Peripheral Nerve: What's New in Basic Science Laboratories

Jae W. Song, MD[a], Lynda J. Yang, MD, PhD[b], Stephen M. Russell, MD[a,*]

KEYWORDS

- Nerve regeneration • Allotransplantation
- Electrical stimulation • Preferential motor reinneravation
- Transgenic mice

Peripheral nerve regeneration research reflects the historical events that have evolved understanding of this complex phenomenon. Over time, the works of clinicians and scientists have contributed insights into ways of optimizing repair. Two such examples are Sunderland and Rays'[1,2] seminal work in intraneural anatomy, which provided a map for the surgeon performing intrafascicular nerve repair, and on a cellular level, Cajal's contribution to neurohistology, much of which has been repeated, verified, and expanded upon by more sophisticated molecular and cellular biology techniques. Hence, research today aims to combine clinical expertise with the basic sciences. The group of investigators assembled during the Second World War by the Medical Research Council exemplifies the success of multidisciplinary investigations. Headed by Hugh Cairns and Herbert Seddon, professors of surgery at Oxford University, the team was joined by J.Z. Young,[3] a neuroscientist, who then recruited Ernst Gutmann, a Czech physician, Ludwig Guttmann, a neurologist, and Peter B. Medawar, a scientist who subsequently went on to win the Nobel Prize for his work on transplant research. Together, this multidisciplinary group, along with others, went on to make significant contributions in understanding function and pathology of peripheral nerves, highlighting the importance of translational basic science research.

In this article, the authors have chosen to discuss some current translational research in peripheral nerve regeneration. The article summarizes the research of nerve allotransplantation, which is founded upon principles of immunology and transplant biology.[4–6] It also discusses brief electrical stimulation after nerve repair as a new clinical therapy aimed to increase the rate of axonal regeneration. Lastly, it discusses current tools generated by neuroscientists that enable physicians to observe dynamic neurobiological processes at the cellular level, which may enable practitioners to answer clinical questions that were not answerable before.

PERIPHERAL NERVE ALLOTRANSPLANTATION

Nerve repair techniques in the early 19th century reflected the lack of biological understanding of nerve regeneration. Nerves, treated like elastic rubber bands, either were stretched[7] or patients' limbs specifically positioned[8] or shortened to approximate two nerve stumps. Later, secondary explorations of stretched nerves[9] and the lack of functional recovery suggested that better methodology was necessary. Philipeaux and Vulpian[10,11] are known to be the first to try both nerve allograft and nerve autografts in dogs. They described failure in allograft but some success with nerve autograft. In subsequent decades and with the

[a] Department of Neurosurgery, New York University School of Medicine, 462 First Avenue NB 7S-4, New York, NY 10016, USA
[b] Department of Neurosurgery, University of Michigan School of Medicine, 3552 Taubman Center, 1500 East Medical Center Drive, Ann Arbor, MI 48109, USA
* Corresponding author.
E-mail address: russes01@yahoo.com (S.M. Russell).

Neurosurg Clin N Am 20 (2009) 121–131
doi:10.1016/j.nec.2008.07.026
1042-3680/08/$ – see front matter

casualties of World War I, autografts, xenografts, and allografts were re-evaluated extensively, and in the late 1940s, autogenous nerve grafts were established as the gold standard for nerve repair.[12] This procedure necessitates donor site morbidity, however, and hence other modalities have been explored, one of which is allografts. Peripheral nerve allotransplantation solves the problem of creating another intentional site of morbidity and provides, in theory, a limitless supply of nerve graft material. The first human nerve allotransplantation was performed by Albert in 1885[13] to reconstruct a large median and ulnar nerve gap from an amputated foot and leg. This unsuccessful attempt[14] was followed by many more disappointing results,[15] largely because of the lack of knowledge on transplantation immunology.

In the 1940s, Medawar and colleagues'[16] work on immunologic tolerance laid the groundwork for understanding the mechanism behind graft rejection, and with the advent of microneurosurgical techniques,[17] an interest in nerve allotransplantation was revived. First, various nerve allograft pretreatment and preservation methods were investigated. In the 1960s, systemic immunosuppression was introduced with the first clinical success of azathioprine for renal transplantation. Exploring combinations of different therapeutic modalities (ie, pretreatment and systemic immunosuppression) demonstrated synergistic advantages. Donor-specific tolerance (inducing tolerance specifically to allograft antigens in an otherwise immunocompetent patient) is a very attractive therapeutic modality that is being investigated.

Pretreatment and Storage

Storage methods were of interest, particularly during periods of war, because the possibility to preserve allograft material would provide the surgeon with ample material to treat war casualties in the time of need. With better understanding of the immune response, pretreatment methods aimed to preserve the graft for a length of time and reduce immunogenicity. Pretreatment methods that have been investigated include:

- Irradiation[18,19]
- Cryopreservation (deep-freezing)[20]
- Lyophilization (freeze-drying)[21,22]
- Freeze–thawing (repeated cycles of freezing [-70°C] and thawing [37°C] to render the graft acellular, leaving behind only the Schwann's cell basal lamina as a scaffold)[23]
- Predegeneration (allow donor nerve to degenerate in situ and allow time for Wallerian degeneration to optimize neurotropic factors before harvest)[24,25]

