Chapter 8

Bridging Defects in Chronic Spinal Cord Injury Using Peripheral Nerve Grafts: From Basic Science to Clinical Experience

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Additional information is available at the end of the chapter

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Abstract

Nerve grafting of the injured spinal cord should pursue a sixfold attack: lysing the fibrosis/gliosis to an extent that allows settling of the basal lamina preventing meanwhile collapse of the neural tissue matrix; supplying the tissue matrix with a suitable scaffold, on which the basal lamina can settle; basal lamina synthesis; seeding the basal lamina with cell adhesion molecules; providing the axonal growth cone with neurite outgrowth promoting factors that allow its distal progression; supplying the axonal growth cone with neurotrophic factors that power its continued growth. In addition to this, the intrinsic properties of the neurons should be stimulated, possibly through modulating the function of astrocytes by heparin, aspirin and other factors. Nerve side grafting of the cord increases the incidence of nerve regeneration by applying additional grafts extending from the side of the donor end of the cord to the side of the recipient end. Also, it allows the surgeon to enhance regeneration through a partially regenerated cord. During surgery, after establishment of CSF circulation, a long-lasting indwelling catheter has to be inserted for postoperative drug and cell delivery. This allows for continual lysis of the gliosis by chondroitinase ABC, sialidase and other factors.

Keywords: spinal cord injury, nerve grafting, indwelling intrathecal catheter implantation, lysis of the gliosis, chondroitinase ABC

1. Introduction

Since 1903, when Tello and Cajal demonstrated that the central nervous system (CNS) could regenerate [1, 2], experimental neuroscience has advanced our knowledge repairing the injured

spinal cord. Several cellular transplantation strategies have been recommended with some clinical success [3–6]. Clinically, however, the injured spinal cord is usually extensively gliotic, cystic, even disrupted, necessitating bridging the injury zone first before contemplating cellular transplantation. Placing peripheral nerve grafts to bridge the injury zone has been successful experimentally [7–13], yet only anecdotal clinical evidence supports it [14, 15]. In a review article on bridging spinal cord injuries, Fawcett [16] commented on this disparity between experimental and clinical neuroscience: 'Sadly, we have yet to achieve a treatment that is licensed for this purpose in human patients'. The aim of this review is to enable clinicians to put the findings made by neuroscientists into clinical practice and to provide neuroscientists with upcoming ideas investigating the clinical issues physicians face.

2. Changing concepts of nerve grafting

2.1. Basic concepts of nerve grafting

Autogenous nerve grafting is the standard for repair of irreducible nerve gaps [17]. The basic principles of nerve grafting include the following (**Figure 1**):

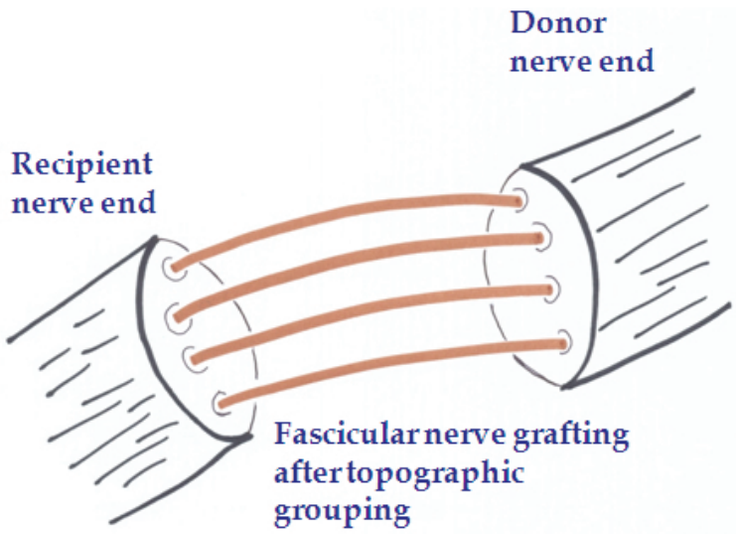

Figure 1. The basic principles of conventional end-to-end grafting include trimming both proximal and distal nerve ends up to healthy nerve fascicles; avoiding any tension at the repair site, avoiding any shearing stress at the repair site, fascicular grafting, end-to-end grafting suturing fascicles at proximal nerve ends to their counterparts at distal nerve ends after grouping them topographically, using small caliber sutures (9/0 or 10/0 sutures), and healthy vascular bed.

- trimming both proximal and distal nerve ends up to healthy nerve fascicles;

- avoiding any tension at the repair site, for even minimal tension, can end up with fibrosis, hampering progression of regeneration;

- avoiding any shearing stress at the repair site because this incites an inflammatory reaction ending up with fibrosis and hampering progression of regeneration;

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- fascicular grafting because autogenous nerve grafts derive their nutrition from the extracellular matrix; using large diameter grafts instead of small diameter fascicles can produce central necrosis of the graft;

- end-to-end grafting suturing fascicles at proximal nerve ends to their counterparts at distal nerve ends after grouping them topographically, in order to avoid aberrant nerve sprouting;

- using small caliber sutures (9/0 or 10/0 sutures) because large caliber sutures may produce fibrosis;

- healthy vascular bed because a fibrotic bed may prevent progression of regeneration through the grafts.

In the absence of a proximal nerve end, such as in brachial plexus avulsions, nerve transfer (neurotisation) refers to using an expendable nearby donor nerve as a substitute, grafting it to the original recipient. The principles of nerve transfer include:

- donor nerve of high axonal load,

- single donor to single recipient to prevent cocontractions.

Autogenous grafts act as immunogenically inert scaffolds, providing appropriate neurotrophic factors and viable Schwann cells for axonal regeneration [17].

2.2. Molecular aspects of peripheral nerve regeneration

Advances in the understanding of molecular pathways and their physiological role have provided us with new insights as to the mechanism of axonal (peripheral nerve) regeneration [18, 19].

Fibrous tissue and chondroitin sulphate proteoglycans secreted by astrocytes provide the necessary scaffold for settling of the basal lamina and subsequent basement membrane synthesis. *Neurite outgrowth promoting factors* are basement membrane-related extracellular matrix proteins (such as laminin (LN), fibronectin (FN), heparin sulphate proteoglycans (HSP) and tenascin), which pave the proper path by supplying orientation and adhesiveness for axons. *Neurotrophic factors* are specific trophic agents that power peripheral nerve regeneration. They include (a) the neurotrophins (nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5(NT-4/5)); (b) the neurokines (ciliary neurotrophic factor (CNTF) and leukaemia inhibitory factor (LIF)); (c) the transforming growth factor (TGF)-b family (TGF-b1, TGF-b2, TGF-b3, glial cell line¦derived neurotrophic factor (GDNF)). *Schwann cells* secrete (a) cell adhesion molecules (CAMs), such as N-CAM, Ng-CAM/L1, N-cadherin and L2/HNK-1; (b) produce basement membrane that contains many extracellular matrix proteins, such as laminin (LN), fibronectin (FN), heparin sulphate proteoglycans (HSP) and tenascin; (c) secrete neurotrophic factors and their receptors. In addition to this, axonal regeneration is determined by trophic factors from activated perineural glial cells (astrocytes), trophic factors from efferent axons by anterograde transport, gene-induced trophic factors by intracrine or autocrine transport, trophic factors from retrograde target cell support, trophic factors from retroaxonal transport and trophic factors

from recruited macrophages (secretory products, cytokines). Activated mesenchymal cells contribute to repair and vascularisation (**Figure 2**).

