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REVIEWS

Advances in Peripheral Nerve Regeneration: Materials, Methods, Techniques

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Abstract

Peripheral nerve injuries have a high incidence in limb trauma and have a devastating impact on the quality of life of the patients. Microsurgical repair of nerves remains the gold standard in severed nerves, but outcomes remain unsatisfactory although this technique has been refined in the last five to six decades. Current medical practice dictates the need for the development and application of novel adjuvant techniques to address the field. Nerve protecting materials, nerve guide conduits (NGCs), autologous nerve conduits, pharmacological agents, growth factor therapies, stem cell therapies, low current nerve stimulation, tissue glue, photochemical tissue bonding are all valuable directions of research with encouraging results. In this review, we are trying to summarize the benefits of each technique and to point out the necessity of a multimodal approach to peripheral nerve regeneration and the opportunity for clinical translation of all the abundant research in current literature.

Keywords: peripheral nerve regeneration, nerve repair, microsurgery, nerve guide conduits, growth factors, stem cells.

Rezumat

Incidența leziunilor nervilor periferici în traumatismele membrelor este crescută și are un impact devastator asupra calității vieții pacienților. Repararea microchirurgicală a nervilor periferici rămâne standardul de aur în cazul nervilor sectionați, dar rezultatele acestor proceduri rămân nesatisfăcătoare în pofida faptului că aceste tehnici au fost continuu rafinate pe parcursul ultimelor șase decenii. Practica medicală actuală dictează necesitatea dezvoltării și aplicării unor tehnici noi și complementare în acest domeniu. Materiale care protejează nervul, tuburi pentru ghidarea regenerării nervilor, tuburi din țesuturi autoloage, medicamente, terapii cu factori de creștere, terapii cu celule stem, stimulare folosind curent de joasă frecvență, adezivi tisulari, adezivi tisulari activați fotochimic reprezintă direcții importante de cercetare cu rezultate încurajatoare. În această lucrare de revizuire a literaturii, autorul încearcă să sumarizeze beneficiile fiecărei tehnici în parte și să sublinieze necesitatea unei abordări multimodale a domeniului regenerării nervilor periferici, dar și necesitatea translației acestor tehnici, larg tratate în literatură, în practica clinică.

Cuvinte cheie: regenerarea nervilor periferici, repararea nervilor, microchirurgie, tuburi pentru ghidarea regenerării nervilor, factori de creștere, celule stem

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INTRODUCTION

Peripheral nerve injuries have a high incidence in limb trauma and have a devastating impact on the quality of life of the patient. Results after surgical treatment are unsatisfactory especially in cases with complete transection of the nerve (neurotmesis) associated with late repair. Thus, the necessity to implement into current practice new techniques, materials and methods for nerve repair.

The majority of peripheral nerve injuries occur in the upper limb and are from traumatic causes. These injuries disproportionately afflict young healthy civilians and military officers who are most at risk of traumatic injuries[1]. Out of 16 million insureds in the database, 220,593 (1.4%) were diagnosed with limb trauma. Eighty-three percent of the patients were less than 55 years old, and 50% were male. The total incidence of nerve injuries within 90 days of upper- or lower-limb trauma was 1.64%. The type of extremity trauma with the highest incidence of nerve injury within 90 days of the diagnosis was a crush injury at 1.9%. Approximately 50% of our sample was selected because of a dislocation, which had an associated nerve injury prevalence of 1.46%[2].

Short history

The study of nerve repair and regeneration potential dates back to ancient times namely to Galen (131-201). Paul of Aegina (525-690) was the first to describe approximation of the nerve ends with wound closure. The first peripheral nerve repair was performed by William de Saliceto in Bologna (1201-1277). In 1850, Waller describes the physiology of distal nerve degeneration. After 24 years, Ranvier further describes the pathophysiology of peripheral nerves and, in 1876, he demonstrates axonal regeneration at the proximal stump of the transected nerve. His studies lie at the forefront of the surgical repair of peripheral nerves. Hueter (1871, 1873) introduced the concept of primary epineurial nerve suture, and Nelaton described secondary nerve repair in 1864. Even at an early time, the idea of decreasing tension on the nerve suture was important.

In 1882, Mikulicz described sutures that reduced tension, and Loebke described bone shortening to decrease nerve tension in 1884. In 1876, Albert described grafting nerve gaps.

World War I and World War II brought about invaluable experiences in terms of classification of nerve injuries and surgical interventions, at the same time, sieving out unreliable techniques and leading to the modern era of direct nerve repair[3].

In 1966, Millessi and Samii pointed out the devastating effect of the presence of tension at the nerve repair site and advise the use of interposition nerve grafts.

In 1985, Rose and Kowalski use vascularized nerve graft by the arterialization of a satellite vein (neurovenous graft) for the repair of digital artery and nerve defects.

In 1985, M. Merle and De Medinaceli report their first results concerning brachial plexus surgery using nervous graft and tissular sealants.

In 1989, MacKinnon and Delon use bioabsorbable polyglicolic acid microtubes for digital nerve repair.

