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Gene Therapy in Rodents Models of Traumatic Peripheral Nerve Injury

Julia Teixeira Oliveira[1], Fatima Rosalina Pereira Lopes[1], Fernanda Martins de Almeida[1,2] and Ana Maria Blanco Martinez[1,3]*

[1]Laboratory to Neurodegeneration and Repair, Institute of Biomedical Sciences, Center for Health Sciences, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
[2]Federal University of Rio de Janeiro, campus Macae, Rio de Janeiro, Brazil
[3]Department of Pathology, Faculty of Medicine, Federal University of Rio de Janeiro, Brazil

Abstract

Although the peripheral nervous system has an inherent capacity for regeneration, injuries to nerves still result in considerable disabilities. The persistence of these disabilities along with the problem of nerve reconstruction has motivated neuroscientists worldwide to seek additional therapeutic strategies. Here we discuss the nerve trauma problem in terms of its molecular aspects and clinical implications with particular emphasis on the current strategies using gene therapy. Experimental models of nerve lesions have been developed for the study of the mechanisms underlying nerve degeneration and regeneration. Transection and crush lesions in rodents have been extensively used for this purpose, giving insights into human nerve regeneration process and also contributing to advancements in the nerve repair field. In recent years, gene-based therapy has emerged as a promising therapeutic tool. This review focuses on the gene therapy currently used on rodent models of nerve injuries for optimizing regeneration, its advantages and pitfalls. Identifying feasible gene delivery systems in basic and pre-clinical trials will hopefully facilitate the translation potential of gene-based therapy into the clinical setting.

Keywords: Gene therapy; Peripheral nerve; Nerve trauma; Wallerian degeneration; Nerve regeneration

Overview of Nerve Degeneration and Regeneration

Nerve damage produces a well characterized cascade of cellular and molecular events, described as Wallerian degeneration, throughout the distal stump. This process involves several phases, some concurrent, others consecutive, in which the distal stump degenerates; the myelin associated with degenerating axons are degraded and removed by Schwann cells (SC), and blood-recruited macrophages. SC can dedifferentiate, proliferate and align within the basal lamina tubes, called Bügner bands, which may subsequently be penetrated by regrowing axons. Once the SC-axon attachment is re-established, the remyelination process begins [1].

Growth cones emerge from the proximal stump of severed axons, induced by local factors released in response to the injury [2-4] and elongate if they find a favorable environment. It is known that regeneration of injured axons following trauma depends on a delicate balance between growth-promoting and growth-inhibiting factors. Several neurotrophic factors, cytokines, extracellular matrix molecules and hormones are secreted by neurons, SC, macrophages, the target tissue and cells present in the injury site, promoting neuronal survival, thus creating a permissive microenvironment for axon regeneration [5]. In this sense, SC in the vicinity of the transected axon synthesize a variety of growth factors including glial-derived neurotrophic factor (GDNF), insulin-like growth factor (IGF-1), and nerve growth factor (NGF), which have effects on axon growth [6]. The neurotrophins (NTs) family, which includes the brain-derived neurotrophic factor (BDNF), NGF and NTs 3 and 4/5, also play a crucial role in the regeneration process, serving as molecular cues and activators of the key signaling pathways that will support neuronal survival and growth [7].

In the absence of guiding cues, regenerating axons make a tortuous course and form a neuroma which is composed of immature axonal sprouts and connective tissue. However, if regenerating axons gain the distal nerve stump, they elongate within the endoneurial tubes, in association with the SC and the basal lamina, seeking the reinnervation of their original targets organs [8].

Traumatic Nerve Lesion Models in Rodents

Nerve injury is one of the most challenging reconstructive problems in the field of restorative medicine and neurosurgery. Peripheral nerve injuries are common, but the treatment success will depend on some limiting factors such as: age, the wound itself, nerve repair technique, injury site, and time between injury and surgical intervention. Nerve injuries can be described by Seddon classification [9] as neurapraxia, axonotmesis and neurotmesis, according to the disruption of the nerve structure and the possible prognosis. Neurapraxia is a reversible state where there is no anatomical rupture in the injured neuron. In this situation there is no need for a surgical intervention, and function is completely recovered. Axonotmesis occurs when there is a complete interruption of the axons, but the nerve supportive connective tissue is preserved. Neurotmesis is a complete damage of the entire nerve, including axons and supporting tissue, which requires surgical intervention [9]. Some years later, Sunderland [10] subdivided neurotmesis into three additional grades, adding useful information for prognosis and treatment strategies.