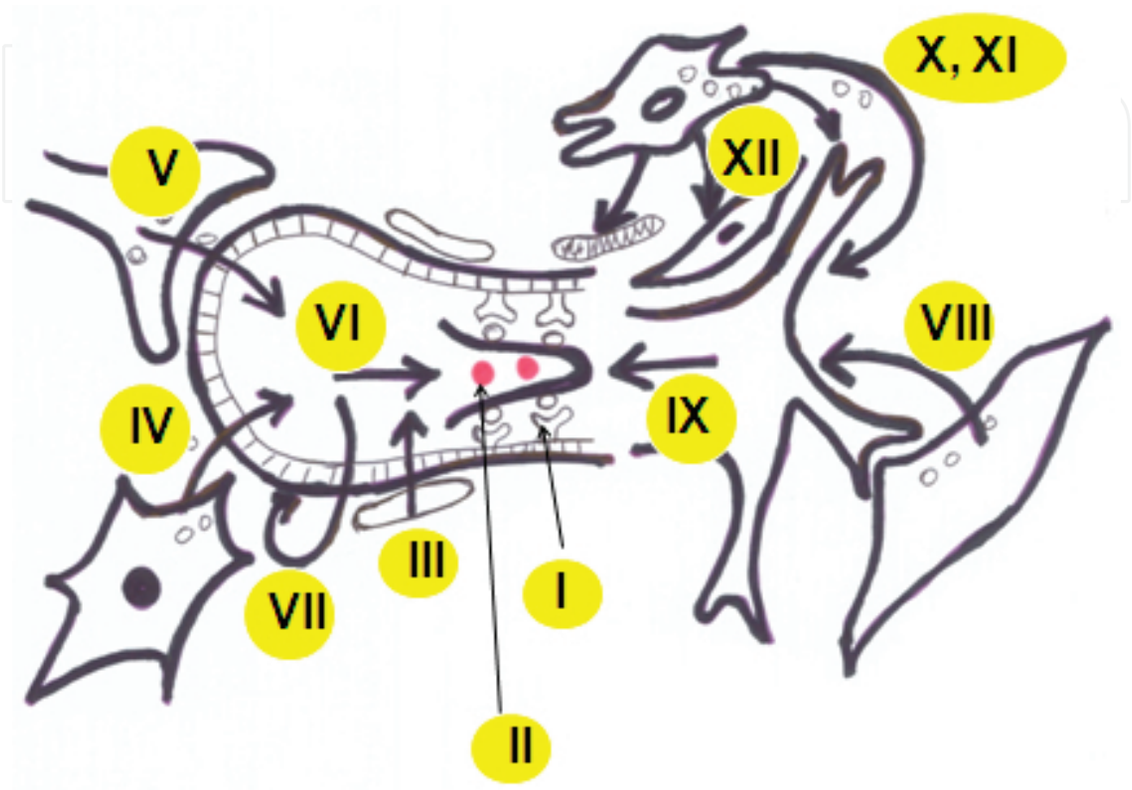

Figure 2. Molecular aspects of axonal regeneration. (I) *Neurite outgrowth promoting factors* [such as laminin (LN), fibronectin (FN), heparin sulphate proteoglycans (HSP) and tenascin] pave the proper path by supplying orientation and adhesiveness for axons. (II) *Neurotrophic factors* are the specific trophic agents that power peripheral nerve regeneration. They include (a) neurotrophins (NGF, BDNF, NT-4/5); (b) neurokines (CNTF, LIF); (c) (TGF)-β family (TGF-β1, TGF-β2, TGF-β3, GDNF). (III) *Schwann cells* secrete (a) cell adhesion molecules (CAMs), such as N-CAM, Ng-CAM/L1, N-cadherin, andL2/HNK-1; (b) basement membrane that contains many extracellular matrix proteins, such as laminin (LN), fibronectin (FN), heparin sulphate proteoglycans (HSP) and tenascin; (c) neurotrophic factors and their receptors. In addition to this, axonal regeneration is determined by trophic factors from activated perineural glial cells (astrocytes) (IV), trophic factors from efferents by anterograde transport (V), gene-induced trophic factors by intracrine (VI) or autocrine (VII) transport, trophic factors from retrograde target cell support (VIII), trophic factors from retroaxonal transport (IX), trophic factors from recruited macrophages (secretory products X, cytokines XI). Activated mesenchymal cells contribute to repair and vascularisation (XII). Among other molecules, heparin and aspirin modulate astrocytic function stimulating them to secrete axonal trophic factors (IV).

Based on the previous considerations, axonal sprouting and nerve grafting are based on a sixfold attack (**Figure 3**):

– lysing the fibrosis/gliosis in the injury zone to an extent that allows settling of the basal lamina preventing meanwhile collapse of the neural tissue matrix; or excision of the fibrotic segment and replacing it with nerve grafts;

– supplying the tissue matrix with a suitable scaffold, on which the basal lamina can settle,

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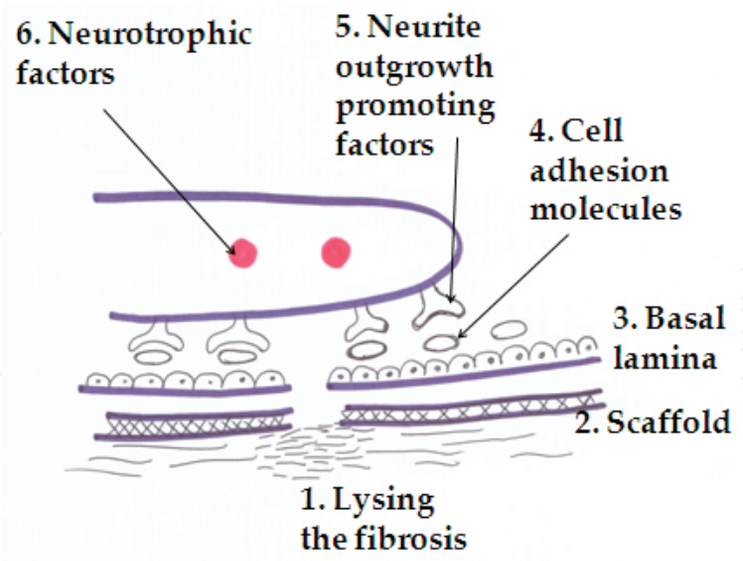

Figure 3. The sixfold attack: lysing the fibrosis/gliosis to an extent that allows settling of the basal lamina preventing meanwhile collapse of the neural tissue matrix; supplying the tissue matrix with a suitable scaffold, on which the basal lamina can settle; basal lamina synthesis; seeding the basal lamina with cell adhesion molecules; providing the axonal growth cone with neurite outgrowth promoting factors (FGF) that allow its distal progression; supplying the axonal growth cone with neurotrophic factors that allow its continued growth.

– basal lamina synthesis;

– seeding the basal lamina with cell adhesion molecules;

– providing the axonal growth cone with neurite outgrowth promoting factors that allow its distal progression;

– supplying the axonal growth cone with neurotrophic factors that power its continued growth.

2.3. Changing concepts of nerve grafting: side grafting

The previous conditions prevailing, 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, the so-called recipient end to donor side coaptation. Described independently by Balance and Harris over a century ago (in 1903), interest in end-to-side coaptation has been rekindled by Viterbo et al. [17]. In its essence, it involves grafting donor side to recipient end after stimulating donor side collateral sprouting by mechanical trauma or axotomy (**Figure 4(a)**). An indirect application of it is increasing the incidence of nerve regeneration 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 [20] (**Figure 4(b)**). 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 and caudal cord to cauda equina) [20] (**Figure 4(c)**). 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 4(d)**).

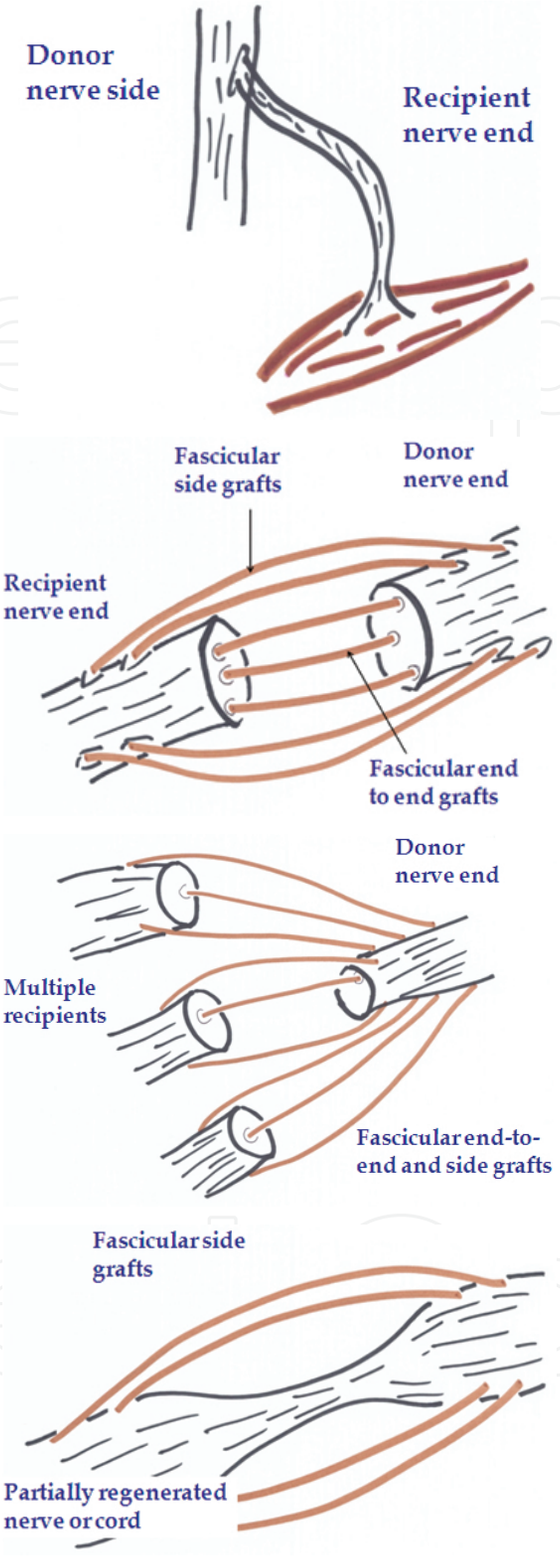

Figure 4. (a) End-to-side grafting involves grafting donor side to recipient end after stimulating donor side collateral sprouting by mechanical trauma or axotomy. (b) An indirect application of side grafting is increasing the incidence of nerve regeneration after conventional end-to-end grafting by applying additional grafts extending from the side of the donor end to the side of the recipient end. (c) Side grafting allows the surgeon to use a single high axonal load donor for multiple recipients without producing cocontractions. (d) Partially regenerated nerves cannot be surgically cut and nerve grafted sacrifying already regained function; side grafting allows the surgeon to enhance regeneration through them.

