Peripheral nerve injuries affect up to 5% of patients admitted to trauma centres\(^1\),\(^2\). These injuries represent an important issue in medicine because patients with peripheral nerve trauma are often at the peak of their employment productivity and any loss or decrease of function is particularly devastating.\(^3\) Injuries to peripheral nerves are challenging surgically. Despite advancements in microsurgical techniques, complete recovery of nerve function after repair is almost never achieved.\(^4\) Peripheral nerve repair has reached a plateau today, with functional recovery still unsatisfactory and surgical techniques can not be further refined. Despite early diagnosis and modern surgical techniques, functional recovery of end organs never reaches the pre-injury level. Poor outcome may be explained by factors both intrinsic and extrinsic to the nervous system, such as the type and level of injury, associated injuries, timing of the surgery, and changes in spinal cord neurons and end organs.\(^5\)\(^7\) Misdirection of regenerated axons at the injury site is still a major problem, resulting in an increased interest in the role of micro-environmental factors in regulating axonal growth and direction. Unlike the central nervous system, the peripheral nervous system has strong potential for regeneration. With an appropriate microenvironment, the regenerating axons can extend their processes into the distal Bungner bands to regain normal function. Traditional epineurium neurorrhaphy induces regeneration by direct nerve contact, which leads to enforced inosculation and inappropriate nerve fascicle coaptation and may result in neuroma. The theory of chemotaxis provides new methods distinct from epineurium neurorrhaphy, thus reducing the likelihood of neuroma while gaining appropriate and concise axon regeneration.

1. Theories related to chemotaxis

Forssman’s 1898 theory of selective peripheral nerve regeneration involved assuming that regenerated axons after nerve injury could recognize the distal stumps of the nerves and...
Mechanisms of nerve chemotaxis still need to be explored. Politis et al. found that the proximal ends of tibial and fibular nerves with a Y-conduit tibial and fibular nerve regeneration model: the regenerating axons is regulated by different concentrations of chemotaxis of neurons. Studies have indicated that the direction and eight weeks, only the motor axons that grew into the same, and thus they have different moderating effects on the quadriceps femoris nerve and saphenous nerve. After two, three, and six weeks later, the number of regenerated myelinated motor and sensory axons was measured and compared in the distal two channels. Motor axons showed no selective regeneration, but sensory axons did (Figure 1). Whether nerve chemotaxis has target organ specificity still needs further evaluation.

2. Mechanisms of chemotaxis

The theory of chemotaxis has been widely accepted, however, its mechanism is still unclear. Hari et al. found that the interaction among neurotrophic factors, basal extra-cellular protein and fibronectin can regulate chemotactic regrowth. The authors assumed that recognition particles (neural cell adhesion molecule, L1 family) are the basis of nerve chemotaxis. Hoke et al. assumed that recognition particles regulate chemotactic influence on the proximal end of the regenerating nerve. Lundborg et al. inserted the proximal stump of the rat femoral nerve into the proximal end of the Y conduit and the distal stump of the nerves into the quadriceps femoris nerve and saphenous nerve. After two, three, and eight weeks, only the motor axons that grew into the endoneurium of motor nerves had survived (pruning syndrome). However, sensory nerves did not show a pruning phenomenon, suggesting sensory nerves may not have the specificity of selective growth. Maki et al. assessed the selectivity of motor and sensory axon regeneration towards the distal motor and sensory nerve segments that were disconnected from end organs in a rat silicone Y chamber model. Motor axons showed no selective regeneration, but sensory axons did (Figure 1). Whether nerve chemotaxis has target organ specificity still needs further evaluation.

3. Factors that affect chemotaxis

Multiple factors can affect chemotactic regrowth in peripheral nerve repair, including the distance between the nerve stumps, volume of the distal nerve stump, neurotrophic support and structure of the nerve.

