

The neurochemistry of peripheral nerve regeneration

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ABSTRACT

Peripheral nerve injuries (PNIs) can be most disabling, resulting in the loss of sensitivity, motor function and autonomic control in the involved anatomical segment. Although injured peripheral nerves are capable of regeneration, sub-optimal recovery of function is seen even with the best reconstruction. Distal axonal degeneration is an unavoidable consequence of PNI. There are currently few strategies aimed to maintain the distal pathway and/or target fidelity during regeneration across the zone of injury. The current state of the art approaches have been focussed on the site of nerve injury and not on their distal muscular targets or representative proximal cell bodies or central cortical regions. This is a comprehensive literature review of the neurochemistry of peripheral nerve regeneration and a state of the art analysis of experimental compounds (inorganic and organic agents) with demonstrated neurotherapeutic efficacy in improving cell body and neuron survival, reducing scar formation and maximising overall nerve regeneration.

KEY WORDS

Cell body; nerve regeneration; nerve repair; neuron survival; peripheral nerve injury

INTRODUCTION

Peripheral nerve injuries (PNIs) are estimated to occur in 2.8% of multiple trauma patients, or 5% if root and plexus injuries are included. PNI can result from motor vehicle accidents, sharp lacerations, penetrating trauma, stretching or crushing trauma and fractures, and gunshot wounds. In any case, treatment costs, morbidity and loss of function that impact quality

of life are significant.^[1] Novel treatments to restore and augment nerve regeneration and accelerate functional recovery would be greatly beneficial in the management and rehabilitation of PNIs. Identifying strategies that maximise nerve regeneration and enable motor/sensory recovery after injury can help patients to return to functional or employable status while improving performance, morale, mobility, agility and capability.

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OVERARCHING RESEARCH CHALLENGES IN NERVE REGENERATION

The management principles of nerve injuries have not changed in the past 50 years [Figure 1]. Despite substantially increased understanding of neuropathophysiology and experimental evidence of promising neurotherapies, obtaining satisfactory clinical outcomes are challenging in cases of avulsion, crush or blast injuries of peripheral nerves.^[2]

Although active rehabilitation following nerve injury may minimise the risk of disuse atrophy in muscle following nerve transection or trauma, denervation atrophy of muscle targets is a major hurdle to nerve regeneration strategies. Even the best neuroregeneration, outcomes may be compromised due to the lack of a viable neuromuscular junction (NMJ) or muscle target leading to poor functional results.

Any approach to peripheral nerve regeneration must thus be comprehensive and multifaceted, simultaneously facilitating natural host reparative processes and reintegration mechanisms, both proximal and distal to the zone of injury to overcome obstacles to axonal regeneration and successful reinnervation of the target organs. The primary research challenges involve:

- The cell body (withdrawal of target-derived neurotrophic support; alterations in gene/protein expression and antidromic action potentials affecting key neuronal survival pathways in the dorsal root ganglia and cell bodies causing up-regulation in several genes associated with apoptosis; loss of neuroprotection and regeneration in distal segments)
- Zone of injury and distal segment (molecular and cellular changes secondary to Wallerian degeneration [WD]; denervation of Schwann cells [SCs], and down-regulation of structural proteins such as protein zero, myelin basic protein and myelin-associated glycoprotein; slow, insufficient or misdirected axonal outgrowth and damaging

intracellular cascades across and distal to site of injury)

- Target organs (misdirection towards wrong targets, 'pruning' of growth cones that do not reach the correct target or lose the support of their endoneurial tubes chronically denervated SCs in axon cone, down-regulation of growth factors that support axonal progression, exhaustion of trophic factors in denervated target organs leading to NMJ loss and muscle fibre atrophy).

PATHOPHYSIOLOGY OF NERVE DEGENERATION AND REGENERATION

The classifications of Seddon and Maggi *et al.* are based on the anatomical and pathophysiological changes in the nerve following injury that dictate the necessity of surgical repair and impact prognosis of recovery.^[3,4] However, regardless of the type of PNI, neuronal survival is key to overall outcomes.