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2.4. Mathematical modelling and channel-carrying capacity applied to nerve grafting: necessary concepts for subsequent computer-assisted fabrication of artificial nerve grafts

Can we manipulate the molecular mechanisms of the sixfold attack to increase neurite outgrowth into the side grafts? Manipulating molecular mechanisms is based on the sensitivity of the axonal growth cone to spatial molecular concentration gradients [21, 22]. In a study by Rosoff et al. [21], axonal growth has been shown to be enhanced by a steep nerve growth factor (NGF) spatial concentration gradient. There is a narrow range, however, between the lowest NGF concentration necessary for axonal growth stimulation and the highest NCF concentration beyond which axonal growth is competitively blocked. As the lower and upper limits of the concentration gradient should fall within this narrow range, the maximal distance for axonal growth cone progression guided by that gradient would be far less than the length of the neural defect. Utilising synthetic nerve graft scaffolds and observing the sixfold attack, axonal growth can be hypothetically made to bridge the whole length of the neural gap by seeding the scaffolds with multiple NGF spatial concentration gradients [22]. Neurite outgrowth has been modelled as a non-linear partial differential equation, that is solved by an iterative mathematical process suitable for numerical analysis and for subsequent computer-assisted fabrication of artificial nerve grafts [22]. By diffusion, these NGF spatial concentration gradients might also enhance axonal growth within the adjacent natural nerve side grafts.

Alternatively, and also to increase neurite outgrowth into the side grafts and decrease aberrant neural sprouting, multiple microspheres embedding chemical attractive and repulsive cues and placed along nerve side grafts may be used to guide axonal growth [23]. Preliminary experiments conducted with embryonic rat hippocampal neurons and calcium alginate microspheres have been encouraging [24]. A mathematical model has been developed based on the diffusion gradient of the implanted microspheres; a genetic algorithm has been used to study its proper spatial implementation [23].

A more accurate mathematical model for axonal growth cone progression has been provided based on sensory pinch test data [25]. Disadvantages of this model, however, include the assumptions that the initial delay is the major cause of variability, and that delay to scarring of the neural bed lies within the initial delay and that the regeneration rate is linear (constant).

Can we quantify the molecular mechanisms of the sixfold attack so that nearly 100% of all axons sprouting from the proximal spinal cord reach the distal spinal cord through the side grafts and simultaneously minimise the probability of aberrant neural sprouting to nearly 0%? Unless incorporated in information theory, which is a theory based on mathematical probability [26, 27], the mathematical models mentioned above do not provide a numerical solution for this problem. This is imperative, however, for subsequent computer-assisted fabrication of artificial nerve grafts. The Shannon-Hartley channel-carrying capacity principle, a central concept in information theory, 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 minimise the probability of aberrant neural sprouting.

3. Peripheral nerve grafting of the injured spinal cord as an application of the concept of side grafting and the sixfold attack

3.1. Technical aspects of peripheral nerve grafting and repair of the injured spinal cord

An indirect application of side grafting, as mentioned previously, is, first, increasing the incidence of nerve regeneration 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; and, second, preserving partially regenerated nerves which cannot be surgically cut and nerve grafted leading to loss of already regained function [20]. Both of these apply to the spinal cord; compared to its high axonal load, the cross-sectional area of the spinal cord is too small for efficient end-to-end grafting. In addition, some kind of regeneration may have occurred in a contused cord in a completely paraplegic patient; this may take the form of less than Grade 3 motor power improvement, which, according to ASIA standards, is not enough to be considered motor or neurological level progression [28]. Third, side grafting produces less trauma to the spinal cord. In fact, the glial tissue secreted by astrocytes provides the necessary supporting tissue for axons and neurons [2, 29]. In side grafting, fine pial incisions are used. This process is far less traumatic than freshening of the cord ends during end-to-end grafting, a procedure which would lead to excessive glial tissue secretion and subsequent blocking of regeneration.

3.2. Donor nerves

Clinically, the sural nerve is the most commonly used donor nerve, other suitable donor nerves include the medial and lateral cutaneous nerves of the forearm, dorsal cutaneous branch of the ulnar nerve, superficial and deep peroneal nerves, intercostal nerves, and the posterior and lateral cutaneous nerves of the thigh [17].

Pre-degenerated (segments of nerve cut and left in situ for 7–10 days prior to harvesting) peripheral nerves are infiltrated by regenerating axons to a greater extent (both in number and distance) than freshly cut and harvested nerves [30]. The use of pre-degenerated nerves has been contested by other authors, however [31].

3.3. Experimental applications of side grafting

Based on the work of David and Aguayo [5, 7], numerous studies have confirmed axonal outgrowth after nerve grafting of the injured spinal cord [30, 32–38]; axons growing within peripheral nerve grafts have not only retained their physiological properties [39] but have synapsed with neurons near the point of central nervous system re-entry as well [40].

3.4. Re-evaluation of the use of peripheral nerve grafts to bridge spinal cord defects

Nerve grafts supply the injured spinal cord with five factors of the sixfold attack: a suitable scaffold, on which the basal lamina can settle; basal laminae; cell adhesion molecules; neurite outgrowth promoting factors; and neurotrophic factors. Nevertheless, the use of peripheral

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nerve grafts has been challenged [5, 16, 35, 41, 42]. According to experimental observation, damaged spinal cord axons might grow from the cranial cord into peripheral nerve grafts but would not leave them to enter the caudal cord [5]. Schwann cells might even promote gliosis [16, 41].

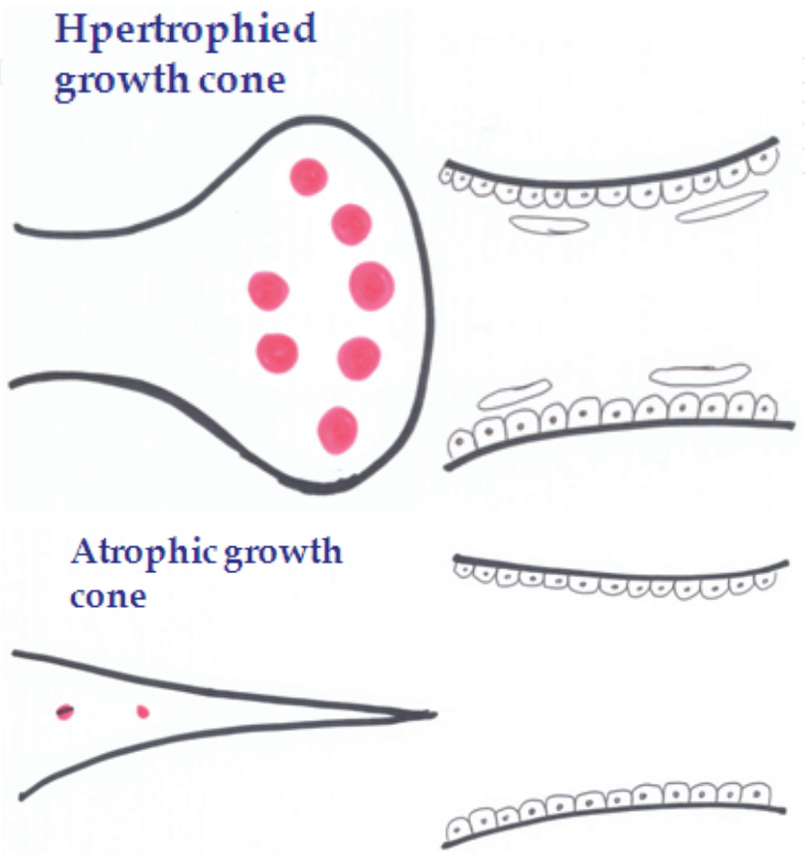

Figure 5. (a) Excessive fibrosis in the presence of neurotophic factors and adequate neuron growth potential leads to hypertrophy of both donor and recipient ends of the damaged neural tissue (excessive neuroma formation; dystrophic growth cones). (b) Excessive fibrosis in the absence of neurotophic factors and adequate neuron growth potential leads to atrophy of both donor and recipient ends of the damaged neural tissue (atrophic neuroma formation; atrophic growth cones).