Most of these pretreatment methods reduced antigenicity; however, nerve regeneration remained inferior compared with autografts.[26] Acellularizing the graft was one of the problems. Sustaining viable Schwann's cells until the graft is repopulated with host Schwann's cells is a critical feature in promoting nerve regeneration across the graft. Although short nerve gaps (less than 3 cm) can be bridged by acellular grafts,[27,28] longer defects are dependent on Schwann's cells and the molecular factors they release, which promote nerve regeneration. Cold preservation (at 2°C in Ringer's solution) of nerve allografts was investigated as a means of storage nearly 50 years ago[8,29] and was observed to sustain some viable Schwann's cells while reducing lymphocytic reaction. More recently, Mackinnon and colleagues[30,31] have extensively investigated cold preservation[32] of nerve allografts in University of Wisconsin (UW) storage solution (supplemented with penicillin, insulin, and dexamethasone). Storage at 5°C for a prolonged period (up to 3 weeks) both minimized antigenicity and sustained some viable Schwann's cells in rats.[31] In vitro assays of human nerve grafts revealed that cold preservation in UW storage solution for 7 days was most optimal for sustaining viable Schwann's cells, with decreasing populations of viable cells with storage longer than 7 days.[31] These are promising results for delineating the conditions with which nerve allografts may be optimally pretreated. For longer peripheral nerve defects, however, it remains a problem to sustain graft Schwann's cells until both the regenerating axon and comigrating host Schwann's cells can repopulate the graft. Current investigations addressing this problem include seeding cultured autologous Schwann's cells within nerve allografts.[33] Preliminary studies report significant regeneration along 6 cm nerve defects in a primate model using this paradigm.

Host Immunosuppression

As opposed to pretreatment methods, an alternative strategy is suppressing the host's immunity, which would leave all components of the graft (cells and structural elements, ie, basal lamina) intact and viable. The success of systemic immunosuppression first was demonstrated in renal transplantation, and its success in the 1960s provided a turning point in transplantation surgery. Soon various immunosuppressant therapies were investigated. Borel and colleagues[34] first introduced cyclosporin A to the field as an

immunosuppressant in 1976. Cyclosporin A prevents interleukin (IL)-2 synthesis, a potent T-cell activator and proliferating cytokine, thus generating a nonspecific immunosuppressed state with less lymphotoxicity than most other immunosuppressant's.[35] It soon became the drug of choice for patients who had allografted organs,[36] and subsequently also was tested for nerve allograft experiments. A minimum effective dose and regimen to prevent graft rejection and its ability to allow regeneration through allografts were assayed in systemically immunosuppressed rodent models.[37–40] Histomorphometric, electrophysiologic, and sciatic nerve functional indices of nerve regeneration were assayed in rats grafted with 3 cm long allografts treated with and without daily doses (5 mg/kg/d) of cyclosporin A, and by 14 weeks after grafting, allografts were statistically indistinguishable from syngeneic (equivalent to receiving an autograft) controls.[39] These studies were repeated in primates,[41,42] and also in sheep using longer (8 cm) nerve allograft material.[43] Cyclosporin A successfully demonstrated histologic evidence of axon regeneration in allografts.

Recipients of organ transplants typically receive life-long systemic immunosuppressant therapy. Unlike transplanted visceral organs, however, peripheral nerve allografts are unique. Ideally, the transplanted allograft only is masked for a temporary period with immunosuppression therapy while providing a scaffold and milieu for host axons and cells to repopulate the graft. With this in mind, short-term immunosuppression was investigated, and in fact, found to be sufficient for axons to regenerate and prevent graft rejection.[44–46] Although cyclosporin A appeared effective for preventing graft rejection, it did not enhance nerve regeneration. For long defects, the ideal agent would promote nerve regeneration and have immunosuppressive properties. Thus, other immunosuppressive agents were investigated. One such drug investigated was FK506 (tacrolimus).

FK506, similar to cyclosporin A, inhibits synthesis of IL-2, resulting in systemic nonspecific immunosuppression. FK506 was first tested by Buttemeyer and colleagues[47,48] and reported as a possible alternative to cyclosporin A. It was discovered that FK506 was significantly more effective in stimulating nerve regeneration while concurrently preventing graft rejection.[49] This study was repeated in a swine model with a longer nerve allograft (8 cm), and similar results were found,[50] verifying FK506 as an immunosuppressant and regeneration-enhancing agent. Studies suggest that FK506 stimulates neural regeneration by increasing GAP-43 mRNA,[51] promoting collateral sprouting,[52] and acting as a neurotrophic agent.[53] Studies are being conducted to investigate whether subimmunosuppressive doses of FK506 can enhance axon regeneration rates or nonimmunosuppressive analogs of FK506 would be effective in enhancing axon regeneration.

Combination therapies also have been explored. The most effective combination therapy was apparent with the combination of cold preservation in UW storage solution and systemic immunosuppression using FK506 in a murine model. Doses of FK506 were reduced, and axonal regeneration was observed to be statistically indistinguishable from autografts. Moreover, regeneration exceeded regeneration in nerve autografts, revealing it as a regeneration-enhancing agent.[54]

Clinical Trials

MacKinnon and colleagues[44,55,56] have applied decades of research on nerve allotransplantation to the clinical setting. In their first case report, an 8-year-old boy received 10 cable nerve grafts (23 cm in length) to his left sciatic nerve.[44] He received oral cyclosporin A and oral prednisone for 26 months after surgery, until functional sensibility in the peroneal and posterior tibial nerve distribution was observed. In this case, some sensory recovery was obtained, but no motor recovery was observed. This was attributed to the lengthy defect the motor axons needed to cross to reach the target muscle, which progressively became denervated. In a subsequent clinical study, seven patients (mean age, 15 years; range 3 to 24 years) were managed with allografts or a combination of autografts and allografts by cable grafting techniques.[56] Allografts were matched for donor and recipient blood types, harvested, and preserved in UW storage solution at 5°C for 7 days to minimize antigenicity. Patients were immunosuppressed with either a combination of prednisone, azathioprine, and cyclosporin A (n = 5) or in place of cyclosporin A, FK506 (n = 2) for an average of 18.5 months (range 12 to 26 months). In one patient, rejection of an allograft occurred because of subtherapeutic levels of cyclosporin A. All other patients, however, demonstrated some sensory and/or motor recovery.

PROMOTING REGENERATION WITH ELECTRICAL STIMULATION

All endeavors to enhance peripheral nerve regeneration must deal with the critical issue of time and distance. Regardless of therapeutic modality used to repair an injured nerve, the proximal end of the nerve stump regenerates at a relatively slow rate. This delay is problematic, with recent studies demonstrating an optimal regenerative

(axon) and receptive (target end organ) time window, after which both components progressively lose their capacity to fully recover function.