NERVE INJURY

Traumatized peripheral nerves are characterized by specific changes both proximal and distal to the site of injury. Proximally, axons retract a variable distance, and, after a brief period of quiescence, elongate as a hydralike regenerating unit in which a single parent axon gives rise to multiple daughter axons. In myelinated nerves, axons sprout at unsheathed gaps known as the nodes of Ranvier, and progress to their sensory or motor targets. Once a functional synapse is made, the remaining daughter axons degenerate, or are "pruned back." In the distal nerve segment, Schwann cells, fibroblasts, myocytes, and injured axons express a host of neurotrophic factors, including glial and brain-derived neurotrophic factors at discrete concentrations and time points as the degrading neural elements are phagocytosed in a process termed wallerian degeneration. Schwann cells assume a pro-regenerative phenotype instrumental in remyelinating and guiding regenerating axons to their appropriate targets along residual endoneurial tubes known as the bands of Bungner. Neurotrophism, which literally means "food for nerves", is the ability of neurotrophins secreted in an autocrine or paracrine fashion to enhance the elongation and maturation of nerve fibers. Functional recovery thus depends on the number of motor fibers correctly matched with motor endplates and the number of sensory fibers correctly matched with sensory receptors[4].

Classification of nerve injury depends upon the nerve components affected, loss of functionality, and the ability to recover spontaneously. Two grading systems are used to stage the extent of nerve injury: Seddon's system and, more recently, Sunderland's system[5].

Seddon proposed a three-tiered model for nerve injury: neurapraxia, axonotmesis, and neurotmesis, in order of increasing severity. According to this system,

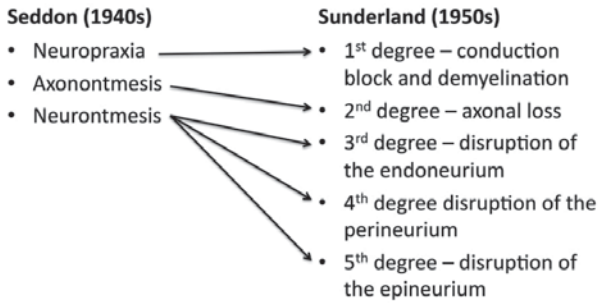

Figure 1. image modified from Mowry, Iowa Grandrounds: Acute Facial Paralysis, 2012.

the neurapraxial stage involves a reversible conduction block characterized by local ischemia and selective demyelination of the axon sheath1. The axon's continuity is retained, and although conduction across the nerve injury is inhibited, conduction within the nerve both proximal and distal to the lesion remains intact[6]. The prognosis for an injured nerve at this stage is good, and recovery occurs within weeks to months[7]. Wrist drop secondary to prolonged external pressure that compresses the radial nerve at the spiral groove of the humerus is a clinical example of neurapraxia[8].

Axonotmesis is a more severe stage of injury, with disruption of not only the myelin sheath, but the axon as well. The epineurium and perineurium remain intact, meaning that there is still some continuity within the nerve[6,7]. Axonotmesis leads to *Wallerian degeneration,* a process whereby the part of the axon that is separated from the neuronal cell body disintegrates distal to the injury[5]. The prognosis for nerves at this stage is fair, and recovery may require months[7]. Axonotmesis is commonly seen in crush injuries and displaced bone fractures[8].

Neurotmesis, the most severe form of nerve injury, is associated with complete nerve division and disruption of the endoneurium[6]. In neurotmesis, the axon, myelin sheath, and connective-tissue components are damaged, disrupted, or transected[5]. As with axonotmesis, neurotmesis initiates Wallerian degeneration, but the prognosis for nerves is poor. Neurotmesis is commonly seen after lacerations or ischemic injuries.

In 1951, Sunderland expanded the classification based on histology to include five injury grades, which broadly correspond to Seddon's three-level classification but with more accurate prognosis of outcomes in axonotmesis injuries[9,10]. Sunderland grades I and II recover completely, grade III recover partially, and grades IV and V usually require surgical intervention. Sunderland grade I injuries are equivalent to neurapraxia. Sunderland grade II injuries have axonal damage but intact endoneurium and hence achieve full recovery. Sunderland grades III and IV will heal spontaneously with increasing degrees of scarring and incomplete recovery due to progressive damage to axons and connective tissue (endoneurium, or endo/perineurium). Scar creates a conduction block and if severe requires excision and nerve reconstruction. Sunderland grade IV injuries usually require surgery due to damage to both axons and all levels of connective tissue (endo/peri/epineurium) with resultant extensive scarring. Sunderland grade V injuries correspond to neurotmesis. This classification has somewhat limited clinical utility as most nerve injuries are of mixed grade and there is no diagnostic test to discriminate between Sunderland grades II and IV. Mackinnon and Dellon modified Sunderland's classification to include a mixed injury pattern better reflecting clinical practice (grade VI)[11].