It might be assumed that failure of growth cone progression is due to missing of the sixth factor in the sixfold attack (lysing the fibrosis/gliosis to an extent that allows settling of the basal lamina preventing meanwhile collapse of the neural tissue matrix). However, failure of axons to regenerate following peripheral nerve grafting may also result from intrinsic properties of the neurons and the absence of neurotrophic factors [43–46]. These considerations help us assess neural tissue during surgery. Excessive fibrosis in the presence of neurotophic factors and adequate neuron growth potential leads to hypertrophy of both donor and recipient ends of the damaged neural tissue (excessive neuroma formation and dystrophic growth cones) [47, 48] (**Figure 5(a)**), whereas excessive fibrosis in the absence of neurotophic factors and adequate neuron growth potential leads to atrophy of both donor and recipient ends of the damaged neural tissue (atrophic neuroma formation; atrophic growth cones) (**Figure 5(b)**). In conclu-

sion, in addition to the sixfold attack, the intrinsic properties of the neurons should be stimulated to produce neurites.

4. Lysing the gliosis in conjunction with nerve grafting

4.1. The gliosis: blocking of regeneration mechanically and through the activation of RhoA

Injured axons encounter a series of inhibitory factors that are non-permissive for growth [4, 29, 41, 49]. These include myelin inhibitors (Nogo-A ,MAG108 (myelin-associated glycoprotein) and OMgp109 (oligodendrocyte myelin glycoprotein)); chondroitin sulphate proteoglycans (neurocan, versican, aggrecan, brevican, phosphacan and NG2); and semaphorins and ephrins. Upregulated in response to spinal cord injury [50–52], they act not only by mechanical blocking of neural regeneration but by inhibiting axon outgrowth neuronal receptors and subsequent activation of signalling pathways known to be involved in the activation of RhoA and the rise in intracellular calcium.

4.2. Lysing the gliosis by chondroitinase ABC

Chondroitinase ABC cleaves the inhibitory chondroitin sulphate glycosaminoglycan chains from the core protein, reducing the inhibition by chondroitin sulphate proteoglycans [53, 54]. Houle et al. [55] have demonstrated CNS axons regenerating through a peripheral nerve graft and entering the caudal spinal cord following chondroitinase ABC treatment.

4.2.1. Chondroitinase ABC in combination with growth factors and scaffolds

Combined with glial-derived neurotrophic factor (GDNF) delivery, chondroitinase ABC promotes axon extension through peripheral nerve bridges [50, 56]. Combined with growth factors, chondroitinase ABC enhances the activation and oligodendrocyte differentiation of endogenous precursor cells after spinal cord injury and attenuates astrogliosis. When added to polycaprolactone or poly (acrylonitrile)/poly(vinyl chloride) (PAN/PVC) scaffolds, chondroitinase ABC allows regenerating axons to exit the distal end of the scaffold and continue on to distal targets [57–60].

4.2.2. Chondroitinase ABC in combination with cell therapies

Chondroitinase ABC has improved recovery of function in synergy with mesenchymal stromal cells [D43], or a Schwann cell bridge and olfactory ensheathing cells or in combination with transplanted neural precursor cells and a growth factor (GF) cocktail containing EGF, FGF2 and platelet-derived growth factor (PDGF)-AA [61, 62].

4.2.3. Thermostabilisation, delivery, dosage and complications of chondroitinase ABC: chondroitinase ABC might be a weak enzyme

Thermostabilisation of chondroitinase ABC with the sugar trehalose can reduce its temperature-dependent loss of activity [50, 63]. Delivery of chondroitinase ABC is predominantly

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intrathecal using osmotic minipumps [30, 55, 64]. Nevertheless, the enzyme can also be loaded into lipid microtubes or possibly poly (lactic-co-glycolic acid) (PLGA), providing a means for its gradual release over 1–2 weeks [65]. It can also be loaded into fibrin gel scaffolds before injecting it intrathecally; this ensures its continuous release for at least 3 weeks [66].

The effect of chondroitinase ABC is dose-dependent [67]. Injected intrathecally in acute injuries at a low dose (1 or 5 IUs), chondroitinase ABC may enhance axonal progression; injected at a high dose (50 IU), it may produce subarachnoid haemorrhage. In subacute or chronic injuries, low-dose injection produces no or limited functional recovery, whilst high-dose injection (50 or 100 IUs) produce lysis of the gliosis [68, 69].

Single-dose chondroitinase ABC is not considered enough. Therefore, multiple single injections at 0, 1, 2 and 4 weeks have been recommended [70]. Daily injections for 2 weeks at 0.06 Units per dose have also been recommended [71]. Four weeks of treatment have promoted recovery more than 2 weeks [72]. One way to ensure the continued release of chondroitinase ABC is neuron transfection with a vector containing the gene encoding chondroitinase ABC [73]. Another way is loading scaffolds with chondroitinase ABC.

Although chondroitinase ABC allows substantial structural plasticity in the spinal cord, it is not sufficient to enhance locomotor recovery unless combined with neural precursor cell transplantation and in vivo infusion of growth factors [74]. In a rat spinal cord injury model, Wilems et al. [62] have used fibrin scaffolds loaded with neurotrophic factors (item I), anti-inhibitory molecules (item II) and encapsulated embryonic stem-cell-derived progenitor motor neurons (pMNs) (item III). Fibrin scaffolds containing items I and II but not item III have had lower chondroitin sulphate proteoglycan levels compared to scaffolds containing items II and III. This shows the importance of combining neurotrophic factors with chondroitinase ABC and cellular transplants. Scaffolds containing item III, but not item II, have shown differentiation into neuronal cell types, axonal extension and the ability to integrate into host tissue. However, the combination of items II and III have led to reduced cell survival and increased macrophage infiltration. This shows that cellular transplants not only claim priority over chondroitinase ABC, but that chondroitinase ABC may be a weak enzyme as well. Because of this fact, a combination treatment of zymosan and chondroitinase ABC has been recommended [75]. Lastly, adipose-derived stem cell transplantation has been found to produce the same effect as chondroitinase ABC administration [76]. Thus, chondroitinase ABC might be a weak enzyme.

4.3. Sialidase as an alternative

In a rat model of spinal cord contusion injury the effects of sialidase (Vibrio cholerae) and chondroitinase ABC (ChABC, Proteus vulgaris) have been tested [77]. Immunohistochemistry has revealed that infused sialidase has acted robustly throughout the spinal cord grey and white matter, whereas ChABC activity has been more intense superficially. Sialidase treatment alone has resulted in improved behavioural and anatomical outcomes.

4.4. Anti-Nogo

Blocking myelin-associated inhibitors with Nogo-A monoclonal antibodies or with Nogo-receptor competitive agonist peptide, NEP1-40 has been shown to increase axonal regeneration [50]. Combination therapies, such as cross-linking the Nogo-66 receptor antibody into a hyaluronic acid hydrogel [50], a combination of methylprednisolone and NEP1-40 and Nogo-receptor vaccination combined with neural stem cell transplantation have improved neural fibre regeneration.

4.5. Rho inhibition

Many of the inhibitory signals described above (ephrins, Nogo) converge on the intracellular molecule Rho-A, which is a key mediator of actin depolymerisation and hence inhibition of axonal elongation. Blocking Rho-A with Rho inhibitor 'cethrin' might overcome its effect. A synthetic membrane-permeable peptide mimetic of the protein tyrosine phosphatase σ (PTPσ), wedge domain can bind to PTPσ and relieve chondroitin sulphate proteoglycan-mediated inhibition [78].

4.6. Reversing the inhibition of phosphoinositide 3-kinase (PI3K) by cell permeable phosphopeptide (PI3Kpep)

Phosphoinositide 3-kinase (PI3K) is a lipid kinase activated by axon growth promoting signals. Chondroitin sulphate proteoglycans inhibit phosphoinositide 3-kinase signalling in axons and growth cones, an effect that can be reversed by cell permeable phosphopeptide (PI3Kpep). The latter acts by R-Ras-PI3K signalling [79].

4.7. Rolipram

Increased intracellular levels of cyclic adenosine monophosphate (cAMP) and protein kinase A have been associated with CNS ability to overcome the gliosis [50]. Rolipram, a phosphodiesterase4 inhibitor, can increase intracellular cAMP levels [50].

4.8. Improving blood vessel formation

Improving blood vessel formation might reduce cell death and promote angiogenesis within the injury zone. Neural stem cells modified to express vascular endothelial growth factor have improved white matter sparing following thoracic contusion spinal cord injury [50]. Biomaterial poly-lactic-co-glycolic acid (PLGA) scaffolds loaded with neural stem cells and endothelial cells have shown increased vessel and neurofilament density at the injury centre [50].

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5. Modulating astrocyte function enhances the intrinsic properties of neurons to stimulate neurite outgrowth into peripheral nerve grafts

In addition to the sixfold attack, the intrinsic properties of the neurons have to be stimulated to produce neurites. This can take place by modulating astrocyte function (**Figure 2**).

5.1. Endogenous inhibitors of axonal regeneration (intrinsic properties of neurons)

Endogenous inhibitors of axonal regeneration include the molecule phosphatase and tensin homologue (PTEN), loss of neuronal cAMP and deactivation/activation of certain transcription factors [46].

