Peripheral nerve reconstruction with autologous vein, collagen, and silicone rubber tubes

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Chapter 1

General introduction

The management of the nerve-injured patient has changed dramatically in the last two decades.\(^1,2\) The results of many investigations have been combined in the last fifty years to provide the foundation for our current management of peripheral nerve injuries. The large amount of traumatic nerve injuries treated during World War II provided information for surgeons interested in the management of the nerve-injured patient.\(^3\) Although review of the functional results achieved by these early peripheral nerve surgeons is generally poor,\(^4\) the clinical material and surgical techniques used at that time provide the important reference point from which our current surgical management has developed. Sunderland's anatomical studies,\(^5\) Millesi's pursuit of tension-free repair,\(^6\) and Moberg's,\(^7\) and Dellon's\(^8\) efforts to quantify the clinical assessment of sensory function have greatly influenced our understanding of nerve injury, regeneration, and recovery.

Nowadays, the presence of several methods of reconstruction of traumatized peripheral nerves indicates, that the discussion on the optimal treatment has not been closed.\(^2\) The introduction of new techniques increased the knowledge of the structure of the peripheral nervous system, varying from the single nerve fiber to the more complex nerve trunks.
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Nerve Fibers

According to Millesi the nerve fiber is the microscopic unit of the peripheral nerve, serving the conduction. It consists of the axon with its axolemma, Schwann cells with or without myelin layers, a basic membrane and a tiny framework of collagen fibers. This structure of the peripheral nervous tissue provides an optimal condition for nerve function, for the integrity of functioning and if necessary for regeneration.

A more detailed description of the structure of the nerve fiber is given by Sunderland.

The perinuclear cytoplasm of nerve cells can form filamentous processes of variable length and thickness. When those processes belong to the neurons of sympathetic ganglia or to the anterior horn of the spinal cord they are called axons. When those processes belong to the posterior root ganglion neurons they are called dendrites. As both these processes are histological indistinguishable, Lundborg suggests to use the general term axon for both types of such processes.

The perinuclear cytoplasm of the nerve cell extends as a filamentous process of axoplasm in the axon. The axoplasm is a viscous fluid in which neurofibrils are present. The connection with the cell body is of importance for the existence of the axon. This important relationship appears to be associated with an intracellular pressure, which causes a proximo-distal flow of axoplasm. This flow is appreciable in the outflow of the axoplasm, which occurs if the nerve is severed.

Surrounding the axon is a multilayered sheath, which presents more complex features in myelinated fibers. In the case of non-myelinated fibers this consists of a chain of Schwann cells external to which is an encircling connective tissue covering, the endoneurium. The boundaries between the Schwann cells are distinct and the relationship to the axon is one in which the cytoplasm of individual Schwann cell surrounds, to a varying degree, one or more commonly several...
In case of myelinated nerve fibers, the multilayered sheath consists of a Schwann cell-myelin complex internally and a connective layer externally. The endoneurial wall differs in no significant respects from that investing the myelinated fiber. One significant difference, however, is that, whereas the endoneurial tube of a myelinated fiber contains only one axon, that associated with non-myelinated fibers may contain several axons. Immediately surrounding the axon is a myelin sheath which, longitudinally, is broken into segments. This segmental arrangement outlines the nodes and internodes of the nerve fiber. The myelin sheath is composed of a complex lipoprotein system. Microscopic and electron microscopic studies have shown a laminated structure of the sheath in which lipid leaflets alternate with thin protein layers. The myelin composition is: phospholipid versus cholesterol versus cerebrosides for 2:2:1.

During development the axon indents the Schwann cells with which it is associated along its course. In the case of the myelinated nerve fiber to be, each Schwann cell establishes a relationship with one axon. These cells gradually envelop the axon, the encircling lips of cytoplasm finally meeting to constitute a mesentery for the axon which is appropriately called mesaxon. Increasing myelinization during development proceeds by an increase in the number of Schwann cell wrappings around the axon.

The layers of the mesaxon, which are just the inturned cytoplasmic surfaces of the Schwann cell, outline a narrow channel containing material which is continuous with that around the axon internally and with the extracellular basement material applied to the exposed surface of the Schwann cell. In that way the axon is surrounded by a very thin space communicating with the exterior. Incisures of Schmidt-Lantermann, representing conical clefts in the myelin extending obliquely between the axon and the external Schwann layer of the fiber, turned out to open when the nerve trunk is stretched, which may be a function to...
prevent abnormal distortion and fracturing of myelin segments. The outer limiting sheath of the nerve fiber is formed by the endoneurium, which is a complex, thin and delicate cylinder of connective tissue.

The diameter of myelinated fibers varies from 2 μm to 30 μm. The variation in diameter is due to both the axoplasm and myelin. The smallest fibers have myelin sheaths with a cross-sectional area exceeding that of the axon, but with axon diameters in excess of 8 μm the axon area is larger than the myelin area. In larger axons the difference between the two grows rapidly.

The myelin thickness is not constant for axons of the same diameter. Along the length of the peripheral nerve fibers they show frequent irregular changes in total fiber diameter, including both axon and myelin and in the area ratio between these two substances. Schwann cell nuclei often cause a decrease in thickness in the myelin sheath and a local reduction in the diameter of the axon. The myelin sheath shows gaps along its path called nodes of Ranvier. In the node of Ranvier a single layer of flattened Schwann cells reaches and embraces the axon, which is constricted at this site. In the normal adult situation this layer has the appearance of a cytoplasmatic wrapping in which the Schwann cell nuclei are embedded. The membrane of the axon (axolemma) at the site of the node shows an inner layer of electron-dense material forming a dense undercoat. The distance between two nodes is called the internode. An internode is built up of myelin and consists of one Schwann cell. In myelinated nerve fibers, local changes occur only at the nodes of Ranvier. At the internodes, the insulating effect of myelin prevents the continuous propagation of the impulse.