Neuronal response to injury

Nerve injury results in both retrograde (cell body and dorsal root ganglia proximal to the site of injury) and anterograde (neuronal elements in the distal segment) effects and causes apoptotic cell death. The extent of neuronal death depends on the developmental age (adult neurons are less likely to die) and level and type of injury (dorsal root avulsion is more destructive than the sharp transection of distal axonal segment) with sensory neurons more affected than motor neurons (10%–30% compared to 0%–10% as seen in sciatic nerve injury models).^[5,6]

Like most cells, neuronal survival depends on an ideal milieu of growth factors, hormones, cytokines and extracellular matrix (ECM) factors. Deprivation of extracellular signals (especially the target-derived neurotrophic support) triggers apoptotic cascades leading to neuronal death.^[7] Programmed neuronal death following injury involves the formation of reactive oxygen species and reactive nitrogen species (ROS/RNS), mitochondrial damage, activation of the Bcl family of proteins and release of apoptosis-inducing factors into the cytosol. Nerve avulsion in rats results in both apoptosis and necrosis,^[1,8] possibly due to the effect of RNS such as peroxynitrite (ONOO⁻).^[9] Depending on the degree of PNI, oxidative stress and dose-dependent activation of ROS or RNS^[10] can significantly influence the mechanism and extent of neuronal death.

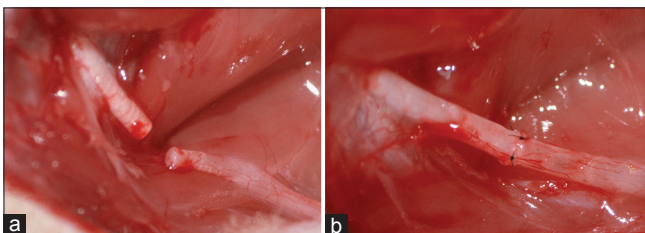


Figure 1: Rat sciatic nerve transection (a) and primary repair with epineurial sutures (b) is seen

After axotomy, injury signals generate a change in gene expression, switching the neuron from its signal transmission to regenerative status. This involves a change in molecular synthesis from neurotransmitters to cytoskeleton and growth associated proteins (GAPs).^[11,12] The action potentials generated by disruption of the neurilemma and the influx of sodium and calcium are amongst the first to determine this change.^[13] Negative signals related to loss of end-organ derived neurotrophic factors (the same loss that triggers apoptosis), paradoxically contribute to the survival of the neuron and regenerative switch.^[14] Different signalling molecules involving the pro-apoptotic Bcl family (BAD, Hrk, Bid, Bik, Bak, Bax), as well as a pro-survival (Bcl-XL, Bcl-2, Bcl-W, Mcl-1) pathways are up-regulated after injury.^[7] For example, a decrease in nerve growth factor (NGF) transport from the periphery to the neuronal cell body is an important 'negative' injury signal that induces axotomy related changes in gene expression and transcription factors.^[14] Nerve injury causes structural alterations in axoplasmic proteins (such as transcription STAT3), which contribute to the regenerative switch after retrograde neuronal transport.^[15]

Changes in the axonal stump

Nerve injury impairs axonal transport, acutely increases intracellular calcium, and activates axonal proteases (calpains) that lead to distal axonal breakdown. This process, called WD^[16] helps the scavenging of myelin and axon growth inhibitors (such as myelin-associated glycoprotein) at the site of injury and promotes a pro-regenerative environment in the distal axonal stump. Degeneration involves also a short segment of the proximal stump, extending retrograde to the first node of Ranvier.^[17] The SCs change from a myelinating to a growth promoting and cytokine-secreting phenotype. The dedifferentiation is induced by the loss of axonal contact and molecules secreted by the injured axon (neuregulin, calcitonin gene-related peptide).^[18] The dedifferentiated SCs support nerve regeneration through two mechanisms. First, SCs initiate myelin clearance and up-regulate cytokines and chemokines for recruiting macrophages that complete removal of axonal and myelin debris. Calpain^[19] and toll-like receptors (TLRs) play an important role in promoting cytokine secretion. TLRs bind to microbial ligands, as well as endogenous molecules (heat shock proteins [Hsps], ECM components) that signal injury and mediate the action of SCs during the inflammatory response.^[20,21]

Second, dedifferentiated SCs multiply and organise within the basal laminar scaffold (that spared by WD in the endoneurial tubes), to form the bands of Büngner, guiding the axonal sprouts and ensuring the neurotrophic and neurotropic support.^[22]