3.1 Distance between the nerve stumps

The theory of chemotaxis suggests that, after nerve injury, distal nerves and target organs have a chemotrophic and chemotactic influence on the proximal end of the regenerating nerves. However, the distance between the nerve stumps is also a factor. If the distance is too large, neurotrophic factors cannot achieve sufficiently high concentrations, and if too small, regenerating axons will grow directly into the distal endoneurium. Lundborg et al. found that a gap of 6 to 10 mm between stumps of rat sciatic nerves showed chemotactic regrowth, but with gaps > 10 mm, growth of the proximal end nerve was prohibited. Politis et al. studying chemotaxis in rat tibial-fibular nerves, chose a distance of 4 to 5 mm, because distances > 6.5 mm showed no significant chemotactic growth.
3.2 Distal nerve stumps

Distal stumps of injured nerves go through a series of cellular and biomolecular changes, including elimination of axons, proliferation of SCs, and Wallerian degeneration. Proliferated but not yet differentiated SCs form a “band of Bungner” in the inner membrane conduits and produce various neurotrophic factors such as nerve growth factor (NGF) and brain-derived neurotrophic factors (BDNF). These molecules promote nerve chemotaxis. After denaturation of the distal nerves, the distal end of the basic membrane conduit remains and can provide a channel and structural basis for the regeneration of nerve axons. Zhang et al. 19 studied the influence of volume of the distal nerves on chemotaxis. They divided 32 rats into 4 groups, proximal nerve stumps were inserted in a nerve tube, and different volumes of nerve grafts were inserted in the distal ends of tubes. After six weeks, the diameter, density, thickness and axonal transport rate of regenerated nerves in the tubes were measured. No significant statistical difference was found between groups, which suggested that the volume of distal nerves does not affect chemotaxis. Takahashi et al. 20 used a rat femoral-nerve Y-conduit regeneration models. Nerve grafts of different volumes were inserted in the distal ends. Axons were counted after six weeks. Regenerated axons in tubes with larger grafts demonstrated more nerve axons, the larger the volume of the graft, the greater the number of regenerated nerve axons. Madison et al. 21 studied a nerve selective regeneration model of rat sciatic nerves and found that chemotactic regrowth of motor nerves is decided by neurotrophic support of distal nerves. Regenerated nerves grew preferentially into the side with rich neurotrophic factors, and the authors assumed that neurotrophic factors were produced by the interaction of distal organs and SCs. Robinson et al. 22 used a rat femoral-nerve Y-conduit model to study the accuracy of motor-axon chemotactic regrowth. The authors found that when sensory and motor axons of similar volume were inserted into the distal end, regenerated nerves grew equally toward the two sides at the early stage but preferentially toward the motor nerve stumps at a later stage. With thick sensory nerves inserted in the far end, regenerated nerve axons grew preferentially towards the sensory nerve stumps. Therefore, regeneration of motor axons is affected by the volume of the distal nerve, which depends on neurotrophic factors secreted by SCs, which can induce the regrowth of proximal nerve stumps. Moradzadeh et al. proposed the theory of nerve architecture: the size of the basal membrane of the conduit of SCs has a strong effect on the regeneration of nerve axons. SCs of motor axons have larger basal conduit membranes than sensory nerve axons, which can facilitate more in-growth of nerves for further repair. Thus, it can be assumed that the chemotactic effect of motor nerves is superior to those of sensory nerve stumps.

3.3 Neurotrophic factors

Recent advances in the understanding of molecular pathways and their physiological role demonstrate that neurotrophic factors play an important role in the development, maintenance, and regeneration of the nervous system. 24-26 Such factors include NGF and the other recently identified members of the neurotrophin family, namely, brain-derived neurotropic factor (BDNF), neurotrophin-3 (NT-3), and NT-4/5. Other factors include the neurokines, ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF), as well as transforming growth factor (TGF)-β and its distant relative, glial cell line–derived neurotrophic factor (GDNF).