MICROSURGICAL NERVE REPAIR

Current peripheral nerve repair practice closely resembles the description by Gabriele Ferrara (1543–1627) of 400 years ago who detailed the procedure consisting of disinfection, appropriate identification of nerve stumps, a gentle suturing technique and limb immobilization[12]. The principles in clinical treatment for nerve injury have not changed in the last 30 years despite substantially increased understanding of neuropathophysiology, and correspondingly clinical outcomes remain poor[13]. The treatment of choice in peripheral nerve injuries is meticulous microsurgical repair by tensionless epineurial sutures. In the presence of a nerve gap, where end-to-end suturing is not possible (usually defects that are greater than 3-4 cm), autologous nerve grafting remains the gold standard[14].

Epineural repair

The purpose of nerve repair is the precise apposition of the two sides of transected nerve using a minimum number of sutures while dissecting nerve ends just to the extent necessary to achieve appropriate alignment with minimal tension[15,16]. The bundles are oriented as well as possible, and the epineurium is united with two lateral 8-0 nylon sutures, the ends of which are left long. Repair of the anterior face is completed with three or four more 7-0 nylon sutures. The nerve is then rotated by manipulation of the lateral sutures so that the posterior epineurium can be united. It is in the fresh wound that the disadvantages of epineurial repair are best shown. The bundles may twist around within the epineurium.

Daniel and Terzis suggest several *advantages* of this technique: technical ease, short execution time, minimal magnification, not invading the intraneural contents, being applicable to both primary and secondary neural repairs and the placement of sutures only in the outer investing sheath, and *disadvantages:* tension from natural retraction despite no loss of nerve tissue, compromising the accurate alignment of fascicles, sutured epineurium and cut axonal interphase being in the same plane, controversial outcome, the need for many sutures in order to achieve structural integrity of the repaired nerve[15,17].

Results of direct epineural repair are best summarized by Sunderland in 1991: end to end repairs proved better results than nerve grafts, early repairs fare better that late ones, younger patients do better than the elderly, distal repairs do better than proximal ones[18]. Mackinnon and Dellon reported in a 40-year compilation of data that after direct nerve coaptation 20–40% achieved very good (M4S3+) recovery after nerve repair, but that few injuries recovered fully[19].

Perineural (fascicular) repair

There has been an ongoing debate with regard to the usefulness of perineural repair, with some authors demonstrating its superiority, while others fully rejecting the technique. This technique was first described by Hashimoto and Langley in 1917, but its superiority is in question. According to them, the advantages of fascicular repair are better recovery of motor and sensory end-organs, greater regeneration of axons entering distal nerves, coaptation of perineurial tubes allowing for more desirable alignment of fascicles and better myelination of the stumps[20]. Sunderland points out the disadvantage: greater fibrosis at the site of suture, increased injury to vessels at the nerve ends, extended operative duration, possibility of compromising the vascular supply of isolated fasciculi, discontinuity of fasciculi on a one-to-one basis and the inability to approximate small funiculi[18]. Although current intraoperative techniques (electrophysiological, immunohistochemical, histochemical, anatomical) can differentiate between sensory or motory fascicle[16], the theoretical advantages of better fascicle alignment with this technique are offset by more trauma and scarring to the healing nerve internally due to the presence of permanent sutures. Despite its anatomical attractiveness, overall group fascicular repair is no better than epineural repair in functional outcomes[37].

There are some scholars that have tried to establish indications for this technique; Jabley states that the technique is contraindicated in multifascicled nerves, but is the treatment of choice in nerve grafting and in nerves with less than five fascicles[22]. Scott W. Wolfe [et al.] states that, in selected cases, in early repair, selective perineural suture is useful because it creates some resistance points within the repaired nerve and prevents the fascicle bundles from twisting around at the time of the repair[15].

Fascicular group repair

This type of neurorrhaphy is feasible especially in the distal trunk of the median and ulnar nerves when there is laceration of the nerve trunk. The surgeon can find a lesion where the fascicular groups present uneven lesions and in this case the motor and sensory fascicles must be correctly identified and matched so that motor-sensory cross innervation is avoided. This technique is also feasible when a nerve trunk is partially severed and it would not be advisable to perform en bloc epineural repair because this would bend the apparently healthy fascicle groups[15,16]. This technique's disadvantages are similar to the perineural suture and, thus, it is currently not very practical due to long operative time and scarring inside the nerve trunk.

Nerve grafting

Autologous nerve grafts fulfill the criteria for an ideal nerve conduit because they provide a permissive and stimulating scaffold including Schwann cell basal laminae, neurotrophic factors, and adhesion molecules[23].

When there is a gap between the nerve ends with excessive tension for direct epineural repair, reversed interposition autologous nerve grafts are required. Human autografts are preferred as the literature is clear that autografting is superior to nerve conduits for longer gaps (>3 cm), more proximal injuries, and critical nerves[24-29]. Wherever possible, we should use cutaneous nerves from the damaged limb for grafting[15], but current workhorses are the medial antebrachial, radial sensitive branch, lateral antebrachial and sural nerve. Remember that nerve grafts should be reversed in the orientation to maximize the number of axons successfully regenerating through the graft (especially when using a sensory nerve graft to repair a motor nerve) and to always properly calculate the number and length of the grafts, authors recommend to harvest a graft that is 15% longer than the gap[15]. Surgeons should also remember a clinical rule of thumb: that there is a 50% loss of axons at each coaptation site. Therefore, for primary nerve repair, approximately 50% of the original axons will successfully regenerate through the repair site. For a nerve graft with two coaptation sites, 25% of axons will successfully regenerate through the

graft. Depending on the distance to the motor/sensory target, there will then be additional axonal loss due to the effects previously discussed of chronic axotomy and muscle fibrosis[30]. Nerve grafts can be single, cable, trunk, interfascicular or vascularized[31].