The growth cone

In the proximal stump, the retrograde morphological changes that reflect nerve regeneration consist of the transformation of the stable axonal segment into a highly active growth cone. The growth cone guides the regenerating neurites to their target end organs according to instructive neural cues made up by gradients of neurotrophic and neurotropic factors.^[23]

The anatomy of the growth cone is divided into three main domains. The peripheral domain (P), highly motile, has a veil-like structure – lamellipodia with actin arranged in a meshwork with finger like extensions-filopodia, containing bundles of actin. The transition zone (T) is set between the peripheral and central domain, rich in myosin that contracts the actin network. The central domain contains microtubules (MTs). As the growth cone advances and the nascent axon elongates, MT becomes incorporated in the newly synthesised cytoskeleton and stabilised by the microtubule-associated proteins (MAPs). GAP-43 is a MAP, its expression being associated with high activity of the growth cone.^[24]

Neurotrophic factors

Polypeptides

Numerous polypeptides, produced by non-neuronal cells after injury, ensure the survival, growth and guidance of regenerating nerves. Amongst the most studied of these factors are NGF, brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), neuregulins, pleiotrophin, insulin-like growth factors-1/2 ([IGF1, IGF2], interleukin-1 [IL-1], IL-6), transforming growth factor-beta 1 (TGF-b1), fibroblast growth factor (FGF), leukaemia inhibitory factor (LIF), vascular endothelial growth factor (VEGF) and sonic hedgehog/shh.^[14,25]

Lipid rafts

Lipid rafts are membrane microdomains enriched in cholesterol and sphingolipids that help growth cone migration and orientation. They are associated with receptors for neurotrophic factors, which on receptor ligation, trigger receptor translocation to the lipid rafts. The asymmetric association and membrane polarisation

of lipid rafts offers directional guidance to the growth cones.^[26]

The extracellular matrix

The ECM, including the basal lamina, plays a very complex role in nerve regeneration. Immediately after injury, laminin, fibronectin, integrins and their ligands on the cell surface undergo upregulation. Apart from acting as a substratum for the migrating SCs, the ECM also possesses chemoattractant and mitogen properties. The ECM levels modulate integrin expression by influencing their rate of removal and recycling. Cell integrins bind to ECM proteins and transmit signals to actin filaments generating mechanical forces.^[27,28]

NEUROINFLAMMATION – BLESSING AND BANE TO NERVE REGENERATION

Inflammation is an important phenomenon in nerve degeneration and regeneration. The timing and degree of inflammation may improve or impede the regeneration process. Cytokines released by macrophages and fibroblasts have a mitotic effect on SCs that facilitate remyelination. Proliferation of fibroblasts in the epineurium, perineurium or endoneurium can result in scar that includes inhibitory proteoglycans (such as chondroitin sulphate) or semaphorins.^[29,30] The scar forms mechanical barrier to the regenerating axons, causes shrinkage of the endoneurial sheaths that contain the axons and contributes to the development of multiple branched axonal terminals to form a neuroma.^[31]

The key for optimal nerve regeneration is to maintain or maximise the pro-regenerative capacity of the de-axonised distal nerve by ensuring the cell body and neuronal survival, to support recipient axonal regeneration (gap crossing and orientation of neuritis) to distal sensory/motor targets (NMJ and target muscle trophicity) as well as prevent neuroma (scar) formation. In addition to overall regeneration, the kinetics of regeneration is critical for functional outcomes as the distal targets need to be reinnervated before denervation atrophy. Various inorganic and organic compounds have been studied thus far to provide optimal nerve regeneration following PNI.

PROMISING INORGANIC AND ORGANIC COMPOUNDS WITH NEUROTHERAPEUTIC EFFICACY

Despite decades of experimental research on the role and relevance or effectiveness and efficacy of various

small molecules, neurotransmitters, biologics and other pharmacologic agents in peripheral nerve regeneration, there are no approved pharmacotherapies for clinical application after nerve injury or repair. An exhaustive review of the literature provides us an understanding of potentially promising agents (inorganic and organic compounds) that could facilitate natural host reparative processes (e.g., SC migration and organisation), promote expeditious and targeted axonal outgrowth, and provide trophic support to maintain the efficacy of the distal pathway beyond the graft/lesion site in a manner consistent with functional reinnervation and recovery.