The etiology of nerve damage includes laceration, avulsion, stretch, compression and contusion [11]. They are frequently seen in the human clinics in cases of, for example, obstetric brachial plexus avulsions and orthopedic traumas. These lesions lead to important functional impairments in most patients; therefore experimental models of nerve injuries have been developed over the last decades in an attempt to mimic the clinical situation and to provide insights into new strategies that can improve nerve regeneration and functional outcomes.

***Corresponding authors:** Ana Maria Blanco Martinez, Assistant Professor, Rodolpho Paulo Rocco 255 - Fundão Island - Health Sciences Center–Block F-Room 12-21941-902, Brazil, Tel: 55-21-2562-6431; E-mail: martinez@histo.ufrj.br

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The two main experimental models of nerve injuries employed in the research are transection, representing severe damage that lead to interruption of nerve continuity, and crushing, representing moderate damage, when nerve continuity is preserved. Ischiatic and median nerves are the most studied types of lower and upper extremity nerves, respectively, due to their large size and, easy surgical accessibility.

The animal models widely used for studies on nerve regeneration have been rodents (mice and rats), probably because they are easy to handle, share higher homology with the human genome and have been extensively used in the field of genetic engineering for a diversity of experimental trials of gain and loss of function as well as reporter assays. Besides, the slow Wallerian Degeneration (Wlds) mutant mouse, which express a chimeric protein that delays axonal degeneration represents a good model for the study of the mechanisms underlying nerve degeneration.

Therapeutic Strategies for Nerve Lesions

A range of degenerative processes as well as traumatic conditions affecting the Peripheral Nervous System (PNS) go untreated. Despite the well-known regenerative capacity of PNS and great advances in microsurgical techniques, regeneration remains a significant clinical problem, leading to functional deficits. Following an injury that interrupts the nerve continuity, the primary repair, or neurorrhaphy, is the standard surgical procedure, when nerve stumps can be approximated without tissue tension. On the other hand, when the lesion causes tissue loss and consequently large gaps, autologous nerve grafting is considered the gold standard for clinical treatments. However this strategy has some limitations, such as the necessity to remove a healthy nerve, which requires an extra surgery, in addition to donor-site morbidity. In an attempt to overcome the drawbacks of the autologous nerve graft therapeutic tool, the development of artificial conduits to bridge peripheral nerve defects has become an attractive field of research in recent years. In addition, despite great advances, refinement of microsurgical nerve repair techniques has probably reached its optimum.

In this way, new approaches for promoting regeneration of the PNS has still been investigated, such as, stem cell transplantation [12-16] and the use of gene-based therapies [15,17]. Some studies also associate cell and gene therapies with positive results. These strategies may interfere at different stages of the regeneration process, showing promising results, as they can act on the refined molecular microenvironment. To date, there is no therapeutic strategy alone or combined that leads to complete functional recovery following nerve trauma. Gene therapy has emerged as a novel and alternative therapeutic strategy for treating peripheral nerve disorders.

Gene Therapy for Nerve Lesions

Gene therapy is the insertion, alteration or removal of genes within living cells with the aim to treat a disease. The most common form of gene therapy involves the insertion of functional genes into a genomic location, referred to as transgenes, making the transduced cells express continuously the protein of interest. The genetic modification can be accomplished *in vivo* as well as *in vitro*, being the last one followed by transplantation to the tissue of origin [18].

Viral vectors have emerged as the most efficient way to express a potentially therapeutic gene into the nervous system. Initially, Herpes Simplex Virus (HSV) and Adeno Virus (AdV) were used as vectors for gene transfer in the nervous system, however, currently, vectors based on Adeno-Associated Virus (AAV) and Lenti Virus (LV) are the two most commonly used systems [18].

