Laser-assisted nerve repair. An experimental study
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Citation for published version (APA):

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The peripheral nervous system comprises all nervous tissue outside of the brain and spinal cord. Peripheral nerves consist of many hundreds of nerve fibres ensheathed by connective tissue. Each nerve fibre is composed of an axon and enclosed by a sheath of Schwann cells, which surrounds the axon almost from its beginning at the neuron to its peripheral termination in the end organ, such as muscle or skin (BABEL, 1970; GRAY, 1970; LANDON, 1976). The larger peripheral axons are also enveloped in a myelin sheath (myelinated axons), within the sheath of Schwann. The myelin sheath is part of the Schwann cell and consists of concentrically wrapped layers (consisting of a few to 50 or more turns around the axon) of the plasma membrane of the Schwann cell (GRAY, 1970; BISCHOFF, 1975). The smallest axons of the peripheral nerves lack a myelin sheath (unmyelinated axons), but the axons occupy recesses in the surface of the same Schwann cell. The presence or absence of a myelin sheath exerts an important influence on the physiological properties of the neuron. The sheath of Schwann and the myelin sheath are interrupted at regular intervals by a node of Ranvier (RANVIER, 1871/1872), which is a point of discontinuity between successive Schwann cells along the length of the axon. Schwann cells are indispensable for the life, function, and regeneration of the axons of peripheral nerve fibres (SPENCER, 1981).

The nerve fibres of peripheral nerves are grouped in fascicles and held together by connective tissue surrounded by blood vessels and lymphatics, forming a peripheral nerve. It has become customary to classify nerve fibres according to their diameter, varying between 1 and 20 μm, because the speed and amplitude of action potential vary with the diameter of the fibre (HURSCH, 1939; LLOYD, 1943; BISCHOFF, 1975; WHITWAM, 1976). Motor nerve fibres supplying skeletal muscles are thick and heavily myelinated, those of visceral smooth muscle are thin, lightly myelinated, or without myelin. Tactile fibres are medium-sized and moderately myelinated, pain and taste fibres are thinner, with less myelin or no myelin at all.

The neurons of the axons have an unique system for intracellular transport, making an adequate supply of necessary substances to the distal axons parts possible. By this axonal transport, a wide variety of materials synthesised in the cell body, move along the axon at different flow rates (OCHS, 1969; GRAFSTEIN, 1980). Slow transport (1-6 mm/day) involves subunit proteins of the axon's cytoskeletal elements (microtubules, neurofilaments) and fast transport (400 mm/day) involves mainly plasma membrane constituents (glycoproteins, lipids, transmitter storage vesicles).
Fascicles
The individual nerve fibres in the peripheral nerve form large or small bundles, called fascicles. Each fascicle may contain motor, sensory, or sympathetic fibres in various numbers. The number and size of the fascicles may vary in any given nerve and even along the course of the nerve. Also, there is a cross migration of nerve fibres from one fascicle to another along the length of a nerve and fascicular plexi can form throughout the course of the nerve (SUNDERLAND, 1945A). Each fascicle is surrounded by the perineurium.

Endoneurium
The endoneurium is the supporting connective tissue that fills the fascicle and provides the packing between individual nerve fibres (KEY, 1876; GAMBLE, 1964A & 1964B; SUNDERLAND, 1965). The endoneurium has a special relationship to the nerve fibres in which the collagen fibrils are closely packed around each nerve fibre to form the supporting wall of what is conveniently referred to as the endoneurial tube, which is occupied by a cylinder of tissue comprising the axon, Schwann cell sheath, and the myelin. The main function of the endoneurium is to resist elongation under tension and protecting the nerve fibres.