Antioxidants

Oxidative stress is considered to be one of the main causes of neural damage after injury. Previous studies have shown that PNI induces the production of ROS and nitric oxide in axotomised neurons. Cells and tissues are equipped with a variety of enzymatic and non-enzymatic antioxidants to remove excess ROS and protect against oxidative injury. These substances involved in scavenging free radicals are used experimentally to promote nerve regeneration. Glutathione and ascorbic acid (Vitamin C) are the most well-known and frequently studied anti-oxidants used for enhancing nerve regeneration.^[32,33]

Melatonin

Melatonin is an indoleamine synthesised from tryptophan and pass freely through lipid-rich membranes and to cross blood-brain, blood-testis and maternofetal barriers.^[34] Melatonin's main superpower is cell protection from ROS and RNS. At the mitochondrial level, it can reduce the ROS/RNS formation by decreasing electron leakage from the respiratory chain; it also reduces the release of mitochondrial pro-apoptotic factors by regulating the mitochondrial permeability transition pores.^[35] In terms of nerve injury and regeneration, melatonin has neuroprotective effect on injured neuron, anti-inflammatory effects at the injury site, scar-reducing effect on coaptation site, proliferative effects on SCs and stimulating effects on axonal regeneration.^[36-40] All these studies show that melatonin is an important molecule in nerve regeneration and exerts its effects more than one mechanism.

N-acetylcysteine

N-acetylcysteine (NAC) is a neuroprotective agent and a cysteine donor in the synthesis of glutathione, the free-radical scavenger. It stabilises the mitochondrial membrane by up-regulating antiapoptotic family of

proteins Bcl-2 and down-regulating Bax and caspase 3, increasing survival of the primary sensory afferent neuron population.^[41] Its neuroprotective effect on motor neurons also was evidenced by Zhang *et al.* on ventral root avulsion and ventral rhizotomy injury model in rats.^[42]

Acetyl-L-carnitine

Acetyl-L-carnitine is a peptide with anti-oxidant and anti-apoptotic activity.^[43] In the central nervous system (CNS), it has been shown to prevent cell damage by ROS and cell apoptosis by preventing failure of mitochondrial oxidative metabolism.^[44] In the peripheral nervous system, it was also shown to reduce neuron death and to eliminate sensory neuron loss after peripheral nerve transection, most probably involving the same extracellular signal-regulated kinase (ERK) pathway.^[45] Wilson *et al.* proved acetyl-L-carnitine's efficiency in improving sensory and motor neuron regeneration.^[46]

FK506 (tacrolimus), cyclosporine A and non-immunosuppressant analogues

FK506 is an immunosuppressant agent, used to help nerve allografts in-take. It also possesses neuroprotective and neurotrophic properties. Its actions are receptor dependent. These receptors called immunophilins or FK506 binding proteins with different effects: (1) Immunosuppressant (2) neuroregenerative.^[47]

The immunosuppressant role is exerted by calcineurin inhibition;^[48] however, the neuroprotective effect is independent of the calcineurin pathway. It is due to FK506 anti-oxidative/antinitrosative and anti-apoptotic features mediated by thioredoxin and glutathione redox systems and anti-apoptotic mediators (Bcl-2).^[49] By reducing inflammation, FK506 reduces also scar formation, promoting nerve regeneration, increasing the density of myelinated axons, with larger diameters and thicker myelin sheath.^[50] FK506 enhances nerve regeneration by binding to Hsp-90. By binding to Hsp-90, FK506 causes dissociation of p23 and activation of transcription, with increased ERK $\frac{1}{2}$ phosphorylation and GAP-43 synthesis.^[51] The use of FK506 in peripheral nerve repair and regeneration is restricted by its immunosuppressant effect. Inhibiting inflammation may cause a slowing down in myelin debris removal and in neurite outgrowth.^[52] Efforts have been made in finding FK506 analogues deprived of immunosuppressive undesirable effect.^[47,53,54] Cyclosporine A, an immunophilin ligand to cyclophilin,

sharing similar immunosuppressant properties as FK506, through the same calcineurin inhibiting mechanism, fails to enhance nerve regeneration.^[55]