The molecule phosphatase and tensin homologue (PTEN) on chromosome 10 is a tumour suppressor. Its deletion has been shown to increase post-embryonic neural regeneration after injury. Its inhibition–mediated regeneration is partly mediated by the inhibitor of the mechanistic target of rapamycin (mTOR) pathway; it is also mediated by glycogen synthesis kinase GSK-3β.

When levels of cAMP at the growth cone are high, the effect on the growth cone is chemoattraction, whereas when they are low, the effect is chemorepulsion.

Certain transcription factors are positive regulators of axonal growth (e.g., members of the Krüppel-like factors (KLFs) present in retinal ganglion cells (RGCs); STAT3 a transcription factor, part of the JAK-STAT signaling pathway; members of the Jun and Fos families, components of the transcription factor AP-1 and ATF3). Other transcription factors are negative regulators of axonal growth. Nuclear factor IL-3 (NFIL3) represses CREB-mediated transcription and expression of regeneration-associated genes such as arginase and GAP-43.

It follows that combatting endogenous inhibitors of axonal regeneration include the following:

– Inactivation of GSK-3β by neurotrophins. This increases collapsin response mediator protein-2 (CRMP-2) stabilisation of microtubules and increases axon elongation in developing neurites.

– Local administration of taxol. Microtubules and actin microfilaments are critical for regeneration [80]. They potentiate the effect of GAP-43. Thus, local administration of taxol, a microtubule-stabilising agent, increases neurite outgrowth [81].

– Elevating cAMP levels by local injection of a phosphodiesterase inhibitor. This improves axonal regeneration.

– Conditioning lesions. Conditioning lesions [46] are based on the observation that double level nerve lesions regenerate better than single level nerve lesions. Thus sciatic nerve transection prior to a spinal cord lesion improves regeneration within the injured spinal cord.

– Cell adhesion molecules. Their synthesis is increased after peripheral nerve injury but not after CNS injury [82, 83]. Expression of cell adhesion molecules by neurons can be induced by virally mediated vectors or by injecting them into the CNS injury site [84, 85].

Many of these functions can be activated by modulating astrocyte function.

5.2. Astrocyte trophic effects on neurite outgrowth and neurogenesis

Astrocytes release a variety of trophic factors [86–88]. These trophic factors include nerve growth factor, basic fibroblast growth factor, transforming growth factor-β, platelet-derived growth factor, brain-derived neurotrophic factor, ciliary neurotrophic factor and others. Reactive astrocytes increase the expression of several of these, notably nerve growth factor, basic fibroblast growth factor, brain-derived neurotrophic factor and neuregulins, which can stimulate neurite outgrowth. Reactive astrocytes also overexpress neuropilin-1 and vascular endothelial growth factor, which act in concert to promote angiogenesis after cerebral ischemia. Hevin (SPARC-like protein 1), a synaptogenic protein released by astrocytes, forms a relay between a neurexin (pre-synaptic) and a neuroligin (post-synaptic); this relay is crucial for the synaptogenesis [89].

5.3. Modulating the function of astrocytes and heparin

5.3.1. Role of heparin in lysing the gliosis

Both unfractionated and low molecular weight heparins inhibit thrombin activation [90]. In addition, they have a fibrolytic (gliolytic) effect and can modulate astrocyte function.

Clinically, perineural application of condensed polytetrafluoroethylene-extractum cepae-heparin-allantoin gel during peripheral nerve surgery improves functional recovery [91]. The anti-fibrotic effects of heparin are well documented after flexor tendon surgery of the hand [92], in the resolution of intraperitoneal fibrosis [93, 94] and in improving various scar types [95].

Among other actions, heparan sulphate/heparin influences fibroblast growth factor responsible for cell proliferation, differentiation, signal transduction and angiogenesis [96, 97]. Heparin is known to inhibit fibroblast growth factor (FGF)-2-stimulated DNA synthesis as well as gene expression of FGF-2 and its receptor in AT2 pneumocytes [98]. Probably via syndecan-1, the presence of heparin at high concentrations reduces the activity of FGF-7, which is responsible for enhancement of keratinocytes migration and proliferation. Heparin enhances the action of FGF-1, which regulates the proliferation of fibroblasts, endothelial and epithelial cells, and influences angiogenesis via effect on the activity of endothelial cells. Heparin can enhance the stability of FGF-1 and might determine the formation of FGF1-FGFR (fibroblast growth factor receptor) active complex.

5.3.2. Possible role of heparin in modulating astrocyte function

Astrocyte stress response and trophic effects are mediated by the FGF family member, on which heparin exerts a profound influence [96, 99]. Fibroblast growth factor 1 (FGF1) has been shown to maintain the survival of neurons and induce neurite outgrowth [100]. Basic fibroblast growth factor (FGF-2) has been found to increase neuronal survival and neurite extension in foetal rat hippocampal neurons when bound to heparin substrates [101]. The length and

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sulphated position of heparin regulate FGF-2-dependent astrocytic transformation (stellation), native heparin significantly promoting FGF-2-dependent astrocytic transformation, whereas heparin hexasaccharide and 2-O-, 6-O- and N-desulphated heparins inhibit it [102]. Heparin affin regulatory peptide (HARP, pleiotrophin, heparin-binding growth-associated molecule) promotes neurite outgrowth and synaptic development. High levels of heparin affin regulatory peptide HARP mRNA and protein are induced in transformed astrocytes [103, 104]. Glypican-1 is a major high-affinity ligand of the Slit proteins, both of which are strongly upregulated in reactive astrocytes, suggesting their possible role in the inhibitory environment preventing axonal regeneration after injury. Heparins inhibit glypican-Slit interactions [105, 106].

5.4. Possible role of aspirin in modulating astrocyte function

Ciliary neurotrophic factor (CNTF) is a promyelinating trophic factor. Acetylsalicylic acid (aspirin) increases mRNA and protein expression of CNTF in primary mouse and human astrocytes in a dose- and time-dependent manner. Aspirin-induced astroglial CNTF is also functionally active; supernatants of aspirin-treated astrocytes of wild type, but not Cntf null, mice increase myelin-associated proteins in oligodendrocytes and protected oligodendrocytes from TNF-α insult [107].

5.5. Possible role of hyaluronic acid salts in modulating astrocyte function

The presence of high molecular weight hyaluronic acid (hyaluronic acid with limited degradation) after spinal cord injury decreases glial scarring. High molecular weight hyaluronic acid stabilised against degradation mitigates astrocyte activation in vitro and in vivo. Therefore, hyaluronic-acid-based hydrogel systems hold great potential for minimising undesired scarring as part of future repair strategies after spinal cord injury [108].

6. Combining peripheral nerve grafts with scaffolds: the scaffold as a drug release system

The defect within the spinal cord is too large to be bridged by nerve grafts alone; besides, the myelin sheath within them is inhibitory to axonal growth. The rationale for polymer implants is twofold, to replace a damaged area of the cord with just such a structural matrix [109] and to provide it with a synthetic scaffold, in which myelin is absent. Combining peripheral nerve grafts with scaffolds has gained more acceptance because of the importance of seeding the scaffolds with multiple nerve growth factor (NGF) spatial concentration gradients in order to promote axonal growth both within the scaffolds and the nerve grafts [22].

Biomaterial scaffolds in spinal cord injury have been reviewed by Madigan et al. [109] and Straley et al. [110]. Commonly used scaffolds include natural polymers (in vivo extracellular matrix polymers, polymers derived from blood, and polymers from marine life) and synthetic polymers (poly-hydroxy acid polymers and synthetic hydrogels). Examples for in vivo

extracellular matrix polymers are collagen solutions and the glycosaminoglycan hyaluronic acid. Examples for polymers derived from blood are plasma-derived polymers, fibronectin and fibrin. Examples for polymers from marine life are agarose, alginate and chitosan. Synthetic polymers include poly-hydroxy acid polymers and synthetic hydrogels. Compared to natural polymers, they offer wider scope to design and control the characteristics of the material. Poly-hydroxy acid polymers are biodegradable materials based on polyesters of lactic and glycolic acid (PLA and PGA) and their co-polymer PLGA. Synthetic hydrogels are based on polyethylene glycol, a biodegradable synthetic polymer of ethylene oxide units. Poly(2-hydroxyethyl methacrylate) (pHEMA) compounds and pHEMA-co-methyl methacrylate (pHEMA-MMA) are used as spinal cord scaffolds.

Whatever macroengineering and microengineering procedures scaffolds are subjected to allow for axonal growth, this will not occur unless the scaffold is seeded with basal lamina and supplied with neurite outgrowth promoting factors and neurotrophic factors (**Figure 3**). These factors can be released from the scaffold material itself, from integrated micro- or nano-spheres or tubules of a different material, or by means of a scaffold's capacity to support a genetically modified cell line in vivo [58, 109, 110].

7. Augmentation of peripheral nerve grafts with cellular transplants

Nearly half of the spinal cord injuries occur at the thoracolumbar junction (D12-L1), the site of the conus medullaris. We could potentially nerve graft the spinal cord (part of the CNS) directly to the cauda equina (part of the peripheral nervous system) without resorting to cellular transplants to restitute the neuronal and astrocytic components of the CNS. There is mounting evidence, however, that cellular transplants potentiate the effect of other factors and might even recruit endogenous neural precursor cells [62, 76].