The effects on functional recovery of prolonged motor neuron axotomy and prolonged muscle denervation were evaluated independently after delayed (up to one year) nerve repair.[57,58] These authors used nerve cross-anastomosis in the rabbit hind limb, an experimental procedure first introduced by Holmes and Young (1942). In brief, to evaluate the ability of chronically axotomized regenerating motor neurons to reinnervate freshly denervated muscle, they first transected the posterior tibial nerve and sutured it to the innervated lateral gastrocnemius muscle, to preclude regeneration. A second surgery was performed after varying time intervals (up to 1 year), where the previously ligated tibial nerve was cross-sutured to the distal end of a cut common peroneal nerve, innervating the tibialis anterior muscle. Alternatively, to evaluate the effect of chronic muscle denervation, the common peroneal nerve was transected, and regeneration was prevented by suturing it to the biceps femoris. The tibialis anterior muscle was left denervated for up to 1 year before the tibial nerve was cut and a tibial-common peroneal cross-anastomosis was performed. Nerve regeneration and muscle reinnervation were evaluated using electrophysiologic and histologic methods to quantify muscle and motor unit forces. Chronically denervated muscles (ie, longer than 6 months) became progressively less receptive to regenerating axons. Theoretically, the lack of neurotrophic factors after a prolonged delay may prevent maintenance of functional contacts or synapses. This translated into a 90% reduction of the number of functional motor units after 6 months of denervation. In addition to the deleterious effects of chronic muscle denervation, the Schwann's cells from the distal nerve branch also progressively become chronically denervated.[59] Typically after nerve injury, the distal Schwann's cells clear the distal pathways of degenerating axon and myelin debris by phagocytosis and digestion. These cells then proliferate in alignment using a configuration known as ''Bands of Bunger'' and await regenerating axons. During this regenerative interim, these Schwann's cells up-regulate a molecular array of growth-associated proteins that promote axon growth.[60] Unfortunately, this period of receptiveness is limited. In vivo studies in rodents reveal that chronically denervated Schwann's cells eventually atrophy and become progressively refractory to the milieu of regenerating axons.[60] After prolonged axotomy, (especially beyond 3 months) a significantly reduced number of motor axons regenerated.[58] The axons that did regenerate, however, tended to reinnervate muscle fibers by expanding their motor unit size. In summary, these studies revealed a limited time period after which functional recovery was limited severely. Thus, efforts should be dedicated to increasing the number of regenerating motor neurons or sustaining the receptiveness of the denervated muscle and Schwann's cells to improve regeneration and functional recovery. In reality, these two phenomena occur in parallel. Moreover, in people, the large distance that axons must traverse to reach the target end organs is prohibitive,[61] further emphasizing the critical issue of time.

Rate of Nerve Regeneration

Interest in the rate of nerve regeneration first was addressed histologically by Cajal[62] and independently described in the clinical setting in 1915 by Tinel[63,64] and Hoffman.[65] In the 1940s, a more complete and systematic study of the rate of nerve regeneration in rabbits[66] and people[67] was investigated. Regeneration is described as occurring in three steps:[67]

(1) An initial delay at the suture or scar site (comprising the latent or lag period).
(2) Mature axons traversing the distance toward the end organ.
(3) The time required for the fibers to reestablish functionality at the muscle or skin.

Although it generally is accepted that nerves regenerate at a rate of 1 mm/d, recent studies have demonstrated a much more protracted period of regeneration.[68] In a rodent model, the femoral nerve was transected and repaired, and the muscle nerve branch was back-labeled with neurotracers after a period of 2 to 10 weeks to identify regenerating motor neurons. The neurotracers were applied 25 mm from the original repair site. If all nerves regenerated at 1 mm/d, by 4 weeks, most of the regenerating motor neurons should be back-labeled. Surprisingly, 8 to 10 weeks passed before most of the motor neurons traversed the length of the defect. This dramatic result was putatively attributed to characteristic collateral sprouting that occurs with regenerating axons.[69] These sprouts appear to emerge from nearby nodes of Ranvier.[70,71] As nutrient resources are distributed down each of these sprouts, the rate of regeneration may be slowed until these collaterals are pruned, and material is focused down the regenerating parent axon. Second, the suture site, where there is considerable scarring, appears to significantly contribute to this delay.[66,72,73] Axons regenerate

asynchronously across this suture site, and a significant amount of wandering, both laterally and retrograde into the proximal nerve stump, occurs at the scarred region before axon collaterals enter a distal endoneurial tube.[62,74] Studies demonstrated a differential regenerative ability among motor neurons, with only 25% of motor axons traversing the suture site by 7 days. This largely asynchronous and variable ability of motor axons to regenerate has been described as staggered regeneration.[75–77]

In efforts to promote regeneration and compress this period of delay, the influence of electrical stimulation in promoting axon growth has been evaluated.[68,73,78] Results revealed that the delay in nerve regeneration could be reduced to 3 weeks after 1 hour of 20 Hz continuous electrical stimulation, in striking contrast to the 8 to 10 weeks required without electrical stimulation.[68] Using neurotracers to label regenerated axons that had just crossed the surgical repair site, a follow-up study demonstrated an accelerated recruitment of regenerating motor axons across the injury site when electrical stimulation was used. There was, however, no increase in the rate of slow axonal transport, which reflects the regeneration rate.[73] When tetrodotoxin was applied to prevent action potential transmissions, the effects of electrical stimulation on regeneration vanished, suggesting the mechanism was mediated in the cell body, perhaps by gene transcription regulation.[68] Using semiquantitative in situ hybridization, indeed a more rapid and robust mRNA expression of the neurotrophin, brain-derived neurotrophic factor (BDNF) and its high-affinity receptor trkB were observed in regenerating motor axons after 1 hour of 20 Hz electrical stimulation, in contrast to controls not receiving electrical stimulation.[79] Moreover, this transcriptional response was followed by an increase in mRNA expression levels of regeneration-associated genes (eg, alpha1-tubulin and GAP-43), which also were elevated significantly by electrical stimulation compared with controls. This result suggests that BDNF/trkB signaling regulates gene expression of cytoskeletal proteins, which acts to promote the outgrowth of growth cones in regenerating motor axons.[80]