During peripheral nerve regeneration, the neurotrophins NGF, NT-3 and BDNF have a well-defined and selective beneficial effect on the survival and phenotypic expression of primary sensory neurons in spinal-cord dorsal root ganglia and motoneurons. Other neurotrophic factors such as CNTF, GDNF and ILGF 27 may also facilitate the chemotactic growth of neuronal cells.

Nerve growth factor maintains activity of neurons, induces and promotes the sprouting of axons, and provokes SCs to express neural-cell adhesion molecules. Utly et al. 28 used BDNF and collagen matrix to repair defects in rat sciatic nerves and found that BDNF significantly accelerated the chemotactic regrowth of peripheral nerves.

To improve the nerve regeneration effect, neurotrophic factors have been added into conduits. Adding exogenous NGF locally has been adopted. This method seems useful in repairing long nerve defects and improving the nerve regeneration effect. HEK-293 cells can be genetically modified to release and regulate NGF in vitro. A 13-mm sciatic nerve gap was bridged with silastic conduits in 120 nude rats, and transfected HEK-293 cells were added and induced with ponasterone A to secrete bioactive NGF. The process increased bioactive NGF expression, enhanced macroscopic nerve growth, and improved functional recovery and regeneration seen on histology from seven days to four months. 29

4. Use of nerve chemotaxis

The theory of chemotaxis has provided new ways to promote peripheral nerve regeneration. 30 The influencing factor in postoperative functional recovery is the correct matching of the proximal sensory or motor tracts with the corresponding tracts in the distal stumps. With a small gap between the ruptured mixed nerve, the proximal sensory and motor nerve fibers grow through the gap and selectively find their counterparts in the distal nerve stumps. 31 Small gaps can form a microenvironment suitable for chemotactic factors from the distal stumps to affect the proximal stumps. Preserving a cavity of a specific size between the proximal and distal stumps, called small-gap bridging, may be beneficial in regenerating mixed nerve fibers. The technique drives more regenerating nerve fibers into the distal stumps than with epineurium neurorrhaphy and provides a relatively close microenvironment to facilitate regeneration because cells are protected against invasion. As well, neurotrophic factors are enriched for the regenerating nerve, and as long as enforced coaptation of nerve stumps is avoided by use of the bridging technique, scar formation is minimized. 32, 33

Small-gap sleeve bridging based on the chemotaxis theory has had satisfactory results in both experimental and clinical studies. The technique might replace epineurium neurorrhaphy as the gold standard for repairing peripheral nerve injury. With tibial nerves of rats, Jiang et al. found small-gap sleeve bridging more effective than direct epineurial suturing.
Figure 2: Small gap bridging technique (2A) prevents enforced coagulation and creates a favorable microenvironment that enhances accurate nerve coaptation, while direct epineural suturing (2B) may result in enforced coagulation and inaccurate nerve coaptation.

4.1 Nerve conduits

Autologous nerve grafting is the golden standard for treating peripheral nerve injury. However, autograft harvesting requires a second operative site with the sacrifice of a functional nerve, which results in donor sensory loss, potential formation of neuroma and neuropathic pain. The theory of nerve chemotaxis has provided the method of nerve conduit bridging to treat large-fragment nerve injury. With such bridging, an appropriate microenvironment is provided to promote the chemotactic regrowth of nerve axons. The conduit provides both stumps with trophic support and prevents the invasion of surrounding tissues. The regenerating stumps are prevented from growing randomly in different directions but not toward their original pathways, which may have developed neuroma. Moreover, nerve conduits enrich the neurotrophic factors secreted from the distal stumps, which influences proximal stumps in building a microenvironment beneficial for the regeneration of nerves (Figure 2).