7.1. Types of cellular transplants used to augment peripheral nerve grafts

In a meta-analysis reviewing cellular transplantation strategies in spinal cord injury, Tetzlaff et al. [111], have come to the following conclusions. Schwann cells are the most extensively studied cell type. They are reported both to remyelinate-injured spinal cord axons and to form a permissive substrate for their regeneration [112]. However, compared to neural precursors such as oligodendrocyte precursors or neural precursor/stem cells, they provoke a more robust astrocytic reaction, resulting in less effective integration into the host spinal cord [113]. Olfactory ensheathing cells demonstrate good integration into host spinal cord [114]. However, there is no robust evidence of improvement after their transplantation [115]. They also appear to require adjuvant treatment to increase efficacy (e.g., Schwann cells, Matrigel, rolipram, cAMP and neurotrophic factors) [116,117]. Neural stem/progenitor cells appear to integrate well into the host spinal cord with improved outcomes [118]. They differentiate mostly into astroglial cells, less so oligodendrocytes seen but rarely into neurons [119]. Suspicion has been raised as to their role in axonal regeneration [120, 121]. Fate-restricted neural and glial precursor have the potential to remyelinate injured axons [122]. More evidence is needed,

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however, to confirm this observation [123]. Bone marrow stromal cells have some bridging capacity in sharp transaction models [124]. However, their integration in the injured spinal cord is very limited. There is no convincing differentiation into neural cells despite claims to the contrary [125]. They are reported to stimulate neurite outgrowth over neural proteoglycans, myelin-associated glycoprotein and Nogo-A [126, 127].

7.2. Cellular transplants in combination strategy

Because of the previous controversies, the use of a combination strategy including Schwann cells has been recommended by Bunge [128]. The following combination strategy has been suggested [128]: Schwann cells, neuroprotective agents and growth factors administered in various ways, such as, olfactory ensheathing cell (OEC) implantation, chondroitinase addition or elevation of cyclic AMP. A targeted approach has been proposed by Kadoya et al. [43]. It includes the following: a peripheral conditioning lesion (bilateral sciatic nerve crush), mesenchymal stem cell transplantation mixed with neurotrophin-3 (NT-3) and creating a neurotrophic factor gradient by injection of lentivirus expressing neurotrophin-3 (NT-3) just proximal to the site of the lesion.

7.3. Number of cellular transplant injections

A third unresolved issue is the number of injections that the patient has to receive. Mackay-Sim et al. [129] have used a single intraoperative injection. Multiple injections have been recorded by other authors [130, 131].

7.4. Inducing mobilisation of neural precursor cells

Stem cell transplantation has the potential to recruit endogenous neural stem cells [132]. Neural stem cells exist in the mammalian developing and adult nervous system (mainly in the hippocamous and subventricular area). Multiple cell-intrinsic regulators coordinate neural stem cell maintenance, self-renewal and migration into injured areas. Essential intracellular regulators include the orphan nuclear receptor TLX, the high-mobility-group DNA binding protein Sox2, the basic helix-loop-helix transcription factor Hes, the tumour suppressor gene Pten, the membrane-associated protein Numb and its cytoplasmic homolog Numblike. Manipulating these factors among others [133–135] by injecting them through indwelling catheters might induce mobilisation of neural stem cells to the injured spinal cord area.

8. Clinical application

8.1. Pre-operative assessment (neurological and radiographic evaluation)

Patients should be evaluated pre-operatively and at monthly intervals. Motor power and sensation should be evaluated using ASIA standards [28]. Confounding factors during motor power evaluation include fake muscle contractions produced by movements of the trunk and cocontractions between abdominal muscles and different muscle groups. Optional elements

of ASIA neurologic impairment assessment should be included because the abdominal muscles and medial hamstrings are the first muscles to regain power.

Radiographic evaluation should include plain anteroposterior and lateral radiographs and pre-operative magnetic resonance imaging (MRI). The injury zone on the MRI is determined by the superior and inferior extents of the gliosis; nerve grafts have to be extended beyond the injury zone [136] (**Figure 6**).

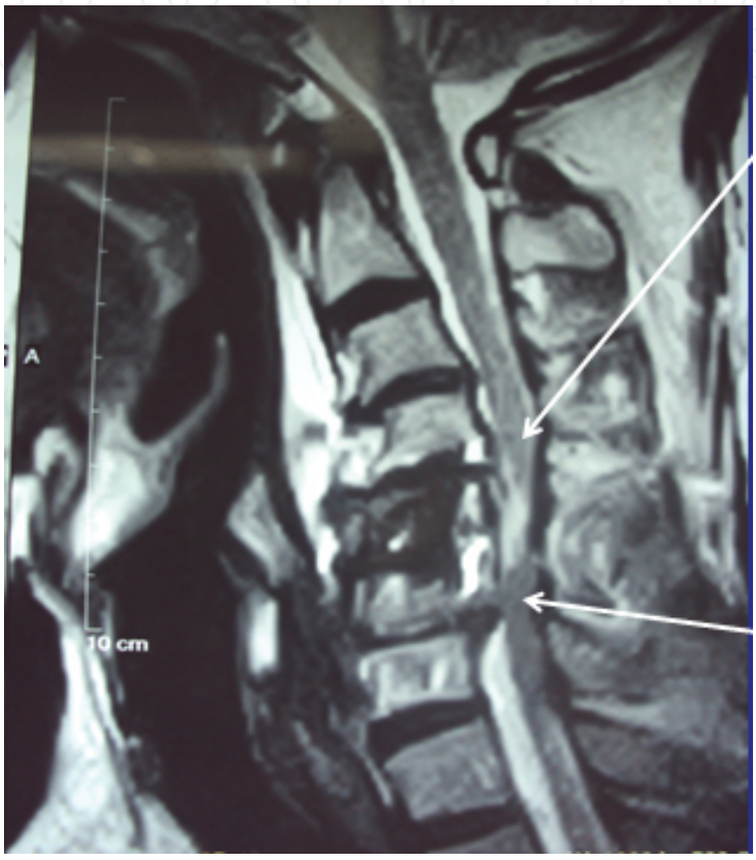

Figure 6. Cervical vertebral C5,6 fracture dislocation in a quadriplegic patient; the gliosis extends from the inferior border of cervical vertebra C4 to the superior border of cervical vertebra C6. The injury zone on the MRI is determined by the superior and inferior extents of the gliosis; nerve grafts have to be extended beyond the injury zone.

8.2. Timing of grafting

Spinal cord injury is considered chronic months to years after injury [6]. At this stage, the primary and secondary injuries have ceased. As the zone of gliosis may extend superiorly or inferiorly during the secondary injury phase, the patient should be operated upon at least 2 months after the injury, i.e., when it has become chronic.

Improvement is also independent of the time delay between the date of injury and the date of definitive surgery. This is supported by the observations made by Li and Raisman [137], who have noted that sprouts from cut corticospinal axons persist despite the presence of astrocytic scarring in long-term lesions of the adult rat spinal cord.

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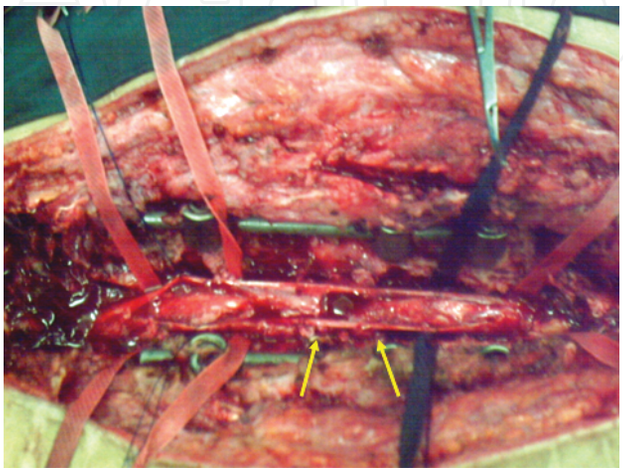
8.3. Operative technique, establishment of CSF circulation and establishment of a continuous drug delivery system (indwelling catheter implantation for post-operative drug and cell administration)

After exploring the injured cord through a posterior spinal laminectomy incision, sural nerves are side-grafted to the cord, especially on its ventral aspect [20, 138] (**Figures 7**(**a** and **b**) and **8**). The cord graft construct invariably adheres to the dura preventing CSF circulation. The latter is important for nutrient, cell and growth factor transport [139, 140]. To prevent the cord adhering to the dura, it has become the author's practice to wrap the cord graft construct with a silicone membrane (**Figure 9**). In fact, silicone chambers or tubes have been used as scaffolds for peripheral nerve regeneration [141–143]. Interest to use them as scaffolds for cellular growth has been rekindled [144, 145]. They have been modified physically to increase porosity or coated to allow for growth of mesenchymal stem cells [146–148] or even to allow neuron-like differentiation of mesenchymal stem cells [149]. They have been modified physically to increase porosity or chemically (with hyaluronic acid and hyaluronic acid--collagen conjugate) to allow for growth of neural cells [150, 151] or to inhibit glial tissue formation [152, 153]. To establish a continuous drug and cell delivery system, an indwelling percutaneous catheter is placed in the interstitium between the membrane and the dura; the dura is finally closed (**Figure 9**). **Figure 10** is a schematic drawing of the hypothetical spinal cord-graft-scaffold-catheter construct.



