

The Impact of Neuroscience on the Evolution of Decision-Making in Brachial Plexus Surgery. Part II: Nerve Grafts Act as Operator Channels (How the New May Meet the Old)

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Abstract

Nerve grafts could be conceived of as operator channels (rather than anatomical guidance channels), proximal nerve stumps as operator donors, and distal nerve stumps and the muscles supplied by them as operator recipients. *First*, consequences of nerve grafts acting as molecular operator channels include recipient end to donor side nerve grafting; donor end to recipient side grafting; donor side to recipient side coaptation; recipient/donor end to donor/recipient side grafting would better be assisted by recipient/donor side to donor/recipient side grafting, augmenting not yet regenerated neuromas in continuity by bypass grafts; augmenting end-to-end repairs/grfts by side grafts; augmenting late neuromas in continuity; preserving neuromas at proximal and distal nerve stumps of injured nerves; assisting nerve transfer (neurotization); and assisting side grafting of end-to-end grafts/repairs. *Second*, nerve grafts act as operator channels responding to a cellular membranous bed and incremental neurolysis of fibrosis by heparin and chondroitinase ABC. *Third*, subjecting intact nerve endings to tensile forces to promote axonal sprouting while simultaneously relieving tensile and shear forces off the grafted or sutured area has led to the following consequences: mobilization of the brachial plexus along its whole length during surgery; not completely excising the fibrosis but leaving a posterior fibrous segment to help unload the grafted segment from tensile and shearing forces; using epiperineurial 2/0 vicryl tension sutures to both intact nerve endings to apply tensile forces to them while unloading the grafted segment; loop grafting; continual tensile forces (dynamic tension devices, magnetic nanoparticles); revision brachial plexus surgery; free muscle transplantation as internal splints; dealing with neuromata of Types 1A, 1B, 2A, 2B, 3A, and 3B (so-called neuromata in continuity) by a shortening/tensioning/loop grafting procedure; widening the indications for surgery in obstetric brachial plexus; humeral shortening osteotomy; and inciting axonal sprouting from the spinal cord into avulsed brachial plexus roots by magnetic nanoparticles. *Fourth*, distal nerve transfer techniques may act as conditioning lesions enhancing direct

cord implantation and brachial plexus grafting, a plea for reverting to full exploration of the brachial plexus. *Fifth*, in chronically denervated muscles, the motor end plate can regenerate even years after nerve injury, provided muscle mass has been preserved, and the muscle has not been replaced by fibrous tissue. This has paved the way for many reconstructive procedures including direct muscle implantation.

Sixth, axonal cone progression can be stimulated through partial inhibition of actin retrograde axonal transport and by cellular transplantation strategies into target muscles.

Keywords: nerve grafting, brachial plexus, nerve injury

1. Introduction

Brachial plexus surgery has progressed through four eras of development, the pre-microsurgery era, the era of introduction of microsurgical techniques as applied to peripheral nerves, the era of refinement of microsurgical techniques, and the era of neuroscience. In the first part of this article, we have revised the basic concepts of brachial plexus surgery, the foundations of which have been laid during the second and third eras [1]. Surgical decision-making in both of these eras has been dominated by the neuroscientific concept of nerve grafts acting as anatomical guidance channels. Notwithstanding this, neuroscience has come up with many revelations pertaining to anatomical neuroscience, neurohistochemistry, neurophysiology, molecular neuroscience, physics as applied to neuroscience, mathematical neuroscience, and tissue engineering as applied to neuroscience. Many of these revelations have been experimentally proven. Putting them into clinical practice is still anecdotal, however. Based on these revelations, nerve grafts could be conceived of as operator channels (rather than anatomical guidance channels), proximal nerve stumps as operator donors, and distal nerve stumps and the muscles supplied by them as operator recipients.

The operator concept has reverted surgical thinking toward full exploration and grafting of the brachial plexus, allowing new thinking to meet old thinking. It is hoped that it will help the brachial plexus surgeon to devise animal experimental trials to apply neuroscientific findings clinically. It is further hoped to help him put these findings into clinical practice.

2. Nerve grafts as molecular operator channels responding to molecular cues

2.1 Insights gained from molecular neurobiology: the neurotrophic and contact guidance theory for axonal guidance

Nondiffusible and diffusible (permissive or inhibitory) molecules influence axonal growth [2–4]. *Neurite outgrowth-promoting factors* are nondiffusible cell adhesion molecules (CAMs) and extracellular matrix (ECM) molecules (such as laminin (LN), fibronectin (FN), heparin sulfate proteoglycans (HSP), and tenascin), which pave the proper path by supplying orientation and adhesiveness for axons, the so-called axonal guidance. *Neurotrophic factors* are diffusible signals powering peripheral nerve regeneration.

Neurotrophic factors, laminin, Schwann cells, and stem cells have been administered experimentally to promote axonal growth. Other factors include local

administration of taxol. Microtubules and actin microfilaments are critical for regeneration [5]. They potentiate the effect of GAP-43. Thus, local administration of taxol, a microtubule-stabilizing agent, increases neurite outgrowth [6]. By increasing the intracellular levels of cyclic adenosine monophosphate (cAMP) and protein kinase A activity, neurons have been shown to overcome myelin-based inhibition [7]. Intracellular cAMP levels can be elevated using a non-hydrolyzable analogue of cAMP, dibutyryl cAMP (dbcAMP), or rolipram, a phosphodiesterase4 inhibitor [7].

2.2 Manipulating molecular mechanisms to increase neurite outgrowth into nerve grafts

Manipulating molecular mechanisms is based on the sensitivity of the axonal growth cone to *spatial molecular concentration gradients* [8, 9]. Utilizing synthetic nerve graft scaffolds, axonal growth can be hypothetically made to bridge the whole length of the neural gap by seeding the scaffolds with multiple nerve growth factor (NGF) spatial concentration gradients [9]. These may be seeded with or without microspheres [10, 11].

2.3 Recipient end to donor side nerve grafting: a first consequence of the neurotrophic and contact guidance theory

End-to-side neurorrhaphy was first described by L  ti  vant in the “Traite des Sections Nerveuses” in 1873 [12] followed by Balance [13] and Harris [14] in 1903. It involves grafting donor side to recipient end after stimulating donor side collateral sprouting by mechanical trauma, or axotomy, with [15, 16] or without [17] removing the epineurial sheath (**Figure 1**). Interest in end-to-side coaptation has been rekindled by Lykissas et al. [12] and Viterbo et al. in 1994 [18] as well as by others [19–22]. From a *molecular neurobiologic* point of view, this phenomenon may result from collateral sprouting induced by neurotrophic factors released from the

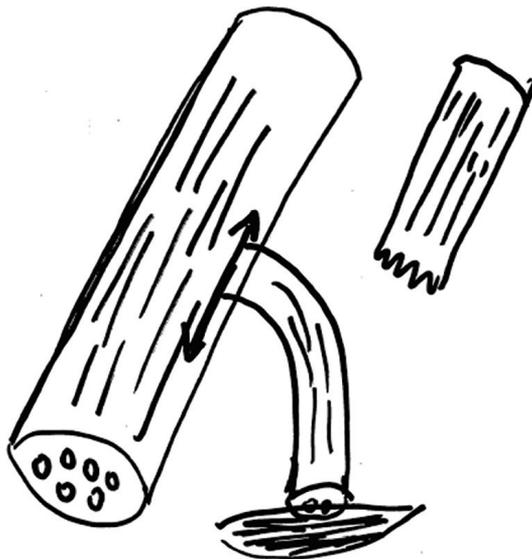


Figure 1. End-to-side neurorrhaphy, the first consequence of the neurotrophic and contact guidance theory. Originally, it involves grafting donor side to recipient end after stimulating donor side collateral sprouting by mechanical trauma, or axotomy, with or without removing the epineurial sheath.

end-to-side implanted nerve stump [20]. Now, end-to-side neurorrhaphy has gained widespread use. Its clinical applications include digital nerve and other sensory nerve injuries, brachial plexus injuries, mixed nerve injuries, facial nerve injuries, and painful neuromas. These have been reviewed elsewhere [12].

2.4 A second consequence: donor end-to-recipient side grafting, so-called reverse end-to-side nerve suture

Early experimental evidence for successful donor end to recipient side neurorrhaphy has been provided by Adelson et al. [23]. These authors have used “jump grafts” bypassing a neuroma in continuity. Similar attempts have included suturing the distal end of the cut rat peroneal nerve to the side of the transected and repaired tibial nerve [24, 25]. Proper donor end to recipient side neurorrhaphy has been successfully carried out experimentally by other authors [26, 27]. Early sensory protection in reverse end-to-side neurorrhaphy has improved the functional recovery of chronically denervated muscle in rat [28, 29]. Coaptation of the anterior interosseous nerve to the side of distal ulnar nerve by donor end to recipient side neurorrhaphy technique has been postulated as an effective therapeutic intervention for high ulnar nerve injuries in human [30]. *A good example of donor end to recipient side grafting (so-called reverse end-to-side nerve suture) is spinal accessory distal nerve transfer (donor end) to the suprascapular nerve, via an anterior or posterior approach, to augment a partially regenerated nerve (in obstetric palsy), to assist grafting of a C5,6 rupture (assisted nerve transfer or neurotization), or as a conditioning lesion (vide infra) (Figure 2).*

2.5 A third consequence: donor side to recipient side coaptation

The feasibility of donor side to recipient side grafting has been investigated in the rat [19]. Results have been in favor of end-to-end repair, followed by the side-to-side repair. Results of side-to-side technique have been superior to the end-to-side technique.

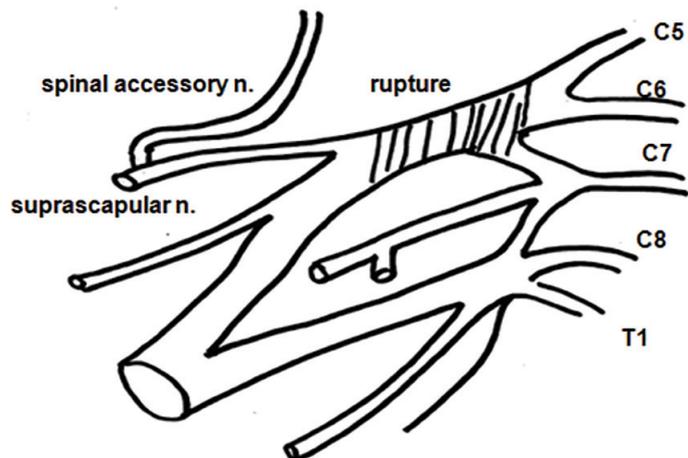


Figure 2. Donor end to recipient side grafting (so-called reverse end-to-side nerve suture), the second consequence of the neurotrophic and contact guidance theory. A good example is terminal spinal accessory distal nerve transfer (donor end) to suprascapular nerve side, via an anterior or posterior approach, to augment a partially regenerated nerve (in obstetric palsy), to assist grafting of a C5,6 rupture (assisted nerve transfer or neurotization), or as a conditioning lesion (vide infra).

Clinically, in facial reanimation surgery, hypoglossal-facial nerve (HN-FN) neuroorrhaphy is used to treat facial palsy when the proximal stump of the injured facial nerve is unavailable. This technique, however, is not suitable for incomplete facial palsy. Hypoglossal-facial side-to-side neuroorrhaphy has been investigated as a solution [31]. Also clinically, in traumatic brachial plexus palsy, biceps muscle reinnervation has occurred after side-to-side anastomosis of an intact median nerve to a damaged musculocutaneous nerve [32] (**Figure 3**). At 9 months postinjury, the 34-year-old patient with right upper and middle trunk injury has had the damaged upper trunk of the brachial plexus repaired with an end-to-end graft. Failing biceps recovery at 8 months postoperatively, the intact donor median nerve has been side grafted to the side of the recipient musculocutaneous nerve via epineurial windows.

2.6 A fourth consequence: recipient/donor end to donor/recipient side grafting had better be assisted by recipient/donor side to donor/recipient side grafting

Under Section 2.5, we have demonstrated the superiority of side-to-side grafting to end-to-side grafting [19, 31]. In a rat experimental study, Seif-el-Nasr [33] has investigated three groups: end-to-end sciatic nerve repair (control group), reverse end-to-side femoral nerve (donor end) to sciatic nerve (recipient side), and reverse end-to-side plus side-to-side femoral nerve (donor end and side) to sciatic nerve (recipient side). Results have been superior in the third group. It has been concluded that the more the side contact area between donor and recipient, the more collateral axonal sprouting occurs (**Figure 4**).

This has been confirmed in another rat study [34].

2.7 A fifth consequence: nerve augmentation: augmenting not yet regenerated neuromas in continuity by bypass grafts

The term augmentation refers to adding grafts/local neurotrophic agents to the already established neuroma in continuity/end-to-end repair or graft/side graft.

The brachial plexus surgeon may be faced with the following situation. A patient presents 3–12 months postinjury with a complete brachial plexus palsy. An

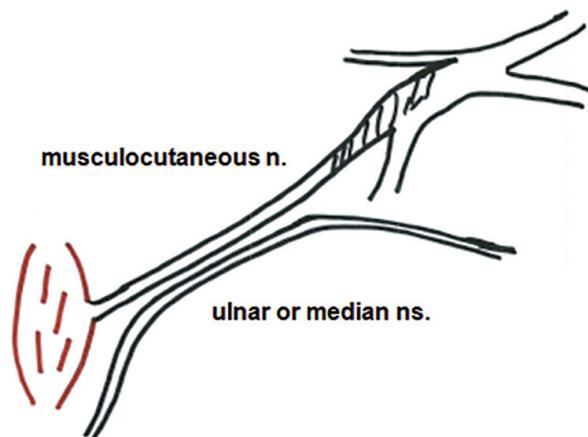


Figure 3. Donor side to recipient side grafting, the third consequence of the neurotrophic and contact guidance theory. A good example is median nerve (donor side) or ulnar nerve (donor side) grafting to augment a partially regenerated musculocutaneous nerve (e.g., in obstetric palsy), to assist a grafted musculocutaneous nerve (assisted nerve transfer or neurotization), or as a conditioning lesion (vide infra).

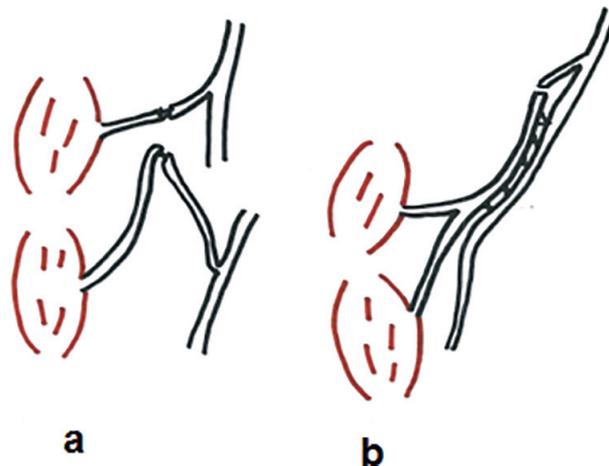


Figure 4. Recipient/donor end to donor/recipient side grafting had better be assisted by recipient/donor side to donor/recipient side grafting, the fourth consequence of the neurotrophic and contact guidance theory. The more the side contact area between donor and recipient, the more collateral axonal sprouting occurs. To improve elbow flexion, a double nerve transfer has been described (6.8 Part I), involving ulnar nerve fascicular transfer to the motor branch to biceps and median nerve fascicular transfer (fascicle to flexor carpi radialis or to flexor digitorum superficialis) to the motor branch to brachialis (a). The use of median nerve fascicular transfer may be avoided by assisting Oberlin's transfer by ulnar/musculocutaneous side grafting (b).