Antineoplastic agents

Chemotherapy drugs have also been tested hoping to reduce scar formation by inhibiting fibroblast activation and collagen formation. Local administration of doxorubicin (DXR) at the site of neurolysis of a sciatic nerve in rats and extraneural injury by scraping the surface of biceps femori muscles was the model used. Light and electron microscopic evaluation revealed a lower scar tissue formation index and scar density scores in the DXR than in the control group.^[56] The same injury model was successfully used to test mitomycin C for its antiscarring properties.^[57] Geldanamycin is an antibiotic and anti-neoplastic agent, analogue to FK506 but without its immunosuppressant effect. It was shown to accelerate nerve regeneration compared to FK506, *in vivo*, in rats.^[58,59]

Rolipram and chondroitinase ABC

Rolipram is an inhibitor of cyclic adenosin monophosphate (AMPC) phosphodiesterase Type IV receptor, preventing the levels of AMPC to decline after neuronal injury. Among other, rolipram has a powerful anti-inflammatory action.^[60] Udina *et al.* evidenced rolipram's utility in the accelerating nerve regeneration of both motor and sensory components of a transected and directly repaired common peroneal nerve in rats. The results were compared to a group receiving chondroitinase ABC (ChABC) instead of rolipram. Proteoglycans like chondroitin sulphate proteoglycan (CSPG) inhibit neurites' elongation. ChABC creates a permissive distal stump environment by degrading CSPG. The results were similar. No cumulative effect was noted in the group receiving both rolipram and ChABC.^[61]

Corticosteroids

Steroid medication, due to its property to limit inflammation and consequent scar formation was investigated to evaluate any beneficial effect on nerve regeneration and repair. Karlidag *et al.* conducted a comparative study, on a facial nerve transection and direct coaptation injury model in rabbits and compared methylprednisolone (MP) and NAC. Compared to the control group, the best regeneration was achieved in the NAC group. The highest degree of degeneration was observed in the MP group.^[62] The results come in contradiction with the findings of an earlier study, that of Becker *et al.*, who showed that scarring was less,

and better results were obtained after local application of corticoids.^[63] In another comparative study MP was without effect in the section injury model and it reduced axonal and myelin degeneration alongside oedema in the compression group.^[64] Seth *et al.* compared the effects of local versus systemic administration of dexamethasone on a model of facial nerve axotomy and direct coaptation. Both the treatment groups failed to achieve statistical significance over the control group.^[65]

Calcium channel blockers

Calcium channel blockers are thought to have antiscarring effects. By blocking movement of Golgi-derived vesicles, an intracellular accumulation of procollagen proteins occur. This triggers a negative feedback on procollagen gene transcription. Based on these findings, Xue *et al.* showed that Type I collagen was less in the group receiving verapamil; and transmission electron microscopy showing less collagen fibres and structurally uniform in the treatment group.^[66]

Hyaluronic acid

Hyaluronic acid (HA) is a mucopolysaccharide, a basic component of the ECM. It favours cell proliferation and migration, it reduces the scar formation by inhibiting lymphocyte migration, proliferation and chemotaxis, granulocyte phagocytosis and degranulation, macrophages motility.^[67]

Several experimental studies confirm that HA reduces perineurial nerve tissue adhesion formation, without impairment of the wound healing process [Figure 2].^[68,69] The evaluation of scar formation and adhesion was done macroscopically (Petersen's grading scheme) and histologically (scar tissue formation index). The axon counts, axon diameter and mean fibre diameter were all better in the HA treatment group.

A comparative study between FK506 and HA was conducted. Electrophysiological studies and functional evaluation (toe-spreading index) revealed similar results for FK506 and HA.^[70] Zor *et al.* investigated the effect of HA (film sheath at the site of neurorrhaphy), VEGF (VEGF gene in plasmid injection) and combined therapy, on axonal regeneration. The group subject to combined therapy had well myelinated, compact fibres aligned with SCs.^[71] HA-carboxymethyl cellulose was proved by Agenor *et al.* to prevent aberrant outgrowth of regenerating axons, thus avoiding painful neuroma formation.^[72]