In addition to viral vectors system, there are other vehicles that have been exploited to deliver genetic material to the peripheral nervous system. The employment of these alternatives transfection systems may represent an attempt to overcome the citotoxicity elicited by some virus vector systems. Some of these biotechnologies are based on cationic liposome transfection by means of the use of hemagglutinating virus of Japan-liposome/DNA complex, lipofectamine/DNA complex, polysaccharide and polyamine/DNA complex and polyethylenimine/DNA complex. Some vector constructs are made of the plasmid and they may be associated with electroporation, sonoporation, dextran or calcium phosphate in order to increase gene transfection. When gradual releasing is desirable, they can be delivered in association with polymer matrixes.

Unfortunately, there are still some limitations in gene therapy for regenerative medicine, including nerve injuries. Some important safety-related issues have to be solved as well as vector systems optimizations should be performed. First, although non-viral methods are considered safer than viral methods for gene transfer, the efficiency of the first is reduced relative to viral methods. Second, hazardous effects, such as immune responses and systemic reactions, should be strongly avoided. Filtration methods have been optimized leading to less contaminant from viral stocks, consequently decreasing but not ending the possibility of immune system reaction. In addition, targeting wrong cells and insertional mutagenesis (a mutation caused by the insertion of an exogenous DNA into a genome) also represent important issues concerning biosafety that should be circumvented.

Advances have been made on the design of cell-selective promoters and transcriptional activators, driving the effective expression of selective cell types, but there is still a lack of studies investigating this issue, specially, in the nerve repair field (e.g. SC-selective promoter). Although lentiviral vectors, which have been extensively used for treating nerve disorders, offer safer integration sites, they do not yet confer full protection against insertional mutagenesis. Apart from biosafety issues, the ideal vector system for targeting the injured peripheral nervous system should be better established. Moreover, the ideal target/site of gene transfer (SC, DRG neurons, endoneurial fibroblasts, stem cells, skin, muscle or nerve), the transgene (therapeutic protein of interest) to be delivered and the appropriate time of transduction should still be defined.

This review presents an overview of nowadays gene therapy for peripheral nerve injury. The use of this therapeutic strategy holds great hope in the nerve repair field because it lies mainly in providing prolonged extra bioactive trophic factors support, within the site of injury, resulting in the improvement of survival of sensory and motor neurons and SCs; increased SC motility; axon regrowth and remyelination; reinnervation; and, ultimately, functional recovery [15,17,19-33], as shown in Table 1.

Targets for Gene Transfer on Rodent Models Of Nerve Lesions

Gene-based therapy to treat nerve disorders brings the possibility of optimizing nerve regeneration, mainly through the introduction of genetic material into the cells, by several injection routes, such as, Dorsal Root Ganglion (DRG), intramuscular, intranerve, intrathecal, intraperitoneal, subcutaneous or intravenous. The genetic modification can be accomplished *in vivo* as well as ex vivo. However, *ex vivo* approach

Transgene	Vector	Transfected Tissue/Cell	Animal	Reference
NT-3	HSV	Subcutaneous	Rat	[19]
GDNF	Adenovirus	Laryngeal nerve	Rat	[20]
VEGF	Plasmid	Muscle	Mouse	[21]
FGF-2	Plasmid	Scwann Cells	Rat	[22]
VEGF	Plasmid	Muscle/Nerve	Rat	[23]
VEGF	Plasmid	Muscle	Rat	[24]
HGF	Adenovirus	Muscle	Rat	[25]
NGF+GDNF	Lentivirus	Nerve	Rat	[26]
VEGF	Plasmid	Bone Marrow	Rat	[27]
FGF-2	Plasmid	Scwann Cells	Rat	[28]
VEGF	Plasmid	Muscle	Mouse	[15]
BDNF	Adenovirus	Nerve	Rat	[29]
NGF	Lentivirus	Schwann Cells	Rat	[30]
GDNF	Lentivirus/ Plasmid	Schwann Cells	Rat	[31]
VEGF	AAV	Muscle	Rat	[32]
VEGF+G-CSF	Plasmid	Muscle	Mouse	[17]
CDNF	Lentivirus	Nerve	Rat	[33]