Brushart et al.¹³ found, using rat sciatic nerves, that the microenvironment produced by nerve conduit bridging is beneficial for autoselection and functional regeneration. Nakamura et al.¹⁶ reported better results using PGA tubes filled with collagen sponge than autografts on 15-mm gap in the peroneal nerve of beagle dogs. Weber et al.³⁷ compared polyglycolic acid conduit (PGA) bridging and direct suturing in a randomized controlled study of 136 patients with peripheral nerve injury in five US medical centers. The authors found 91% satisfactory healing with PGA bridging and 49% with direct suturing. They suggested that conduit bridging is more effective with defects > 8 mm. Taras et al.¹⁸ performed peripheral nerve conduit repair on 73 patients. Except for two patients with scar formation, all other patients achieved satisfactory recovery. Lohmeyer et al.¹⁹ used nerve conduits to repair nerve defects of the forearms in 14 patients: four patients achieved excellent recovery, five satisfactory recovery, one unsatisfactory improvement and two no improvement. Therefore, small-gap sleeve conduit bridging is effective in treating nerve defects, but excellent microscopic surgical skill is vital. To evaluate the clinical application of nerve conduits, Meek et al.⁰ compared nerve tubes approved by the EU and US Food and Drug Administration, favoring the PGA nerve conduit for repair of peripheral and cranial nerve defects because of its advantages in length, price, and availability of clinical data.

Nerve conduit bridging creates nerve regeneration chambers, which are beneficial for tissue engineering. Bioabsorbable conduits and compound conduits (consisting of neurotrophic factors, nerve supporters, SCs and nerve stem cells) have promoted chemotactic growth of peripheral nerves and enhanced nerve repair. Various non-neural conduits have been studied, both synthetic and biological, including silicone tubes, fibroconnectin mats, denatured skeletal muscle or muscle basal lamina, human amniotic membrane, veins, and polyglycolic acid-collagen tubes. Some of these have been more potent in promoting peripheral nerve regeneration with the introduction of SCs. The silica gel canal was the earliest artificial conduit. Its use avoided the harvesting of autologous tissue, which resulted in a positive regeneration effect but was verified to be inferior to the use of veins and needed secondary removal surgery to avoid chronic nerve compression syndrome or inflammatory reaction. Hence, a biodegradable and bioabsorbable artificial conduit is required. Many scholars have used autogeneic epineurium or normal nerve-trunk autogeneic veins, small arteries and even muscle fibers to promote peripheral nerve selective regeneration.

Common biodegradable materials include collagen, chitin, polylactic acid (PLA), polyglycolic acid (PGA), poly(lactide-co-glycolide) (PLGA), poly(caprolactone) (PCL/PCA). These materials can provide initial support for the nerve, and degrade to an innocuous product after the nerve completes the regeneration. (Table 1)

4.2 Schwann cells

SCs are one of the key elements in peripheral nerve regeneration. After nerve injury, SCs in the distal stump undergo proliferation and phenotypical changes to prepare the local environment for axonal regeneration. SCs direct peripheral nerve regeneration by Netrin-1 receptors and Unc5H2. SCs in the peripheral nerve trunk exist in two forms: myelin and nonmyelin forming cells, both differentiating from a common pool of immature SCs derived from the neural crest via intermediate cells called SC precursors.

Modifying nerve conduits by implanting SCs has become a hot topic in research of the nerve gap bridging technique. Siemonow et al. transplanted 24 epineurial tubes as a conduit to bridge 20-mm nerve-gap defects in two experimental groups. For group 1, the tube was filled with saline and for group 2, with...
isogenic bone-marrow stromal cells (BMSCs) prestained with PKH-dye. Twelve autograft sciatic nerve repairs served as a control. After 12 weeks, the epineural tube/BMSC construct was comparable to autograft repair in sensory and motor recovery.

