylation of S6 ribosomal protein and 4EBP-1 to initiate protein synthesis.[36] Since GAP-43 plays a key role in axon sprouting and outgrowth in regenerating axons,[37] it is possible that GAP-43 is involved in estrogen-induced upregulation of mTOR signaling and myelination. Progesterone may act in an independent manner following binding to its specific progesterone receptor, which in turn induces myelination via upregulation of Krox20 (also termed early growth response gene: Egr2),[38] a transcription factor that regulates PNS myelination.[39] This activation directly drives expression of myelin proteins like MPZ and PMP22, both of which are primary constituents of the myelin sheath.[34,38] However, both estrogen and progesterone suffer from well-documented side-effect profiles which can differ from patient to patient depending on the dose and duration of drug treatments. The United States Food and Drug Administration mandated a “black box” warning on all estrogen products based on the results of the Women’s Health Initiative (WHI) because unopposed estrogen use can increase the risk of

endometrial cancer in intact uteri, invasive breast cancer in post-menopausal women, and deep vein thrombosis (DVT).[40] Additional risk factors for estrogen include hypertension, obesity, diabetes mellitus, tobacco use, and/or history of venous thromboembolism. Estrogen receptor signaling are also involved in prostate carcinogenesis.[41] In WHI studies, an increased risk of DVT, pulmonary embolism, stroke, and myocardial infarction was observed in women ≥65 years with daily conjugated estrogens combined with medroxyprogesterone.

On the other hand, corticosteroids are sometimes used as a treatment option for nerve injury, including acute spinal cord injury, facial nerve paralysis, compressive neuropathy, and neuropathic pain,[42–45] A recent clinical study also demonstrated an important role for oral corticosteroids in the recovery of motor and sensory function following iatrogenic nerve injuries in total hip and knee arthroplasty.[46] However, several retrospective reviews have shown that long-term glucocorticoid use, even in low doses, is a significant independent predictor of diverse adverse effects, including bone loss or fracture, serious infections, gastrointestinal bleeding or ulcer, and cataracts.[47–50] The risk incurred with glucocorticoid use is both dose- and duration-dependent. Although the generalized use of hormonal therapies has not been common in the treatment of TPNI, several pre-clinical studies have shown that topical steroids can improve functional recovery and morphometric indices of the injured peripheral nerve[51–53] and this may have clinical implications after nerve transection because of its fewer or no adverse effects compared to chronic systemic administration. Therefore, the potential clinical usefulness of topical steroids to reduce post-injury nerve dysfunction warrants further investigations.

Erythropoietin

Erythropoietin (EPO), an endogenous hormone and FDA-approved drug for the treatment of anemia, has neuroprotective properties in both the central and peripheral nervous system.[54–57] Although originally discovered as hematopoietic agent, EPO has been extensively studied in translational neuroscience and its role in neuroprotection/neuroregeneration has been elegantly reviewed by several authors.[27,58–60] It is documented that EPO and its receptor (EPOR) are present in a wide variety of non-erythroid cells throughout the body and may impact many biological functions. In the nervous system, EPO is produced and secreted by the neurons of hippocampus, cortex, internal capsule, midbrain, and nervous system tumors.[61–63] The EPOR is also expressed on the myelin sheath of radicular nerves in human PNS.[64] We and others have shown the presence and upregulation of EPOR at the site of peripheral nerve injury in mice.[65,66] EPO improves the sciatic function index of mice and rats after crush injury,[57,65–68] and this functional improvement was observed with EPO administration prior to injury, immediately after injury, and after 1 week suggesting that the timing is not critical.[57,65–68] Importantly, EPO mediated functional improvement can be demonstrated from single doses up to days after injuries in mice and seems to correlate with injury.[65] Although the mechanisms by which EPO works are still poorly understood, it is thought to work by promoting the expression of myelin genes, MPZ and PMP22.[69] We have shown that mice which received systemic EPO following nerve crush injury maintained more myelinated axons at the site of injury.[57] *In vitro* EPO treatment also promoted myelin formation and protected myelin from the effect of nitric oxide (NO) exposure in co-cultures

of Schwann cells and dorsal root ganglion cells.[57] In addition to the fundamental myeloprotective role of EPO, these findings also demonstrated an anti-oxidative effect of EPO at the site of nerve injury which is consistent with the direct role of EPO against oxidative stress.[59] Given EPO's widespread and frequent use to treat anemia in humans and its favorable side-effect profile,[70,71] early clinical trials have already begun investigating EPO's therapeutic potential treating human peripheral nerve trauma associated with joint replacement surgery.[46,72] It is also noteworthy that clinical use of EPO is not without problems, in particular the route of administration and the potent side-effect profile. Aside from blood hyper-viscosity from increased RBC production, hypertension and its related problems,[73,74] the most common side effects of EPO therapy are headache and an influenza-like syndrome.[75,76]