Table 1: An overview of studies on gene therapy for nerve regeneration over the last decade.

requires the transduction of cells *in vitro* followed by transplantation to the injured nervous system, which could represent some advantages over *in vivo* approach. First, in some cases, *ex vivo* techniques allow a better control of the type and number of transduced cells before they can be delivered as therapeutic agents in injured nerves. Second, this technique opens up the possibility of using a diversity of genetically modified cell types, in particular the stem cells, apart from transducing resident cells. In this context, cell and gene therapy, which have both emerged as the most promising area of medical science today, can be associated, potentially contributing to the regenerative medicine field. Although transduced stem cells represent a novel and alternative source for gene transfer with the aim to repair the peripheral nervous system, the three main cellular targets for this purpose are still SC, injured neurons and muscle fibers.

Following a peripheral nerve injury, SC play a main supportive role in promoting tissue regeneration, by secreting growth-promoting molecules, guiding the regenerating axons toward target organs and myelinating regenerated axons [34,35]. However, under conditions of chronic injury, denervated SC can lose the capacity of expressing growth-promoting molecules. Therefore, maintaining the supportive role of SC is crucial for the success of nerve regeneration, in particular in cases of traumatic lesions that are close to neuronal cell bodies or when there is a large gap between nerve stumps, conditions that are associated with a gradual decrease on axonal growing rate. Thus, Schwann cells might be ideally suited as cellular platforms for driving prolonged expression of neurotrophic factors and extracellular matrix molecules [36]. Moreover, in addition to local releasing of regeneration-promoting recombinant proteins, transgene delivery to SC can also indirectly influence nerve regeneration process, either by enhancing survival and proliferation or by increasing its motility capacity [18].

The preferred viral vectors for SC transduction are different pseudotypes of lentiviruses, which are described in the literature as having the ability to infect glial cells [18,37,38], and different adeno-associated vectors pseudotypes, such as adeno-associated vector 8, which preferentially transduces SC [39]. However, several researchers use other viral as well as non-viral transfection systems on SC, showing satisfactory results in the regeneration process after nerve crush or transection lesion models.

In a study using *ex vivo* retrovirally transduced SC with GDNF gene, delivered into a silicon conduit, to bridge a rat 10-mm ischiatic nerve defect, the *in vitro* expression of GDNF mRNA was significantly enhanced and the protein secretion was increased more than 5-fold in transduced cells. Moreover, the released protein was biologically functional as demonstrated by the capacity to improve motor neuron survival. Most importantly, the *in vivo* assay showed improved nerve regeneration by histological and electrophysiological parameters [40]. Controversially, another work demonstrated that SC infected with lentiviral vector to over express GDNF and seeded into an acellular nerve allograft, to bridge a 14-mm rat ischiatic nerve gap defect, resulted in poor functional recovery. The local continuous expression of GDNF, which is an important component of the regenerative process after nerve injuries, led to entrapment of axons in the graft, thus preventing axon regeneration. This study highlights the importance of turning off trophic factor signal to allow the axons to regenerate toward the target-organ [31]. Since both lentivirus and retrovirus gene transfer systems lead to long-term expression of the protein of interest, it is likely that the successful nerve regeneration in the first aforementioned study was possible because of the smaller nerve gap, while the nerve gap challenge in the second study was 4 mm higher.

Another important trophic factor for promoting nerve regeneration following an injury is the fibroblast growth factor-2 (FGF-2), which consists of low or high molecular weight isoforms. Transfected SC with either 18-kDa-FGF-2 or 21-/23-kDa-FGF-2 isoforms were seeded into nerve allografts to bridge a 15-mm rat ischiatic nerve defect, giving different results. While the 18-kDa-FGF-2 isoform inhibited myelination of regenerating axons, 21-/23-kDa-FGF-2 isoform mediated early recovery of sensory functions and stimulated myelination especially in longer distances from the proximal nerve stump [22].