A single nerve graft is used when there is little diameter difference between the donor and recipient.

Trunk grafts use a donor segment from a large nerve interposed to repair a gap in a proximal nerve. There has been poor success with this method as large diameter donor nerves fibrose internally due to poor vascularity before axons are able to regenerate across the graft[31].

During the early 1970s, Millesi recommended nerve grafting for any nerve gap larger than 2 cm and achieved good results in median, ulnar and radial nerve injuries[24-28]. He first described the interfascicular nerve graft: strands of grafted nerves are interposed between carefully dissected fascicle groups, matching the groups from the proximal and distal stumps and using 5-6 autografts for the median nerve and 4-5 for the ulnar and radial nerves. To date, this is the most accepted technique for bridging median, ulnar and radial nerve defects of more than 3-4 cm.

Vascularized nerve grafts (the term graft is improperly used in the literature, since the surgical procedure is a free composite flap) are indicated when large nerve defects exist and where the bed for nerve reconstruction is poorly vascularized. Although several studies have been performed with vascularized nerve grafts for bridging large defects, there is no clear indication of their superiority vs interfascicular nerve grafting. In 2010, Terzis and Kostoupulos demonstrated that vascularized nerve grafts for complex upper extremity injuries provided good to excellent sensory return in severely scarred beds in patients in whom conventional nerve grafts had failed. They have also provided relief of causalgia after painful neuroma resection and rewarding degree of motor recovery in selective cases even for above the elbow injuries. Vascularized nerve grafts can also be used successfully to bridge long nerve gaps (10-30 cm) and also for patients who present late for treatment. As long as preoperative needle electromyography indicates that the denervated targets are still present (fibrillations)[32]. Comparative studies between vascularized and non-vascularized nerve grafting are scarce because of the impossibility to design such a study. Experience is mostly based on case reports.

Nerve grafting remains the golden standard in nerve defects larger than 3 cm, however, autografts sacrifice the sensory function of the nerve, with sensory loss and scarring at the donor site and a potential for neuroma formation[33].

ALTERNATIVE SURGICAL PROCEDURES FOR REANIMATION OF DAMAGED NERVES

Nerve transfer

The definition of this surgical procedure is the coaptation of a healthy donor nerve (whose function needs to be sacrificed) to a nerve stump of higher functional importance. This technique has been successfully used for distal median to ulnar nerve transfer in timely restoration of critical intrinsic muscle function in isolated ulnar nerve injuries, but not for combined ulnar and median nerve injuries. This method safely and effectively restored intrinsic function before terminal muscle degeneration[34]. Brown and Mackinnon successfully used anterior interosseous nerve to deep motor branch of ulnar nerve, third webspace sensory contribution of median nerve to volar sensory component of ulnar nerve, and end-to-side reinnervation of ulnar dorsal cutaneous to the remaining median sensory trunk in order to restore ulnar nerve function in the hand in a series of cases[35]. Also, nerve transfers can provide an alternative and consistent means of reestablishing volitional control of upper extremity function in people with cervical level spinal cord injury. Early outcomes provide evidence of substantial improvements in self-reported function despite relatively subtle objective gains in isolated muscle strength[36].

The benefits of nerve transfers are well described. In most cases, there is only one neurorrhaphy site; with nerve grafts, there are two. In addition, nerve transfers minimize the distance over which a nerve has to regenerate because it is closer to the target organ and is more specific[37]. The goal is to maximize functional recovery with fast reinnervation of denervated motor targets[38]. The most common applications of motor nerve transfers include restoration of elbow flexion, shoulder abduction, ulnar innervated intrinsic hand muscles function, radial nerve function and facial nerve palsy[38].

Free functional muscle flap transfer

The definition of this procedure is to transplant (and sacrifice) a fully functional muscle with its vascular pedicle and motor nerve to a donor site where there is a recipient vascular pedicle, viable motor nerve, but lack of functioning muscle due to atrophy or trauma. Free flap transfers have been in the recent decades the workhorses in plastic and reconstructive surgery along with the advancements of microsurgical techniques, widespread availability of operating loupes and microscopes and trained surgeon teams. A very good exam-

ple for this is the field of breast reconstruction, where the gold standard nowadays are free DIEP, SIEA and gluteal flaps compared to muscle transposition flaps some decades ago.

The indications for free transfers in nerve reanimation are severe and delayed nerve injuries or nerve injuries that have had poor results after primary reconstruction[32].