4-aminopyridine

4-aminopyridine (4-AP), a broad-spectrum potassium (K^+) channel blocker and FDA-approved drug for the symptomatic treatment of multiple sclerosis (MS),[77,78] that has been shown to improve neuromuscular function in patients with other demyelinating disorders including myasthenia gravis,[79] spinal cord injury,[80] and Lambert-Eaton syndrome.[81,82] The neurological benefits of 4-AP are believed to result from increases in action potential duration, calcium influx, neurotransmitter release, synaptic transmission, and direct effects on muscle.[83–85] 4-AP may enhance cell-membrane excitability and impulse conduction, and we observed that clinically relevant doses of 4-AP treatment beginning shortly after injury enhance global functional recovery, promote remyelination and improve nerve conduction velocity in an established mouse model of peripheral nerve crush injury.[86] We also showed that 4-AP can distinguish or classify a crush injury from a transection injury by transient motor function recovery,[86–88] an effect most likely related to its nerve conduction restoration properties because the time course of recovery was far too rapid to be explained by regenerative mechanism and it is demonstrable at time-points far earlier than those allowed by EDX (see above). Importantly, at the tissue level, 4-AP also appears to be myeloprotective and possibly axonoprotective.[86,87] Early studies with 4-AP showed an increase in action potential duration and amplitude when measured on experimentally demyelinated mammalian peripheral nerve fibers.[89,90] These findings highlight a possible diagnostic and therapeutic contribution to recovery and prognosis owed to excitatory molecules that may stabilize impulse conduction within hours to days after injury.[91–93] Ongoing work on clinical translation of these findings may reveal clinical indications to TPNI, both in diagnosis and treatment.[94] However, despite the beneficial effects, the clinical ability of 4-AP to restore function has been limited because of its narrow therapeutic window, the need for frequent dosing throughout the day, and significant adverse side effects such as anxiety, tremors and seizures.[95–97] Clinical trials have shown that the efficacy of 4-AP is related to the total drug exposure whereas the toxicity is related to the peak serum levels.[98,99] We have demonstrated that long-term treatment is beneficial with 4-AP following acute TPNI improving functional recovery, myelin thickness, as well as important parameter of EDX, such as NCV.[86,87] Our findings with 4-AP convincingly demonstrated that it can be used either as local, transdermal or injectable form to exert its beneficial effect on TPNI recovery.[86–88] Given the interplay between clinical efficacy, safety profile, and

patient compliance, further developments of safe methods of administering 4-AP are a reasonable focus in the near term to allow treatment of the demyelinating component of TPNI, despite its promise as a regenerative medicine agent.

Electrical stimulation

Electrical stimulation (ES) represents a promising non-pharmacological approach to accelerate and promote recovery following peripheral nerve injury.[100–105] Studies in animals and humans have shown that ES promotes preferential reinnervation of both motor and sensory neurons, allowing for a faster recovery.[100,106,107] In addition to promoting reinnervation, ES also aids in the remyelination process following peripheral nerve injury.[108] ES is also reported to provide benefit on nerve injury-induced muscle atrophy and function.[109–111] Like ES, treadmill running is reported to exert positive effects on nerve regeneration and functional recovery.[112,113] Moreover, the combination of these two activity-dependent therapies, ES and treadmill running, has been shown to exert positive synergistic effects on nerve regeneration and muscle reinnervation.[114,115] While post-operative direct low-frequency (20 Hz) ES of the proximal nerve stump for 1 hour (2- to 3- fold threshold current) is a standard regimen,[116] experimental studies have demonstrated that the same ES parameters seven days prior to axotomy (conditioning ES, CES) can promote functional nerve regeneration with upregulation of regeneration-associated genes.[117,118] Although the exact mechanisms by which ES and exercise enhance nerve regeneration are poorly defined, cyclic adenosine monophosphate (cAMP) and brain-derived neurotrophic factor (BDNF) are reported to play key roles.[119–121] ES causes an increased influx of calcium into the neurons followed by an increase in intracellular cAMP levels,[120,122] and the downstream signaling molecule of cAMP, protein kinase A, promotes the expression of regeneration-associated genes for axonal growth.[120] Calcium-induced phosphorylation of extracellular signal regulated kinase, ERK, has been shown to cause increased expression of BDNF and myelination in the peripheral nervous system.[123,124] Despite the reported benefits with ES, the clinical use of implantable electrical devices remains a challenge because of the nature of invasive procedures, the lack of proper controls, and patient dissatisfaction with discomfort.[125]