Perineurium
Each fascicle is surrounded by the perineurium which is formed of several lamellae consisting of closely packed perineurial cells and collagen fibres (RÖHLING, 1961; SUNDERLAND, 1965). The thickness of the perineurium ranges from 1.3 µm to 100 µm, and there is a relationship between the diameter of the fascicle and the thickness of the perineurium. The perineurium has several functions. First, it serves to protect the nerve fibres within its boundaries and to maintain an intrafascicular pressure, which is important in promoting the axonal transport from proximal to distal. Second, the perineurium serves to protect the fascicle from stretch injury, and the elastic properties and integrity of a nerve undergoing elongation are retained as long as the perineurium remains intact. Third, the perineurium acts as a diffusion barrier (similar to the blood-brain-barrier), which blocks the passage of a wide range of macromolecular substances and acts as a barrier to the spread of infections.

Epineurium
The external epineurium is the connective tissue that encircles the nerve and delineates it from surrounding tissue to which it is loosely attached so that the nerve enjoys considerable mobility. The internal epineurium separates the fasciculi and also binds them loosely together. The connective tissue from the external epineurium is condensed at the surface of the nerve to form a definitive investing sheath. It consists of collagen tissue and the internal epineurium can account for 25% to 75% of the cross-sectional area of a given peripheral nerve (SUNDERLAND, 1949). The epineurium responds to damage and will react with collagen deposition during the healing process.
Moreover, the epineurium allows the nerve to be stretched without the fasciculi being strained and protects the fasciculi from external mechanical forces by providing the packing around it. The thickness of the external epineurium varies between individual nerves and also within the same nerve (Sunderland, 1965). Figures 2.1 and 2.2 show the various parts of a peripheral nerve.

**Mesoneurium**

The mesoneurium, also called the paraneurium, is a fine sheet of undifferentiated connective tissue that connects the epineurium to the neighbouring connective tissue, contains the feeding blood vessels, and permits gliding of the nerves during limb movements (Millesi, 1990). The mesoneurium is inserted on the nerve along a straight line and is merged into the epineurium-connective tissue complex. The width of the mesoneurium varies, but it tends to be greater in the vicinity of the joints where the nerve is more mobile.

**Vascularization of peripheral nerves**

The peripheral nerve receives its blood supply at regular intervals through vessels originating from larger limb arteries and their collaterals (Sunderland 1945b; Lundborg, 1979 & 1988). The pattern of blood supply varies among individuals and also between the two extremities in the same individual. All blood vessels approach the nerve through the mesoneurium, which enable the nerve to move without impairing its blood supply. There exists a dual vascular system, an extrinsic (perifascicular) and an intrinsic (intrafascicular) one, which communicate with each other. In the extrinsic system, the arteries run longitudinally in the epineurium, accompanied by venules and a variable number of capillaries. The longitudinal vessels give off collaterals, which are distributed around the fascicles within the perineurium. The intrinsic system lies within the endoneurium throughout the length of the nerve and it consists of capillaries that run mostly longitudinally. The venous system closely parallels the arterial system.

**Degeneration and regeneration of peripheral nerves**

When a nerve has been injured, a sequence of degenerative and subsequent regenerative processes occur in the nerve, both proximal and distal to the lesion. The lesion in the nerve can be caused by mechanical trauma (transection, crush, compression), physical trauma (hyperthermia, coagulation, radiation), chemical trauma (toxic substances), and ischaemia. The light microscopic features of the degeneration of axons distal to the lesion were described by Augustus Waller in 1850. His investigations proved to be important and since then degenerative changes occurring in the peripheral nerve have been called Wallerian degeneration. Subsequently, several studies have described degenerative and regenerative aspects of peripheral nerves (Cajal, 1928; Ohmi, 1961; Barton, 1962; Dixon, 1963).
Chapter II

Fig. 2.1. Schematic view of a peripheral nerve (from SMITH, 1978, printed with permission).

Fig. 2.2. Cross-section of the rat sciatic nerve (toluidine blue, original magnification x 100). Note a capillary filled with erythrocytes in the centre of the nerve.
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Degeneration
When a nerve is transected, trauma occurs at both cut edges. In the proximal segment, degeneration occurs only for short distances. In the distal nerve segment however, the axon, its terminal endings, and the myelin sheath completely disintegrate and the Wallerian degeneration takes place throughout the whole length of the distal part of the nerve (WALLER, 1850; BARTON, 1962).