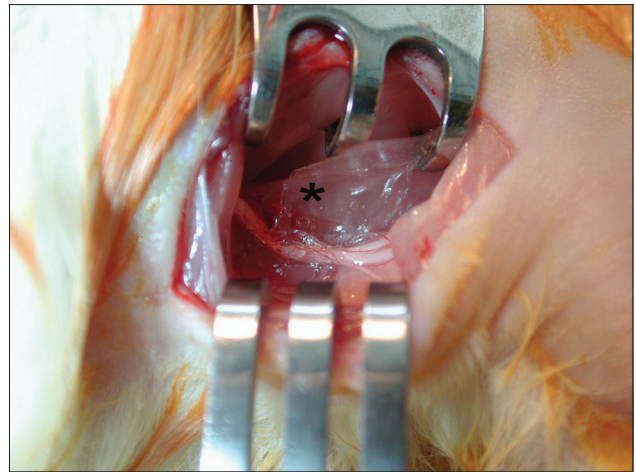


Figure 2: An easy and effective method of local drug delivery at the nerve repair site is the film sheath application. Following nerve repair, hyaluronic acid film sheath (*) is applied at the transection and repair site

Citicoline

Together with choline and cytidine, its metabolites, citicoline represents a substrate for phosphatidylcholine, a primary component of neuron membrane. Citicoline is a membrane stabilizer, reducing membrane breakdown and toxic products secondary to ischaemia.^[73] Functional, electrophysiological and histological evaluation showed that citicoline improved functional recovery and neuronal regeneration and reduced scar formation, following primary repair.^[74,75]

Chitooligosaccharides

Lowmolecularweightchitooligosaccharideshasantioxidative, anti-inflammatory, antiproliferative and antibacterial properties,^[76] and also proven to have neuroprotective effects and to enhance nerve regeneration.^[77,78]

Fasudil

Fasudil is an inhibitor of the Rho kinase. Rho is a major signalling pathway in the CNS. It blocks neuron regeneration by blocking neurites' outgrowth. In PNI models, it causes collapse of the growth cones by hindering the actin cytoskeleton dynamics.^[79,80] Rho kinase inhibition by fasudil was shown to be associated with an increase in neurite outgrowth *in vitro* and *in vivo*, in both sensory and motor neurons.^[81,82] Ibuprofen, due to its anti-inflammatory and Rho inhibitory actions, has a positive effect on nerve regeneration functionally and electrophysiologically.^[83]

Arachidonic acid derivatives

WD is in part mediated by neuroglia as well as infiltrating inflammatory cells and regulated by inflammatory

mediators. Part of this neuroimmune signalling is mediated by the innate immune system, including arachidonic acid derivatives such as prostaglandins and leukotrienes. The enzymes responsible for their production, cyclooxygenases and lipoxygenases, also participate in nerve degeneration and regeneration.^[84-87] The findings point towards a new avenue in the pharmacological treatment of nerve injury, based on a pathophysiologically relevant paradigm.

Gels

Aprotinin (trasyolol), was tested by Görgülü *et al.* to see the antiscarring effects on PNI: external neurolysis, abrasive injury, direct coaptation. Aprotinin was effective in reducing epineural scar formation in all three types of injury.^[88] ADCON-T/N, a glycosaminoglycan polymer gel was tested by several authors. Compared to control gel, it reduced scar formation in all three types of nerve injury described above, without impeding neuronal regeneration.^[89-91] Other bioabsorbable adhesion barrier gel solutions such as hyaloglides-gel and auto-cross-linked polysaccharide proved to be just as efficient as ADCON-T/N.^[92]

Growth factors and cytokines

Over the years, the possibility of enhancing the regenerative potential of lesioned neurons by exogenous application of growth-promoting molecules has been addressed in a number of studies. These molecules include neurotrophins (NGF, BDNF), GDNF family members (GDNF, neurturin, persephin, artemin), FGF-1, FGF-2, IGF-I, IGF-II, neuregulins (glial growth factor), neuropeptides (galanin, vasoactive intestinal peptide, pituitary adenylate cyclase-activating peptide), and neuropoietic cytokines belonging to the gp130 family (ciliary neurotrophic factor, LIF, cardiotrophin-1, oncostatin M, IL-6, IL-11) in the lesioned peripheral nervous system.^[93-95]

Growth factors elicit a response via receptor tyrosine kinases at the plasma membrane of neurons. The main signalling cascades required for axon growth are the Ras/ERK and the phosphatidylinositol-3 kinase/Akt pathways.^[96] Another important growth factor, VEGF, increases neovascularisation at the nerve repair site and improves nerve regeneration.^[71]

Although there are very encouraging results, usage of growth factors and cytokines in peripheral nerve regeneration has two major drawbacks. First, it is possible

that growth factors may cause neuronal apoptosis. Increased FGF levels, for example, may lead to neuron death.^[97] Second, these are peptide structures and have a short half-life.