The majority of peripheral-nerve regeneration studies still use the ischiatic-nerve model, perhaps because of the large size of this nerve. Clinically, however, upper-extremity lesions, such as brachial plexus avulsion or trauma, are more common and can lead to disorders with emotional, social, work-related, and economic aspects [41,42]. In this context, studies on upper-extremity nerves injuries models are gaining more attention from neuroscientists worldwide [13,43,44]. The rat musculocutaneous nerve, which arises from the lateral cord of the brachial plexus, has been used on studies of severe nerve injury and repair associated with the gene transfer therapeutic approach. In one of these studies the nerve stumps were transfected with Vascular Endothelial Growth Factor (VEGF) plasmid and repaired by End-To-End (ETE) or End-To-Side (ETS) neurorrhaphy microsurgical techniques [45]. Although a non-viral method of transfection was used in this study, the authors reported increased levels of VEGF in SC. Despite the fact that the treatment did not affect the number of neurons that reinnervated the musculocutaneous stumps after ETE neurorrhaphy, it resulted in higher quality axon reinnervation after both ETE and ETS neurorrhaphy and improved muscle weight index and function [45].

Several studies on nerve regeneration relied on the association of different therapeutic tools for getting improved outcomes. Interestingly, Gravvanis and coworkers [46] used the gene therapy combined with two microsurgical repair strategies, tubulization and ETS, on a rat peroneal nerve transection model with a 10-mm nerve segment removal. In this work, SC transduced with the pREV-HW3 retrovirus, encoding for sialyl-transferase-X, the polysialylated form of the neural cell adhesion molecule, presented increased motility *in*

vitro, which is considered a prerequisite for myelination process. The *in vivo* assay demonstrated improved fiber maturation, as indicated by the increased axon diameter and myelin thickness, which was attributed to SC migrational behavior. In another work using polysialyltransferase ST8SiaIV it was demonstrated that this molecule impairs the initiation of remyelination by the SC but it can also act by improving selective reinnervation of motor endplates [47], suggesting that a transient expression of this molecule by glial cells might exert beneficial effects on nerve regeneration. This is an important issue because the forced expression of this molecule by SC in gene transfer approaches might be turned off at some point to allow axon remyelination and an appropriate reinnervation of target-organ.

As aforementioned, the majority of studies on peripheral nerve injuries are on the ischiatic nerve, which is a mixed nerve. However, it is also interesting to investigate pure sensory or motor nerves, as it represents the clinical practice and these studies can shed some light on the biological and physiological mechanisms of specific molecules in functionally different types of nerve fibers. Recently, *in vivo* recombinant NGF delivery to SC on a rat crush-injured mental nerve (a nerve that carries sensory information to the central nervous system) using recombinant replication-deficient adenoviral vectors was capable of improving sensory recovery. The choice for mental nerve as a sensory nerve was due to the easy accessibility and exposure of about 2-cm length for treatment and evaluation. In addition, the use of this nerve represents an attempt to reproduce mental nerve damage, a condition frequently seen in the field of dental and maxillofacial plastic surgery (dental implant or orthognathic surgery) [48].

Gene transfer of adenoviral bone morphogenetic protein 7 (BMP7), a member of transforming growth factor-β (TGF-β) superfamily of ligands, was examined on both crush and transection models of peripheral nerve injury [49]. Interestingly, injection of the adenovirus into the rat ischiatic nerves led to extensive expression of transgene green fluorescent protein in the lumbar spinal cord, DRG and ischiatic nerves, suggesting that transduction was mediated by synapse transport; it also indicated that adenoviral transduction was achieved by retrograde transport. In this study, the effects of adenoviral BMP7 transduction on ischiatic nerve explants and SC cultures were analyzed and the results showed enhanced cell proliferation in both cultures. In addition BMP7 over expression protected axon and myelin degeneration and improved functional recovery [49].