Cell-based therapy

Some of the severest injuries to peripheral nerves, such as avulsions, lacerations, and contusions may suffer from the additional loss of Schwann cells needed to mediate regeneration. Such injuries may fail to regenerate even at the accepted rate of 1 mm/day.[16] With this slower rate of regeneration, end organ reinnervation may take months or years or may fail eventaully.[16,19] In the chronic axotomy state, the denervated Schwann cells eventually lose their capacity to support growth of the neurons and the lack of healthy Schwann cells is an important issue in nerve regeneration, which spurs interest in cell-based therapy.[16,19,126,127]

Cell-based therapy is a promising branch of regenerative medicine and Schwann cell cultures have demonstrated favorable results in the experimental model of TPNI with regeneration and remyelination.[128,129] However, the process of human Schwann cell

collection involves invasive nerve biopsy and the culture also has limited *in vitro* expansion.[130] In search for a suitable cell line, stem cells (SCs) have garnered substantial interests as candidate transplant cells because of their availability, rapid in vitro expansion, survival and integration within the host tissue.[131] Based on the differentiation potential, there are three categories of SC therapy: totipotent, pluripotent, and multipotent stem cells. Pluripotent and multipotent stem cells have been the focus of most research to date.[132]

Embryonic stem cells (ESCs) are pluripotent SCs that can differentiate into all three germ layers and Schwann cells can be generated from ESCs with 60% efficiency.[133] In an animal model, injection of ESCs translated to improved nerve repair and functional ability.[134] ESCs are limited in their therapeutic potential as they are in short supply, owing to their source in the human embryo and they also carry a risk of teratoma formation.[135] Human induced pluripotent stem cells (hPSCs) have shown some promise in the regeneration and protection of myelin, possibly by providing an exogenous source of self-renewing Schwann cells.[136] Multipotent somatic stem cells from bone marrow, mesenchymal stem cells (MSCs), have also shown some promise with greater number of myelinated axons when used in combination of artificial conduits and acellular grafts,[131,137,138] and they are also capable in myelinating cultured PC12 cells *in vitro*.[139]

In addition, adipose tissue and skin have been reported to provide easily accessible and less invasive potential sources of SCs. Adipose-derived stem cells (ADSCs) are a form of multipotent stem cells that can differentiate into a Schwann-like cell with similar functional properties.[140] It is possible that ADSCs may exert their effects via the release of growth factors, such as nerve growth factor, vascular endothelial growth factor, and BDNF, as well as through the recruitment of endogenous Schwann cells.[141,142] Although ADSCs are one of the more attractive SC therapies due to their availability in the adipose tissue, one restriction is their tendency to differentiate toward adipocytes, which can hinder nerve recovery.[143] The skin and associated structures have a highly available supply of transplantable cells due to their ability to differentiate into Schwann-like cells.[7,131] Skin-derived precursor cells (SKPCs) are neural crest-related precursor cells found in the dermis, which are capable of *in vitro* differentiation into neural crest cells, including those with the features of Schwann cells and peripheral neurons.[144–146]

While there is still much to learn about the role of SCs and their therapeutic potential in TPNI, there is now exciting *in-vivo* and *in-vitro* evidence indicating that they may be efficacious myelinating phenotypes and localization can be maintained. Most importantly, clinical studies exploring the feasibility of cell-based strategies as an adjunct therapy in chronic nerve injury will require careful investigation to determine the amount and method of cell delivery and the fate (cell survival and differentiation) of transplanted cells for safety, reliability, and maximum efficacy.