The initial changes in axonal structure consist of localised accumulations of mitochondria a few hours after injury (Figs. 2.3a and b). Within one day, neurofilaments and mitochondria of axons disintegrate. At about two days, the axonal cylinder starts to disintegrate. Concomitantly, myelin degeneration begins by breaking down into fatty droplets and concentric lamellar structure. Macrophages, derived from the endoneurial and Schwann cells, progressively remove the cell debris in the first weeks after injury. The Schwann cytoplasm remains relatively intact and, after axonal and myelin degeneration, forms a tube surrounded by the endoneurium and filled with fluid and scattered fragments. In the next weeks, the wall of the tube thickens and the tube shrinks to even less than half of its original diameter (SUNDERLAND, 1950). Ultimately, these changes produce a solid, nucleated band of Büngner (BÜNGNER, 1891). Meanwhile, the Schwann cells proliferate (SPENCER, 1981; PELLEGRINO, 1985). As the myelin disappears, the endoneurial content condenses into a dull grey, hardened, and rounded cord. The bands of Büngner may remain for many months, awaiting reinnervation. If regeneration does not occur, the bands gradually become reduced by proliferating connective tissue.

Regeneration
The localised degeneration of the proximal segment of a peripheral nerve is followed within one to two weeks by axonal regeneration, in the form of fine axonal sprouts (CAJAL, 1928; OHMI, 1961; BARTON, 1962; DIXON, 1968). The regenerating axons in the proximal segment give rise to a great number of axonal sprouts which proceed distally (Figs. 2.3c and d). In order to reach the distal nerve segment, the axonal sprouts have to cross the critical area between the proximal and distal nerve segments. Like any surgical wound, the repair site is characterised by local wound healing processes, such as proliferation of endothelial cells, fibroblasts, and Schwann cells, and synthesis and deposition of collagen. The success of nerve regeneration depends to a large extent on what happens at this repair site (LUNDBORG, 1987).

The anatomical reunion of a transected nerve is the result of intense cellular activity at the cut surfaces of both nerves. The Schwann cells multiply vigorously in the distal nerve segment (SCHRÖDER, 1968; PELLEGRINO, 1985) and the fibroblasts of the endoneurium, perineurium, and epineurium are equally active. The Schwann cell proliferation in the distal segment is associated with production of some diffusible substances that attract axons and the regeneration process is therefore to a large extent regulated and modified by the distal nerve segment, in particular by the Schwann cells (SPENCER,
Fig. 2.3. Schematic view of nerve degeneration (a and b) and regeneration (c to e) after transection of a myelinated nerve fibre (from MUMENTHALER, 1987, printed with permission). (a) 1-4 days: Wallerian degeneration starts. (b) 10-21 days: Wallerian degeneration proceeding throughout the whole distal nerve and forming of bands of Büngner. Macrophages remove the myelin debris and axonal sprouting occurs from the proximal segment. (c) > 1 month: Regenerating sprouts proceeding distally. (d) Reinnervation of the end organ (muscle) and progressive remyelination of the axons. (e) If the axons do not reach the distal segment, such as due to connective tissue proliferation at the repair site, the axons form a neuroma together with proliferating Schwann cells and fibroblast. The distal nerve is degenerated and the muscle shows marked atrophy.

1 Neuron, 2 Axon, 3 Schwann cell, 4 Basal membrane, 5 Mitosis of Schwann cell, 6 Myelin sheath, 7 Myelin debris, 8 Macrophages, 9 Band of Büngner, 10 Muscle
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1981; Pellegrino, 1985; Wong, 1995). The perineurial cells, derived from the perineurium, are one of the first cells participating in the anatomical reu­nion of the nerve (Schröder, 1993).