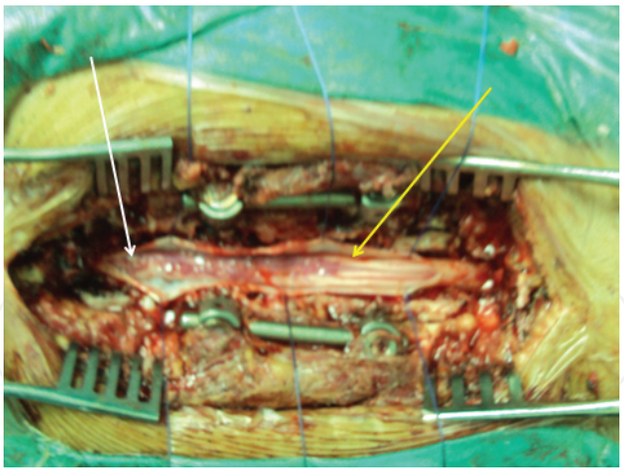


Figure 7. (a) Through a midline dorsal incision, a formal laminectomy is performed preserving the facet joints and pedicles. The dura is exposed. The dura is incised longitudinally and held with stay sutures exposing the cord lesion. The yellow arrows points to the defect in the cord. (b) Spinal cord lesion without defect but with a completely gliotic segment in a paraplegic patient suffering from a dorsolumbar fracture dislocation. The gliotic segment extends from the cord (white arrow) up to the cauda equine (yellow arrow).

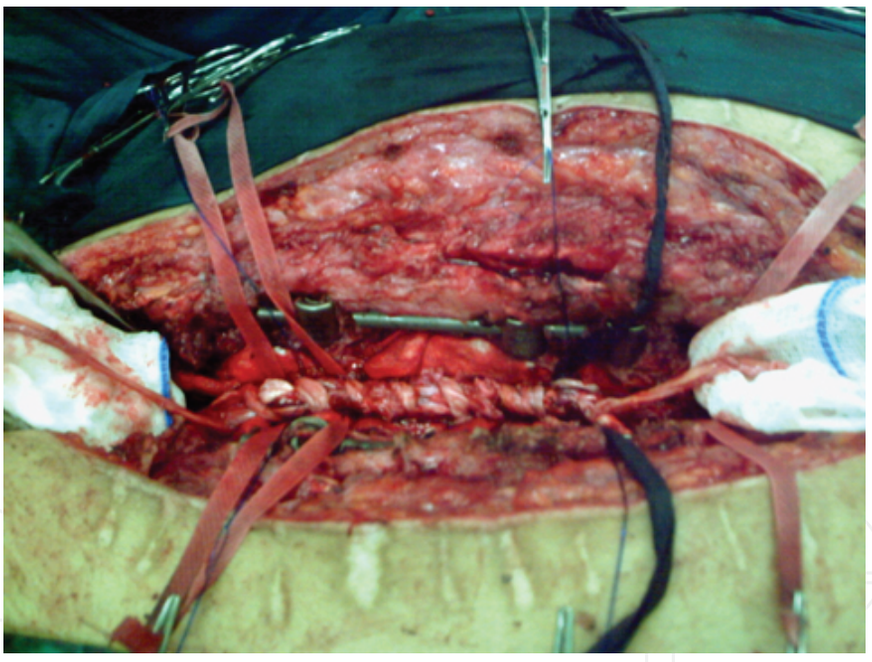


Figure 8. Sural nerve grafts having been side-grafted to the cord.

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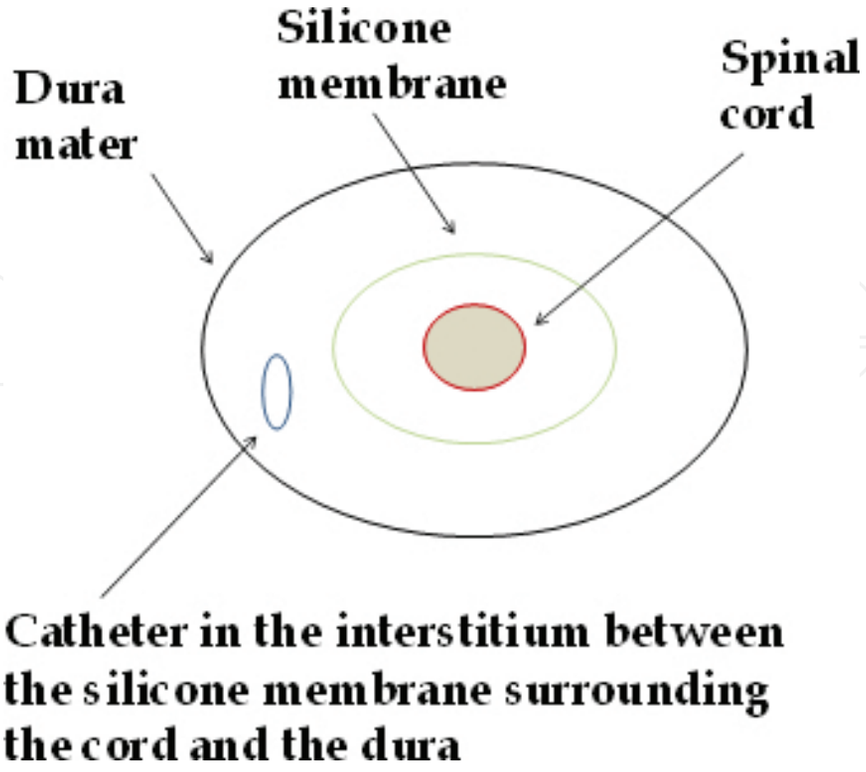

Figure 9. To prevent the cord adhering to the dura, it has become the author's practice to wrap the cord graft construct with a silicone membrane. To establish a continuous drug and cell delivery system, an indwelling catheter is placed in the interstitium between the membrane and the dura; the dura is finally closed.

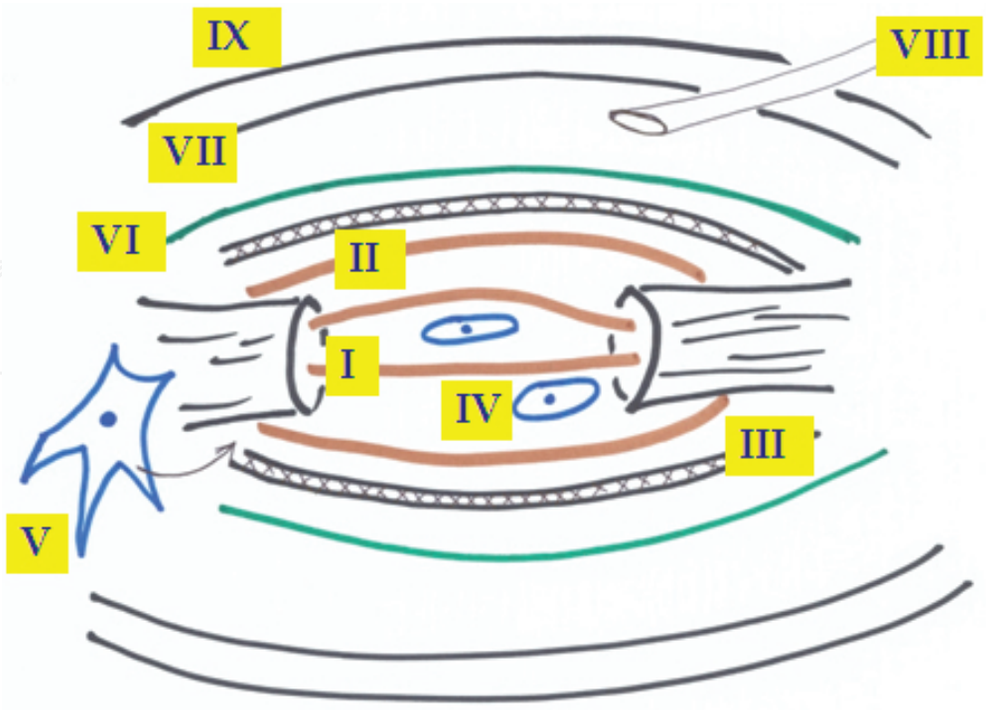

Figure 10. A schematic drawing of the hypothetical spinal cord-graft-scaffold-catheter construct: (I) end-to-end grafts; (II) side grafts; (III) synthetic scaffolds; (IV) cellular transplants; (V) modulated astrocytes; (VI) silicone membrane; (VII) dura mater; (VIII) indwelling catheter for post-operative delivery of neurolyzing agents (chondroitinase ABC, heparin), neurotrophic factors, neurite outgrowth promoting factors, injectable scaffolds and cellular transplants; (IX) skin.