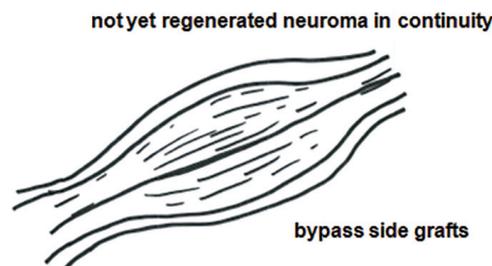


Figure 5. A fifth consequence of the neurotrophic and contact guidance theory: nerve augmentation, augmenting not yet regenerated neuromas in continuity by bypass grafts.

electromyogram shows only scattered motor unit action potentials on voluntary contraction, no summation potentials. The surgeon explores the brachial plexus surgically; a neuroma-in-continuity is found. Nerve “bypass” grafts may be used instead of neurolysis or segmental resection with interposition grafting (**Figure 5**). This has been investigated in the rat [35, 36] and confirmed in a rabbit model [37].

2.8 A sixth consequence: nerve augmentation: augmenting end-to-end repairs/grafts by side grafts

Based on the evidence mentioned under Sections 2.6 and 2.7, nerve end-to-end repairs/grafts had better be augmented by side grafts (**Figure 6**).

2.9 A seventh consequence: nerve augmentation: augmenting late neuromas in continuity, the nerves and muscles of which have partially regenerated but are not expected to regenerate completely

Late neuromas in continuity, the nerves and muscles of which have partially regenerated but are not expected to regenerate completely are a challenge to the

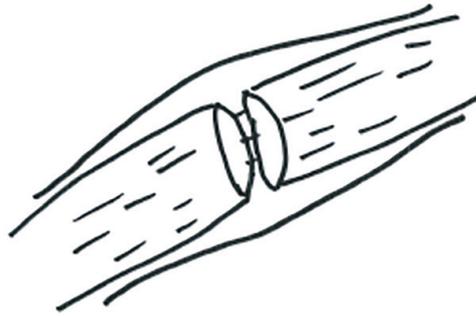


Figure 6.

A sixth consequence of the neurotrophic and contact guidance theory: nerve augmentation, augmenting end-to-end repair by bypass grafts.

brachial plexus surgeon. In late obstetric palsy cases (presenting after the age of 3 years), e.g., nerves have partially regenerated leading to variable recovery of motor power. It should be noted that partially regenerated nerves cannot be cut and grafted, in order not to lose already regained motor power and preserved muscle mass, but they can be augmented by side grafting (**Figure 7**).

Experimentally [38, 39] augmentation by side grafting has the potential to prevent the progressive deterioration of the Schwann cell support of regenerating nerves. An electrophysiologic study has been conducted to evaluate the origin of regenerated axons after bypass grafting (end-to-side coaptation) and the utility of nerve bypass grafting for peripheral nerve injury [40]. It has been concluded that collateral sprouting across end-to-side sutures is the chief means of axonal outgrowth in nerve bypass grafts and that functional recovery can be expected in bypass grafting to nearly the same extent as in cable grafting. These findings have also been confirmed by other authors [41]. It has also been confirmed that end-to-side neurorrhaphy, which is an axonal provider, could be useful as a Schwann cell provider to support axonal elongation in acellular nerve allografts [42].

Clinically, in late obstetric brachial plexus lesions [43], it has been concluded that nerve augmentation improves cocontractions and muscle power in the biceps, pectoral muscles, supraspinatus, anterior and lateral deltoids, triceps, and in Grade

late neuroma in continuity with partially regenerated nerves **bypass side grafts**

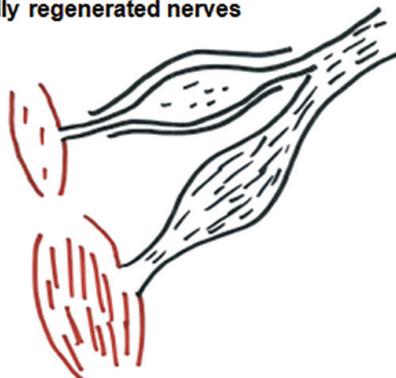


Figure 7.

A seventh consequence of the neurotrophic and contact guidance theory: nerve augmentation, augmenting late neuromas in continuity, the nerves and muscles of which have partially regenerated but are not expected to regenerate completely by bypass grafts.

2 or more forearm muscles. It is less expected to improve infraspinatus power or Grade 0 or 1 forearm muscles. Nerve augmentation of partially regenerated nerves by side grafting has also been an established procedure in partial facial palsy [44]. Cross-face sural nerve side grafts sutured to the contralateral donor facial nerve have been used to augment the partially injured and regenerating facial nerve.

2.10 An eighth consequence: dealing with neuromas at proximal and distal nerve stumps of injured nerves: a plea not to excise them up to healthy fascicles

Classical microsurgical teaching recommends excision of neuromas at proximal and distal nerve stumps of injured nerves up to healthy nerve fascicles. This allows fascicular suturing that obeys topographic nerve anatomy and thus averts aberrant axonal sprouting. Neuromas at proximal and distal nerve stumps of injured nerves, however, contain an increased number of Schwann cells, neurotrophic factors, neurite outgrowth-promoting factors and cell adhesion molecules. According to the neurotrophic and contact guidance theory, neurite outgrowth-promoting factors and cell adhesion molecules pave the proper way for axonal sprouting, thus minimizing aberrant sprouting. Neurotrophic factors fuel the process of axonal cone progression. Thus, neuromas at proximal and distal nerve stumps of injured nerves ought not to be excised up to healthy fascicles, because regeneration within them might be superior to regeneration across healthy fascicles (Figure 8a–i).

In fact, there is ample evidence confirming this.

In an attempt to bridge nerve defects through an acellular homograft seeded with autologous Schwann cells, Schwann cells have been obtained from a regeneration neuroma of the proximal stump [45]. Thus, obviating the need for in vitro expansion of extracted Schwann cells, the proximal stump neuroma has served as a rich biological resource for autologous Schwann cells.

The regenerative potential of neuromas is indicated by retrograde growth of myelinated fiber sprouts. This has been investigated following section of mouse sural nerves [46]. The regenerative potential of neuromas is partly due to the trophic effects of nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF) [47]. Nerve growth factor inhibition has prevented traumatic neuroma formation in the rat [48–50].

The regenerative potential is preserved even in longstanding neuromas, as late administration of anti-NGF therapy has attenuated tumor-induced nerve sprouting, neuroma formation, and cancer pain [51]. NGF influences the mechanical or histological properties of reinnervated motor units [52]. A regenerative potential has been ascribed to ankyrin G, a multifunctional transmembrane protein of the axolemma and a key protein in neuroma formation. It binds Na⁺ channels in the initial segments of a regenerating axon and links with neuronal cell adhesion molecules [53].

Evidence comes also from vestibular schwannomas [54]. The continued growth of vestibular schwannoma may be explained by the presence of p75^{NTR}, which is highly expressed in vestibular schwannomas and promotes cell survival by activating nuclear transcription factor κ B [55]. Vestibular schwannomas have formed the basis for studying the growth factors responsible for the regenerative potential of neuromas [54, 56]. These include transforming growth factor (TGF)-beta 1 and glial cell line-derived neurotrophic factor (GDNF), nerve growth factor (NGF), intracellular adhesion molecule-1 and vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), neuregulin (NRG) and erythropoietin (EPO), interleukin (IL)-1 β , IL-6, and tumor necrosis factor- α (TNF- α).

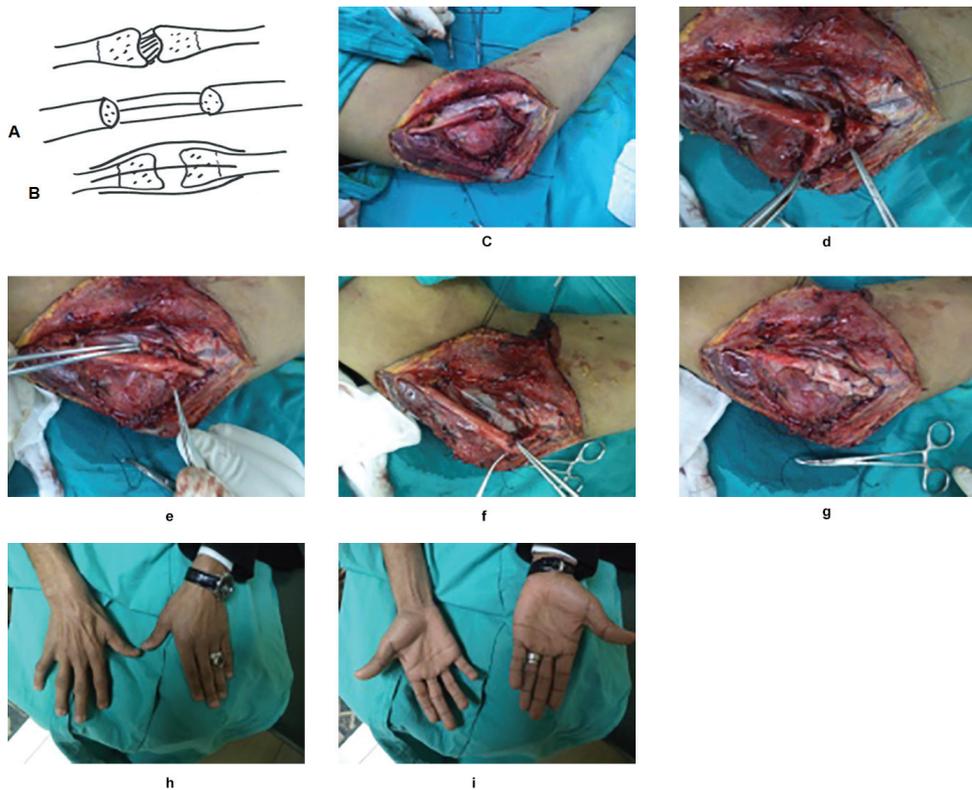


Figure 8. Dealing with neuromas at nerve stumps: an eighth consequence of the neurotrophic and contact guidance theory. (a) Nerve grafts as anatomic guidance channels: cutting stumps up to healthy nerve fascicles and grafting. (b) Neurotrophic and contact guidance theory: excising fibrotic segments and preserving gliotic segments and repair/side grafting. (c) The right ulnar nerve of a 30-year-old man was cut at the medial epicondyle during a motor car accident. The ulnar nerve has been transposed anteriorly. (d) Excising the fibrotic segments, preserving the gliotic segments, and tensioning/shortening (vide infra) using 2/0 vicryl sutures. (e) The effect of tensioning/shortening: a fibrous segment has been left at the posterior wall to minimize shearing stress (vide infra); a thin silicon sheet has been left posteriorly to create a cellular membranous bed (vide infra). (f) Microsutures having been placed. (g) Augmentation of end-to-end repair by bypass side grafts (vide supra) via loop grafting (vide infra). (h) Dorsal view of both hands 9 months postoperatively: the lumbrical minus deformity has disappeared; the dorsal interossei have regenerated. (i) Palmar view of both hands 9 months postoperatively: incomplete regeneration of the palmar interossei, of the hypothenar muscles, and of the adductor pollicis.

2.11 A ninth consequence: assisted nerve transfer (neurotization): assisted side grafting of end-to-end grafts/repairs

Assisted neurotization involves transferring a suboptimal proximal donor nerve to the injured recipient nerve pending arrival of regenerative signals from an optimal distal donor. The proximal nerve transfer assists the distal nerve transfer until its regenerative signals arrive.

Assisted neurotization (nerve transfer) is well known in facial reanimation surgery [44]. The nerve to masseter muscle (a branch of the maxillary nerve and a nearby donor) or the hypoglossal nerve (nearby donor) is used to power the ipsilateral paralyzed facial nerve pending signals through cross-face sural nerve grafts from the contralateral facial nerve (optimal distant donor). The latter signals add harmony, spontaneity, and emotional expression to the movements already regained by nerve to masseter transfer (**Figure 9**). Assisting cross-facial nerve grafting by minihypoglossal to facial nerve transfer has been called the “babysitter” procedure by Terzis [57]. The introduction of side neurorrhaphy later on has made

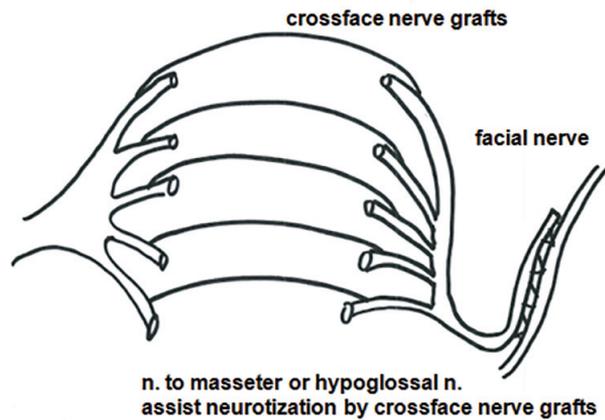


Figure 9.

A ninth consequence of the neurotrophic and contact guidance theory: assisted nerve transfer (neurotization) and assisted side grafting of end-to-end grafts/repairs. The nerve to masseter muscle or the hypoglossal nerve (nearby donors) is used to power the ipsilateral paralyzed facial nerve pending signals through cross-face sural nerve grafts from the contralateral facial nerve (optimal distant donor). The latter signals add harmony, spontaneity, and emotional expression to the movements already regained by nerve to masseter transfer.

it possible to avoid cutting the hypoglossal nerve completely [58, 59]. These authors [58] have modified the conventional technique of hypoglossal-facial nerve anastomosis by means of sectioning one third of the hypoglossal nerve area, thus avoiding dysfunction of this nerve. In both of these studies, the use of an interposition graft and its end-to-side anastomosis to the hypoglossal nerve has allowed the preservation of tongue function but has required two anastomoses and a free second donor nerve. This hypoglossal-facial-jump-anastomosis has been further modified [60], obviating the use of an interposition graft. Following mastoidectomy, the facial nerve has been mobilized in the fallopian canal down to its bifurcation in the parotid gland and cut in its tympanic portion distal to the lesion. Then, a tensionless end-to-side suture to the hypoglossal nerve has been performed.

2.12 A tenth consequence: augmentation of end-to-end grafts/repairs/side graft by growth factors and cellular transplantation is controlled by hormones and other agents, all of which can be administered locally to improve neural recovery

According to the neurotrophic and contact guidance theory, axonal contact guidance is provided by cell adhesion molecules and neurite outgrowth factors, while neurotrophic factors fuel axonal cone growth and progression. Local administration of these factors (as discussed under item 6, tissue engineering, *vide infra*) will augment nerve grafting. Doping acellular, chondroitinase-treated nerve grafts with growth factor (NGF), e.g., has augmented axonal ingrowth [61]. As regards cellular transplantation, embryonic spinal cord fetal cells and cultured neural progenitor cells from different spinal cord segments have been injected into the transected musculocutaneous nerve of the rats, and atrophy in biceps brachii has been assessed [62].

The whole process is controlled by hormones, especially growth hormone, thyroid hormones, and sex hormones [38, 63–75]. A multitude of other agents have also been investigated. Betamethasone, vitamin E, pyrroloquinoline quinone, erythropoietin, and immunophilins (FK506, FK1706, FK506-binding protein) have been shown to improve neuronal recovery.

Interestingly, botulinum neurotoxin A promotes functional recovery after peripheral nerve injury by increasing regeneration of myelinated fibers [76].