CELLULAR AND TISSUE ENGINEERED THERAPIES

Although the primary aim of this manuscript is the chemical substances that are used for enhancing nerve regeneration, cellular and tissue engineered therapies should be briefly mentioned.

Nerve tissue engineering refers to the fabrication of biocompatible constructs with or without cellular components that enhance nerve regeneration. Axonal elongation has a close relation with ECM and SC. Thus, tissue engineered scaffolds or nerve conduits can be used for SC migration with minimising significant fibrosis.^[98] A new technology, 3D printing technology, has been combined with micro-computed tomography to both demonstrate and replicate the internal structure of acellular nerve allografts.^[99]

Cellular therapies in nerve regeneration may be performed with stem cells or SCs. Stem cells can differentiate into glial fibrillary acidic protein-positive SCs and can support myelination and the repair process.^[100] Stem cells may also differentiate into fibroblast-like cells capable of producing neurotrophic factors in addition to ECM proteins.

CLINICAL APPLICATIONS AND FUTURE PERSPECTIVES

Despite the progress in surgical techniques, today it is clear that peripheral nerve repair is a matter of multidisciplinary and integrated treatment rather than surgical repair. This multidisciplinary approach includes not only surgical repair but also tissue engineering, cellular therapies, nerve regeneration enhancing strategies.^[101] Translational and clinical studies are needed to transfer these basic information to standard clinical practice. It is known that each substance has different sites of action. Thus, new experimental and clinical studies which aim to investigate the effects of combined therapy of these substances, are required the further increase the functional outcome.^[102] Multicentered studies will also help to determine the ideal substance or substance

combination, which will be accepted as standard of care in peripheral nerve regeneration.

New animal studies showed reported that motor and sensory nerves have different denervation and regeneration features. Moreover, the time passed from initial nerve injury is an important factor and different cellular or chemical substances may act differently in acute or chronic injuries.^[103] Thus, the regeneration and time relation should be also needed to be evaluated.

There are still basic research, which is dedicated to the identification of new molecules that might promote nerve regeneration, for example, inflammatory cytokines, growth factors and Lipotropic factors, as well as their controlled local release.^[104] The list of chemicals used to reduce inflammation and scarring is still opened. Substances such as halofuginon (a Type I collagen synthesis inhibitor) proven to reduce fibrosis and adhesion in abdominal, pelvic and tendon surgery, are still to be tested on PNI and regeneration.^[105,106]

Decorin another antifibrotic and anti-inflammatory agent which traps TGF- β in the ECM before it can bind to its receptors on the cell surface.^[107] It has been shown to promote axon growth across adult rat spinal cord injuries.^[108] These *in vitro* studies on neurons belonging to the CNS could be taken one step further, by testing decorin's antiscarring effects *in vivo* and on PNI models also.

CONCLUSIONS

Due to the varied changes in nerves following different types of PNI, the key is to maintain or maximise the pro-regenerative capacity of the de-axonised distal nerve, to support recipient axonal regeneration to distal sensory/motor targets and to achieve functional neuro-integration and neuro-rehabilitation.

Treatment paradigms that have been tested and proven in experimental models have included selective neurotrophic agents (drugs/biologics/growth factors) or cellular neurotherapies (SC/mesenchymal stem cell/adipose-derived stem cell), when delivered in a targeted fashion act through multiple, non-redundant cellular/molecular mechanisms or pathways and have a global, complementary impact on the cellular, scaffold, signalling, inflammation, vascularisation process critical for nerve regeneration.

We have summarised promising inorganic and organic compounds that may have clinical, translational relevance in nerve regeneration. These agents may have multifaceted effects on neuroprotection (pharmacological prevention of some of the damaging intracellular cascades that lead to secondary tissue loss), axonal regeneration (increase of growth factors, neutralisation of inhibitory factors, reduction in scar formation), and help maintain distal neuronal pathways or targets.

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Conflicts of interest

There are no conflicts of interest.

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