When the axonal sprouts arrive at the cut surfaces of a transected nerve they are free to wander anywhere (Fig. 2.4). If obstructed, some turn in their tracts and grow back into the proximal part of the nerve. Others turn laterally away from the line of the nerve, escaping into the tissue surrounding it, forming a neuroma (Fig. 2.3e). This neuroma is characterised histologically by numerous minifascicles, growing in all directions, containing only few or a small group of collateral axonal sprouts encased by a multi layered perineural covering (Badalamente, 1985). Axonal loss at the repair site may be considerable, but if the surgical repair has been well performed, a considerable number of the axons can proceed into the distal nerve segment. In the early stage of nerve regeneration, the formation of numerous miniature fascicles is often observed. This compartmentation expresses the need for restoration of the normal endoneurial environment around nerve fibres by restoring the perineural barrier. The stimulus for the compartmentation results from damage of the perineurium exposing the nerve fibres to a foreign environment (Morris, 1972). As a consequence, this reaction is mostly expressed at the periphery of the nerve.

The numerous axonal sprouts arising from the proximal nerve segment have an affinity for the surfaces of the Schwann cells and grow along the outer edge of a band of Büngner and on the inside of the basal lamina. Although the distal endoneurial tubes are packed with proliferating Schwann cells, the stream of axoplasm squeezes in between them and many axonal sprouts may be present in one tube (Holmes, 1942). In the next stage, they become progressively enfolded by Schwann cells in a fashion similar to the multiple envelopment found in unmyelinated nerves. One axonal sprout may be centrally placed and rapidly develop a myelin sheath; others are peripherally but still surrounded by Schwann cell cytoplasm and several branches in one tube may become myelinated. At this stage, the distal nerve segment contains an overpopulation of axons, as groups of these sprouts are derived from single axons above the level of the lesion. These branches continue distally but the number of branches is smaller in the most distal part of the nerve than in the vicinity of the lesion. The growth rate of the sprouts is approximately three to four millimetres per day in mammals (Danielsen, 1986a), after an initial delay of several days before the axons cross the repair site (Danielsen, 1986b). The further regeneration advances, the fewer the number of branches (Mackinnon, 1991). The reduction in the number of branches or sprouts occurs when some of them make peripheral connections and then subsequently mature at the expense of disappearing sprouts, which have not established peripheral contacts.

Thereafter, a process of maturation takes place, including axonal enlargement and myelination (Fig. 2.5). Even with optimum axonal regeneration the endoneurial tubes contract and re-expand to only 60%-80% of their normal cross-sectional area (Sunderland, 1950). The smaller axons in the dis-
Fig. 2.4. Schematic view of nerve regeneration after transection (from CAIL, 1928). The axonal sprouts proceed from proximal to distal. Some of the sprouts get lost by escaping from the repair site and either turning backwards or going laterally or distally outside the original nerve. Other sprouts can proceed distally, however, normal axonal architecture is not accomplished.

Fig. 2.5. Transmission electron micrograph of peripheral nerve regeneration 16 weeks after transection and end-to-end FGNR (rat oculomotor nerve, original magnification x 4,300). Note axons and myelin sheaths of various diameter associated with Schwann cells.
tal segment probably explain the slower physiological conduction times observed. The functional recovery depends on reestablishment of appropriate sensory and motor connections at the endorgans. The abundance of regenerative sprouts from the original neuron and the capacity of bands of Büngner to accommodate hundreds of fibres favour successful reinnervation. In addition to the abundance of sprouts, peripheral deletion of maladaptive terminations and central remodelling of reflex arcs offer additional possibilities for functional recovery after the anatomical nerve regeneration has been completed (Wall, 1986; Bach, 1990).

**Injury to the peripheral nerve**

Although there are several causes of peripheral nerve injuries, only the mechanical (i.e. traumatic) injuries will be outlined. There are two classifications of mechanical nerve injury, one described by Seddon (1942a & 1943) and one described by Sunderland (1951). Seddon's classification is mostly used because it is simple and practical. The injury to the nerve is followed by various degrees of functional loss depending on the pathoanatomical basis of the lesion.