3. Nerve grafts as operator channels responding to a cellular membranous bed and incremental neurolysis of fibrosis

3.1 Limitations of vascularized nerve grafts

Under 5.7 Part I, we have demonstrated the superiority of vascularized nerve grafts in scarred vascular beds, in bridging long defects and as salvage grafts in revision surgery. Nevertheless, the use of vascularized nerve grafts has been contested [77, 78]. It has been shown that between 4 and 6 days after operation, blood flow at both the fascicular (endoneural) and the nerve-sheath (epineural) levels is significantly greater in nonvascularized than in vascularized nerve grafts [77]. In a second study [78], 5 months after grafting there has been no significant difference between the conventional and vascularized nerve autografts. In a third study [79], vascularized nerve grafts have shown a better axonal organization and a significantly higher number of regenerated axons in the early phases (on day 30). On day 90, however, the difference in axonal number has not been significant. In a fourth study [80], acetylcholine transferase activity has been statistically higher in vascularized nerve grafts at 4 weeks postoperatively. At 6 weeks postoperatively, this difference has disappeared. Thus, although vascularized nerve graft diminishes endoneurial scarring early after their application [81], some degree of scarring eventually occurs in both vascularized and nonvascularized grafts. This late scarring leads to both of them achieving similar results.

3.2 Nerve cuffing as a measure to limit fibrous tissue reaction and to prevent aberrant axonal sprouting

Surgical cuffing (by plasma clot, Millipore, and Silastic; less so by tantalum, gold, autografted and homografted blood vessels, muscle, Surgicel, surgical tape, liquid plasticizers, collagen) shields the surgical site from ingrowth of connective tissue [82]. If a nerve cuff is to be used, (1) the cross-sectional area of the cuff should be two to three times that of the nerve trunk (smaller cuffs may constrict the anastomotic site; larger cuffs may invaginate and constrict the nerve trunk), (2) the length of the cuff should not exceed 8–10 mm (greater lengths inhibit collateral circulation to the nerve trunk; shorter lengths do not provide adequate shielding at the surgical site), and (3) placement of the cuff onto a nerve stump prior to anastomosis is required.

3.3 Creating a cellular membranous bed rather than cuffing

Another attempt to enhance vascularization of the grafts and prevent endoneurial fibrosis is creating a cellular membranous bed for them. The omentum has been used for this purpose [83, 84]. In a third study [85], to create a membranous bed for nerve grafts, a silicone sheet has been applied between both cut ends of the median nerve in Wistar rats. After removal of the silicone sheet at 5 weeks, a nerve graft has been anastomosed inside the neofomed biological membrane. Application of the biological membrane has not only enhanced nerve regeneration but has also facilitated surgery and has reduced operative time (requiring small incisions at both nerve ends). This has been confirmed in a fourth study [86].

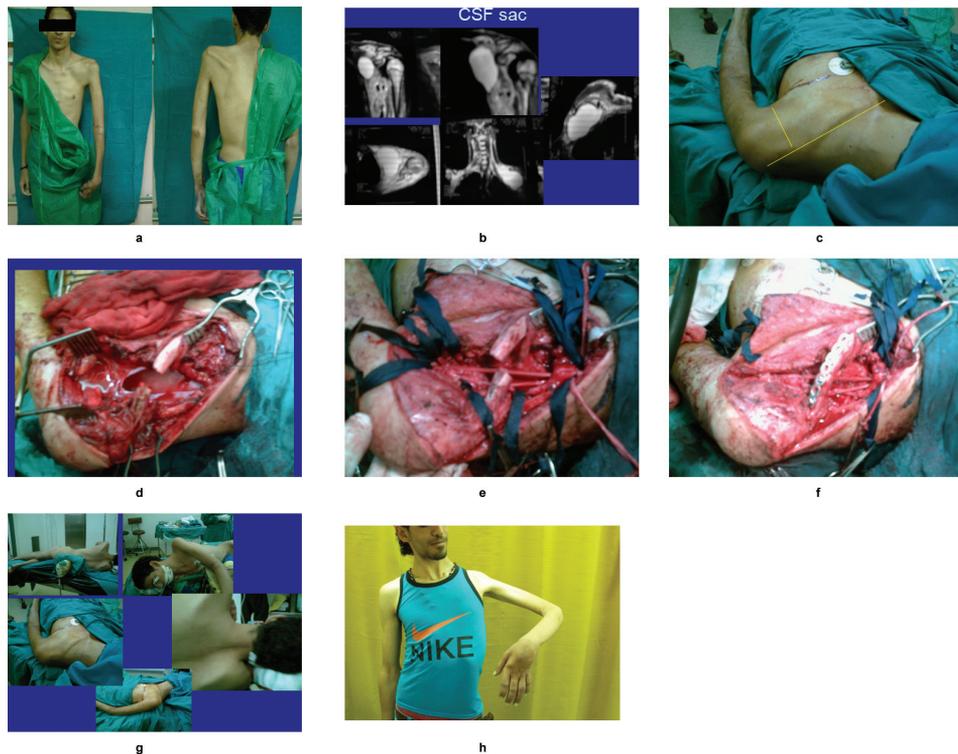


Figure 10.

Creating a membranous bed for nerve grafts from a huge meningocele in a case of direct cord implantation. A 27-year-old male had sustained a complete root avulsion of the left brachial plexus dating 8 months back. The brachial plexus had become retracted to the deltopectoral groove. He had also suffered a neglected rupture of the subclavian artery and vein, both of which had been tamponaded by a huge retroclavicular CSF sac. The patient was explored through a T-shaped supraclavicular incision. Because the CSF sac was huge, the clavicle had to be osteotomized. Grafts were placed into the brachial plexus and embedded into the CSF sac after closing its connection to the cervical cord. The patient was next placed laterally, the cervical cord was exposed through a laminectomy, and the grafts were implanted into its ventral aspect. (a) Preoperative picture of the patient. (b) MRI appearance of the CSF sac. (c) T-shaped supraclavicular incision. (d) The clavicle having been osteotomized and the sac exposed. (e) Nerve grafts having been embedded into the sac. (f) Plating of the clavicle. (g) The patient having been placed laterally to expose the cervical cord via a laminectomy. (h) Postoperative picture 3 years after surgery. The patient regained full shoulder flexion/abduction/adduction, full elbow flexion/extension, forearm pronation/supination, and grade 2 wrist flexion. Shoulder external rotation was possible only with gravity eliminated.

Clinically, in direct cord implantation, the CSF sac formed by the pseudomeningocele may form a healthy cellular membranous bed for placing nerve grafts between the cord proximally and the roots/trunks/divisions/cords of the brachial plexus distally (**Figure 10a–h**). It has been our practice to create a membranous bed using a silicon sheet wrapped partially around the brachial plexus after the procedure of tensioning/shortening/loop grafting (*vide infra*) (**Figure 11a–g**). Problems with silicone sheet insertion include a foreign body reaction and late extrusion in some subjects.

3.4 Endoneurial fibrosis: collapse of internal architecture

In a mouse model of traumatic peripheral nerve injury, the role of nerve ischemia has been studied [87]. Interruption of endoneurial blood supply (microvascular dysfunction) leads to persistent endoneurial hypoxia and subsequent reduction in levels of the Na₂/K₂ ATPase ion transporter. The end result is endoneurial fibrosis. Thus, hypoxia and the Na₂/K₂ ATPase ion transporter may be a target for the

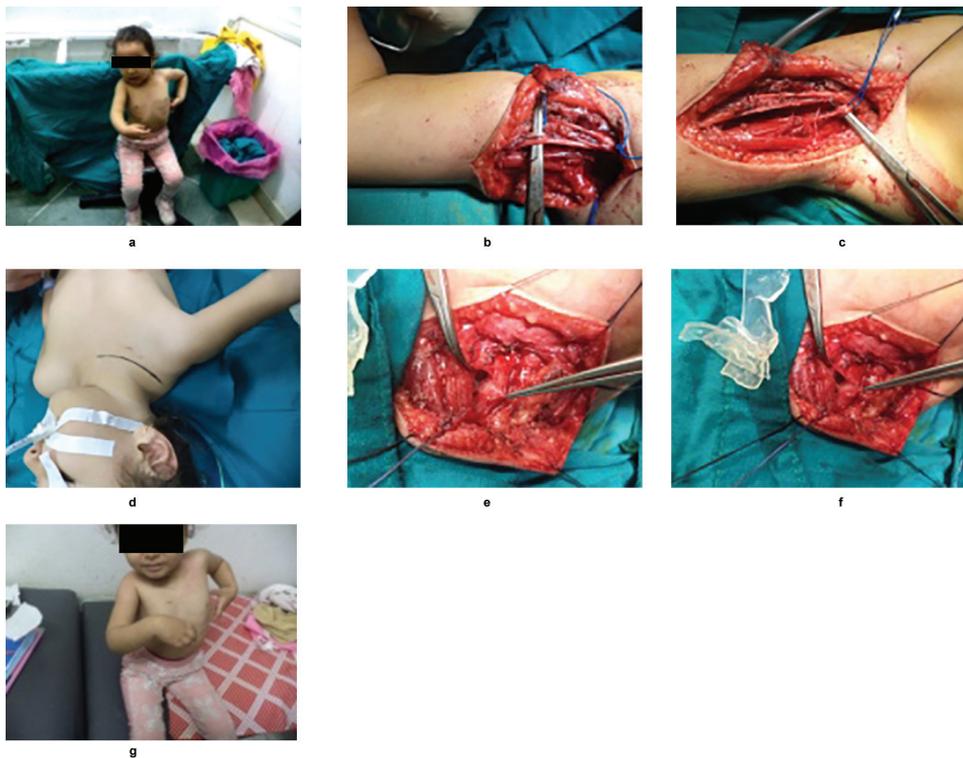


Figure 11.

Creating a membranous bed using a silicon sheet in obstetric brachial plexus palsy. A 4-month-old female infant had presented to us with a C5,6,7 rupture. She had been operated upon using the procedure of tensioning/shortening/loop grafting (vide infra). At the termination of the procedure, a thin silicon film had been placed posterior to the plexus to create a cellular membranous bed. In 2 years, she regained shoulder abduction/flexion, elbow extension, and wrist and finger extension. As elbow flexion was absent, the musculocutaneous nerve was side grafted to the ulnar nerve (donor) (Figure 3). To strengthen shoulder abduction, the axillary nerve was side grafted to the nerve to long head of triceps. In order to restore full function (particularly shoulder external rotation), the brachial plexus was exposed, the silicon sheet was removed, the cellular membranous bed was located, and the brachial plexus was revised by the mobilization/tensioning/shortening procedure (vide infra). (a) Preoperative picture at 2 years/4 months. (b) Axillary dissection: the musculocutaneous nerve having been side grafted to the ulnar nerve (donor). (c) Axillary dissection: the axillary nerve having been side grafted to the nerve to long head of triceps (donor). (d) Supraclavicular incision. (e) The cellular membranous bed after extracting the silicon sheet. (f) The silicon sheet placed beside the membranous bed. (g) Postoperative picture at 4 months after surgery: elbow flexion has been regained; shoulder external rotation has been regained to less than half of the full range.

treatment of nerve injury. Human neuroma contains also increased levels of semaphorin 3A (but not semaphorin 3F) that is associated with inhibition of neurite outgrowth [88].

3.5 Incremental neurolysis of fibrosis and neurogenesis: the roles of heparin and chondroitinase ABC

Both unfractionated and low-molecular-weight heparins inhibit thrombin activation [89, 90]. By partially inhibiting the coagulation mechanism, they can combat hypoxia by improving endoneurial blood flow. In addition, they have a *fibrolytic* (*glycolytic*) effect and can be used for incremental neurolysis of endoneurial fibrosis. The antifibrotic effects of heparin are well-documented after flexor tendon surgery of the hand [91], in the resolution of intraperitoneal fibrosis [92, 93] and in improving various scar types [94]. In peripheral nerve surgery, perineural application of condensed polytetrafluoroethylene-extractum cepae-heparin-allantoin gel

during peripheral nerve surgery improves functional recovery [95]. Heparin (as well as acetylsalicylic acid (aspirin) and high-molecular-weight hyaluronic acid) not only influences fibroblast growth factor 1 and fibroblast growth factor 2 activity [96–98], but they also have a *neurogenic effect* through modulating astrocyte function [96–106].

For the non-anticoagulant effects of heparin, the reader is referred to the review articles by Mulloy *et al.* [89] and Olczyk *et al.* [97]. Similar roles as heparin have been ascribed to chondroitinase ABC in peripheral nerve regeneration [107–127] with consequent improvement of cocontractions (aberrant sprouting) [121].

Although the blood flow promoting, endoneurial neurolysing, and neurogenic effects of heparin and chondroitinase ABC are well established, clinical experience with both of them in brachial plexus surgery is still anecdotal. In brachial plexus injuries associated with subclavian artery injury, early administration of Clexane in therapeutic dose by the vascular surgeons after synthetic grafting of the subclavian artery at day 1 after injury leads to early regain of motor power, when the brachial plexus has been explored and grafted later on at 3 months after injury (**Figure 12**). Heparin may be administered late (months) after successful brachial plexus grafting, when clinical progression has come to a halt (**Figure 13**). Also, it may be



Figure 12.

Effect of institution of heparin therapy immediately after injury in a left C5,6,7 rupture associated with subclavian artery injury in a 30-year-old man. After grafting of the subclavian artery, Clexane had been administered in therapeutic dose by the vascular surgeon (Clexane 80 IU three times daily for 8 days). He had then been turned to Clexane 60 IU twice daily. Brachial plexus grafting was performed 4 months after the initial injury. After brachial plexus surgery, Pradaxa and Clexane were administered as described under Section 3.5. Motor power improvement started to occur 3 months after brachial plexus surgery. (a) Postoperative picture 3 months after brachial plexus surgery: biceps function has been regained. (b) Postoperative picture 3 months after brachial plexus surgery: shoulder abduction has been regained up to half of the full range. (c) Postoperative picture 9 months after brachial plexus surgery: shoulder abduction has been regained more than half of the full range; grade 2 wrist extension has been regained.



Figure 13.

Effect of institution of heparin therapy 1 year after nerve grafting of a C5,6,7,8 T1 rupture in a pediatric subject. (a) Picture 1 year postoperatively: no recovery of motion. (b) Picture 2 years after institution of heparin therapy.



Figure 14. Effect of institution of heparin therapy immediate postoperatively. Postoperative picture at 5 years after surgery and heparin therapy. This patient has regained shoulder abduction/flexion/less than half of range of external rotation, elbow flexion/extension, forearm pronation/supination, and full hand function. Hand grip is still weak (30 mmHg compared to 95 mmHg on the contralateral side).

administered immediate postoperatively (**Figure 14**). Patients respond best to intermittent oral ultrafractionated heparin therapy in high dose combined with ultrafractionated local heparin injection around the brachial plexus at the supraclavicular area. It has been the author's experience to use Pradaxa (Boehringer Ingelheim) 400 IU for 10 days followed by a hiatus of 7 days, to be repeated again in 10-day/7-day cycles. Oral therapy is aided by Clexane 20 IU injection at the supraclavicular area every 14 days. A common complication of this therapy is sinus formation due to local Clexane injection; otherwise the therapy is tolerated well.

3.6 Heparin and muscle regeneration

Heparin stimulates muscle growth and regeneration. This is supported by the following evidence.

Skeletal muscle regeneration involves muscle precursor proliferation and differentiation and probably requires the participation of heparin-binding growth factors such as fibroblastic growth factors (FGFs), hepatic growth factor (HGF), and transforming growth factor (TGF)-beta. Heparan sulfate proteoglycans are expressed in forming muscle masses during the development and in cell culture, suggesting their participation in the regulation of myogenesis. Heparin itself has been widely utilized for growth factor delivery due to its electrostatic nature and specific affinity with heparin-binding growth factors. Heparin-containing polymeric particulates are utilized as functional carriers to deliver growth factors in a controlled manner [128]. Heparan sulfate proteoglycans are increased during skeletal muscle regeneration [129]. It has been shown that four major species—perlecan, glypican, syndecan-3, and syndecan-4—are transiently upregulated. The first three are detected at the surface or basement membranes of newly formed myotubes. Thus, *heparin and heparan sulfate proteoglycans are required for successful skeletal muscle regeneration.*

In addition, *muscle reinnervation and insulin growth factor (IGF)-I synthesis are affected by exposure to heparin*, an effect which is partially antagonized by anti-growth hormone-releasing hormone [130].