8.4. Post-operative drug and cellular transplant administration through the catheter

The catheter can be used for post-operative administration of growth factors, neurolyzing agents, cellular transplants or even scaffolds. The author's practice has been as follows. Starting from the fifth post-operative day calcium heparin (5000 IU) is injected every second day through the catheter. Chondroitinase ABC (5 IU, Sigma) is dissolved in 2 cc normal saline and injected on a weekly basis.

8.5. Catheter-related complications: keeping the indwelling catheter for 18 months or more around the spinal cord as a means of establishing a continuous spinal drug delivery system

Catheter-related complications include tension headache, meningitis, fibrous track formation, catheter slippage, difficult catheter insertion and catheter blockage. Fibrous track formation is noted by increased pressure on injection through the catheter, associated with increased serosanguinous discharge from the catheter skin exit site due to drug extrusion. Tension headache can be avoided by decreasing the volume of injection; meningitis and early catheter blockage and slippage are avoided by proper catheter care. Complications associated with fibrous track formation, such as difficult catheter insertion, late catheter blockage and slippage (during months 9–18) can only be avoided by inserting the catheter in the interstitium between the silicone membrane and the dura. In this way, the catheter need not be exchanged over 18 months.

8.6. Author's clinical experience with heparin and chondroitinase ABC: its clinical safety and limitations

Delayed wound healing and sinus formation is related to repeated calheparin injection. Its incidence decreases when calheparin is administered every other day.

A vasovagal reaction occurs, when chondroitinase ABC is rapidly injected intrathecally. Its manifestations are cough, hypotension, general irritability and spinal cord irritability manifested by lower limb twitches. A vasovagal reaction does not occur, when the enzyme is injected extradurally or slowly intrathecally.

8.7. Results of surgery

In a clinical study [14], the right and left antero-lateral quadrant of the cord at T7-8 levels have been nerve grafted to homolateral L2-4 lumbar ventral roots. Eight months after surgery, voluntary contractions of bilateral adductors and of the left quadriceps have been observed.

Similar improvements have been observed in another study [15] after nerve side-grafting and augmentation by single-stage mesenchymal cell transplantation. Improvement has been

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hampered by cocontractions between abdominal muscles and different muscle groups. It has also been hampered by spasticity

In a not yet published study, the author has observed that repeated heparin, chondroitinase ABC and cellular transplant injection through an indwelling catheter placed in the interstitium between the membrane and the dura has led to the disappearance of cocontractions between abdominal muscles and different muscle groups. All patients have had pre-operative bouts of a moderate dull aching pain in the abdomen, back and both legs caused by adherence of the cord to the dura and the bony spinal canal. It has been completely resolved by inserting the silicone barrier membrane in the interstitium between the spinal cord and the dura.

Studies using cellular transplantation alone in spinal cord injuries have reported similar motor and sensory score improvement [154–157]. In all these studies, spontaneous or treatment-induced anatomical neural plasticity as well as the adaptive reorganisation of the neural pathways occurring after injury and acting to restore some of the lost function have to be taken into consideration [4].

8.8. False positive results

On evaluating results of surgery after grafting the cord to the cauda equina in thoracolumbar lesions, it should be noted that false positive results could be obtained from intercostal nerves (peripheral nerves) regenerating into the cauda equina (peripheral nerves) via nerve grafts (**Figure 11**).

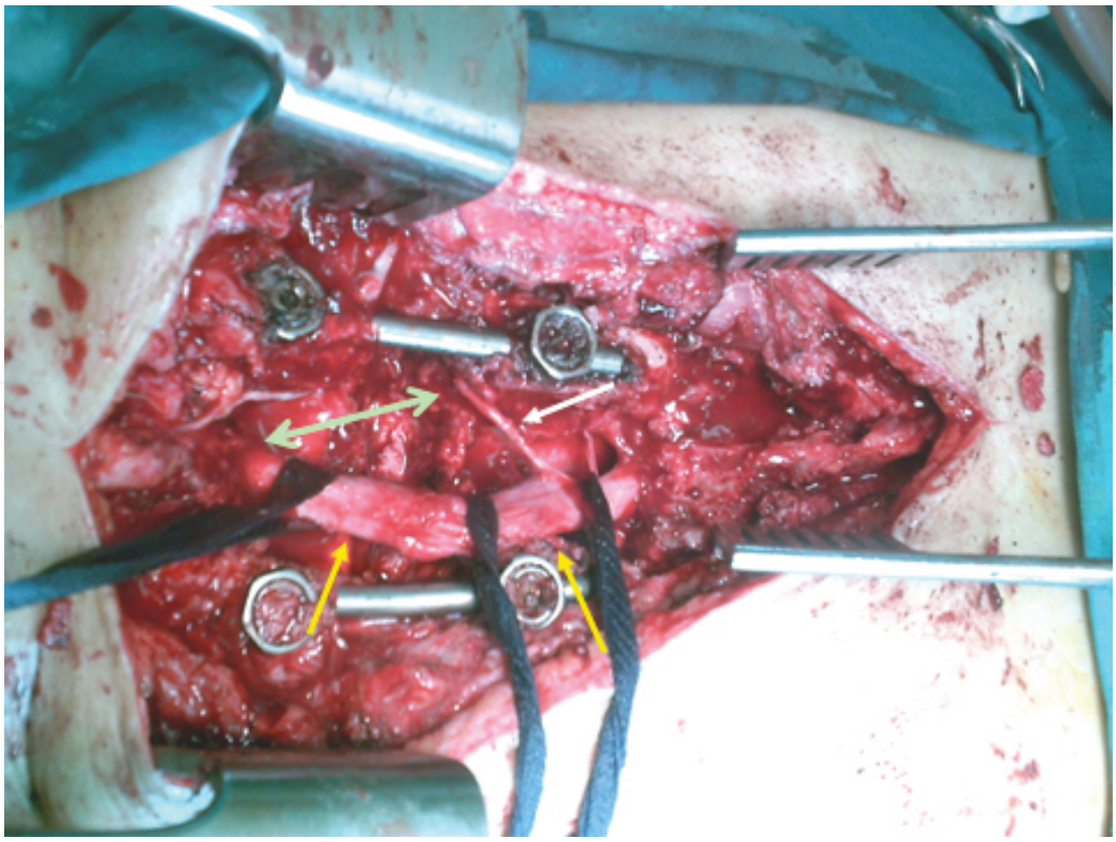

Figure 11. In thoracolumbar lesions, false positive results could be obtained from intercostal nerves (peripheral nerves) at the cranial cord (white arrow) regenerating into to the caua equina (peripheral nerves) (yellow arrow) via nerve grafts.

9. Post-operative target organ derived trophic support

Target organ derived neurotrophic factors, the so-called neurotrophins (nerve growth factor(NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3(NT-3) and neurotrophin-4/5(NT-4/5)), are transported by retrograde axonal via an endosomal mechanism involving dyneins [158, 159]. Neurotrophins contribute a lot to axonal progression; in the injured spinal cord, this stimulus is lost. After grafting the spinal cord, an important question is whether this stimulus can be restituted by injecting neurotrophins into target organs post-operatively, or whether axonal progression into specific nerves can be restituted by injecting neurotrophins into the specific muscles supplied by them. Experimental evidence points to this [158, 159]. By the same token, it can be questioned whether other neurotrophic factors and neurolyzing agents can be similarly injected into target muscles. Both in vitro and in vivo local infusion of fibroblastic growth factors (FGFs) have been found to rescue motoneuron death induced by spinal cord injury [160]. However, evidence for retrograde axonal transport of heparin-binding growth factors is lacking [161].

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10. Conclusions

We have outlined current experimental and clinical experience applying nerve side grafts to the injured spinal cord. Nerve side grafting increases the incidence of nerve regeneration by applying additional grafts extending from the side of the donor end of the cord to the side of the recipient end. A partially regenerated cord cannot be surgically cut and end grafted; nerve side grafting can enhance regeneration through it without incriminating already regained function. Nevertheless, side grafting will fail, unless the gliosis is counteracted or lysed by chondroitinase ABC, sialidase, anti-Nogo, Rho inhibitors and other factors. Side grafting will also fail unless neurons are stimulated to produce neurites. Modulating the function of astrocytes by heparin, aspirin and other factors is one method to stimulate the intrinsic properties of the neurons to produce neurites. Side grafting should be augmented by artificial scaffolds and cellular transplants. Clinically, to prevent the cord adhering to the dura and re-establish CSF circulation, it has become the author's practice to wrap the cord graft construct with a silicone membrane. To establish a continuous drug and cell delivery system, an indwelling catheter is placed in the interstitium between the membrane and the dura; the dura is finally closed. Post-operative injection of paralysed muscles with neurotrophic factors stimulates neurite outgrowth by target-organ-derived neurotrophic support.

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