**Neuropraxy**

In this type of injury, the nerve is contused but the continuity of the nerve is not interrupted. This type of lesion is mostly caused by pressure or stretching of the nerve. Histologically, there is some local myelin damage, but the axonal continuity is preserved and only slight degeneration takes place. The lesion is mostly reversible within weeks or months and the return of function is in most of the cases complete.

**Axonotmesis**

A more severe compression or stretching of the nerve may result in loss of axonal continuity at the level of the lesion, however, still with intact endoneurial tubes. In this type of lesion, the axon degenerates, but the prognosis is good because the preserved endoneurial tubes guarantee that axons regenerate in their original endoneurial tubes (Haftek, 1968). This type of lesion results in less successful recovery than axonotmesis, but returned function can still be favourable.

**Neurotmesis**

Neurotmesis includes lost continuity of all the elements of the peripheral nerve. If untreated, the gap between both nerve segments becomes filled with connective tissue and neural elements. The resulting neuroma can cause unpleasant complaints at exposed sites which may arise spontaneously or after mechanical irritation (Burchiel, 1991). The mechanism of neurotmesis is usually a penetrating injury due to gunshot wound or knife. Surgical repair is required and the functional regeneration after surgery is not complete and sometimes even very unsatisfactory.
Interest in peripheral nerve repair dates to 1800 years ago. Galen (200 AD) is believed to be the first physician who discussed the possibility of nerve regeneration. He prescribed local medications to promote ‘agglutination’ in nerve injuries. The first documented surgical repair is attributed to Paulus of Aegina around 600 AD (Ochs, 1980). He used sutures in nerve injuries in addition to agglutination and advised to apply the sutures deeply. Roger of Parma (13th century) used egg albumin to repair nerves in a similar fashion. In the 14th century, Guy de Chauliac observed excellent functional regeneration after suture repair in a young man (Brennan, 1923). Although Avicenna (1564) tried to suture nerves in the 16th century, Ferrara (1596) was the first to give a precise description of suturing of the segments of a transected nerve using a special needle with an eye. Florens (1828) performed the first end-to-end epineurial suture followed by Nelaton in 1863 (Ochs, 1980). After excision of a tumour of the median nerve, the defect of 3 cm was treated by suturing the nerve segments using silver wire.

Waller’s work in 1850 led to rediscovery of the nature of peripheral nerve degeneration and subsequent regeneration after several hundred years of general belief that nerves did not regenerate. The greatest progress in the treatment of nerve injuries came during wars, when the large amount of nerve injuries gave the surgeons a tremendous experience. The American Civil War resulted in the work of Mitchell (1964 & 1874). The First World War provided material for the work of Dejerine (1915), Tinel (1917), and Foerster (1929).

The Second World War brought the work of Seddon (1942a, 1944 & 1948), Zachary (1946), Woodhall (1956), and Sunderland (1968). A large clinical series of peripheral nerve injuries sutured by epineurial repair was reported by Seddon (1944). Subsequently, Seddon (1948) introduced the use of cable nerve grafts to overcome the poor results achieved when loss of nerve tissue required an end-to-end neurorrhaphy under considerable tension. Highet (1942) was among the earliest to stress the importance of eliminating tension in peripheral nerve repair, and this has been later confirmed by Samii (1972) and Millesi (1972a). Sunderland (1968) made several important contributions to the understanding of nerve physiology and repair. Peripheral nerve injuries during the Korean and Vietnamese Wars resulted in the work of Omer and Spinner (1980).