Heparin and heparan sulfate synthetic mimetic polymers (RGTA(11)) have exerted similar effects on muscle regeneration [131]. Extracellular matrix histone H1 is an endogenous extracellular ligand for muscle cell heparan sulfate proteoglycans. It binds to perlecan, it is present in regenerating skeletal muscle, and it

stimulates myoblast proliferation [132]. Midkines are a family of developmentally regulated neurotrophic and heparin-binding growth factors that are stimulated by heparin and are involved in muscle development [133].

Under Section 3.5, we have shown how oral heparins can be aided by local administration of Clexane around the brachial plexus. Based on the evidence aforementioned, an interesting speculation is whether local administration of Clexane into the common flexor and extensor muscle mass can aid oral heparins in restoring hand function. It has been the author's practice to inject Clexane 20 IU into the common flexor and extensor muscle mass; the results as to restoring hand function have been disappointing. However, Clexane thus administered has preserved muscle mass (*vide infra*) (**Figure 15**). This has taken place apart from the volume effect produced by repeated injection.

3.7 Heparin and obstetric palsy

It is also interesting to speculate whether heparin can correct fibrous tissue deformity, restore muscle balance, and help nerve regeneration in obstetric brachial plexus palsy. It has been the author's postoperative practice after obstetric brachial plexus nerve grafting to inject Clexane 20 IU supraclavicularly every 14 days. In infants, this has kept the joints supple. Its effect on restoring muscle balance and on nerve regeneration takes years, however, appearing at preschool age (**Figure 16**).

3.8 Schwartz-Jampel syndrome: a good model for studying the effect of heparin in obstetric brachial plexus palsy

Studying Schwartz-Jampel syndrome serves as a good model for studying the effect of heparin in obstetric brachial plexus palsy. Schwartz-Jampel syndrome (SJS), first described in the United States in 1962, is a hereditary disorder characterized by myotonia, facial dysmorphism, and skeletal deformities [134, 135]. Patients have a short neck, blepharophimosis, flattened face, hypertrichosis of the eyelids, prominent eyebrows, high-arched palate, low-set ears, micrognathia, short stature, and skeletal deformities. They have proximal muscle hypertrophy, distal muscle wasting, and generalized hyporeflexia.

A mutation in the perlecan gene is its cause [136]. Dyssegmental dysplasia and Silverman-Handmaker type are due to its functional absence.

Perlecan is the major heparan sulfate proteoglycan of the pericellular matrix, a component of the extracellular matrix that is found immediately surrounding individual chondrocytes in developing and adult cartilage, and is rich in the proteoglycan perlecan [137]. Experimental perlecan knockdown has altered matrix



Figure 15. *Intramuscular heparin has preserved forearm muscle mass in a right C5,6,7,8 T1 rupture nerve grafted 3 years back. (a) Whole rt. upper limb 3 years after surgery. (b) Right forearm 3 years after surgery.*



Figure 16. Heparin in obstetric palsy. This male patient was operated upon at the age of 4 months for a left C5,6,7 rupture. After surgery, Clexane 20 IU was injected supraclavicularly every 14 days. (a) Postoperative picture at 2 years/4 months: less than half of the range of shoulder external rotation; Mallet score III. (b) Postoperative picture at the age of 4 years: more than half of the range of shoulder external rotation has been regained; Mallet score IV. (c) No deltoid/biceps cocontractions. (d), (e) Wrist extension has been regained. (f) Finger extension is still weak.

organization and has significantly decreased the stiffness of both chondrocytes and interstitial matrix as a function of age and genotype, implicating a role for outside-in mechanical signals from the pericellular in regulating the intracellular mechanisms required for the overall development of cartilage.

Schwartz-Jampel syndrome is also characterized by distal peripheral nerve hyperexcitability resulting from synaptic acetylcholinesterase deficiency, nerve terminal instability with preterminal amyelination, and subtle peripheral nerve changes, all as a result of perlecan deficiency [138]. Muscle biopsies containing neuromuscular junctions have shown well-formed postsynaptic element, synaptic acetylcholinesterase deficiency, denervation of synaptic gutters with reinnervation by terminal sprouting, and long nonmyelinated preterminal nerve segments.

Reasoning the other way round, Schwartz-Jampel syndrome shows the profound changes heparin (a proteoglycan like heparan sulfate) may have on modulating joint, muscle, and nerve function, all of which are implicated in obstetric brachial plexus palsy.

4. Nerve grafts as physical operator channels responding to mechanical forces and electric stimulation

4.1 Nerve response to tension

In the previous section, we have introduced the neurotrophic and contact guidance theory for axonal growth. It is based on molecular neurobiology.

The process of “stretch growth of integrated axon tracts” or tension, however, is perhaps the most remarkable axon growth mechanism of all [139]. This process can extend axons at seemingly impossible rates without the aid of chemical cues or even growth cones. Growth cone-mediated neuronal elongation occurs through cytoskeletal dynamics involving the polymerization of actin and tubulin subunits at the

tip of the axon. However, axons and growth cones also generate forces (through cytoskeletal dynamics, kinesin, dynein, and myosin), forces induce axonal elongation, and axons lengthen by stretching [140]. This has been confirmed in nerve chamber experiments [141]. These experiments have also demonstrated that regeneration is downregulated by circumferential pressure; formation of a contractile cell (myofibroblast) capsule around regenerating nerves restricts growth by application of circumferential mechanical forces.

The concept of subjecting intact nerve endings to tensile forces to promote axonal sprouting while simultaneously relieving tensile and shear forces off the grafted or sutured area [142, 143] to combat fibrosis (**Figure 17**) has many consequences:

1. During surgery, the brachial plexus has to be mobilized along its whole length, because its adhesion to surrounding structures imparts compressive forces to its intact ends and tensile forces to the diseased (nerve grafted) segment (**Figure 18**).
2. After mobilizing the whole plexus, the fibrous segment had better not be fully excised. Leaving a posterior fibrous segment helps unload the grafted segment from tensile and shearing forces (**Figure 19**).

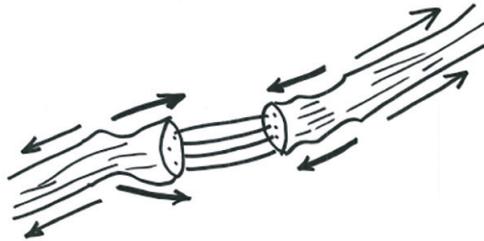


Figure 17. *Subjecting intact nerve endings to tensile forces to promote axonal sprouting while simultaneously relieving tensile and shear forces off the grafted or sutured area to combat fibrosis.*

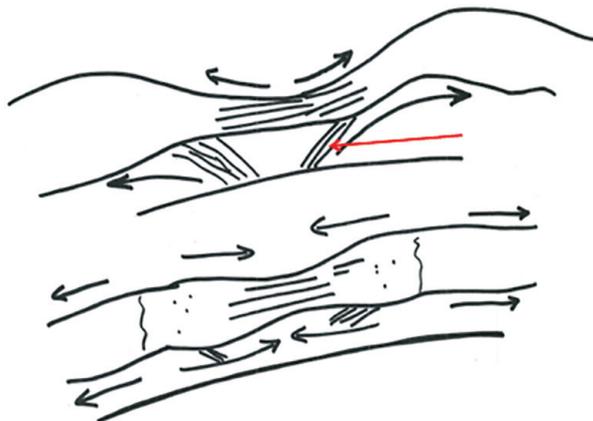


Figure 18. *During surgery, the brachial plexus has to be mobilized along its whole length, because its adhesion to surrounding structures imparts compressive forces to its intact ends and tensile forces to the diseased (nerve grafted) segment (red arrow).*

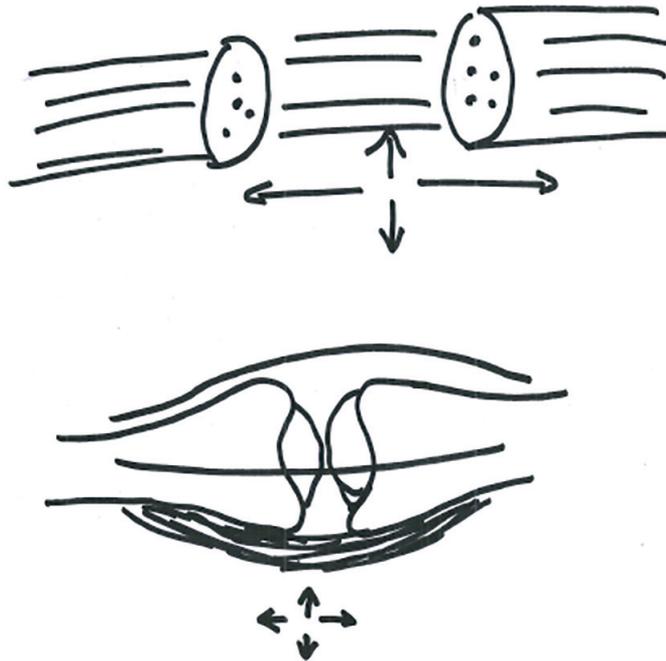


Figure 19.
After mobilizing the whole plexus, the fibrous segment had better not be fully excised. Leaving a posterior fibrous segment helps unload the grafted segment from tensile and shearing forces.

3. After completion of grafting, epiperineurial 2/0 vicryl tension sutures have to be applied to both intact nerve endings to apply tensile forces to them while unloading the grafted segment (**Figure 20**).
4. The same effect just described is produced by loop grafting [144]. In addition, they serve as side grafts, enhancing nerve regeneration through the neurotrophic and contact guidance theories for axonal growth. As they do not damage potentially partially regenerating/regenerated axons, such as in obstetric brachial plexus palsy, they can be applied to them (**Figure 21**)

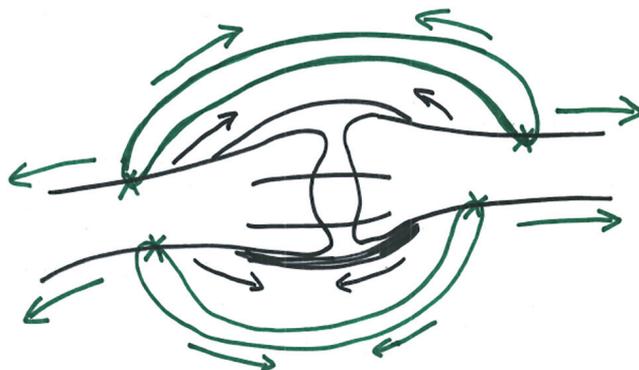


Figure 20.
After completion of grafting, epiperineurial 2/0 vicryl tension sutures have to be applied to both intact nerve endings to apply tensile forces to them while unloading the grafted segment.

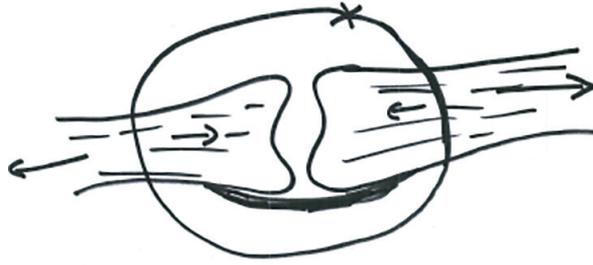


Figure 21.

The same effect just described is produced by loop grafting. In addition, they serve as side grafts, enhancing nerve regeneration through the neurotrophic and contact guidance theories for axonal growth. As they do not damage potentially partially regenerating/regenerated axons, such as in obstetric brachial plexus palsy, they can be applied to them.

5. Continual not single tensile forces have to be applied, so dynamic tension devices [145, 146] have to be improved to meet these requirements. By the same token, just as Ilizarov external fixation produces bone by tension, it also promotes nerve regeneration by tension (**Figure 22**).
6. For the same reason just mentioned, and because it is difficult to apply dynamic tension devices to brachial plexus lesions, axonal elongation may be likewise induced by magnetic nanoparticles [147, 148] applied parenterally to both nerve ends and subjected to pulsed electromagnetic fields (**Figure 23**).
7. Because subjecting intact nerve endings to tensile forces to promote axonal sprouting while simultaneously relieving tensile and shear forces off the grafted or sutured area can be applied at any time to stimulate axonal elongation, there is always a place for revision surgery. During revision surgery, the whole plexus has to be mobilized and the procedures described under 3 and 4 have to be applied. Because these procedures do not affect the integrity of the previously repaired brachial plexus, they can be applied to partially regenerated lesions (**Figure 24**).
8. Free muscle transplantation spanning shoulder, elbow, and wrist/fingers as described under 3.6 Part I acts within few months. It serves as a good internal splint stabilizing the elbow and wrist pending return of function as described under 7.

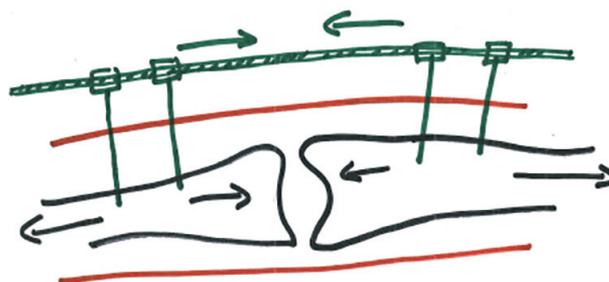


Figure 22.

Continual not single tensile forces have to be applied, so dynamic tension devices have to be improved to meet these requirements.

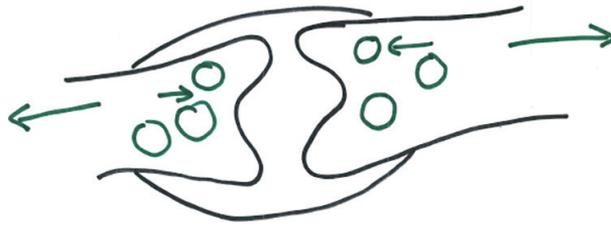


Figure 23.

For the same reason just mentioned, and because it is difficult to apply dynamic tension devices to brachial plexus lesions, axonal elongation may be likewise induced by magnetic nanoparticles applied parenterally to both nerve ends and subjected to pulsed electromagnetic fields.

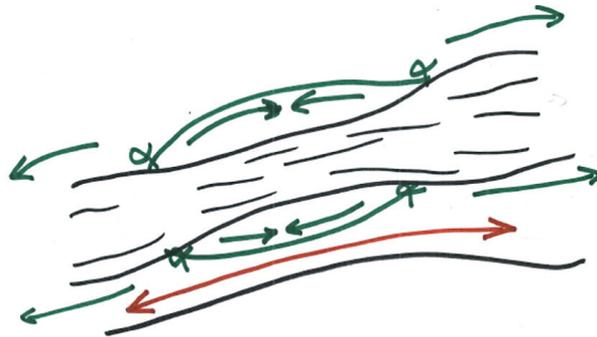


Figure 24.

Revision surgery on the brachial plexus.

9. Under 4.3 Part I, we have mentioned that “neuromata of Types 1A, 1B, 2A, 2B, 3A, and 3B (so-called neuromata in continuity) are all treated by neurolysis (Table 3 Part I). Neuromata of Types 3C, 4N, and 4S (so-called complete neuromata) are treated by excision and nerve grafting. A Typ. 5 neuroma includes roots, trunks, divisions, or cords with interrupted continuity and root avulsions. The formers are treated by excision and nerve grafting, whereas the latter are treated by neurotization.” Also, under 12.2.3 Part I, we have referred to the controversy over neurolysis versus excision and grafting in obstetric brachial plexus lesions. Both concepts need revision, however. In Types 3A, 3B, 3C, 4N, and 4S, the axons and endoneurium are cut and retracted, but the epineurium is intact. Because the epineurium is intact, the injured brachial plexus enjoys a considerable degree of mobility, being not adherent to surrounding structures. Consequently, the brachial plexus can undergo a shortening/tensioning/loop grafting procedure as described under 3 and 4 bringing retracted axons together. This is particularly so in both early and late obstetric palsy cases (**Figure 25**).
10. Item (9) shows the importance of assessing the degree of nerve injury (see 4.3 Part I).
11. Because subjecting intact nerve endings to tensile forces to promote axonal sprouting while simultaneously relieving tensile and shear forces off the grafted or sutured area is a so important concept, in which full exploration of the brachial plexus has to be reverted to.