The senior authors describe that free functional muscle transfers are most commonly used in brachial plexus injuries (BPI) for elbow flexion, but also elbow extension, finger and wrist extension; also a number of original procedures for reconstruction of smile, total lip defects, quadriceps and gluteal function[39]. Yang Y. et al. reported, in 2016, long-term outcomes for 49 gracilis free flaps in 47 patients and that with a well-trained team, free gracilis transfer using an accessory nerve as a donor nerve is a satisfactory treatment to reconstruct the elbow flexion and wrist extension in global BPI patients[40]. Dodakundi et al., in 2013, reported long-term outcomes for 36 double free muscle transfers to restore composite upper limb function after total brachial plexus injury. 70% of patients achieved M4 elbow flexion, with an average total active motion of the fingers of 46 degrees. Importantly, 48% of patients used their injured hand in activities of daily living[39].

MATERIALS AND METHODS FOR PROTECTING SURGICALLY REPAIRED PERIPHERAL NERVES

Nerve healing after microsurgical repair implies scarring of the nerve sheaths and surrounding tissue. This has been proven to be one of the most important factors in the failure of peripheral nerve regeneration after surgical repair, scarring and fibrosis leading to nerve conduction block. Thus, protecting the nerve from surrounding tissue in the process of healing can improve outcomes by providing a protective barrier, with decreased adhesions to surrounding tissue, reducing fibrosis and depending on the type of coverage, providing a vascularized wound bed[41,42]. Several materials and techniques were used to cover and protect the repaired nerves, most of the studies having been conducted on rat sciatic nerves.

Silicon tubes/sheets were assessed because of their simple and cost-effective application, the technique was effective in the repair of peripheral nerve injuries with gaps of up to 3 cm, with better results in the ulnar nerves than in the median nerves, but the material proved to be far too rigid and potentially palpable as a foreign body[43].

Collagen tubes/sheets (Neuragen/NeuraWrap, Neuromed/Neuromatrix/Neuroflex – collagen type I FDA approved) have been proved to permit nutrient exchange and accessibility of neurotrophic factors at the axonal growth zone during regeneration[44]. Kim et al. demonstrated decreased inner epineural connective tissue formation with use of a collagen nerve wrap (NeuraWrap) during primary repair of peripheral nerve transection in a rat sciatic nerve model[45].

Poly-Lactic Acid (PLA) has been used for biomedical applications for over 20 years[46]. PLA film prevented adhesion formation between the nerve and surrounding tissue as well as neuroma formation at the neurorrhaphy site[47].

Coverage with autologous tissue has shown encouraging results, but disadvantages include donor site morbidity, surgical complications and sometimes availability. Vein graft as a protective material for recurrent compressive neuropathy was first reported in the 1990 Masear et al.[48]. Masear et al. applied vein grafts to 145 nerves in 131 patients and concluded that vein grafts are an ideal nerve coverage material in the treatment of the scarred nerves since they are inert and would not elicit an inflammatory response and is also nondegradable[49].

Free fat grafts have an advantage of the availability and accessibility, but using them for covering repaired nerves is unpredictable since it is not yet known how they would promote nerve regeneration and the length of action (absorption of the fat tissue). In the animal model, free fat coverage of the repaired nerves reduced perineural scar formation in some studies, while others showed no benefits or worst, compression neuropathies. It seems that free fat flaps have not established a role in clinical use.

Vascularized free or pedicled fat flaps have been used for coverage of the median nerve in recalcitrant carpal tunnel syndrome. In literature, the workhorses for these techniques are the hypothenar fat pad flap, forearm radial artery flaps, forearm ulnar artery flaps, ulnar fascial fat flaps and a posterior interosseous flap[50-52].

Other local flaps have been described for the coverage of the median and ulnar nerves: abductor digiti minimi muscle flap, palmar brevis flap, pronator quadratus muscle flap, lumbrical flap[53-56]. Clinical results are encouraging, but there is a limited availability of this kind of flaps; there are also no controlled studies to prove the relevance of the clinical outcomes.

Omentum flaps have a large surface are thus can be successfully transplanted to cover brachial plexus injuries. The endothelial cells of the omentum produce

growth factors that are implicated in nerve regeneration[57,58]. Omentum flaps have been proven to decrease or prevent perineural fibrosis[59].

Human amniotic membrane (HAM) allografts are currently used in all fields of medicine since they are nonimmunogenic biological materials. They contain growth factors, anti-inflammatory factors and cytokines which promote wound healing and neovascularization[60]. Meng et al. investigated the HAM allografts as nerve covering materials in a sciatic rat model and found that nerves wrapped with HAM had significantly fewer adhesions and less scar formation than controls. Although the final outcome, both functionally and morphologically, was not significantly improved by wrapping the nerve with HAM, the observed decrease in adhesions and scar formation might help the nerve retain its mobility and thus prevent traction injury and ischemia, which are caused by nerve tethering to the adjacent tissue during the healing process[61]. In October 2016, Gaspar MP et al. concluded that ulnar nerve wrapping with amniotic membrane allograft, when combined with revision neurolysis, was a safe and subjectively effective treatment for patients with debilitating recurrent cubital tunnel syndrome[62].

The HAM allografts have several physiological and anatomical advantages and high availability at a relatively low cost, but their use is subject to special approvals (depending on country) and their usage is at risk from infectious disease transmission.