The introduction of magnification (operating microscope and loupes) in peripheral nerve surgery has led to improved results, reflected in the reports of numerous authors (Smith, 1964; Braun, 1966; Wise, 1969). Delicate instruments originally manufactured for jewellers and diamond cutters have proven satisfactory in the beginning and were later replaced by specially designed microsurgical instruments (Vickers, 1978). Developments in fine suture materials and the use of bipolar coagulators were also of great importance (Malis, 1967). Subsequently, successful intraoperative electrophysiological methods for assessment of nerve function, especially when the nerve
was in continuity, have been developed (Kline, 1969 & 1993). These intraoperative monitoring techniques have increased the incidence of surgical explorations and attempted repairs of lesions previously felt not to be repairable.

Despite numerous attempts, the most efficacious method of nerve repair remains unidentified. Even in ideal cases, where a sharply cut peripheral nerve has been sutured under favourable circumstances, the results show that nerve suturing does not lead to full functional recovery (Young, 1980; MacKinnon, 1988; Kline, 1995; Choi, 1997). When Zachary (1946) published the results of the peripheral nerve sutures in the British Army during World War II, he concluded: "The number of poor recoveries after treatment under good conditions is large enough to be important. This should preclude an attitude of complacency with the present methods of treatment of peripheral nerve injuries and stimulate a search for better ones, both operative and conservative."

Factors influencing the outcome of nerve repair

Many factors relate to the outcome of nerve repair (Steinberg, 1991). In general, the more proximal the lesion the more rapid the initial rate of regeneration, but the poorer the final result because of the longer distance of the axons to be travelled. The likelihood of distal tubal scarring and end organ degeneration is larger as it takes longer before the axons reach their destination. Delay in surgical nerve repair beyond approximately one year will lead to significant atrophy and degeneration of the nerve and end organs (Gutmann, 1944 & 1962). The more severe concomitant injury, the poorer the result. Associate severe local soft tissue injury results in excessive scar production in and around the nerve. Scar tissue within a nerve is a negative factor in influencing the direction of regenerating axon fibres into the distal tubules (Bora, 1967; Samii, 1972; Hudson, 1979). Scar tissue around the nerve can cause constriction and tethering of the nerve to surrounding structures (Hunter, 1991). Pure sensory or pure motor nerves are likely to result in better functional recovery than mixed nerves where the possibility of fibres regenerating down inappropriate sheaths exists (Steinberg, 1991).

One of the most important factors influencing the functional nerve regeneration after repair is the age of the patient. In the clinical series, the results in children are usually better than those at a more older age (Tajima, 1989; Kline, 1995; Koller, 1998). However, there is no scientific evidence for increased quality or rate of regeneration of the younger, growing peripheral nervous system (Lin, 1994) and it is assumed that superior results in children are due to the greater cerebral adaptation to the injury (Leonard, 1973; Steinberg, 1991). Another factor is the generally shorter distances axons have to grow in young individuals to reinnervate target organs.

At last, peripheral reinnervation leads to a new pattern of impulses in afferent nerve fibres and to a new cortical projection of peripheral sensory and motor representation (Wall, 1986; Bach, 1990). Thus, although the
complexity of peripheral factors influencing axonal regeneration is striking, the central nervous component of the problem is important. Sensory reeducation, implying a detailed programme for cerebral adaptation to the new situation, represents an important component of rehabilitation following peripheral nerve injuries (DELLON, 1974; KLINE, 1995).

Surgical technique
Since the introduction of the surgical microscope, it is clear that microscopic repair leads to better functional results than a macroscopic repair (SMITH, 1964; BRAUN, 1966; WISE, 1969). Nerve repair must be to a certain degree tension free as tension disturbs healing at the repair site and leads to a marked proliferation of connective tissue (SAMII, 1972; MILLESI, 1972A; MIYAMOTO, 1979). In this way the repair zone becomes occupied by unfavourable scar tissue which both blocks and constricts regenerating axons. When a tension-free repair is not possible, nerve grafting should be performed (MILLESI, 1972B).