Reinnervation Specificity

These studies also addressed another critical issue that frequently complicates complete recovery: the misdirection of regenerating motor and sensory nerves. This random reinnervation, which contributes to poor functional recovery has been documented both experimentally[81] and clinically.[82] Using the femoral nerve paradigm and a double-retrograde labeling technique, Brushart and Seiler[75,77] investigated the specificity with which motor axons regenerated toward their targets. Surprisingly, given equal access to motor and sensory pathways, regenerating motor axons appeared to selectively reinnervate motor pathways. During early stages of regeneration (3 to 4 weeks), motor axons sent an equal number of collaterals to both motor and sensory pathways. During the later stages of regeneration (8 to 10 weeks), however, most motor axons were observed to reinnervate motor pathways, with fewer in the sensory pathways. This pattern was termed preferential motor reinnervation. This specificity is attributed to a pruning mechanism.[77] Motor axons incorrectly innervating sensory pathways somehow are recognized and pruned, whereas motor axons correctly innervating motor pathways are maintained. This specificity was observed even without the presence of the target end organ, suggesting that cues from the regenerating axons and Schwann's cells may be more critical for the emergence of preferential motor reinnervation.[76] The authors suggested that Schwann's cells maintained specific identities associated with their previously innervating axon type and thus were able to promote or maintain the same type of regenerating axon selectively. This was supported further by observing a differential ability of sensory axon-sheathing Schwann's cells versus motor axon-sheathing Schwann's cells to express a carbohydrate epitope (L2/HNK-1 carbohydrate; labeled by anti-L2 and anti-HNK-1 antibodies) when approached by regenerating motor axons. L2/HNK-1 carbohydrate rarely is expressed on Schwann's cells sheathing intact sensory axons, and when incorrectly reinnervated by motor axons, these Schwann's cells previously sheathing sensory axons only weakly express L2 carbohydrate. In contrast, robust L2/HNK-1 carbohydrate expression is seen on Schwann's cells previously innervating motor axons.[83] L2/HNK-1 carbohydrate acting as a possible motor axon specific recognition molecule or having a positive effect on regeneration is consistent with other studies. Schwann's cell-derived L2 promotes neurite outgrowth from motor neuron cultures[84] and is expressed selectively on Schwann's cells of motor axons but not sensory axons.[85] This may provide a mechanism by which regenerating motor axons may be maintained selectively in motor pathways. Moreover, brief electrical stimulation also appeared to improve the accuracy with which motor axons regenerated motor pathways. With brief electrical stimulation, the progressive increase in correctly reinnervating motor pathways was

apparent by 3 weeks versus 8 weeks without electrical stimulation.[68] Electrical stimulation also increased L2/HNK-1 expression in the motor branch but appeared to have no such effect in the regenerating cutaneous branch.[86] This finding correlates with earlier and increased expression of BDNF and TrkB induced by electrical stimulation, suggesting BDNF/TrkB signaling modulates L2/HNK-1 expression in Schwann's cells, influencing preferential motor reinnervation.[86]

These studies demonstrate a potential clinical role for brief electrical stimulation in nerve repair. It appears to promote nerve regeneration across the surgical suture site and thus to decrease the time required for axons to traverse the length of the defect. Clearly, this is an advantage, as axons would reach the distal Schwann's cell tubes and muscles faster, lessening the effect of chronic denervation.[57,58] Gordon and colleagues[87,88] recently translated these findings from rat models to the clinical setting in a randomized– controlled trial of 21 people diagnosed with moderate-to-severe carpal tunnel syndrome who underwent operative carpal tunnel release. The number of reinnervated motor units in the median nerve-innervated thenar muscle before and after carpal tunnel release surgery was measured at varying intervals over the course of 12 months in subjects who had received low-frequency electrical stimulation for 1 hour immediately after the operation. Patients who received electrical stimulation after the surgery demonstrated a significant increase in motor axon regeneration in contrast to control patient groups, measured by the motor unit number estimates (MUNE). Associated with this result was an improvement in manual dexterity (Purdue Pegboard Test), reduced symptom severity (Levine Carpal Tunnel Syndrome Questionnaire), and improved sensation (Semmes-Weinstein Monofilaments).[87] These initial studies demonstrate the feasibility of clinically applying postsurgical electrical stimulation in people to accelerate and thus improve axon regeneration and functional outcomes in a crush injury.

ADVANCES IN NEUROSCIENCE RELEVANT TO PERIPHERAL NERVE

In vivo imaging of the nervous system recently began a new and exciting direction of study, which now enables practitioners to directly observe dynamic behavior of individual cells of the nervous system with higher temporal and spatial resolution. The coadvancement of optical microscopy and sophisticated mouse genetics now allows practitioners to directly observe the nervous system at the level of single cells in their native milieu.[89–92] The recent generation of sophisticated transgenic mouse lines (Thy-1-XFP mice), which selectively express spectral variants of green fluorescent protein in neurons, have provided a new tool to study the nervous system. In these mice, neurons selectively express fluorescent proteins by direct control of a modified Thy-1 promoter.[91] In some of the Thy-1-XFP lines, because of the positional effects of gene insertion, the transgene expression is restricted to a smaller percentage of neurons, which is advantageous for some studies, in particular the densely innervated central nervous system. These lines are called subset lines (ie, Thy-1-YFP-H, Thy-1-CFP-S).[93] These transgenic mice enable practitioners to visualize and reconstruct axons in their entirety,[94] accurately identify the same axon over time,[95,96] and directly observe their dynamism in their native milieu.[97] Furthermore, by crossing mice expressing different colors of fluorescent proteins in a subset of their neurons, interactions between the two different cells are subject to analysis.[96] Previously, with single-color labeling techniques, such analysis was impossible. The discovery of the ability to genetically alter mice to visualize a specific cell type led to the generation of many other transgenic lines, which now include:

- Schwann's cells under the S100-promoter[98]
- Oligodendrocytes under the PLP-promoter[99]
- Macrophages and microglial cells by knocking in green fluorescent protein for the chemokine receptor CX_3CR1[100]
- Mitochondria (exclusively in neurons) under the Thy-1-coxVIII (mitochondrial targeting sequence from human cytochrome oxidase subunit VIII) promoter[91,101]

The ability to directly visualize these specific cell types that comprise the nervous system are an invaluable tool for providing insight into the diseased nervous system (eg, axonal regeneration and degeneration).