Photobiomodulation with laser therapy

Photobiomodulation (PBM) with low-level laser therapy (LLLT) has been extensively studied in many clinical practices including physical medicine and rehabilitation, stroke, degenerative or traumatic brain disorders, and nerve repair.[147–149] Laser therapy is a

noninvasive treatment modality that induces a photochemical reaction in the cell and increases the DNA and RNA synthesis in the cell nucleus, with subsequent cell proliferation and protein synthesis, including changes in nerve cell action potential.[147,148] The precise mechanisms underlying PBM with LLLT and its therapeutic benefits are not fully understood. It is believed that the initial trigger of PBM is the absorption of light (photons) by cytochrome C oxidase in the mitochondrial respiratory pathway. The increased activity of cytochrome C oxidase in turn increases the production of adenosine triphosphate (ATP) and thus modulates the cell functions.[150,151] Both clinical and experimental studies have reported the beneficial effects of LLLT in TPNIs. Animal and *in vitro* studies have shown that LLLT promotes the regeneration and functional recovery of the injured peripheral nerve, accelerates the myelination of regenerated nerves, increases the axonal diameter, and stimulates Schwann cell proliferation.[152–158] Double-blind randomized studies have reported that post-operative LLLT can enhance the regenerative process of the peripheral nerve with increased number of myelinated axons[159] and improve the motor nerve function with functional recovery.[160] However, other studies did not observe any beneficial effects of LLLT.[161,162] Although LLLT has no reported adverse effects, there is no standardization in treatment with LLLT and different irradiation parameters have been used in different models of PNI, and there is also a paucity in the clinical trials with LLLT. Therefore, future studies exploring the effects of different variables, such as wavelengths, dose, continuous or pulsed mode, application site, and type of radiation would verify the usefulness of LLLT in TPNI as an adjunct therapy.

Epigenetics and small molecule alterations in myelination

There is growing evidence that the interplay of environmental risk factors and individual genetic susceptibility modulates disease presentation and therapeutic responsiveness.[163–165] Epigenetics offers a promising link between genetic and environmental influences on phenotype development.[163–167] Epigenetic modifications, which include DNA methylation, posttranslational modifications of histones, and non-coding RNAs, results in the heritable silencing of genes without a change in their coding sequence.[167,168] Such modifications can be induced by stress, tissue damage, and diseases, and can affect both physiological and pathological processes.[166,169–171] Epigenetic mechanisms thus play an essential role in transcriptional control of genes, maintenance of cellular identity, cell activation, and cellular repair processes during stress; with purported roles in many disease processes including autoimmune disease, multiple sclerosis, neuroinflammation, cancer, and normal aging.[169–171]

The ever-evolving field of epigenetics also has the potential to play an important role in peripheral nerve recovery and myelination.[172–175] Myelination by Schwann cells is under strict transcriptional control[176,177] involving sequential, feedforward cascades of promyelinating transcription factors where Sox 10 (SRY-related HMG-box-10) and Oct6 (octamer-binding transcription factor-6) synergistically induce the expression of Krox20 (also termed early growth response gene: Egr2). Krox 20 is considered a master regulator of PNS myelination because it activates many myelin genes, suppresses myelination inhibitors, and maintains the myelinated state.[39] Krox20 together with NAB (NGFI-A/Egr-binding) protein regulates the transcription of myelin structural proteins and biosynthetic components

of myelin lipid layer.[178,179] In addition, several epigenetic and chromatin modifiers are involved in myelination of mammalian nervous system and are crucial for SC differentiation, myelin formation and myelin maintenance.[180,181] For example, histone deacetylase (HDAC) remodels chromatin and condenses chromatic architecture and thus limits DNA access for transcription factors.[182] Recently, an HDAC3-dependent pathway was identified as a potent inhibitor of peripheral myelogenesis.[183] Both *in vitro* and *in vivo* studies demonstrate that addition of HDAC3 inhibitors or the ablation of HDAC3 in Schwann cells significantly increases the production of myelin, conduction velocity, CMAP amplitude, and enhances sensory and motor function.[183] HDAC3 also antagonizes the myelogenic neuregulin-PI3K-AKT signaling axis. While these findings highlight the therapeutic potential of transient HDAC3 inhibition for improving peripheral myelin repair, it is unknown how different members of the HDAC family interact, their potential compensatory mechanisms, and how their expression and activity are regulated.[182]