Exact apposition of the nerve ends is very important implying a satisfactory longitudinal orientation of the fasciculi and a high degree of contact between their ends. EDSHAGE (1964) have shown that a satisfactory macroscopic look of a primary nerve suture does not guarantee an exact microscopic apposition of the cut surfaces. As a consequence, in a mixed nerve the misaligned fasciculi will cause ingrowth of sensory fibres into motor sheaths and vice versa. These fibres are functionally lost. The epineurial vessel pattern along the epineurium can be used for optimal realignment of the nerve ends.

Although various nerve suture techniques have been advocated, all have the common aim of restoring the continuity of the transected nerve. For many years, the sutures were inserted into the periphery of the nerve (Fig. 2.6). This epineurial suture technique is relatively atraumatic and easy to perform and requires less manipulation of the internal neural structures (JABALEY, 1984). However, it does not ensure correct matching of the fascicular internal structures of the nerve, which is specially important for multifascicular nerves. To achieve an optimal fascicular alignment, a fascicular repair has been developed, also called perineurial repair (LANGLEY, 1917; SUNDERLAND, 1968). With this technique, fascicular groups are dissected under magnification, the epineurial tissue is resected over a short distance, and the corresponding fascicular structures in both nerve ends are sutured individually through the perineurium (Fig. 2.7). The advantage of perineurial sutures is improved matching of the fascicles. The disadvantage is the considerable amount of surgical dissection of the nerve with risk of more trauma to the nerve tissue (KLINE, 1961).

Attempts have been made to assess whether perineurial suture techniques have any advantages over epineurial techniques and vice versa. No consistent superiority of one technique over the other has been found in many studies (CABAUD, 1976; LEVINTHAL, 1977; TUPPER, 1983; MURRAY, 1994B). Therefore, there is a place for both techniques and each case of nerve sutu-
Fig. 2.6. Epineurial repair (from SMITH, 1978, printed with permission).

Fig. 2.7. Perineurial repair (from SMITH, 1978, printed with permission).
re should be judged individually. In general, a clean-cut, fresh nerve injury is best treated by a simple epineurial suture, especially at levels where fascicles are closely packed with minimal interfascicular epineurial tissue (Kline, 1995). If the transectional area of the nerve is dominated by epineurial tissue, the epineurial tissue should be resected and fascicles adapted by perineurial sutures.

Suture material
In the past, the selection of nerve suture material was based upon the surgeon's own experience and reports published on tissue reactions in experimental animals after implantation of suture material (Mukherjee, 1951; Nigst, 1963; Edshage, 1964). Nowadays, it is apparent that the optimal material for suturing peripheral nerves is one that elicits the least foreign body reaction. Many surgeons consider fine monofilament nylon thread the suture material of choice as it is easy in use and elicits moderate amount of tissue reaction (Kline, 1995). Opinions differ about the use of absorbable sutures. Although absorbable sutures made of polyglycolic acid (PGA) is considered safe for nerve repair, some authors claim that PGA sutures do not offer advantages in terms of better functional recovery when compared to nonabsorbable sutures such as nylon (DeLee, 1977; Murray, 1994A). In our experience, nerve repair performed with PGA sutures is histologically slightly superior to nerve repair performed with nylon sutures (Chapter VII). No matter what type of suture material is used, sutures have still a negative effect on the regeneration of the axons. As the outgrowth of axons take place through the proliferating connective tissue, an abundant proliferative reaction resulting from sutures will give an increased fusiform spreading of the axons (neuromatous bulb) instead of the desired linear outgrowth. Too many sutures placed in the nerve will result in an extensive cicatrisation, resulting in a scar encircling the nerve. If this scar shrinks, the intraneural space will be reduced and as the quality and quantity of the axons depends on this space, their growth will be retarded (Weiss, 1948).

Sutureless nerve repair
As regenerating axons advance by a push-pull mechanism, it is obvious that productive and blocking changes in the tissue around and between the ends of nerve sutures cause impaired healing. Consequently, repeated attempts have been made to find a method which will allow the nerve ends to be joined without using sutures.