The growth-promotion potential of SC makes these glial cells a suitable choice for cell and gene therapy that aims to promote nerve regeneration following injury. However, *ex vivo* gene transfer approaches on SC leads to donor site morbidity because another nerve has to be sacrificed for harvesting material for *in vitro* transduction, before it can be further transplanted. Moreover, SC have restricted mitotic activity, expanding poorly under culture conditions, which make them an insufficient source for treatments. In order to overcome these technical limitations, studies on vectors carrying cell-specific promoters (e.g. the S100β promoter for SC) should gain more attention allowing the use of *in vivo* approaches with cellular transduction specificity. Therefore, alternatives to SC for gene-based therapy, seeking the improvement of nerve regeneration, have been considered in the nerve repair field.

The injured neurons represent another interesting target for gene transfer in the peripheral nervous system. This therapeutic strategy lies in two main effects: driving expression of genes and transcription factors related to the axon growth program and preventing denervation-induced neuronal atrophy and death. Genetically modified DRG neurons are of particular interest as these sensory neurons have been extensively used as models in neurobiology, providing a suitable model to study the mechanism of neural regeneration [50].

The herpes simplex virus is mostly examined for gene transfer in the nervous system because of its natural neurotropism. In 1988, Geller and Breakefield [51] used herpes simplex virus to infect primary cultures neurons, from rat dorsal root ganglia and superior cervical ganglia, with β-galactosidase gene and they observed stable expression of high levels of this protein without cell death. Although herpes simplex virus has the aforementioned neurotropism advantage for transducing sensory neurons, its clinical prospects in the peripheral nervous system is relatively poor, mainly due to its short duration of expression [18]. Adenovirus and lentivirus vectors are currently employed to investigate whether gene therapy could be a strategy to promote nerve regeneration [18,52,53]. Several naturally occurring adeno-associated virus serotypes are being transformed into vectors that display very interesting nervous system tropism, especially for sensory and motorneurons [52,54,55]

Recently, in a nerve injury model of brachial plexus trauma, a frequent clinical condition, adult rats underwent a dorsal C5–T1 rhizotomy, followed by subcutaneous inoculation of a non-replicating herpes simplex virus-vector carrying the gene coding for bacterial C3 transferase. This therapeutic gene might inhibit Rho signaling by N-ADP ribosylation of Rho GTPase, supporting nerve regeneration. As expected, the treatment resulted in expression of C3 transferase in DRG neurons and blocking of Rho GTPase activity, which was correlated with nerve regeneration and improved sensory-motor coordination of the forepaw [56].

The neuroprotection effect of recombinant adenovirus vector encoding GDNF, BDNF, Ciliary Neurotrophic Factor (CTNF), cardiotrophin-1, IGF-1 and TGF-β was examined in a rat facial nerve avulsion study [57]. The adenovirus was injected directly into the facial canal following nerve avulsion. The authors observed that only GDNF, BDNF and TGF-β were capable of preventing facial motoneurons loss. They also found that the treatment produced an induction of nitric oxide synthase and an improvement on choline acetyltransferase immunoreactivity in the neurons. Although the aim of this work was to investigate motor neuron protection, which is crucial in humans motor neuron diseases such as amyotrophic lateral sclerosis, it can also be useful in the motor nerve trauma field, in particular after severe nerve lesions proximal to neuronal cell bodies (e.g. avulsions) and/or with significant tissue loss, conditions that can be accompanied by significant cell death in the course of time.

Non-viral methods encoding therapeutic genes are also alternatives as gene transfer vehicles for transducing DRG neurons in models of nerve lesions, since their use supports neurite outgrowth *in vitro* [58], as well as the nerve regeneration *in vivo* [59].

Wang and coworkers demonstrated that genes can be efficiently delivered to DRG neurons via lumbar intrathecal injection by means of non-viral methods, such as polyethylenimine (PEI)/DNA and lipofectamine 2000/DNA complexes, as well as viral methods, such as adeno-associated virus vectors and baculovirus vectors [60]. In this study the authors also used a rat ischiatic nerve transection model with a 10-mm nerve defect repaired by tubulization followed by intrathecal injection of PEI/NGF cDNA complexes. This therapeutic strategy resulted in improved nerve regeneration by increasing NGF expression in the DRG of injured nerves, confirming the viability of the chosen route.