Figure 25.

The procedure shortening/tensioning/loop grafting in obstetric brachial plexus cases. In Types 3A, 3B, 3C, 4N, and 4S, the axons and endoneurium are cut and retracted, but the epineurium is intact. Because the epineurium is intact, the injured brachial plexus enjoys a considerable degree of mobility, being not adherent to surrounding structures. Consequently, the brachial plexus can undergo a shortening/tensioning/loop grafting procedure as described under 3 and 4 bringing retracted axons together. This is particularly so in early obstetric palsy cases. In late obstetric palsy, the procedure shortening/tensioning/loop grafting is a good example of nerve augmentation; augmenting late neuromas in continuity, the nerves and muscles of which have partially regenerated but are not expected to regenerate completely by bypass grafts (see Figure 7). (a) Lt C5,6,7 rupture in a 4-month-old female. (b) Operative findings. (c) Status after shortening/tensioning/loop grafting. (d), (e) rt. C5,6,7 rupture in a 4-year-old male. (f) The surgical incision. (g) Operative findings. (h) Status after shortening/tensioning/loop grafting. (i) Application of the silicon sheet.

12. Subjecting intact nerve endings to tensile forces to promote axonal sprouting while simultaneously relieving tensile and shear forces off the grafted or sutured area is a strong stimulus to axonal sprouting. Therefore, the indications for surgery in obstetric brachial plexus cases should be widened (see 12.2.2 Part I) pending their complete recovery. Such cases may also be revised as under item 7.

13. Direct contralateral C7 suturing after a humeral diaphyseal shortening osteotomy as discussed under 7 Part I involves stimulating regeneration by tensioning intact nerve stumps.

4.2 Nerve response to compression

Circumferential pressure hampers axonal growth. This has been confirmed clinically [149] and in nerve chamber experiments [141]. These experiments have also demonstrated that regeneration is downregulated by circumferential pressure;

formation of a contractile cell (myofibroblast) capsule around regenerating nerves restricts growth by application of circumferential mechanical forces.

Hampering axonal sprouting by circumferential pressure in recipient end to donor side neurorrhaphy may stimulate side sprouting.

4.3 Nerve response to electric stimulation

Motor and sensory axon outgrowth is accelerated by brief low-frequency electrical stimulation (even after delayed surgical repair of injured nerves) [38].

4.3.1 Brief low-frequency electrical stimulation accelerates motor and sensory axon outgrowth across the injury site after immediate nerve repair

A latent period precedes the outgrowth of regenerating axons from a proximal nerve stump into a denervated distal nerve stump. This latent period has been estimated to be as short as a day or as long as 3 days, with axons regenerating thereafter at rates of 1–3 mm/day in humans and rats, respectively. Fluorescent dye retracing, however, has shown that it may be longer extending up to 3–4 weeks [38].

Electrical stimulation of the transected nerve proximal to the site of transection and surgical repair reduces the latency of the axons as they regenerate across the suture site. The electrical stimulation does not affect the rate of axonal transport as determined by injecting radiolabeled thymidine into the neurons. The literature is not unanimous as to the frequency or the duration of electrical stimulation (whether at 20 Hz for 30 min/day, for only 20 min or for 10 min). Nevertheless, a 2-week period of continuous stimulation at 20 Hz or even 1 h of stimulation has recommended to promote the outgrowth of regenerating axons across the suture line [38]. The action potentials, generated by the brief electrical stimulation of the axons proximal to the lesion site and transmitted back to the soma of the neurons, are essential for the efficacy of the accelerated axon outgrowth in response to the electrical stimulation; tetrodotoxin blockade of these potentials obliterates the effect of the electrical stimulation.

4.3.2 Brief low-frequency electrical stimulation accelerates motor and sensory axon outgrowth across injury sites after delayed nerve repair

Electrical stimulation has been effective even in chronic axotomy and chronic denervation [38]. Clinically, the 1-h 20-Hz electrical stimulation regimen has been effective in promoting median nerve regeneration after its constriction at the wrist in patients undergoing carpal tunnel release surgery.

4.3.3 Electric stimulation elevates neuronal cyclic adenosine monophosphate and, in turn, the expression of neurotrophic factors and other growth-associated genes, including cytoskeletal proteins

Although findings point to electrical stimulation mediating its effects by elevating neuronal cyclic adenosine monophosphate (cAMP), this theory is not agreed upon unanimously [38]. Growth-associated proteins (GAP-43 and CAP-23, members of a MARCKS-related group of acylated membrane proteins that interact with actin filaments, calmodulin, protein kinase C, and phosphoinositides) are upregulated in response to electric stimulation.

4.3.4 Neurotrophic factors, androgens (and growth hormone, thyroid hormones), and exercise increase the efficacy of electric stimulation

Brief electrical stimulation accelerates the expression of neurotrophic factors and their receptors. Thereafter, the cytoskeletal proteins, actin and tubulin, and GAP-43 are upregulated. However, this expression is transient, declining within days. The administration of androgens in conjunction with electrical stimulation, however, sustains their upregulation. Also, a daily slow training protocol at 10 m/min for 1 h in male mice or a faster 20 m/min training protocol for 2-min intervals four times daily in female mice has resulted in a marked increase in the length of regenerating axons 2 weeks after nerve transaction and repair. Finally, there are several other surgical and pharmacological strategies that are being explored to promote regeneration and to counteract the negative effects of chronic nerve injuries. Surgically, end-to-side or side-to-side nerve autografts can be placed between a donor nerve and a recipient denervated distal nerve stump.

5. Nerve grafts as operator channels responding to tissue engineering as applied to neuroscience: the role of artificial nerve grafts applied as side grafts

In previous publications [150, 151], we have shown that biomaterial neuroscaffolds should be biocompatible, nontoxic, chemically stable, and of known absorption and degradation kinetics matching the degree of in vivo cell/tissue growth and should have adequate surface for cell access, proliferation, and cell differentiation.

Scaffolds should meet macroengineering requirements. Scaffolds should be of proper form, design (cylinder, tube, multichannel, and open-path design with and without a central core), and size (diameter). In addition, they should be supplied with macrogrooves and have reasonable wall thickness (a wall thickness of 0.6 mm, a porosity of 80%, and a pore size range of 10–40 μm).

Scaffolds should meet microengineering requirements. Microengineering refers primarily to microgrooves directing axonal growth. Another microengineering aspect refers to inclusion of filaments.

Scaffolds should fulfill the same mechanical conditions of the recipient nerve. An elastic or shear modulus inequality might produce tension, compression, or shear at the repair site, ending up with fibrosis and hampering progression of regeneration.

Scaffolds should provide adequate space for the interplay and manipulation of the different molecular pathways for axonal regeneration. Biomaterial polymer nerve scaffolds should provide adequate space (80–90% porosity with a pore size of 50 to 250 μm , stabilized by adding glutaraldehyde, polyethylene glycol, heparin, or collagen) and adherence (by cross-linking agents, e.g., genipin) for the components of the neurotrophic and contact guidance theory mentioned under Section 2.1 [2–4].

Scaffolds should provide adequate space for lumen fillers. Methods of lumen filling ((1) combining growth factors with a *growth-supporting matrix* in the lumen, (2) incorporating accessory cells into the lumen matrix, (3) impregnating nerve conduit walls with cells or neurotrophins via crosslinking or immobilization, (4) seeding (genetically engineered) cells that produce growth factors inside the lumen, and/or (5) using microspheres to deliver growth factors or accessory cells to the NC lumen) allow for incorporation of cells and molecular factors.

Scaffolds should meet requirements based on spatial distribution of neurotrophic factor gradients. Because the axonal growth cone is sensitive to spatial molecular concentration gradients as described under Section 2.2 [8], scaffolds should meet

requirements based on spatial distribution of neurotrophic factor gradients. They should also respond to electric stimulation [38] and pulsed magnetic fields [147, 148].

6. The proximal nerve segments (stumps) as motor and sensory operator donors in relation to nerve graft operators

6.1 Regeneration in CNS versus P motoneurons

Although axons can grow [152–155], their growth is inhibited by myelin inhibitors (Nogo-A, MAG108 (myelin-associated glycoprotein) and OMgp109 (oligodendrocyte myelin glycoprotein)), chondroitin sulfate proteoglycans (neurocan, versican, aggrecan, brevican, phosphacan, and NG2), semaphorins, and ephrins. In the central nervous system, laminin is replaced by netrins.

6.1.1 Chondroitinase ABC

Chondroitinase ABC dissolves proteoglycans [156] but should be thermostabilized with trehalose to reduce its temperature-dependent loss of activity; it should be injected in high doses (50 or 100 IUs), at multiple times, and be combined with cell transplantation and growth factor infusion.

6.1.2 Other measures to overcome the gliosis

Sialidase may be superior to chondroitinase ABC; myelin-associated inhibitors may be blocked with *Nogo-A monoclonal antibodies* or with *Nogoreceptor competitive agonist peptide (NEP1-40)*; blocking *Rho-A* with *Rho inhibitor “cethrin”* might overcome its effect; chondroitin sulfate proteoglycan inhibition of phosphoinositide 3-kinase (PI3K) signaling may be reversed by *cell-permeable phosphopeptide (PI3K_{pep})*; *rolipram*, a phosphodiesterase4 inhibitor, can increase intracellular cAMP levels; *taxol*, a microtubule-stabilizing agent, increases neurite outgrowth.

6.1.3 Cell intrinsic mechanisms

In addition to the inhibitors mentioned above, neuronal cell intrinsic mechanisms to regenerate are hampered [157]. The combined action of repressors of axonal growth, the limited injury signaling mechanisms, and the lack of robust expression of regeneration-associated genes (RAGs) results in a restricted potential to regenerate.

6.2 Insights gained from mathematical neuroscience elements of information theory as applied to grafting proximal nerve segments (stumps)

In previous sections, we have detailed the principles of nerve grafting and nerve transfer. In fact, these principles are simple but direct applications of mathematical information theory, a theory that is based on increasing the probability of axonal sprouting from a given donor through a nerve graft channel to a certain recipient. We have also detailed the principles of end-to-side grafting. If the side of a motor nerve is injured, the axonal growth cone may be enticed to grow off motor nerve side to the injured end of another motor nerve, so called recipient end to donor side coaptation. An indirect application of it based on molecular neurobiologic axonal guidance and information theory is increasing the incidence of nerve regeneration

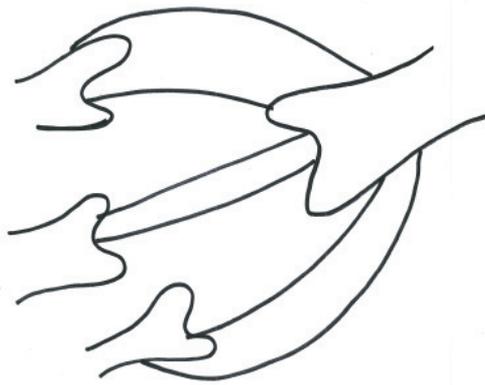


Figure 26.
Single donor to multiple recipient transfer.

after conventional end-to-end grafting by applying additional grafts extending from the side of the donor end to the side of the recipient end [144] (augmented end-to-end nerve grafting) (**Figure 6**). In nerve transfer, the latter technique allows the surgeon to use a single high axonal load donor for multiple recipients without producing cocontractions (e.g., major brachial plexus root to several peripheral nerves) [144] (**Figure 26**). Also, partially regenerated nerves cannot be surgically cut and nerve grafted leading to loss of already regained function; the latter technique allows the surgeon to enhance regeneration through them (**Figure 7**).

The procedure just mentioned has been applied in facial reanimation surgery for multiple branch reconstruction using a rat four-branch facial nerve reconstruction model with end-to-side cross-face nerve graft [158]. This has later on been confirmed clinically in 32 patients who have undergone facial nerve reconstruction [159]. The authors coined the term “axonal supercharging,” namely, the connection of double-donor neural sources to the graft, and “axonal distribution,” namely, the reinnervation of multiple recipient nerve stumps connected to the graft in an end-to-side manner. In mathematical information theory, the Shannon-Hartley channel-carrying capacity principle refers to the intrinsic property of any information channel to accept all information from the donor and transmit it noiseless to the recipient. Applied to nerve grafting, the channel-carrying capacity of a nerve graft scaffold is its ability to transmit all axons sprouting from the proximal cord to the distal cord and simultaneously minimize the probability of aberrant neural sprouting [160, 161].

6.3 Conditioning lesions

6.3.1 The neuroscientific basis of conditioning lesions: the effect of conditioning lesions on the central nervous system versus the peripheral nervous system

The general inability of CNS axons to regenerate can be overcome by a conditioning lesion. Dorsal root ganglion (DRG) neuron peripheral branch injury prior to lesioning the central branch (conditioning lesion) leads to the central axon overcoming the glial scar inhibitory effect [157]. On the contrary, DRG neurons cannot be conditioned by an injury in the central branch [162]. Although a peripheral lesion performed subsequently to the CNS injury does not improve axonal regeneration due to the assembly of a thick glial scar, it still increases the intrinsic regenerative ability of DRG neurons [157]. This evidence strongly suggests that, while peripheral branch injury can increase the intrinsic growth capacity of DRG neurons, the same

is not true for injury of the central branch. The conditioning effect is probably due to the activation of a regenerative process. Several mechanisms coordinate that process (calcium waves, epigenetic modifications, active retrograde macromolecular transport, transcriptional response, and local protein synthesis). *These have been reviewed elsewhere* [157, 162].

6.3.2 *Adding distal nerve transfers to direct cord implantation may enhance the effect of the latter*

First, as direct cord implantation involves axonal growth from the central nervous system to the roots of the brachial plexus (peripheral nerves), it is prudent to enquire whether extraplexal distant nerve transfers have an added benefit of a conditioning lesion. To give an example, a patient with global brachial plexus root avulsion undergoes direct cord implantation. At the time of surgery, the surgeon transfers the spinal accessory to the suprascapular nerve and the intercostal nerves to the musculocutaneous and median nerves. Do both of these transfers enhance recovery of other nerves (e.g., the axillary nerve) by the conditioning lesion effect? This has been observed in a clinical study [163]. It has been concluded that the combination of intra- and extradural neurotizations improves proximal muscle function results.

Second, it is also prudent to enquire whether contralateral nerve transfers (e.g., contralateral C7 transfer) have the same conditioning effect as ipsilateral transfers. Evidence supports this. In an experimental study on the rat sciatic nerve [164], it has been concluded that nerve injury enhances cytokine expression in the contralateral dorsal root ganglion and promotes contralateral nerve regeneration in vivo by shortening the initial delay.

Third, if the surgeon opts to perform these distal transfers not at the time of surgery but as a second procedure, is there a time limit between both surgeries for the conditioning lesion to take effect? We shall provide an answer to this in the following section (Section 6.3.3).

6.3.3 *Adding distal nerve (side) transfers before/after full exploration/grafting/proximal nerve transfer of the brachial plexus may enhance its recovery: a plea for reverting to full exploration of the brachial plexus (Figures 27 and 28)*

Conditioning lesions may also be performed on peripheral nerves to enhance their recovery. This has been addressed in a large series of experiments [165–195] with only one study carried out on the rat optic nerve refuting it [185].