NERVE GUIDE CONDUITS IN PERIPHERAL NERVE REGENERATION

Autologous nerve grafts are the current gold standard for bridging peripheral nerve defects that are larger than 3 cm. This technique has its innate disadvantages and thus has stimulated research for alternative conduits for the bridging of nerve gaps. Nerve conduits can be categorized as autogenous or nonautogenous (biological or non biological)[31].

Nonautogenous nerve guide conduits (NCGs)

Nowadays, hollow NGCs are the clinically approved alternatives to autograft repair. Current clinically translated NGCs are primarily made from synthetic materials such as poly-glycolic acid (PGA), polylactide-caprolactone (PLCL), various combinations of the PGA or PLCL or from animal extracted collagen. These conduits have a number of advantages for nerve repair, including limited myofibroblast infiltration, reduced neuroma and scar formation, reduction in collateral sprouting and no associated donor site morbidity, and facilitates the accumulation of a high concentration of neurotrophic factors; ultimately guiding regenerating nerves to their distal targets[63].

However, the use of hollow NGCs is currently limited to a critical nerve gap of approximately 3 cm and despite some success in nerve repair, these hollow NGCs fail to match the regenerative levels of autograft and show poor functional recovery[64].

PGA nerve conduits have been assessed by a number of clinical studies and demonstrate equivalent results to nerve repairs or autologous grafts for short or moderate digital nerve gaps (less than 3 cm)[65,66].

In a retrospective analysis, in 2010, ninety-six patients underwent 126 repairs using NeuraGen® (collagen) conduits. The retrospective study indicates that collagen conduits were safe to use and were effective in 43% of patients, bridging nerve gaps smaller than 2.5 cm[67].

Since 1995, 11 devices (NGCs and nerve protectant wraps) based on natural and synthetic materials have been approved by the FDA for the repair of peripheral nerve injuries. Whilst autograft remains the gold standard, a large amount of published prospective and retrospective clinical studies have been performed with NeuraGen® (collagen type I NGC) and have demonstrated its comparable efficacy to autograft in discontinuities up to 20 mm. In respect of synthetic materials, and based on the weight of published clinical evidence (including prospective, series, retrospective and RMCTs), Neurotube® (PGA NGC) has the most comprehensive history when compared with other synthetic devices. It should be noted that significant advantages may be derived from other synthetic devices, however limited prospective randomized clinical data is published in the literature to substantiate claims of superior safety and efficacy. It is noted that comparison of devices is difficult given the scope and limitations of each trial, a feature compounded by the lack of clinical evidence which compares a standardized set of testing protocols[68].

Certainly nonautogenous NCGs are an approved and alternative means to bridging shorter sensitive nerve gaps (especially digital nerves), but there is no indication for repairing of functionally important motor nerves. There are many approaches to consider to improve NCGs, but it is certain that a multimodal approach is needed in order to obtain or surpass traditional nerve grafting techniques: the need for intraluminal guidance structures, cell therapy within the NCGs, molecular delivery therapies (growth factors), neurotrophic factors mimetics[69].

Autogenous nerve conduits

In spite of the large quantity of preclinical research with regard to artificial NCGs, the translation of this research to clinical practice is limited. The most frequently used approach is the so called biological tubulisation i.e. using autologous tissue, other than nerve grafts, for bridging nerve defects. Current clinical practice focuses on the use of veins and skeletal muscle grafts.

Vein grafts

Preclinical trials in the rat model showed similar results with the use of vein grafts compared to nerve grafts in bridging small defects. Chiu et al. reported satisfactory functional recovery after sensory nerve repair by means of vein grafts, comparable to traditional autografts in defects smaller than 3 cm[70]. The efficacy of vein grafts in bridging nerve defects in patients has been confirmed in various selected clinical conditions, such as when autologous nerve grafts are insufficient[71], for microsurgical repair of the sural nerve after nerve biopsy[72], and for repair of the inferior alveolar branch of the mandibular division of the trigeminal nerve following iatrogenic damage[73]. A recent prospective randomized study comparing polyglycolic acid and autogenous vein scaffolds for reconstruction of digital nerve gaps showed that recovery after reconstruction with a vein conduit was equivalent to polyglycolic acid conduit repair with fewer postoperative complications[74].

However, most of these studies showed that vein grafts are effective only for short nerve defects, an element that clearly represents a main limiting factor in the employment of this technique[75].

Composite vein-skeletal muscle

Skeletal muscle alone has seen its rise (1940) and fall (in the 1990s). Few clinical trials showed that skeletal muscle tissue is indeed usable in bridging peripheral nerve gaps but there were a lot of inconsistences. In 1993, Brunelli et al. validated the use of veins filled with fresh skeletal muscle: functional results were similar to those found in traditional nerve grafts, but axon number was superior in the veins filled with muscle. This suggests that vein filled with muscle might serve as a grafting conduit for the repair of peripheral nerve injuries and could give better results than traditional nerve grafting[76]. These early results with the technique led to experimental studies in laboratory animal models that have shown that the fresh muscle-vein-combined guides are rapidly colonized by migratory Schwann cells (especially coming back from the distal nerve end) and that these cells maintain the capability to actively proliferate inside the conduit[77-79].