It is evident that epigenetic changes can give rise to several significant disorders and we are just at the beginning of learning and understanding the contributions of these molecular genetic alterations to human diseases. While the reversible nature of epigenetic alterations is encouraging in the effort to find therapies that can reverse the molecular silencing early, it is unknown whether the epigenetic modifications are the cause or the result of disease progression. Future epigenetic studies in TPNIs will certainly enrich our knowledge and pave the way to use epigenetics as a tool to identify a disease biomarker and the potential therapeutic target.

Future directions and conclusions

In the past three decades, tremendous advances were made in TPNI diagnosis and management. However, the failure of well-defined microsurgical techniques to provide satisfactory functional recovery in the presence of complex cellular and molecular events with TPNI raises the question of using more robust therapeutic approaches or combination of new approaches with current strategies to promote functional recovery and quality of life in TPNI patients. Table 1 shows currently used and the potentially novel therapeutic strategies for TPNI management. Future treatments for TPNI will likely require the development of new pharmacologic adjuvant agents as well as uncovering the mechanistic details of those currently available. Key to the successful translation of treatments into widespread use in humans is well-executed clinical trials, which we and others have begun on several adjuvant forms of TPNI treatments. Critical gains in promoting myelin formation or myeloprotection, and allowing early diagnosis will advance treatment substantially.

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Abbreviations

TPNI	traumatic peripheral nerve injury

EDX	electrodiagnostic testing
NCS	nerve conduction study
EMG	electromyography
MUAP	motor unit action potential
CMAP	compound muscle action potential
CNS	central nervous system
PNS	peripheral nervous system
WHI	Women's Health Initiative
DVT	deep vein thrombosis
TCF/LEF	T-cell factor/lymphoid-enhancer
MPZ	myelin protein zero (MPZ)
PMP22	peripheral myelin protein 22
EPO	erythropoietin (EPO)
EPOR	erythropoietin receptor
4-AP	4-aminopyridine
ES	electrical stimulation
cAMP	cyclic adenosine monophosphate
BDNF	brain-derived neurotrophic factor
ESCs	embryonic stem cells
hPSCs	human pluripotent stem cells
ADSCs	adipose-derived stem cells
SKPCs	skin-derived precursor cells
LLLT	low-level laser therapy
PBM	Photobiomodulation
Sox 10	SRY-related HMG-box-10
Oct6	octamer-binding transcription factor-6
Krox20	early growth response gene (Egr2)
NAB	NGFI-A/Egr-binding protein
HDAC	histone deacetylase

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Current surgical and potential therapeutic strategies for traumatic peripheral nerve injury

Strategies	Intervention	Timing of Intervention	Benefit	References	Limitations/Comments/Next step
Surgical:	End-to-end neurorrhaphy Nerve grafting Nerve transfer Conduit repair	Variable (Days to months)	Yes	4,21–24,28,29	For selected nerve laceration injuries Slow rate of nerve regeneration Sub-optimal functional outcome Causes of poor outcome unknown Possible benefit from adjunct therapy
Non-Surgical: **Physical agents**	Physical therapy Electrical stimulation Low-level laser therapy Therapeutic ultrasound	Variable (Days to weeks)	Yes	100–105,107–118,121,123,125,147–149,152–156,159,160	Mainly for post-surgical rehabilitation Device-dependent Compliance issues Requires user-friendly portable devices Large clinical studies required
Pharmacological agents	Steroid hormones Erythropoietin 4-Aminopyridine	Immediate/Early	Yes	30–34,38,44–46,51–53,56,57,60,65–68, 86–90	Mostly pre-clinical findings Effective on crush and laceration injuries Improves motor function Improves myelination Improves nerve conduction Reduces muscle atrophy Proof-of-concept clinical studies required
Miscellaneous	Cell-based therapy Epigenetic target	Unknown	Unknown	131,134,137–141,144,146,170,172–174,176–183	Limited *in vitro* findings Proof-of-concept pre-clinical studies required Proof-of-concept clinical studies required

For more information, please refer to the listed references.