Glues
Several biological and nonbiological glues have been investigated in many experimental and clinical studies. Young (1940) and Tarlov (1942A) introduced fibrin glue for repairing nerves in order "to reduce the difficulties of nerve suture and to minimise the disorganisation of the fibres which is apt to be produced by stitches". The method consisted of nerve segments being
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held together with concentrated coagulated blood plasma. Their opinion was that this method was superior to sutures with regard to fibre alignment and speed of nerve fibre growth across the repair site. Seddon (1942b) and Tarlov (1944) applied this method with success in human nerves. Also Hoén (1946) favoured plasma clot fixation of the fasciculi. Freeman (1965) advocated various adhesive tapes, such as micropore, teflon tapes, or polyurethane but these techniques have not been taken up by others. Nonbiological glues, like histocryl, have been abandoned for nerve repair due to their neurotoxic effects (Kline, 1963; Freeman, 1965).

After this period, fine nylon sutures and the operating microscope were introduced in peripheral nerve surgery and gluing was abandoned. In 1972, the use of fibrin glue revived by Matras using a fibrinogen cryoprecipitate. The clinical use of fibrin glue followed soon (Kuderne, 1975), showing that the fibrin clot method was equivalent to microsurgical techniques for peripheral nerve repair. A highly concentrated human fibrinogen solution with an enriched factor XIII content was introduced in the late 1970’s as a two component fibrin sealant (Tissucol, Immuno AG, Vienna, Austria). In spite of the theoretical advantage of the absence of foreign body in the form of sutures at the nerve repair site, the use of tissue adhesives such as fibrin glue has produced some very controversial results (Becker, 1985; Smahele, 1987; Moy, 1988; Herter, 1989). Nevertheless, fibrin glue is favoured by many European surgeons, like Palazzi (1986), Narakas (1988), and Slooff (1992).

Laser-assisted nerve repair
Laser-assisted nerve repair, as another sutureless technique will be discussed in chapter III.

Experimental methods of nerve repair

Coincident with developments of optimal methods of joining nerves after injury has been investigations into agents that have the ability to enhance the regenerative capacity of nerve tissue. The mechanism of action of the substances includes prevention of the reparative response of fibroblasts, facilitation of the regenerative process of the injured nerve cell, and inhibition of protease activity (Yin, 1998). Some of the factors that have shown promise in nerve repair include triamcinolone, alpha-melanocyte stimulating hormone and its derivatives, leupeptin, alipoproteins, and nerve growth factor.

The concept of providing a conduit through which nerve regeneration can be guided has attracted attention from numerous researchers, starting as early as the 1940’s. Introduced by Weiss (1941), tubulisation of the nerve ends is still an experimental methods of nerve repair (Smahele, 1993). Attempts made to prevent ingrowth of connective tissue by tubulisation of the nerve repair site did not gave the expected results, as there are fibroblasts and Schwann cells within the nerve responsible for intraneural scar tissue formation. Nevertheless, the silicone tube appears to have some potential for...
clinical use, especially in bridging small nerve gaps (Lundborg, 1991 & 1997). Moreover, biocompatible substances like polyglycolic acid tubes, collagen, Millipore, and autologous tissues such as vein and artery are still being experimentally and clinically evaluated as a nerve conduit material (Lolley, 1995).

Conclusions

With recent developments in microsurgery, there has been a significant improvement in suture repair. Many studies have been performed on several suture methods that incorporate epineurial and perineurial repair. Despite some suggestions to the contrary in the literature, epineurial suture repair remains the accepted gold standard, and fine monofilament nylon probably is the suture of choice. However, even epineurial repair poses several disadvantages such as inevitable foreign body reaction, scar and neuroma formation, all of which have negative effects on the histological and functional outcome. Because the limitation of suture repair is the manual skill of the surgeon, the full potential of this technique is probably already reached. It is unlikely that improved clinical results will come from further refinements in microsurgical technique. Therefore, continued investigations to find a sutureless method for joining nerves are of great value.