One of the primary advantages of using these mice is the ability to observe dynamism among the same cells in their native environment over time. A sequence of events in a cellular process can be observed directly rather than extrapolated from single time points using different animals. One study that exemplifies these advantages examined the fidelity with which axons after traumatic crush injury reinnervated the same target muscle fibers.[102] The advantages of using Thy-1 mice for this particular study are manifold. First, because of the bright fluorescence expressed by the axon, an injury inflicted upon the axon is confirmed directly by the lack of fluorescence with

relative ease.[103] Second, the axon of interest is identified quickly and easily at each time point over a period of several days to a month to observe the reinnervation events. This study revealed that after a crush injury, axons reinnervated the same muscle fibers with remarkable precision, and the regenerating axons even branched at the same original branching points. Presumably the endoneurial tubes were intact after such a crush injury. In contrast, after complete transection of the axon (both axons and endoneurial tubes are discontinuous), not only was reinnervation of the muscle fibers incomplete, but regenerating axons were misrouted, often reinnervating synapses that were reinnervated previously by other motor axons. These observations emphasize that in addition to molecular cues, mechanical confinements play a role in directional specificity during regeneration of peripheral motor axons.[102]

Direct imaging of peripheral axons in real time after axotomy has provided more accurate assays of studying degenerating and regenerating nerves.[101] For instance, one now can measure axonal transport rates. Recent advances in visualizing neuronal mitochondrial dynamics in living mice (and hence measure organelle transport in axons) in real time has been accomplished with the generation of new lines of transgenic mice that express fluorescent protein exclusively in axonal mitochondria.[101] The unique polarity of neurons requires these highly specialized cells to have mechanisms that tightly regulate the allocation of nutrients and organelles to axonal branches situated far away from the cell body. Misgeld and colleagues[101] examined changes in axonal transport that accompany axon regeneration in acutely explanted peripheral nerve–muscle preparations. A robust increase in anterograde transport of mitochondria to the proximal end of the transected axon was observed even before visual evidence of axon regeneration. As growth cones advanced, mitochondria rapidly repopulated the distal-most region of the growth cone. This sudden increase in the anterograde transport rate was maintained for 48 hours and only declined minimally in the ensuing weeks. These results suggest the possibility of detecting axonal pathology earlier with changes in transport rates and may reflect a cell body response.[104] Axotomized peripheral neurons undergo immediate changes that are visible at the somal level and collectively are called chromatolysis. It is possible that these changes also are reflected in mitochondria rate of transport. Mitochondria are trafficked to areas of the axon where metabolic demand is high, such as actively growing axons.[105–110] Noting the changes in the rate of transport of organelles is now possible with these transgenic mice and in vivo imaging techniques, which are sensitive enough to acquire images in real-time.

SUMMARY

Currently, there is an emphasis in translating the knowledge of neurobiology obtained in the laboratory into practical clinical applications. The successful transition and application of both nerve allotransplantation and brief electrical stimulation into the clinical arena are examples of how this ultimate goal can be achieved. The vast enrichment of knowledge in the fields of immunology, neurobiology, molecular biology, and imaging techniques over the years provides mechanistic understanding, which is ultimately fundamental for improving potential clinical therapies. New tools generated by basic investigations, such as the Thy-1-XFP transgenic lines, already have become invaluable tools for investigating clinical questions in the field of peripheral nerve regeneration. Other exciting avenues of peripheral nerve regeneration research not discussed in this article include neural stem cells, tissue engineering, neurotrophic factors and pharmacologic agents, and neuroprosthetics. A multidisciplinary approach to research will prove most successful in the evolution of clinical applications with the goal of improving functional outcomes after nerve repair.

REFERENCES

1. Sunderland S. The intraneural topography of the radial, median, and ulnar nerves. Brain 1945;68: 243–98.
2. Sunderland S, Ray LJ. The intraneural topography of the sciatic nerve and its popliteal divisions in man. Brain 1948;71:242–73.
3. Young JZ. Introduction. In: Dyck PJ, Thomas PK, editors. Peripheral neuropathy. 3rd edition. Philadelphia: W.B. Saunders; 1993. p. 2–5.
4. Jensen JN, Mackinnon SE. Composite tissue allotransplantation: a comprehensive review of the literature—part 1. J Reconstr Microsurg 2000;16:57–68.
5. Jensen JN, Mackinnon SE. Composite tissue allotransplantation: a comprehensive review of the literature—part II. J Reconstr Microsurg 2000;16:141–57.
6. Jensen JN, Mackinnon SE. Composite tissue allotransplantation: a comprehensive review of the literature—part III. J Reconstr Microsurg 2000;16:235–51.
7. Richardson MH. Operations on nerves. Boston Medical and Surgical Journal 1886;115:368.
8. Schuller M. Die Verwendung der Nervendehnung zur operativen Heilung von Ubstanzverlusten am Nerven. Wien Med Presse 1888;29:145–52.