*Adding distal nerve (side) transfers to full exploration/grafting/proximal nerve transfer of the brachial plexus may enhance its recovery not only by the conditioning lesion effect but also by their effect on collateral sprouting. Regenerating axons in crushed peripheral nerves grow through their distal nerve segments even in the absence of Schwann cell support, but their elongation rate is reduced by 30% (*vide infra*). It has been examined whether prior exposure of sensory neurons to trophic factors achieved either by collateral sprouting or by regeneration after conditioning lesion could enhance subsequent regeneration of their axons after crush and compensate for loss of cell support. It has been found that prior collateral sprouting for 2 weeks enhances elongation rate of sensory axons regenerating through acellular distal segment of a crushed peripheral nerve [169]. In that experiment, collateral sprouting of the peroneal cutaneous sensory axons in the rat has been evoked by transection of adjacent peripheral nerves in the hind leg. The segment of the peroneal nerve distal to the crush has been made acellular by repeated freezing.*

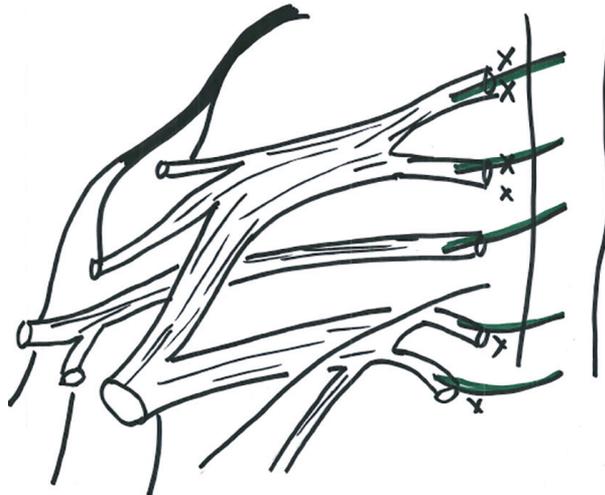


Figure 27.
Adding distal nerve transfer as a conditioning lesion to direct cord implantation.

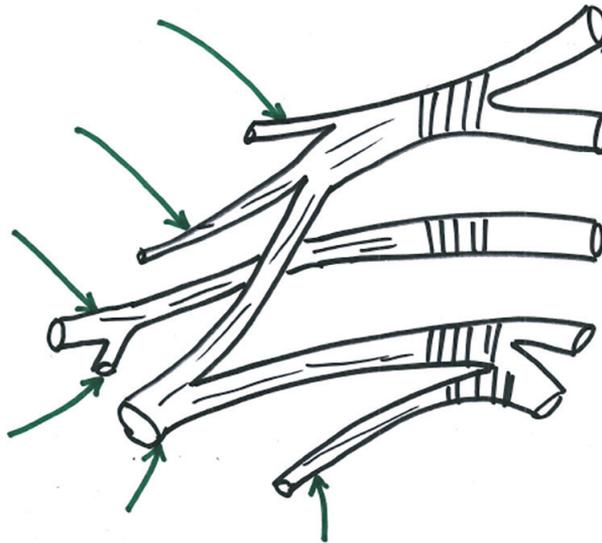


Figure 28.
Adding distal nerve transfer as a conditioning lesion to nerve grafting of the original injury.

The minimum effective conditioning interval (time between conditioning and testing lesions or between distal nerve transfers and repair of the brachial plexus) has to be investigated. (1) The minimum effective conditioning interval has been determined [172]. When the conditioning and testing lesions are made simultaneously (0 day conditioning interval), there has been no conditioning lesion effect. With a conditioning interval of 3 days, there has been a shortening of the initial delay (before the onset of outgrowth) without a change in outgrowth rate. With conditioning intervals of 7, 14, and 21 days, the rates of outgrowth have been increased by 8, 22, and 11%, respectively. In other studies, the minimum effective conditioning interval has been considered to be 5 days [164], 6 days [196], or 7–14 days [165, 177, 184, 188]. Outgrowth accelerates after a single axotomy [172]. The kind of the conditioning lesion influences the intensity and the time course of the conditioning lesion effect

[182]. *Concluding, for distal nerve transfers to act effectively as conditioning lesions, they had better be performed 14–21 days prior to full exploration of the brachial plexus.*

The cell body appears to be the primary locus of the “conditioning effect” [189]. This has been confirmed in a second study [181], detailed in a third one [180], and questioned in a fourth [179].

A conditioning lesion of the peripheral axons of dorsal root ganglion cells has been found to accelerate regeneration of their peripheral axons only [186]. Thus a distal nerve side transfer carried out on the musculocutaneous nerve, e.g., will enhance regeneration of the musculocutaneous nerve only after subsequent full exploration/grafting of the brachial plexus.

The conditioning lesion is not age related [168]; it can be applied in young as well as in aged subjects. The ability of motor axons from mature (6–8 months) and old (22–24 months) Fischer 344 rats to form new axonal sprouts has been compared after subjecting them to a conditioning lesion. There has been no change in the initial delay before sprouting under any condition. Thus, axons from old animals can be stimulated to repair themselves at rates comparable to those seen in younger animals.

Conditioning lesion effects are influenced by electrical stimulation delivered to denervated muscles [176]. Daily electrical stimulation does not modify the increased rate of regeneration but prevents the decrease of the initial delay whatever the type of the prior lesion.

An increased regeneration rate in peripheral nerve axons has been observed following double lesions at the same site [187], so called superimposition lesions [183]. The rate of regeneration of rat sciatic nerve sensory axons has been measured using the pinch-reflex test method and confirmed by studying the transport of labeled protein into the regenerating axons. The regeneration rate of nerves receiving a single test crush lesion has been inferior to that of nerves with a conditioning lesion made at the knee 7 days prior to the test; both regeneration rates have been inferior to that of nerves where both conditioning and test lesions have been made at the same site. This shows that pre-degeneration of the nerve distal to the site of the test lesion increases the rate of regeneration enhancing the conditioning lesion phenomenon. The influence of predegenerated nerve grafts on axonal regeneration from prelesioned peripheral nerves has also been studied in a rat sciatic nerve model [170]. This has been confirmed in a fourth study [175].

Interestingly, in the latter study [175], it has been found that the conditioning interval and not the number of conditioning lesions determines the outgrowth after a test lesion.

In a further study examining the role of migratory Schwann cells in a conditioning effect of peripheral nerve regeneration [166], it has been concluded that both ordinary and reactive types of cells played key roles in initiating and maintaining a conditioning effect, respectively. The reactive type of cells had fewer numbers of branches and higher activity in promoting axonal outgrowth than the ordinary type.

Finally, a conditioning lesion may be produced by external physical factors. Tourniquet compression (120 min of compression with a conditioning interval of 6 days) has been found to have a conditioning lesion effect on peripheral nerve and may enhance nerve regeneration [164]. In a similar study [171], acute nerve compression at low pressure (compression at 30 or 80 mmHg for 2 h) has had a conditioning lesion effect on rat sciatic nerves. The same applies to chronic nerve compression [174].

Vibration exposure ((80 Hz), acceleration (32 m/s² root mean square), and duration (5 h daily, 2 or 5 days)) may also produce a conditioning lesion [173].

A conditioning lesion may be partly responsible for the success of contralateral C7 nerve root transfer in treatment of cerebral palsy in children [197, 198].

6.4 Inciting axonal sprouting from the spinal cord into avulsed brachial plexus roots

Direct root implantation relies on axonal sprouting from the spinal cord into avulsed brachial plexus roots. Can this process be further stimulated? Under Section 2.1, we have shown that progression of axonal cone requires the presence of cell adhesion molecules, *neurite outgrowth-promoting factors*, and *neurotrophic factors*. Schwann cells fuel the whole process. Application of neurotrophic factors in the absence of neurite outgrowth factors will lead to formation of dystrophic growth cones [199–201]. A targeted tissue engineering approach should therefore be followed (see Section 5, *vide supra*). First, however, motoneurons should be incited to grow axons into brachial plexus roots. Just as mentioned under Section 4.1, the main stimulus to this is mechanical. This is proven by the work of Magdesian et al. [202]. Clinically, this stimulation may be applied by means of magnetic nanoparticles. Shin et al. [203] have devised neurospheres derived from human neuroblastoma SH-SY5Y cells labeled with bacterial magnetic nanoparticles that can be guided by a magnetic field and successfully accumulated near the focus site of the magnetic field. These neurospheres may be provided with receptors for motoneuron axons and made to adhere to them. Subjecting them subsequently to magnetic fields may produce targeted sprouting of motoneuron axons into the avulsed roots of the brachial plexus.

7. The recipient nerve stump or recipient muscle as operators in relation to nerve graft operators

7.1 Replacement of chronically denervated muscles by fat or fibrous tissue does not account for poor functional recovery in delayed (more than 1 year after injury) nerve lesion grafting/repair

By the same token, poor hand function after brachial plexus grafting/repair is not due to a long regeneration time associated with regeneration over a long distance. Rather, the basis for poor regeneration is the decline of the transient immediate postinjury expression of growth-associated genes and that is associated with a declining regenerative capacity of neurons and the regenerative support of Schwann cells.

The effect of delayed nerve grafting/repair and poor hand function after brachial plexus grafting/repair (due to a long regeneration time associated with regeneration over a long distance) has been investigated [38]. Contrary to the belief that regenerating nerves cannot reinnervate chronically denervated muscle because the muscle fibers have degenerated and have been replaced by fat, retrograde labeling of axons that have been able to regenerate into the denervated nerve stump has revealed that the chronic denervation has reduced the capacity of freshly axotomized neurons to regenerate their axons through the chronically denervated nerve stump. Hence, chronic denervation of the muscle is not responsible for the low percentage of axons that regenerate through the atrophic Schwann cells. Immediately after nerve injury, there is a rapid upregulation of neurotrophic factors and their receptors in motoneurons and in Schwann cells, but this upregulation is short-lived, declining to baseline levels within a month or more of chronic axotomy and chronic denervation, respectively. Nevertheless, the capacity of chronically denervated Schwann cells to support axonal regeneration can be stimulated by fresh nerve autografts.

The conclusion is, the regenerative capacity of distal nerve stumps especially, less so that of recipient muscles has to be increased (as described under items 3–6) in brachial plexus injuries presenting late or when return of hand function is contemplated.

7.2 Motor endplate regeneration

7.2.1 In chronically denervated muscles, the motor end plate can regenerate even years after nerve injury, provided muscle mass has been preserved and the muscle has not been replaced by fibrous tissue

In an experimental neurophysiologic study [204], it has been proven that motor end plates are in a continuous regenerative process. The authors have induced the formation of ectopic neuromuscular synapses in the rat soleus muscle by implantation of the fibular nerve into the proximal part of the muscle and subsequent sectioning of the soleus nerve. They have shown that motoneurons control the conversion from slow-gating fetal to fast-gating adult-type ACh receptor channels at ectopic end plates in rat soleus muscles. The conversion occurs in the absence of impulse activity provided the nerve continues to be present. However, it also occurs in the absence of the nerve provided the muscle is active and has received an early priming influence from the nerve. Thus, nerve-evoked muscle activity and nerve-released trophic influences complement each other in controlling the gating properties of junctional ACh receptor channels.

7.2.2 First clinical consequence: importance of muscle electric stimulation after brachial plexus surgery should hand function return or should the brachial plexus be operated upon late

Under Section 7.2.1, we have shown that, when the fibular nerve is cut at an early stage of endplate development and the soleus muscle is then stimulated chronically via implanted electrodes, fast-gating channels do not develop in the absence of the nerve terminals within 4–6 days. In spite of evidence to the contrary [205, 206], this and other studies [207–212] show the importance of muscle electric stimulation in brachial plexus injuries immediately after injury and even after grafting/repair. Electric muscle stimulation has been shown to elevate intramuscular BDNF and GDNF mRNA following peripheral nerve injury and repair in rats [213] and to enhance the acetylcholine receptors available for neuromuscular junction formation [214]. Acupuncture plus low-frequency electrical stimulation has attenuated denervation-induced muscle atrophy [215, 216]. Electrical stimulation has been effective after end-to-side neurotomy of the peroneal nerve in rats [217] and has synergistically enhanced functional recovery of chronically denervated muscle combined with sensory nerve cross-anastomosis [218]. Chronic low-frequency stimulation has transformed cat masticatory muscle fibers into jaw-slow fibers [219].

Electric field stimulation has had an effect on cardiac myocyte development. It has promoted cardiomyogenic gene expression in human cardiomyocyte progenitor cells [220] and has promoted maturation of cardiomyocytes derived from human embryonic stem cells [221]; combined with a biomimetic scaffold and growth factor, it has promoted tissue-engineered cardiac development [222]. The electrical stimulation of carbon nanotubes has provided a cardiomimetic cue to mesenchymal stem cells [223]. Radiofrequency energy loop has primed cardiac, neuronal, and skeletal muscle differentiation in mouse embryonic stem cells [224].

Electrical stimulation has influenced cellular transplantation strategies. It has influenced satellite cell proliferation and apoptosis in unloading-induced muscle atrophy in mice [225]. Followed by mesenchymal stem cell transplantation, it has improved anal sphincter anatomy and function in a rat model at a time remote from injury [226]. It has also maximized the beneficial effects of muscle stem cells transplanted into dystrophic skeletal muscle [227]. Electrical stimulation of embryonic neurons for 1 h has improved axon regeneration and the number of reinnervated muscles that function [228]. Electrical stimulation by enzymatic bio-fuel cell has promoted proliferation, migration, and differentiation of muscle precursor cells [229].

In tissue engineering, external physical and biochemical stimulations have enhanced skeletal muscle bioengineering [230, 231]. Electrical stimulation has accelerated motor functional recovery in a rat model of a 15-mm sciatic nerve gap bridged by scaffolds with longitudinally oriented microchannels [232]. Myotube contraction has been controlled using electrical pulse stimulation for bio-actuator [233]. Muscle differentiation and myotube alignment have been influenced by micropatterned surfaces and exogenous electrical stimulation [234].

7.2.3 Second clinical consequence: there is no time limit for grafting the brachial plexus: judgment should be made intraoperatively

Because the motor end plate can regenerate even years after nerve injury, provided muscle mass has been preserved and the muscle has not been replaced by fibrous tissue, the dogma that brachial plexus injuries need not be grafted/repared years after injury is not all through correct. Instead the brachial plexus surgeon should always attempt grafting brachial plexus injuries. He should base his judgment on clinical assessment of the recipient muscles intraoperatively (completely replaced by fibrosis or not). As will be mentioned in the following paragraphs, under certain conditions, even nerves to fibrotic muscles and fibrotic muscles can be grafted. The final conclusion is, the only certain indication for secondary procedures on the brachial plexus is the condition when the brachial cannot be technically grafted (e.g., extensive fibrosis) or nerve transferred.

7.3 Direct muscle implantation

Direct muscle implantation is not only a nerve grafting procedure when the nerve has been avulsed off its recipient muscle. It is also a measure to preserve muscle mass, to enhance distal nerve stump regeneration, and to target reinnervation. Neurotization from muscle to nerve (reverse direct muscle implantation) may help in hand reanimation.

When a nerve has been avulsed off its recipient muscle, it can be implanted directly into the muscle, so called direct nerve implantation.

7.3.1 Experimental evidence and operative technique

Experimentally, restoration of function of denervated muscles has been observed in medium-sized animals by implanting the proximal cut end of the avulsed peripheral nerve directly into both acutely and chronically (10-week to 6-month denervation time) denervated muscles. This has been proven electromyographically, histologically, and histochemically [235, 236]. Similar observations with formation of new motor end plates have been recorded using the anterior tibial muscle of the rat [237]. The proximal stump of the posterior tibial nerve has been

severed at the ankle level and embedded into the transected distal 1/5 (5 mm) of the endplate-free segment of the muscle.