For repair of a long-gap (15-mm) rat sciatic nerve defect model, Lin et al.63 divided 30 rats into four groups for treatment with silk conduits with empty silk microspheres, silk microspheres with GdNF uniformly distributed in the conduit wall, silk microspheres with the highest GdNF concentration at the distal end, and isografting. At six weeks, histological analysis revealed that porous-silk fibroin–based nerve conduits with controlled GdNF infusion had the best curative effect. Di Summa et al.64 compared the efficacy of fibrin conduits implanted with primary sCs, sC-like differentiated bone marrow-derived mesenchymal stem cells (dMSCs), and sC-like differentiated adipose-derived stem cells (dASCs) to bridge a 1-cm rat sciatic nerve gap in a

Table: Some of the studies on the application of conduits for peripheral nerve injury

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Subject</th>
<th>Nerve</th>
<th>Length</th>
<th>Conduit Material</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhang 41</td>
<td>2010</td>
<td>SD Rats</td>
<td>sciatic</td>
<td>1 cm</td>
<td>acellular nerve matrix with stem cells</td>
<td>Stem cells promote nerve regeneration.</td>
</tr>
<tr>
<td>Nakamura 42</td>
<td>2004</td>
<td>beagle dogs</td>
<td>peroneal nerve</td>
<td>1.5 cm</td>
<td>PGA with collagen sponge</td>
<td>The myelinated axons in the PGA side are larger in diameter than the autograft side.</td>
</tr>
<tr>
<td>Weber 43</td>
<td>2000</td>
<td>clinical trial</td>
<td>digital nerve</td>
<td></td>
<td>PGA conduit</td>
<td>Excellent results were obtained in 43 percent of repairs, good results in 43 percent, and poor results in 14 percent.</td>
</tr>
<tr>
<td>Xu 47</td>
<td>2011</td>
<td>SD rat ,</td>
<td>sciatic</td>
<td>1 cm</td>
<td>PDLLA/Chondroitin sulfate/Chitosan</td>
<td>Rapid functional recovery achieved in the experimental group.</td>
</tr>
<tr>
<td>Williams 49</td>
<td>1984</td>
<td>rat</td>
<td>sciatic nerves</td>
<td>1 cm</td>
<td>silicone chamber</td>
<td>Nerve regeneration promoted in the conduit group.</td>
</tr>
<tr>
<td>Whitworth 50</td>
<td>1995</td>
<td>inbred Lew rats</td>
<td>sciatic nerve</td>
<td>1 cm</td>
<td>orientated strands of the cell adhesive fibronectin,</td>
<td>Fibronectin supported a significantly faster rate of growth and amount of axons than the freeze-thawed muscle grafts.</td>
</tr>
<tr>
<td>Glasby 51</td>
<td>1986</td>
<td>marmosets</td>
<td>ulnar nerve</td>
<td></td>
<td>autogenous skeletal muscle</td>
<td>Normal hand function had returned and the grafts were shown to transmit normal compound extracellular action potentials in both directions by six months,</td>
</tr>
<tr>
<td>Fawcett 52</td>
<td>1986</td>
<td>rat,rabbit</td>
<td>sciatic nerves</td>
<td>Rat: 0.5 cm Rabbit: 4 cm</td>
<td>muscle basal lamina grafts</td>
<td>Satisfactory results achieved functionally, electrophysiologically, and anatomically</td>
</tr>
<tr>
<td>Tang 54</td>
<td>1993</td>
<td>clinical trial</td>
<td>digital nerve defects</td>
<td>0.5– 5.8 cm</td>
<td>autogenous vein graft taken from forearm</td>
<td>Follow-up revealed excellent recovery in two digital nerves, good in nine, fair in five and poor in two</td>
</tr>
<tr>
<td>Ashley 55</td>
<td>2006</td>
<td>New born infants</td>
<td>brachial plexus</td>
<td></td>
<td>collagen matrix tubes</td>
<td>Most patients achieved good recovery</td>
</tr>
<tr>
<td>Kiyotani 56</td>
<td>1996</td>
<td>cats</td>
<td>sciatic nerves</td>
<td>2.5 cm</td>
<td>PGA mesh coated with collagen</td>
<td>16 months after implantation of the tube revealed regeneration of well vascularized nerve tissue.</td>
</tr>
<tr>
<td>Brown 57</td>
<td>1996</td>
<td>rabbit</td>
<td>hind-limb nerve</td>
<td>3 cm</td>
<td>polyglycolic acid filled with gelatin/Schwann-cell suspension</td>
<td>No significant difference between experimental and a control group in which the nerve gap was reconstructed by using a PGA conduit filled with gelatin only</td>
</tr>
<tr>
<td>Ayhan 59</td>
<td>2000</td>
<td>Wistar rats</td>
<td>sciatic nerves</td>
<td>1 cm</td>
<td>epineurial sheath tube</td>
<td>Sciatic functional indices and histomorphometric analyses revealed statistically significant improvement in experimental group</td>
</tr>
<tr>
<td>Karacaoglu 60</td>
<td>2001</td>
<td>rat</td>
<td>sciatic nerves</td>
<td>1 cm</td>
<td>epineurial tubes</td>
<td>Morphometric analysis showed significant improvement in experimental group</td>
</tr>
<tr>
<td>Geuna 62</td>
<td>2004</td>
<td>Inbred Lew rats</td>
<td>sciatic nerve</td>
<td>1 cm</td>
<td>muscle-vein combined conduits</td>
<td>Experimental group showed significant improvement by total number, mean density and mean size of myelinated nerve fibers,</td>
</tr>
<tr>
<td>Varejao 63</td>
<td>2003</td>
<td>rat</td>
<td>sciatic nerve</td>
<td>1 cm</td>
<td>Poly (DLLA-epsilon-CL) conduit enriched with fresh skeletal muscle</td>
<td>α-GFAP (glial fibrillar acid protein) immuno-labelling of Schwann cells showed progression inside muscle-enriched tubes</td>
</tr>
<tr>
<td>Barcelos 65</td>
<td>2003</td>
<td>Wistar rats</td>
<td>sciatic nerves</td>
<td>1 cm</td>
<td>inside-out vein grafts(IOVG) and inside-out artery grafts (IOAG)</td>
<td>IOVG presented a closer-to-normal nerve organization than IOAG</td>
</tr>
</tbody>
</table>