9. Stookey B. The futility of bridging nerve defects by means of nerve flaps. Surg Gynecol Obstet 1919; 29:287–311.
10. Philipeaux JM, Vulpian A. Recherches experimentales sur la reunion bout a bout de nerfs de fonctions differentes. Jounral Physiologie de L'Homme et L'Animal 1863;6:474–516.
11. Philipeaux JM, Vulpian A. Note sur des essais de greffe d'un troncon du nerf lingual entre les deux bouts du nerf hypoglosse, apres excision d'un segment de ce dernier nerf. Arch Physiol Norm Pathol 1870;3:618–20.
12. Seddon HJ. The use of autogenous grafts for the repair of large gaps in peripheral nerves. Br J Surg 1947;35:151–67.
13. Albert E. Einige operationen am nerven. Wien Med Presse 1885;26:1285.
14. Huber GC. A study of the operative treatment for loss of nerve substance in peripheral nerves. J Morphol 1895;11:629–735.
15. Sanders FK. The repair of large gaps in the peripheral nerves. Brain 1942;65:281–337.
16. Billingham RE, Brent L, Medawar PB. Actively acquired tolerance of foreign cells. Nature 1953; 172:603–6.
17. Millesi H. Microsurgical nerve grafting. Int Surg 1980;65:503–8.
18. Marmor L, Foster JM, Carlson GJ, et al. Experimental irradiated nerve heterografts. J Neurosurg 1966; 24:656–66.
19. Easterling KJ, Trumble TE. The treatment of peripheral nerve injuries using irradiated allografts and temporary host immunosuppression (in a rat model). J Reconstr Microsurg 1990;6:301–7.
20. Zalewski AA, Gulati AK. Evaluation of histocompatibility as a factor in the repair of nerve with a frozen nerve allograft. J Neurosurg 1982;56:550–4.
21. Weiss P. Functional nerve regeneration through frozen-dried nerve grafts in cats and monkeys. Proc Soc Exp Biol Med 1943;54:277–9.
22. Weiss P, Taylor C. Repair of peripheral nerves by grafts of frozen-dried nerve. Proc Soc Exp Biol Med 1943;52:326–8.
23. Wang GY, Hirai K, Shimada H. The role of laminin, a component of Schwann's cell basal lamina, in rat sciatic nerve regeneration within antiserum-treated nerve grafts. Brain Res 1992;570:116–25.
24. Bentley FH, Hill M. Nerve grafting. Br J Surg 1936; 24:368–87.
25. Trumble TE. Peripheral nerve transplantation: the effects of predegenerated grafts and immunosuppression. J Neural Transplant Plast 1992;3: 39–49.
26. Mackinnon SE, Hudson AR, Falk RE, et al. Peripheral nerve allograft: an assessment of regeneration across pretreated nerve allografts. Neurosurgery 1984;15:690–3.
27. Strauch B, Ferder M, Lovelle-Allen S, et al. Determining the maximal length of a vein conduit used as an interposition graft for nerve regeneration. J Reconstr Microsurg 1996;12:521–7.
28. Sanders FK, Young JZ. The degeneration and reinnervation of grafted nerves. J Anat 1942;76: 143–66.
29. Gutmann E, Sanders FK. Recovery of fibre numbers and diameters in the regeneration of peripheral nerves. J Physiol 1943;101:489–518.
30. Evans PJ, Mackinnon SE, Best TJ, et al. Regeneration across preserved peripheral nerve grafts. Muscle Nerve 1995;18:1128–38.
31. Levi AD, Evans PJ, Mackinnon SE, et al. Cold storage of peripheral nerves: an in vitro assay of cell viability and function. Glia 1994;10:121–31.
32. Belzer FO, Southard JH. Principles of solid-organ preservation by cold storage. Transplantation 1988;45:673–6.
33. Hess JR, Brenner MJ, Fox IK, et al. Use of cold-preserved allografts seeded with autologous Schwann's cells in the treatment of a long-gap peripheral nerve injury. Plast Reconstr Surg 2007; 119:246–59.
34. Borel JF, Feurer C, Gubler HU, et al. Biological effects of cyclosporin A: a new antilymphocytic agent. Agents Actions 1976;6:468–75.
35. Ruhlmann A, Nordheim A. Effects of the immunosuppressive drugs CsA and FK506 on intracellular signaling and gene regulation. Immunobiology 1997;198:192–206.
36. Cohen DJ, Loertscher R, Rubin MF, et al. Cyclosporine: a new immunosuppressive agent for organ transplantation. Ann Intern Med 1984;101:667–82.
37. Zalewski AA, Gulati AK. Survival of nerve allografts in sensitized rats treated with cyclosporin A. J Neurosurg 1984;60:828–34.
38. Zalewski AA, Gulati AK. Failure of cyclosporin A to induce immunological unresponsiveness to nerve allografts. Exp Neurol 1984;83:659–63.
39. Bain JR, Mackinnon SE, Hudson AR, et al. The peripheral nerve allograft: an assessment of regeneration across nerve allografts in rats immunosuppressed with cyclosporin A. Plast Reconstr Surg 1988;82: 1052–66.
40. Bain JR, Mackinnon SE, Hudson AR, et al. The peripheral nerve allograft: a dose–response curve in the rat immunosuppressed with cyclosporin A. Plast Reconstr Surg 1988;82:447–57.
41. Bain JR, Mackinnon SE, Hudson AR, et al. The peripheral nerve allograft in the primate immunosuppressed with cyclosporin A: I. Histologic and electrophysiologic assessment. Plast Reconstr Surg 1992;90:1036–46.
42. Fish JS, Bain JR, McKee N, et al. The peripheral nerve allograft in the primate immunosuppressed with cyclosporin A: II. Functional evaluation of