In a fourth experimental study on the rat soleus muscle, the distance between the site of nerve implantation and the site of the native motor endplate zone has been found to influence the morphology of reinnervation [238]. The transected tibial nerve has been implanted directly into the motor endplate zone in one group and far from the motor endplate zone in another group. Frozen sections have been processed to demonstrate both axons and motor end plates; a combined silver-acetylcholinesterase stain has been used to identify reinnervated motor end plates and to quantify motor end plates reinnervated by the neurotization process. Significantly more ectopic motor end plates have been generated by far implantation, and native motor end plates have been increased by near implantation. The total number of motor end plates has been found to be independent of implant location.

Technically, the native motor zone (NMZ) within a given skeletal muscle is the best site for direct nerve implantation. This has been proven in an experimental rat model, in which the proximal stump of the severed sternomastoid nerve has been implanted into a muscle slit made in the NMZ of the muscle where denervated motor end plates are concentrated. The outcome has been evaluated 3 months after surgery [239].

7.3.2 Technical modifications and measures to improve outcome

A first modification of the direct nerve implantation technique has been the *transfer of a neuromuscular pedicle* from a nearby muscle. This has found application in surgery of the face and larynx. In a rabbit model, both techniques have been compared to each other, both immediately after denervation and after a delay period [240]. Both methods have produced consistently functional neuromuscular units; return of function has been apparent within 6 weeks of both nerve implantation and neuromuscular transfer; neither method has shown a clear advantage over the other. However, in another model using the rabbit's denervated mentalis muscle [241], the neuromuscular pedicle method has achieved more rapid reinnervation and produced stronger contractions than direct nerve implantation. The latter observation has been confirmed in a further study [242] using the rabbit ansa hypoglossi nerve and sternothyroid to contralateral sternohyoid neuromuscular pedicle transfer. In a third study, the peroneal nerve of the rat has been transected and implanted/neuromuscular-pedicle-transferred into the gastrocnemius muscle of the hind limb [243]. Reinnervation has been quicker with the neuromuscular pedicle technique; both methods guarantee only a partial recovery of the function of the paralyzed muscle.

A second modification of the direct nerve implantation technique has been *combining it with a nerve graft, sutured with end-to-side neurorrhaphy* [244]. The peroneal nerve of the rat has been sutured to the tibial nerve with end-to-side neurorrhaphy; the terminal branches of the tibial nerve have been subsequently implanted in the anterior tibial muscle (ATM).

In a third modification of the direct nerve implantation technique, transection of the donor nerve is avoided [245]. The donor nerve is inserted within the denervated muscle following segmentary epineurectomy (*lateral muscular neurotization*). In this rat study, lateral neurotization group has been successful in preventing muscle atrophy and gaining reinnervation in electromyographic, histological, and weight parameters.

In an attempt to improve surgical outcome of direct nerve implantation, *adding nerve growth factor* (NGF) has been investigated in a rat experimental study [246]. The denervated soleus muscle has been neurotized via peroneal nerve implantation,

and NGF administered additionally. Electromyographically and in terms of muscle weight, this has led to better reinnervation. Histologically, an increase in the density of motor end plates has been observed. In a further attempt, *Schwann cell transplantation* has enhanced reinnervation after direct nerve implantation in the rat [247]. The denervated anterior tibial muscle has been neurotized by tibial nerve implantation, and a Schwann cell suspension has been injected at the implantation site. A large number of regenerating axons have grown for a longer distance throughout the muscle, and reinnervated motor end plates have been significantly more abundant. There has been a significant increase in compound muscle action potential and in muscle weight.

7.3.3 Clinical experience

In a clinical series of 12 patients treated by direct motor nerve implantation (direct muscular neurotization), motor function of the neurotized muscles improved to grades M3 and M4 at 12 months postoperatively [248]. Similar observations have been made in another case series, in which 42 out of 47 cases have improved [249]. Direct nerve implantation has also been used as a measure to reduce pain. In a prospective study involving 13 patients, the authors have evaluated the results of treating recurrent “Morton’s” neuroma by a technique that combined resecting the interdigital neuroma through a plantar approach and implantation of the proximal end of the nerve into an intrinsic muscle in the arch of the foot [250]. It has been concluded that recurrent pain after a dorsal interdigital neurectomy can be treated successfully through a plantar approach with implantation of the proximal end of the nerve into an intrinsic muscle.

7.3.4 All technical modifications are measures not only to preserve muscle mass but also to enhance distal nerve stump regeneration and target reinnervation

All technical modifications mentioned under Section 7.3.2 are clinical applications of Tessa Gordon’s recommendation [38] of enhancing regeneration in chronically denervated nerves by adding autologous nerve grafts. They should be added to the armamentarium described under items 3–6.

7.3.5 Reverse direct muscle implantation: surrounding a non-regenerated nerve with an intact muscle over a long length to enhance side neurotization from the intact muscle to the non-regenerated nerve

The brachial plexus surgeon is commonly faced with the following situation. *A patient presents with total brachial plexus palsy. He undergoes surgery on the brachial plexus. In 2 years, he recovers shoulder and elbow functions but not hand function. A worthwhile question is the following. Can the surgeon use the already recovered latissimus dorsi muscle as a pedicled transfer and wrap its distal extension into the elbow around the radial, ulnar, and median nerves to neurotize them to aid recovery of hand function? This appears to be possible.* In facial reanimation surgery, recovering a certain degree of mimicry after sacrifice of the facial nerve is a clinically recognized finding in facial suggesting a phenomenon of neurotization from muscle to nerve. This has been confirmed by Taupin et al. [251] in a case of hemifacial reanimation. They have reported on a female patient who has undergone parotidectomy with sacrifice of the left facial nerve for a recurrent parotid tumor. The distal branches of the facial nerve, isolated at the time of resection, have been buried in the masseter muscle underneath. The patient has recovered voluntary hemifacial function. The electromyographic analysis of the motor activity of the zygomaticus major before

and after block of the masseter nerve has shown a dependence between facial muscles and the masseter muscle.

7.4 The muscle mass preserving effect of nerve side coaptation (grafting)

In reconstructive plastic surgery, free muscle transplantation can be used to restore function (functional muscle transplantation), but it can also be used to cover soft tissue and bone defects. In the latter case, anastomosing the nerve to the free muscle transplant to a donor nerve is not necessary. Nevertheless, it may be anastomosed to a motor or sensory donor to preserve muscle mass. Muscle mass preservation occurs even in the case of end-to-side (or side-to-side) coaptation.

This has been proven in several experimental studies. In one study, the preservation of rat gracilis muscle flap mass after motor and sensory end-to-side neurotomy has been examined [252]. It has been shown that muscle mass can be preserved by end-to-side nerve repair and that motor nerve reinnervation is able to better arrest atrophic changes of the muscle flaps. In another study, sensory reinnervation with end-to-side neurotomy has preserved muscle mass in pedicled muscle flaps [253].

7.5 Nerve augmentation of partially regenerated nerves by side grafting

The muscle mass preserving effect occurs naturally in muscles of motor power grade 2 or less of partially regenerated major nerves. The individual nerves supplying these muscles cannot/need not be cut, but can be augmented by side grafts or other measures (nerve augmentation, supercharging).

Under Section 2.9, we have discussed the role of bypass grafting in augmenting late neuromas in continuity of partially regenerated nerves. Experimentally [38] augmentation by side grafting has the potential to prevent the progressive deterioration of the Schwann cell support of regenerating nerves.

In this section, we shall outline its role in muscles of motor power grade 2 or less of partially regenerated major nerves. In late obstetric palsy cases (presenting after the age of 3 years), e.g., nerves have partially regenerated leading to variable recovery of motor power. Muscle mass may have been preserved in those muscles which have not recovered clinical motor power. This can be detected by running an electromyogram. Muscle mass preservation may be built upon to restore clinical motor power to these muscles. It should be noted that partially regenerated nerves cannot be cut and grafted, in order not to lose already regained motor power and preserved muscle mass, but they can be augmented by side grafting.

In a clinical study on improving motor power in late obstetric brachial plexus lesions (nerve augmentation) [43], Grade 0 muscles have been neurotized, if the electromyogram has shown scattered motor unit action potentials on voluntary contraction without interference pattern. Differential regeneration of muscles having different preoperative motor powers has been noted; improvement to Grade 3 or more has occurred more in Grade 2 than in Grade 0 or Grade 1 muscles. It is less expected to improve infraspinatus power or Grade 0 or 1 forearm muscles [43]. They should be aided by the armamentarium described under items 3–6 and Section 7.3.

Nerve augmentation can also take place by nerve growth factors and hormones as mentioned under item 3, by neurolyzing agents as mentioned under item 4, by tissue engineering constructs as mentioned under item 6, and by modifications of the direct muscle implantation techniques as mentioned under Section 7.3.

7.6 Assisted neurotization involves transferring a suboptimal proximal donor nerve to the injured recipient nerve pending arrival of regenerative signals from an optimal distal donor

It preserves muscle mass, it is a conditioning lesion, it prevents degeneration of the distal nerve stump, and it is a kind of distal nerve transfer.

Under Section 2.11, we have shown that assisted neurotization involves transferring a suboptimal proximal donor nerve to the injured recipient nerve pending arrival of regenerative signals from an optimal distal donor. The proximal (to target muscle) nerve transfer assists the distal (to target muscle) nerve transfer until its regenerative signals arrive. We have detailed its use in facial reanimation surgery. In brachial plexus surgery, assisted neurotization has mainly preserved muscle mass in grade 0 or grade 1 muscles and has prevented degeneration of the distal nerve stump. In addition to this, it acts as a conditioning lesion, and it is a kind of distal nerve transfer. Eleven brachial plexus lesions have been repaired using end-to-side side-to-side grafting neurotization in root ruptures, in phrenic and spinal accessory nerve neurotizations, in contralateral C7 neurotization, and in neurotization using intact interplexus roots or cords [139]. The recipient nerves have been (side) neurotized or grafted to donors of dubious integrity or to nerves with a suboptimal number of fibers. After this, the same recipient nerves have been (side) neurotized to distant donors (e.g., contralateral C7).

7.7 Chronically denervated muscle fibers can survive and can be induced to regenerate

Chronic denervation in muscle or muscle flaps leads to myofiber atrophy, fibrosis, and fatty tissue infiltration. An interesting question is whether we can induce such muscles to regenerate [254].

In a review article [255] examining the survival of chronically denervated muscle fibers, the following has been observed. Despite long-term denervation there is structural and ultrastructural evidence for survival of muscle fibers in mammals, with some fibers surviving at least 10 months in rodents and 3–6 years in humans. Thus, human denervated muscle fibers survive years of denervation and can be rescued from severe atrophy by home-based functional electrical stimulation.

These authors have even listed the options to substantially increase the regenerative potential of skeletal muscle in those patients who have experienced denervation of muscles “too late to be recovered by home-based functional electrical stimulation,” that is, (1) induction and separation of autologous myogenic cells, either (i) by *in vivo* marcaine infiltration of an expendable muscle tissue (latissimus dorsi) and grown *in vitro* or (ii) derived from autologous adipose tissue by *in vitro* induction and cell-sorting selection of myogenic stem cells able to replicate *in vivo*; (2) multiple injections of the autologous myogenic stem cells, followed by their proliferation, fusion, and differentiation into adult-like muscle fibers; and finally, (3) their tetanic contractions induced by surface electrodes and an external neuromodulator [255].

The extensive regenerative capacity of skeletal muscles has been reviewed in another study [256]. Satellite cells, quiescent myogenic precursor cells, become activated following muscle injury: they divide and form myoblasts, fuse into myotubes, and finally mature to myofibers.

Clinically, the technique of *neuromuscular pedicle* transfer, a modification of direct nerve implantation, supplies degenerated muscles with myocytes, nerve supply, and vascularity. As detailed before under Section 7.3, this technique has been successfully applied in surgery of the face and larynx [240–243].

Cellular transplantation holds promise for inducing degenerated muscles to regenerate. They can provide myoblasts or even stimulate available myoblasts to regenerate. Human myogenic reserve cells (myoblasts, muscle progenitor cells, satellite cells (SC)) have been observed to act as quiescent stem cells that contribute to muscle regeneration after intramuscular transplantation in immunodeficient mice [257–259]. Muscle-derived satellite cells have been used for treating type 1 diabetes in rats (*Rattus norvegicus*) [260]. Fluorescence-activated cell sorting (FACS) isolated muscle stem cells have been engrafted into injured skeletal muscle [261]. Autografting satellite cells after their *in vitro* expansion could improve the regenerative efficiency to repair large clusters of the damaged myofibers induced by repeated compression such as occurring in compartment syndrome [262].

Allogenic-derived muscle stem cells reside in human skeletal muscle and display a long-term ability to proliferate, allowing generation of a clinically relevant amount of cells [263].

The fate of intramuscular transplantation of muscle precursor cells between the time of administration and the time at which the graft is considered stable has been studied in macaques [264]. The cell suspension has been observed to leak from the muscle bundles during injection toward the epimysium and perimysium, where most cells accumulate after transplantation. After 3 weeks, they migrate centrally to form myofibers.

Hepatocyte growth factor (HGF) is required for the activation of these cells. It plays a role in the migration of proliferating SC (myoblasts) and is present as a soluble factor during muscle regeneration, along with extracellular matrix (ECM) molecules [265].

The development of cell therapy for repairing damaged or diseased skeletal muscle has been hindered by the inability to significantly expand immature, transplantable myogenic stem cells (MuSCs) in culture. Methyltransferase Setd7 facilitates such transition by regulating the nuclear accumulation of β -catenin in proliferating myogenic stem cells. Genetic or pharmacological inhibition of Setd7 promotes *in vitro* expansion of myogenic stem cells and increases the yield of primary myogenic cell cultures [266].

Oxidative stress preconditioning of mouse perivascular myogenic progenitors has been found to select a subpopulation of cells with a distinct survival advantage *in vitro* and *in vivo* [267]. Neuromuscular electrical stimulation has promoted the development of mature human muscle from immortalized human myoblasts (immortalized human myogenic precursor cells) in mice [268]. Thyroid hormone receptor alpha has been deemed essential to maintain the satellite cell niche during skeletal muscle injury and sarcopenia of aging [269].

Chronic inflammation in skeletal muscle impairs satellite cells' function during regeneration [270]. Macrophages seem to play an important role in impaired muscle regeneration since these cells are associated with skeletal muscle stem cell (namely, satellite cells) activation and fibro-adipogenic progenitor cell (FAP) survival. Specifically, an imbalance of M1 and M2 macrophages seems to lead to impaired satellite cell activation, and these are the main cells that function during skeletal muscle regeneration, after muscle damage. Additionally, this imbalance leads to the accumulation of FAPs in skeletal muscle, with aberrant production of pro-fibrotic factors (e.g., extracellular matrix components), impairing the niche for proper satellite cell activation and differentiation. Chronic physical activity and exercise restores M1 and M2 macrophage balance, thus improving skeletal muscle regeneration after injury.

Adult muscle stem cells (satellite cells) are indispensable for adult skeletal muscle repair and regeneration [271].

Mesenchymal stem cells have been successfully differentiated into myogenic lineage both in mono- and in cocultures independent of hepatocyte growth factor (HGF) and insulin-like growth factor-1 (IGF-1). They have expressed desmin, myocyte enhancer factor 2, myosin heavy chain 2, and alpha-sarcomeric actinin. An increased expression of different myogenic key markers could be observed under HGF and IGF-1 stimulation. Three-dimensional cultivation in fibrin-collagen-I gels induced higher levels of myogenic differentiation compared with two-dimensional experiments. Cultivation on poly- ϵ -caprolactone-collagen-I nanofibers has induced parallel alignment of cells and positive expression of desmin [272].

Human cardiac muscle patches of clinically relevant dimensions (4 cm \times 2 cm \times 1.25 mm) have been generated by suspending cardiomyocytes, smooth-muscle cells, and endothelial cells that had been differentiated from human-induced pluripotent stem cells (hiPSCs) in a fibrin scaffold and then culturing the construct on a dynamic (rocking) platform [273]. In vitro assessments of these patches have suggested maturation in response to dynamic culture stimulation. In vivo assessments conducted in a porcine model of myocardial infarction have been successful.