More recently, gene transfer to denervated target tissues, such as denervated muscle, has attracted more attention from neuroscientists. After peripheral nerve injuries, anatomical and functional connections between motoneurons and skeletal muscle fibers are lost, inducing progressive muscle atrophy. The time between nerve trauma and reinnervation is a key determinant of functional recovery. In this context, improving regeneration rate is pivotal for restoring neuromuscular function.

The intramuscular injection of GDNF encoding adenovirus in a rat model of constriction-induced ischiatic nerve injury demonstrated the efficacy of the growth factor delivery method, which possibly led to the improved myelination and better behavioral outcomes [61]. However, it is important to highlight that there are still some limitations in the use of adenoviral vectors for gene transfer and therapy, such as inflammatory response and short-term expression. A study on rat median nerve transection model with a 10 mm-segment defect investigated the effects of VEGF delivered by two routes, intramuscular or directly injected inside a conduit made with vein filled with muscle fibers, on the muscle trophism and nerve regeneration process [32]. Interestingly, adeno-associated virus 2-VEGF directly injected into the denervated skeletal muscle, but not those delivered into transplanted muscle-vein conduit, significantly attenuated denervation-related atrophy. It was hypothesized that failure of axonal regeneration observed when the muscle-vein conduit was used could be explained by the fact that muscle fibers in the conduit might mimic their original end target, and therefore become reinnervated by sprouting axons. Although the nerve regeneration process was only investigated on animals treated with muscle-vein conduit injection, the authors suggested that local delivery of adeno-associated virus-VEGF in denervated muscle should be seen as a rational strategy for limiting atrophy progression, thus improving functional recovery. Intramuscular injection of a VEGF plasmid has also been successfully used to treat ischemic neuropathy in animal models [62] and human subjects [63]. Recently, our group also investigated the effects of VEGF on nerve regeneration, by delivering a non-viral plasmid vector into the muscle through electroporation, on a mouse ischiatic nerve transection model. In this work we combined the gene transfer with nerve repair microsurgery by means of a biodegradable conduit to bridge a 3 mm-nerve gap. We observed that VEGF expression was increased in VEGF-treated regenerating nerve, demonstrating the successful of the transfection. This therapeutic strategy increased blood-vessel formation, enhanced nerve regeneration, supported the survival of DRG and motor neurons and also improved the functional performance of treated-animals [15]. Taken into account these promising findings for encouraging nerve regeneration, our group further investigated the effects of gene therapy by using Granulocyte Colony-Stimulating Factor (G-CSF), which is also a trophic factor, alone or in association with VEGF on a similar mouse model of ischiatic nerve injury [17]. The best outcomes observed, such as increased number of myelinated fibers (Figure 1, authors´ unpublished figure), blood vessels, and neurons in the DRG and motoneurons in the spinal cord, were found when both gene therapy strategies were used together. In addition, the combined gene transfer treatment was able to accelerate functional recovery and to prevent muscle atrophy, suggesting an improvement of reinnervation and muscle activity. It is possible that a synergistic action of these