Due to its efficacy, the muscle-vein-combined nerve repair technique has already been applied in clinical case series in selected conditions such as when autograft repair was not possible[80], primary crush injuries81 and digital nerve repair[82,83]. All clinical reports have consistently shown good clinical outcome in most cases with percentage of functional recovery similar to autologous nerve grafting. In addition, if nerve reconstruction is performed soon after lesion, the possibility of delayed autograft repair is still possible in case of failure of regeneration[81].

Indeed the use of autologous conduits shows more promise in clinical practice than NCGs, but the final outcomes of the clinical trials show comparable results to nerve grafting, which we already know are suboptimal. Several lines of research need to be followed in order to address the problem of nerve regeneration in a multimodal approach. The current line of research consists of intricating the surgical approach with a molecular one: enriching the autologous nerve conduits with growth factors, stem cells or even gene transfer.

PHARMACOLOGY, HORMONES AND GROWTH FACTORS

Currently there are no clinically available pharmacological treatments for nerve injury. Recently two pharmacological agents, N-acetyl-cystein and acetyl-L-carnitine, have been shown to offer almost complete neuroprotection experimentally within a clinically pragmatic time-frame, and both are established as safe clinical pharmaceutical agents[84-85]. Sildenafil, PDE5 inhibitor, has been demonstrated to promote a neurotrophic phenotype in a rat model[87].

Hormones seem to be a promising pharmacological intervention in nerve injuries. Progesterone or allopreganolone modulate Schwann cells (SC) physiology through action on the expression of myelin proteins and SC differentiation[88,89]. Thyroid hormone and growth hormone have been shown to improve axonal myelination, myelin thickness and functional recovery in rat sciatic nerve injury models[90,91].

Neurotransmitters such as GABA, ATP, glutamate and acetylcholine play key roles in neuronal-ganglia interactions. They and their receptors have been proposed for pharmacotherapies for nerve repair[92].

Last but not least, research, with regard to growth factors (GF) and their ways of administration in nerve regeneration, is abundant. NGF, GDNF, CTNF and IGF-1 are shown to improve regeneration distances, re-myelination, increase axonal regeneration speed and

functional recovery in preclinical trials[93-96]. But translation of this research to clinical practice is faced with different problems: timing and dosage of treatment, method of administration and release, interaction with other molecules, downregulation of receptors; all of which are almost impossible to control and predict in a clinical setting. Possible solutions for this problems might be external modulation of endogenous GF[97], delivery of GF by controlled release systems[98] or transplantation of GF expressing cells – stem cells.

THE ROLE OF STEM CELLS IN PERIPHERAL NERVE REGENERATION

In the complex and incompletely understood mechanisms of peripheral nerve regeneration at the injury site, Schwann cells (SC) have emerged as the key player. The optimal environment for axonal regeneration relies on the synthesis and release of many biochemical mediators that are temporally and spatially regulated with a high level of incompletely understood complexity[99].

The ideal stem cell for clinical application, including peripheral nerve repair, must be easily accessible, rapidly expandable in culture, capable of in vivo survival and integration into host tissue and must be amenable to stable transfection and expression of exogenous genes[100]. Emphasis has been placed on the importance of stem cell type, differentiation, cell scaffold and method of cell delivery[101].

Stem cells have the potential to replace lost neurons or increase the number of glial support cells. In the peripheral nervous system, emphasis has been placed mostly on increasing SC number and activity[99]. Taking into account the difficulty of autologous SC culture, this approach is considered to be impractical. But exogenous stem cells can differentiate into SC-like phenotype and assume their role in the nerve stumps. Transplanted cells will differentiate and enhance growth factor secretion and extracellular matrix (ECM) components thus creating the best environment for nerve regeneration[102].

Adipose derived stem cells represent nowadays the most practical source of cells for transplantation. In light of easier harvest, superior stem cell fraction, differentiation potential and proliferation capacity, they have supplanted bone marrow derived cells. The beneficial effect of these cells on regeneration has been widely reported and, until clear evidence emerges that differentiation is superior, the use of undifferentiated cells is the most pragmatic and clinically translatable option[99].

Optimizing the effect of the transplanted stem cell is heavily reliant on appropriate delivery and support. In cases where nerves are repaired primarily or when autograft or allograft nerve is going to be used, stem cells can be delivered by a variety of different methods: 1) cells suspended in culture medium can be microinjected into nerve ends or grafts, 2) cells suspended in fibrin matrix that are injected around repair sites, 3) cells can be injected within the lumen or in the matrix of a conduit, 4) systemic administration of stem cells following peripheral nerve injury and 5) injection at the level of neuromuscular junction or even distributed within denervated muscle.

Despite *in vitro* and pre-clinical success, the application of stem cells to peripheral nerve repair has yet to make an impact in the clinical arena. Translation is currently limited by justified concerns regarding genetic manipulation, cell instability and the risks of tumorigenesis. However, perhaps the most pertinent issue is that despite great effort to manipulate the regenerative process, the use of stem cells simply has not led to outcomes that significantly and consistently surpass those achieved with conventional techniques[99].