reinnervated muscle. Plast Reconstr Surg 1992;90: 1047–52.
43. Matsuyama T, Midha R, Mackinnon SE, et al. Long nerve allografts in sheep with cyclosporin A immunosuppression. J Reconstr Microsurg 2000;16:219–25.
44. Mackinnon SE, Midha R, Bain J, et al. An assessment of regeneration across peripheral nerve allografts in rats receiving short courses of cyclosporin A immunosuppression. Neuroscience 1992;46:585–93.
45. Midha R, Mackinnon SE, Evans PJ, et al. Comparison of regeneration across nerve allografts with temporary or continuous cyclosporin A immunosuppression. J Neurosurg 1993;78:90–100.
46. Atchabahian A, Doolabh VB, Mackinnon SE, et al. Indefinite survival of peripheral nerve allografts after temporary cyclosporin A immunosuppression. Restor Neurol Neurosci 1998;13:129–39.
47. Buttemeyer R, Jones NF, Rao UN. Peripheral nerve allotransplant immunosuppressed with FK 506: preliminary results. Transplant Proc 1995;27: 1877–8.
48. Buttemeyer R, Rao U, Jones NF. Peripheral nerve allograft transplantation with FK506: functional, histological, and immunological results before and after discontinuation of immunosuppression. Ann Plast Surg 1995;35:396–401.
49. Myckatyn TM, Ellis RA, Grand AG, et al. The effects of rapamycin in murine peripheral nerve isografts and allografts. Plast Reconstr Surg 2002;109: 2405–17.
50. Jensen JN, Brenner MJ, Tung TH, et al. Effect of FK506 on peripheral nerve regeneration through long grafts in inbred swine. Ann Plast Surg 2005; 54:420–7.
51. Gold BG, Yew JY, Zeleny-Pooley M. The immunosuppressant FK506 increases GAP-43 mRNA levels in axotomized sensory neurons. Neurosci Lett 1998;241:25–8.
52. Udina E, Ceballos D, Gold BG, et al. FK506 enhances reinnervation by regeneration and by collateral sprouting of peripheral nerve fibers. Exp Neurol 2003;183:220–31.
53. Steiner JP, Connolly MA, Valentine HL, et al. Neurotrophic actions of nonimmunosuppressive analogues of immunosuppressive drugs FK506, rapamycin, and cyclosporin A. Nat Med 1997;3:421–8.
54. Grand AG, Myckatyn TM, Mackinnon SE, et al. Axonal regeneration after cold preservation of nerve allografts and immunosuppression with tacrolimus in mice. J Neurosurg 2002;96:924–32.
55. Mackinnon SE. Nerve allotransplantation following severe tibial nerve injury. Case report. J Neurosurg 1996;84:671–6.
56. Mackinnon SE, Doolabh VB, Novak CB, et al. Clinical outcome following nerve allograft transplantation. Plast Reconstr Surg 2001;107:1419–29.
57. Fu SY, Gordon T. Contributing factors to poor functional recovery after delayed nerve repair: prolonged denervation. J Neurosci 1995;15:3886–95.
58. Fu SY, Gordon T. Contributing factors to poor functional recovery after delayed nerve repair: prolonged axotomy. J Neurosci 1995;15:3876–85.
59. Sulaiman OA, Gordon T. Effects of short- and long-term Schwann's cell denervation on peripheral nerve regeneration, myelination, and size. Glia 2000;32:234–46.
60. Hall SM. The biology of chronically denervated Schwann's cells. Ann N Y Acad Sci 1999;883:215–33.
61. Hoke A. Mechanisms of disease: what factors limit the success of peripheral nerve regeneration in humans? Nat Clin Pract Neurol 2006;2:448–54.
62. Ramon Y, Cajal S. Degeneration and regeneration of the nervous system [reprinted] 1991 edition. Oxford (UK): Oxford University Press; 1928.
63. Tinel J. Le signe du fourmillementdans les lésions des nerfs périphériques. Presse Medicale 1915; 47:388.
64. Tinel J. Tingling signs with peripheral nerve injuries. 1915. J Hand Surg [Br] 2005;30:87–9.
65. Hoffmann P, Buck-Gramcko D, Lubahn JD. The Hoffmann-Tinel sign. 1915. J Hand Surg [Br] 1993;18:800–5.
66. Gutmann E, Guttmann L, Medawar PB, et al. The rate of regeneration of nerve. J Exp Biol 1942;19: 14–44.
67. Seddon HJ, Medawar PB, Smith H. Rate of regeneration of peripheral nerves in man. J Physiol 1943; 102:191–215.
68. Al-Majed AA, Neumann CM, Brushart TM, et al. Brief electrical stimulation promotes the speed and accuracy of motor axonal regeneration. J Neurosci 2000;20:2602–8.
69. Aitken JT, Sharman M, Young JZ. Maturation of regenerating nerve fibres with various peripheral connexions. J Anat 1947;81:1–22.
70. Morris JH, Hudson AR, Weddell G. A study of degeneration and regeneration in the divided rat sciatic nerve based on electron microscopy. IV. Changes in fascicular microtopography, perineurium, and endoneurial fibroblasts. Z Zellforsch Mikrosk Anat 1972;124:165–203.
71. MacKinnon SE, Dellon AL, O'Brien JP. Changes in nerve fiber numbers distal to a nerve repair in the rat sciatic nerve model. Muscle Nerve 1991;14: 1116–21.
72. Forman DS, Wood DK, DeSilva S. Rate of regeneration of sensory axons in transected rat sciatic nerve repaired with epineurial sutures. J Neurol Sci 1979;44:55–9.
73. Brushart TM, Hoffman PN, Royall RM, et al. Electrical stimulation promotes motoneuron regeneration without increasing its speed or conditioning the neuron. J Neurosci 2002;22:6631–8.