Minced muscle graft progenitor cells have been found to contribute to muscle fiber formation after volumetric muscle loss injury in wild-type and immune-deficient mice [274, 275]. Co-delivery of micronized urinary bladder matrix, however, has damped the regenerative capacity of minced muscle grafts in the treatment of volumetric muscle loss injuries [276].

7.8 The importance of retrograde axonal transport: insights gained from neuroproteomics

7.8.1 Scientific basis of retrograde axonal transport

Pivotal to muscle regeneration and to motoneuron preservation is retrograde axonal transport [152, 157]. To fully understand this, Massing et al. [277] have conducted several experiments on the rodent femoral nerve that have culminated in proteomic investigations to identify specific biochemical mediators that may be the underlying mechanisms that direct accurate axon regeneration.

The rodent femoral nerve gives two terminal branches, one to the skin and the other to the quadriceps muscle. These authors have modified the original experiment by Weiss and Edds [278] by giving regenerating axons equal access to the terminal cutaneous and muscle branches, rather than being forced into foreign nerve stumps. Motor axons have initially grown equally into both nerve branches but over time have been preferentially retained in the muscle branch, thus resulting in preferential motor reinnervation. Conversely, when muscle contact has been denied but the cutaneous branch has remained intact, the cutaneous branch has become the preferred terminal nerve branch for motor neuron projections, suggesting that regeneration accuracy is highly dependent on the accessibility options allowed by a particular surgical preparation. Thus, both the end organ and the pathway can influence preferential motor reinnervation. To assess the relative roles of each, the same authors have developed surgical models that manipulate relative levels of trophic support in the muscle and cutaneous nerve branches. In all of these studies, regeneration accuracy has been measured by the number of motor neurons that are double retrogradely labeled from just one of the two terminal pathways or simultaneously from both pathways. Thus, target organ trophic support leads to preferential regeneration of motoneurons compared to sensory neurons, so-called *trophomorphism*. It has also been found that preferential projections develop as early as 2 weeks following the initial surgery.

Target-derived trophic support implies the presence of soluble factors migrating from end organs in a retrograde fashion. In fact, an increased endocytotic/exocytotic activity occurs at the postsynaptic density, the endplate region of the muscle, where muscle-derived factors would pass across the neuromuscular junction to the preterminal motor axon. The expression levels of several neurotrophic factors are also increased following either nerve or muscle damage [e.g., hepatocyte growth factor (HGF), fibroblast growth factor (FGF), brain-derived neurotrophic factor (BDNF)]. It remains first, to identify whether these factors or other factors are present in the postsynaptic density and second, to elucidate their function. A neuroproteomic experiment generally consists of several successive steps [279].

Microtubule-associated proteins, or MAPs, are bound along the length of axonal and dendritic microtubules [280]. Breakthrough experiments using nerve ligation assays have identified kinesin as a major motor for anterograde transport along the axon and dynein as the motor for retrograde transport. Neurotrophins (NGF, BDNF, NT3/4) bind to and activate neurotrophin receptors (TrkA, TrkB, TrkC, p75NTR). Following receptor-mediated endocytosis, these receptor-ligand complexes are sorted into compartments called signaling endosomes for transport toward the cell soma. There is evidence for an early endosomal lineage for signaling endosomes, since these organelles are positive for EEA1 and Rab5B, but they may mature to Rab7-positive compartments. Ligand-receptor complexes can be sustained during transport, resulting in activated Trk receptors (pTrks) and downstream signaling molecules (e.g., pERK1/2, B-Raf, and p-p38) in both the axon and cell body. Precise spatial and temporal resolution of signaling endosome dynamics has been revealed with NGF-coated quantum dots, which exhibit pronounced unidirectional motility toward the cell soma interspersed with frequent pauses; average speeds range from 0.2 to 3 $\mu\text{m/s}$. This retrograde transport depends on dynein-dynactin as inhibition of this motor complex prevents activated neurotrophin receptors from exiting the distal axon, thereby decreasing neuron viability.

The current exception to the paradigm of kinesin-3-dependent transport of dense core vesicles (DCVs) is BDNF transport. The neurotrophin BDNF is stored in dense core vesicles (DCVs) and trafficked within axons to the presynaptic site. However, the axonal transport of BDNF is regulated by huntingtin, which scaffolds both kinesin-1 and dynein motors. The phosphorylation of huntingtin through the IGF-1/Akt pathway acts as a molecular switch to regulate the transport of BDNF-containing vesicles in axons. Phosphorylation of huntingtin at S421 promotes anterograde transport, while dephosphorylation of huntingtin promotes retrograde transport. Biochemical studies indicate that phosphorylation of S421 enhances the recruitment of kinesin-1 to BDNF transport vesicles and enhances the association of kinesin-1 motors with microtubules, leading to increased anterograde flux and BDNF release [280].

7.8.2 Stimulating axonal cone progression through partial inhibition of actin retrograde axonal transport

The brachial plexus surgeon is faced with the following situation. Distal muscles regenerate less than proximal ones. Also, there is differential regeneration of certain groups of muscles. In obstetric palsy, this leads to muscle imbalance and subsequent glenohumeral deformity. *The question is whether we can inject these muscles with guidance cues that are passively or actively transported to the nerves to these muscles stimulating axonal progression within them.*

In fact axonal branching and growth cone structure depend on target cells [281]. Ciliary neurons have been cocultured with myotubes and antibodies to GAP-43 (a neuron-specific, growth-associated phosphoprotein) and MAP-2 (a cytoskeletal

marker for dendrites) and have been utilized together with immunofluorescence microscopy to characterize the changes in patterns and polarity that ciliary nerve growth cones undergo when they contact a “proper” target. Ciliary neurons plated alone or cocultured with fibroblasts have developed one or two axons, but, when cocultured with myotubes, most cells have developed four or five axons showing GAP-43 immunoreactivity. These results indicate that muscle cells or the factors they release can regulate the growth and topography of axons and their growth cones.

Growth cones interact with the extracellular matrix (ECM) through integrin receptors at adhesion sites termed point contacts. “Point contact adhesions link extracellular matrix proteins to the actin cytoskeleton through numerous adaptor and signaling proteins. One presumed function of growth cone point contacts is to restrain or ‘clutch’ myosin-II-based filamentous actin (F-actin) retrograde flow (RF) to promote leading edge membrane protrusion. In motile non-neuronal cells, myosin-II binds and exerts force upon actin filaments at the leading edge, where clutching forces occur.” In an experimental study, the authors [282] have sought to determine whether similar F-actin-clutching forces affect axon outgrowth and guidance in growth cones. In *Xenopus* spinal neurons, they have shown that retrograde flow is reduced in rapidly migrating growth cones on bound laminin (LN) compared with non-integrin-binding poly-d-lysine (PDL). Opposing effects on retrograde flow rates have also been observed in growth cones treated with chemoattractive and chemorepulsive axon guidance cues that influence point contact adhesions. On bound LN, brain-derived neurotrophic factor (BDNF) promotes point contact formation and enhances neurite outgrowth, whereas semaphorin 3A had the opposite effect. Treatment with 100 ng/ml BDNF for 15 min has not affected the RF rates in growth cones on PDL but has caused a marked decrease in RF on LN. Moreover, acute stimulation with LN has accelerated axon outgrowth over a time course that correlates with point contact formation and reduced retrograde flow. Thus both bound and soluble laminins stimulate axonal cone progression. However, in sharp contrast to bound LN, *excessive* soluble LN activates actin network contractility, suggesting that, without clutching by point contacts, soluble LN strongly stimulates RF and induces axon retraction. Pretreatment with 2 µg/ml heparin significantly inhibits LN binding leading to its excessive solubility. Finally, the authors have shown that RF is significantly attenuated *in vivo*, suggesting that it is restrained by molecular clutching forces within the spinal cord. The conclusions are the following. Point contact adhesions are correlated directly with clutching of filamentous actin retrograde flow (RF), which guides developing axons. Acute assembly of new point contact adhesions is temporally and spatially linked to attenuation of RF at sites of forward membrane protrusion. Importantly, clutching of RF is modulated by extracellular matrix (ECM) proteins and soluble axon guidance cues, suggesting that it may regulate axon guidance *in vivo*. *Laminin an extracellular matrix protein clutches RF, reducing it, thus promoting axonal cone progression. This applies to both bound and soluble laminin. However, excessive soluble laminin, mobilized by heparin, increases retrograde axonal flow, inhibiting axonal progression. Under Section 3.4, we have referred to the neurogenic effect of heparin. Thus, to get the utmost benefit of it, heparin should be administered in a pulsatile form (high dose over a few days followed by a hiatus of several weeks). This will limit excessive mobilization of laminin. BDNF promotes axonal cone progression and can be administered intramuscularly to enhance axonal cone progression preferentially to specific muscles. Excessive mobilization of laminin may also provide a reasonable explanation, why in one study [283], heparin has been found to inhibit skeletal muscle growth in vitro.*

The results stated above have been confirmed by other authors [284–295].

7.8.3 *Stimulating axonal cone progression by cellular transplantation strategies into target muscles*

Under Section 7.8.2, we have described the effect of injecting guidance cues into specific target muscles to promote axonal regeneration into them. The same effect can take place by cellular transplantation strategies.

Bone marrow mesenchymal stem cell injection in hindlimb skeletal muscle has enhanced neurorepair in mice with spinal cord injury [296].

The expression of chemokine receptor 2 (CCR2) on bone marrow-derived cells regulates macrophage recruitment into injured muscle, numbers of myogenic progenitor cells, and the extent of regenerated myofiber size, all of which are independent of CCR2 expression on host-derived cells. This emphasizes the role of bone marrow-derived cells, possibly macrophages, in CCR2-dependent events that regulate effective skeletal muscle regeneration [297].

Local and noninvasive transfection with neurotrophin-3 (NT-3) can be performed using ultrasound with microbubbles (MBs) [298]. Transfected cells can be subsequently transplanted into specific target muscles, not only to attenuate the atrophy of denervated muscle but also to induce motor endplate regeneration.

8. Conclusions

- The neuroscientific concept of nerve grafts acting as anatomical guidance channels is the basic concept that dominates surgical decision-making in brachial plexus surgery. Nevertheless, the operator concept is an aid to the brachial plexus surgeon; nerve grafts could be conceived of as operator channels (rather than anatomical guidance channels), proximal nerve stumps as operator donors, and distal nerve stumps and the muscles supplied by them as operator recipients.
- Based on the neurotrophic and contact guidance theory for axonal guidance, nerve grafts act as molecular operator channels responding to molecular cues. This has led to the following consequences: recipient end to donor side nerve grafting; donor end to recipient side grafting; donor side to recipient side coaptation; recipient/donor end to donor/recipient side grafting would better be assisted by recipient/donor side to donor/recipient side grafting; augmenting not yet regenerated neuromas in continuity by bypass grafts; augmenting end-to-end repairs/grafts by side grafts; augmenting late neuromas in continuity; preserving neuromas at proximal and distal nerve stumps of injured nerves; assisting nerve transfer (neurotization); and assisting side grafting of end-to-end grafts/repairs.
- Nerve grafts act as operator channels responding to a cellular membranous bed and incremental neurolysis of fibrosis. Because endoneurial scarring limits vascularized nerve grafts, nerve cuffing has evolved as a measure to limit fibrous tissue reaction and to prevent aberrant axonal sprouting. However, it is rather creating a cellular membranous bed that promotes neural vascularization. The importance of incremental neurolysis of fibrosis and neurogenesis has revealed heparin and chondroitinase ABC as therapeutic agents.
- Nerve grafts act as physical operator channels responding to mechanical forces and electric stimulation.

The concept of subjecting intact nerve endings to tensile forces to promote axonal sprouting while simultaneously relieving tensile and shear forces off the grafted or sutured area has led to the following consequences: mobilization of the brachial plexus along its whole length during surgery; not completely excising the fibrosis but leaving a posterior fibrous segment to help unload the grafted segment from tensile and shearing forces; using epiperineurial 2/0 vicryl tension sutures to both intact nerve endings to apply tensile forces to them while unloading the grafted segment; loop grafting; continual tensile forces (dynamic tension devices, magnetic nanoparticles); revision brachial plexus surgery; free muscle transplantation spanning shoulder, elbow, and wrist/fingers as internal splints; dealing with neuromata of Types 1A, 1B, 2A, 2B, 3A, and 3B (so-called neuromata in continuity) by a shortening/tensioning/loop grafting procedure; widening the indications for surgery in obstetric brachial plexus pending their complete recovery; humeral shortening osteotomy [299]; and reverting to full exploration of the brachial plexus has to be reverted to.

Brief low-frequency electrical stimulation accelerates motor and sensory axon outgrowth across injury sites (even after delayed surgical repair of injured nerves in animal models and patients) and enhances nerve regeneration and target reinnervation.

- Nerve grafts act as operator channels responding to tissue engineering as applied to neuroscience, thus opening the vista for the role of artificial nerve grafts applied as side grafts.

The proximal nerve segments (stumps) act as motor and sensory operator donors in relation to nerve graft operators. Based on principles of information theory, additional grafts can be applied extending from the side of the donor end to the side of the recipient end (augmented end-to-end nerve grafting). Based on the same principles, in nerve transfer, a single high axonal load donor may be side grafted to multiple recipients. Because conditioning lesions promote axonal sprouting, adding distal nerve transfers to direct cord implantation may enhance the effect of the latter. Also, adding distal nerve (side) transfers before/after full exploration/grafting/proximal nerve transfer of the brachial plexus may enhance its recovery, a plea for reverting to full exploration of the brachial plexus. Because neurite outgrowth responds to mechanical stimulation, inciting axonal sprouting from the spinal cord into avulsed brachial plexus roots (in direct cord implantation) may be produced by magnetic nanoparticles.

- The recipient nerve stump or recipient muscle acts as operators in relation to nerve graft operators. Chronically denervated muscle fibers can survive and can be induced to regenerate. Replacement of chronically denervated muscles by fat or fibrous tissue does not account for poor functional recovery in delayed (more than 1 year after injury) nerve lesion grafting/repair. Rather, the basis for poor regeneration is the decline of the transient immediate postinjury expression of growth-associated genes and that is associated with a declining regenerative capacity of neurons and the regenerative support of Schwann cells. In chronically denervated muscles, the motor end plate can regenerate even years after nerve injury, provided muscle mass has been preserved and the muscle has not been replaced by fibrous tissue. As a first clinical consequence, muscle electric stimulation after brachial plexus surgery is important should hand function return or should the brachial plexus be operated upon late. As a second clinical consequence, there is no time limit for grafting the brachial plexus; judgment should be made intraoperatively. As a

third clinical consequence, direct muscle implantation is not only a nerve grafting procedure when the nerve has been avulsed off its recipient muscle. It is also a measure to preserve muscle mass, to enhance distal nerve stump regeneration, and to target reinnervation. Neurotization from muscle to nerve (reverse direct muscle implantation) may help in hand reanimation. The muscle mass preserving effect of nerve side coaptation (grafting) is the fourth consequence. Nerve augmentation of partially regenerated nerves by side grafting is the fifth consequence. The muscle mass preserving effect occurs naturally in muscles of motor power grade 2 or less of partially regenerated major nerves. The individual nerves supplying these muscles cannot/need not be cut, but can be augmented by side grafts or other measures (nerve augmentation, supercharging). The sixth consequence is assisted neurotization. This procedure involves transferring a suboptimal proximal donor nerve to the injured recipient nerve pending arrival of regenerative signals from an optimal distal donor. It preserves muscle mass, it is a conditioning lesion, it prevents degeneration of the distal nerve stump, and it is a kind of distal nerve transfer.

Insights gained from neuroproteomics have revealed mechanisms of retrograde axonal transport. Axonal cone progression can be stimulated through partial inhibition of actin retrograde axonal transport and by cellular transplantation strategies into target muscles.

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