https://doi.org/10.1017/S0317167100014220 Published online by Cambridge University Press
long-term experiment (16 weeks). Control groups were fibrin conduits without cells and autografts. Conduits with SCs, dMSCs, and dASCs demonstrated better regenerative qualities in terms of functional and morphological properties of regenerated nerves than did conduits without cells. MSCs are abundant and can be easily collected, which solves the problem of the SC source and allows for wide clinical application of SCs. Furthermore, small-gap bridging of rat nerves with conduits including MSCs conferred a better effect than with simple conduits. Matsuse et al. induced human umbilical cord Y-derived mesenchymal stromal cells (UC-MSCs) to differentiate into cells with SC properties by treatment with A-mercaptopethanol then retinoic acid and a set of specific cytokines and used them in nerve conduits as neurotrophic factors: functional recovery of injured nerves was significantly promoted, which indicates that cells with SC properties and the ability to support axonal regeneration and reconstruct myelin can be successfully induced from UC-MSCs to promote functional recovery after peripheral nerve injury.

CONCLUSIONS

Studies have shown that nerve regeneration has target tissue specificity. However, whether such regeneration has topographic or target organ specificity is not clear. The mechanism of chemotactic regeneration still needs further study. The use of small-gap conduit bridging creates a beneficial microenvironment for chemotactic nerve growth and is important in the functional recovery of end organs. Although the theory of chemotaxis and small gap bridging technique seemingly has promising effects, there are still huge challenges to overcome to find a perfect combination of nerve tubes, cells, cellular matrix, neurotrophic factors as well as appropriate distance between stumps to create a favorable microenvironment for chemotactic nerve regeneration to achieve full and precise nerve repair.

REFERENCES