74. Witzel C, Rohde C, Brushart TM. Pathway sampling by regenerating peripheral axons. J Comp Neurol 2005;485:183–90.
75. Brushart TM, Seiler WA 4th. Selective reinnervation of distal motor stumps by peripheral motor axons. Exp Neurol 1987;97:289–300.
76. Brushart TM. Motor axons preferentially reinnervate motor pathways. J Neurosci 1993;13:2730–8.
77. Brushart TM, Gerber J, Kessens P, et al. Contributions of pathway and neuron to preferential motor reinnervation. J Neurosci 1998;18:8674–81.
78. Nix WA, Hopf HC. Electrical stimulation of regenerating nerve and its effect on motor recovery. Brain Res 1983;272:21–5.
79. Al-Majed AA, Brushart TM, Gordon T. Electrical stimulation accelerates and increases expression of BDNF and trkB mRNA in regenerating rat femoral motoneurons. Eur J Neurosci 2000;12:4381–90.
80. Al-Majed AA, Tam SL, Gordon T. Electrical stimulation accelerates and enhances expression of regeneration-associated genes in regenerating rat femoral motoneurons. Cell Mol Neurobiol 2004;24:379–402.
81. Brushart TM, Mesulam MM. Alteration in connections between muscle and anterior horn motoneurons after peripheral nerve repair. Science 1980;208:603–5.
82. Thomas CK, Stein RB, Gordon T, et al. Patterns of reinnervation and motor unit recruitment in human hand muscles after complete ulnar and median nerve section and resuture. J Neurol Neurosurg Psychiatr 1987;50:259–68.
83. Martini R, Schachner M, Brushart TM. The L2/HNK-1 carbohydrate is preferentially expressed by previously motor axon-associated Schwann's cells in reinnervated peripheral nerves. J Neurosci 1994; 14:7180–91.
84. Martini R, Xin Y, Schmitz B, et al. The L2/HNK-1 carbohydrate epitope is involved in the preferential outgrowth of motor neurons on ventral roots and motor nerves. Eur J Neurosci 1992;4:628–39.
85. Martini R, Bollensen E, Schachner M. Immunocytological localization of the major peripheral nervous system glycoprotein P0 and the L2/HNK-1 and L3 carbohydrate structures in developing and adult mouse sciatic nerve. Dev Biol 1988;129:330–8.
86. Eberhardt KA, Irintchev A, Al-Majed AA, et al. BDNF/TrkB signaling regulates HNK-1 carbohydrate expression in regenerating motor nerves and promotes functional recovery after peripheral nerve repair. Exp Neurol 2006;198:500–10.
87. Gordon T, Amirjani N, Jones KE, et al. Brief electrical stimulation accelerates axon regeneration and muscle reinnervation in humans. Neuroscience Meeting Planner, Online: Society for Neuroscience; 200. Program No. 872.9/D16.
88. Gordon T, Brushart TM, Amirjani N, et al. The potential of electrical stimulation to promote functional recovery after peripheral nerve injury—comparisons between rats and humans. Acta Neurochir Suppl 2007;100:3–11.
89. Conchello JA, Lichtman JW. Optical sectioning microscopy. Nat Methods 2005;2:920–31.
90. Lichtman JW, Conchello JA. Fluorescence microscopy. Nat Methods 2005;2:910–9.
91. Misgeld T, Kerschensteiner M. In vivo imaging of the diseased nervous system. Nat Rev Neurosci 2006;7:449–63.
92. Helmchen F, Denk W. Deep tissue two-photon microscopy. Nat Methods 2005;2:932–40.
93. Feng G, Mellor RH, Bernstein M, et al. Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. Neuron 2000;28: 41–51.
94. Keller-Peck CR, Walsh MK, Gan WB, et al. Asynchronous synapse elimination in neonatal motor units: studies using GFP transgenic mice. Neuron 2001;31:381–94.
95. Kerschensteiner M, Schwab ME, Lichtman JW, et al. In vivo imaging of axonal degeneration and regeneration in the injured spinal cord. Nat Med 2005;11:572–7.
96. Walsh MK, Lichtman JW. In vivo time-lapse imaging of synaptic takeover associated with naturally occurring synapse elimination. Neuron 2003;37:67–73.
97. Bishop DL, Misgeld T, Walsh MK, et al. Axon branch removal at developing synapses by axosome shedding. Neuron 2004;44:651–61.
98. Zuo Y, Lubischer JL, Kang H, et al. Fluorescent proteins expressed in mouse transgenic lines mark subsets of glia, neurons, macrophages, and dendritic cells for vital examination. J Neurosci 2004; 24:10999–1009.
99. Hirrlinger PG, Scheller A, Braun C, et al. Expression of reef coral fluorescent proteins in the central nervous system of transgenic mice. Mol Cell Neurosci 2005;30:291–303.
100. Jung S, Aliberti J, Graemmel P, et al. Analysis of fractalkine receptor CX_3CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. Mol Cell Biol 2000;20:4106–14.
101. Misgeld T, Kerschensteiner M, Bareyre FM, et al. Imaging axonal transport of mitochondria in vivo. Nat Methods 2007;4:559–61.
102. Nguyen QT, Sanes JR, Lichtman JW. Pre-existing pathways promote precise projection patterns. Nat Neurosci 2002;5:861–7.
103. Steward O, Zheng B, Tessier-Lavigne M. False resurrections: distinguishing regenerated from spared axons in the injured central nervous system. J Comp Neurol 2003;459:1–8.
104. Baloh RH. Mitochondrial dynamics and peripheral neuropathy. Neuroscientist 2008;14:12–8.
105. Povlishock JT. The fine structure of the axons and growth cones of the human fetal cerebral cortex. Brain Res 1976;114:379.

106. Chada SR, Hollenbeck PJ. Mitochondrial movement and positioning in axons: the role of growth factor signaling. J Exp Biol 2003;206:1985–92.
107. Hollenbeck PJ, Saxton WM. The axonal transport of mitochondria. J Cell Sci 2005;118:5411–9.
108. Pan YA, Misgeld T, Lichtman JW, et al. Effects of neurotoxic and neuroprotective agents on peripheral nerve regeneration assayed by time-lapse imaging in vivo. J Neurosci 2003;23:11479–88.
109. Hayashi A, Koob JW, Liu DZ, et al. A double-transgenic mouse used to track migrating Schwann's cells and regenerating axons following engraftment of injured nerves. Exp Neurol 2007; 207:128–38.
110. Magill CK, Tong A, Kawamura D, et al. Reinnervation of the tibialis anterior following sciatic nerve crush injury: a confocal microscopic study in transgenic mice. Exp Neurol 2007;207:64–74.