ALTERNATIVE TECHNIQUES FOR AUGMENTATION OF PERIPHERAL NERVE REGENERATION

Low frequency electrical stimulation

There are limited reports of postoperative electrical stimulation of the transected nerve in animal studies demonstrating that applying a low frequency electrical current can speed up axonal regeneration. Although still in its early stages, its potential for clinical translation has been demonstrated in two recent studies on patients with compressive neuropathy and digital nerve laceration. One hour of electrical stimulation was applied to 21 patients undergoing carpal tunnel decompression that also had thenar muscle atrophy. The electrically stimulated group showed evidence of accelerated axonal regeneration and target reinnervation[103]. Both pre-clinical and clinical experiences show this could be a valuable option in peripheral nerve regeneration, yet more studies are needed.

Photochemical tissue bonding (PTB)

PTB creates a covalently bonded nerve wrap around a nerve coaptation, using an Nd/YAG laser, photoactive dye, and a nonimmunogenic amnion wrap. Animal studies in rat sciatic nerve and rabbit common peroneal nerve models have demonstrated improved axon

counts and gait function after end-to-end coaptation with a PTB nerve wrap[104-106].

Laser welding

Although CO_2 laser-welded nerve adhesion has demonstrated favorable results in animal models, its clinical use can be cumbersome and its versatility is limited. Concerns remain about the high rate of nerve dehiscence and thermal injury to axons and nerve tissue[107].

Tissue glue repair

Fibrin sealants have a proven track record as a safe and effective nerve glue. The longest and greatest experience with nerve glue is in brachial plexus reconstruction. In this setting, fibrin glue has been indispensable. Most found fibrin glue repair to be equal or superior to suture repair[107].

Another biocompatible glue is PEG hydrogel, which demonstrates stronger adhesion than fibrin glue without being neurotoxic; PEG may be superior to fibrin glue because of its greater tensile strength and longer duration before breakdown (4 weeks). PEG is nontoxic and biocompatible and does not induce a significant inflammatory response. What may be an additional advantage is that it may have adhesion inhibiting properties that prevent perineural scarring. PEG hydrogel is therefore a promising candidate as a nerve glue[107].

Tissue glue is and will be a promising field for nerve repair techniques since it is a relatively cheap option for direct coaptation of nerve ends.

CONCLUSIONS

Outcomes following peripheral nerve injuries and repair have slowly improved since the development of microsurgical techniques five to six decades ago, but still remain frustratingly poor.

When possible, early (maximum of 48-72 hours) end-to-end neurorrhaphy remains the best option for a trauma patient; an early correct microsurgical neurorrhaphy giving him/her the best chances to recover motor and/or sensitive function. Still the long term prognosis of the early microsurgical repair depends on a lot of factors: type of injury and injury to adherent structures, associated trauma, the qualification of the surgical team, patient adherence to rehabilitation.

It is generally accepted that direct neurorrhaphy remains the golden standard for nerve lesions with no defect; while in the case of nerve defects larger than 3 cm nerve grafting should be the norm.

But let us not forget that Mackinnon and Dellon reported in a 40-year compilation of data that, after direct nerve coaptation, 20-40% achieved very good results and that complete recovery is not to be expected. These percentages decrease when there is a nerve defect that requires grafting. Rehabilitation remains a very important part for preserving muscle function while the nerve regenerates.

Nerve transfers can safely and effectively restore intrinsic function before terminal muscle degeneration and are a good option for salvaging functionally important motor units, when primary repair has failed. Their main disadvantage is that the surgeon sacrifices part of a functional nerve.

When nerve repair either by direct coaptation or nerve grafting is not indicated or has failed and there is loss of a motor unit (muscles suffer from atrophy and fibrosis at 6-12 months after being denervated), the surgeon has to opt for functional muscle free flaps for reanimating the injured site.

Nerve guidance conduits (NGCs) are at the present date the only clinically approved substitutes for nerve grafts, but their proven clinical use is limited to small defects in sensory digital nerves. Composite autologous nerve conduits (veins filled with fresh skeletal muscle fibers) show benefits compared with NGCs, but their clinical superiority compared with the established nerve suture techniques have yet to be proved in comparative studies.

Stem cell therapy and growth factors have proven to be good tools for promoting nerve regeneration in the preclinical setting. Translation of this abundant research in the clinical use remains a desire that faces numerous problems: complexity of interactions, cell instability, risks of tumorigenesis, means of administration, correct dosing, uncontrollable gene expression.

Nerve regeneration research nowadays follows an abundancy of paths, but few that can be clinically translated. This is why nerve repair and regeneration outcomes have not improved dramatically over the last five decades. There seems to be a great need for multimodal approaches using combined surgical and adjuvant techniques. Current lines of research are trying to do that. They consist of intricating the surgical approach with a molecular one: enriching the autologous nerve conduits with growth factors, stem cells or even gene transfer.

New and current fields of research have yet to be further explored, but translation to clinical practice seems to be the heart of the matter.

Or couldn't we just simply bypass nerve regeneration and replace damaged nerves with myoelectric prostheses wirelessly linked to the brain?

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