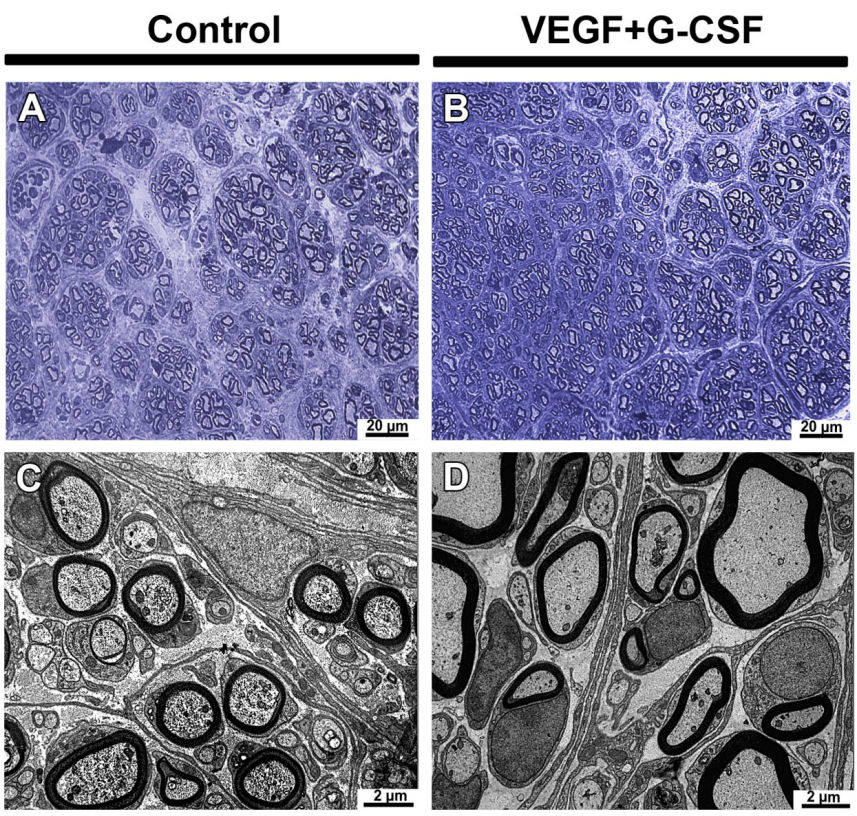

Figure 1: Gene therapy with VEGF and G-CSF enhances sciatic nerve regeneration. Semithin (A and B) and ultrathin (C and D) cross sections of regenerating sciatic nerves 6 weeks after injury. Observe in A, the control, dispersed groups of myelinated fibers, while treated animals (B) present a more organized regenerating nerve with several myelinated fibers. (C) Control group exhibits smaller myelinated fibers in comparison to gene therapy treated group (D).

two factors accounted for the optimization of the nerve regeneration process, since G-CSF can indirectly promote angiogenesis and vasculogenesis by increasing the production of VEGF [64]. In a novel paradigm, genetically modified stem cells can represent an alternative source for improving nerve regeneration. The use of combined stem cell and gene therapies lies mainly in the capacity of these cells to function as naturally physiological "mini-pumps" in addition to the forced expression of growth-promoting molecules. GDNF–modified human amniotic fluid–derived mesenchymal stem cells embedded in matrigel and delivered into the crushed ischiatic nerve was capable of enhancing nerve regeneration. Moreover, this "supplementary" treatment was also able to display effects on SC, by decreasing apoptosis [65]. Recently, GDNF or BDNF genes were transfected by a non-viral system into neural stem cells followed by transplantation into a conduit on a rat ischiatic transection nerve model with a 15 mm-segment defect [66]. Both gene transfer treatments led to an improvement of myelination and increased size of regenerated nerve, which correlated with functional recovery.

In this review we provided an overview of nowadays gene therapy for mild and severe peripheral nerve injuries, addressing the types of vectors systems used, carrying a diverse range of therapeutic proteins, in different targets/sites of nerve transfer. Knowledge of current pre-clinical research status of gene therapy for different nerve injuries, in addition to its limitations and strengths, might contribute to clarify suitable gene transfer protocols. In this way, this study gives an insight into feasible candidates of nerve transfer for targeting human peripheral nervous system following different traumatic injuries. Finally, we hope this study facilitates the clinical translation potential of genetic approaches for treating peripheral nerve injuries in a near future, thus, collaborating with nerve surgeons and contributing to the regenerative medicine field.

Conclusion

In summary, despite the fact that gene transfer for optimization of nerve regeneration is still in its infancy, there is a growing body of evidence that this therapeutic tool yields promising outcomes. Gene therapy can be considered a feasible candidate for promoting nerve regeneration following trauma, but the translation of this promising therapeutic tool to the clinical set remains a challenge.

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