Therefore, the impulse jumps from one node to the other. This type of conduction is called saltatory conduction and is faster than continuous conduction. Axonal conduction of the impulse is progressively faster in axons with larger diameters and thicker myelin sheaths. At the nodes of Ranvier a constriction is reported with
a reduction of the diameter to 50 percent of its average internodal size.\textsuperscript{28,30} There is also a reduction of the axon diameter at the Schmidt-Lantermann clefts. There are at random constrictions present along the nerve fiber, increasing in number when the fiber is stretched providing the fiber with a beaded appearance.\textsuperscript{24,31-33} Independent of the presence of clefts of Schmidt-Lantermann or nodes of Ranvier, there are irregular changes in axon diameter and myelin thickness along the length of individual nerve fibers.\textsuperscript{10} Sunderland found in human nerves that axon diameters varied from 3.25 μm to 11.75 μm and the total diameters from 6.5 μm to 16.0 μm. The total myelin thickness varied between 0.5 μm and 6.0 μm. The ratio myelin area/axon area varied along the course of the fiber between 6.68 and 0.11. The ratio axon diameter/total diameter remained relatively constant for comparatively long stretches of the fiber but elsewhere varied between 0.36 and 0.95. Non-myelinated fibers did not show that wide range of variation in diameter, it did not exceed 3 μm.\textsuperscript{10}

According to their function, the nerves are classified as motor, sensory and (para)sympathetic fibers. Motor nerve fibers originate in the anterior horn neurons of the spinal cord and terminate in the neuromuscular endings of the skeletal muscle. Motor nerve fibers range in thickness from 2 μm to 20 μm. Mostly, they are divided in 2 groups; those with a size range of 10 μm to 17 μm, and those with a size range of 2 μm to 8 μm.\textsuperscript{34}

Sensory nerve fibers comprise the peripheral dendrites of posterior root ganglion neurons. The fibers end either freely or in a wide variety of specialized end organs or receptors. Sensory nerve fibers consist of myelinated and non-myelinated ones. Myelinated ones have a thickness ranging from 2 μm to 30 μm. In the peripheral sensory system the presence of non-myelinated and fine myelinated fibers is dominant. Both of these types of nerves are represented in the whole group of sensory nerve fibers, which are also divided in cutaneous fibers, and deep-lying fibers. The
first terminate in the skin and tissues superficially to the fascia. They register sensations of touch, pressure, pain, warmth and cold. The deep-lying fibers terminate in muscles, tendons, articular and periarticular structures, connective tissue and bone. They register sensations of pressure, pain, temperature and stretch. The cell bodies of the autonomic nerves of the peripheral nerve system are present in the pre- and paravertebral ganglia for the orthosympathetic fibers and in the intra- and juxtamural ganglia for the parasympathetic ganglia.

Sympathetic nerve fibers of the peripheral nerve system are generally non-myelinated, but few medullated fibers are present in mammals, like a few in the rabbit and a considerable number in the cat. Sympathetic nerve fibers terminate in the vessels, hair muscles and glandular structures of the skin, travelling by cutaneous nerves and by the deep branches of the main nerves. Most nerves have both motor and sensory types of fibers and are called mixed nerves. These nerves have both myelinated and unmyelinated fibers.

The establishment of variation in the form of the action potential among the fibers of a nerve trunk, immediately suggested the possibility of a relationship between action potential and nerve fiber morphology. Quantitative birefringence studies, using polarised light, have disclosed that the axon sheaths of a wide variety of fiber types differ chiefly with respect to the reactive amounts of oriented protein and lipid present. This difference is observed not only between typical invertebrate and vertebrate fibers, but also when the fibers of a single vertebrate nerve are compared, and on the whole the velocity of conduction is more greatly affected by sheath structure than by diameter. In 1942, Taylor found that the structure of the sheath is as important as fiber diameter in determining the order of magnitude of conduction velocity when widely different fiber types are compared. Lillie demonstrated in 1925 a greater conduction velocity in a wire enclosed by an interrupted myelin tube than in one enclosed by a continuous tube. Considering this
variation in total fiber diameter, myelin thickness, axon diameter and internodal length along individual nerve fibers, the disagreement among investigators concerning possible mathematical relationships between these data and conduction velocity is not surprising.\textsuperscript{10}

The working hypothesis that an axon has a constant diameter and a myelin sheath of uniform thickness along its length has been fallacious.\textsuperscript{27} Discrete physiological functions may be subserved by fibers with a considerable range of diameter.\textsuperscript{10} A further observation of interest in this connection is that the structure of fibers is constantly changing along their length, while obviously retaining the same function.\textsuperscript{10} In the more proximal part of the nerve the conduction velocity is higher than it is in the more distal part. From their studies Erlanger and Gasser\textsuperscript{41,42} arrived at a classification of nerve fibers into three groups, namely: an A-group of large thickly myelinated fibers with long internodes and a high conduction velocity (15 - 120 m/sec), a B-group of small thinly myelinated fibers with short internodes and a mean conduction velocity (3 - 14 m/sec) and a C-group of non-myelinated fibers (0.2 - 2 m/sec).

**Nutrition Of Nerves**

The state of the cell body of the neuron is responsible for the survival and efficient functioning of nerve fibers. However, it is not clear whether the nutrition of the entire length of long axons is dependent on this cell body. There is evidence that the supplying blood vessels along the course of the nerve fiber may take care for the nutrition of both the nerve fiber as well as the supporting tissue.\textsuperscript{10} However, in experimental studies in nerve regeneration using tendon nerve autografts for bridging a nerve defect in the rat, the onset of vascularization appeared to coincide with axonal regeneration into the grafts.\textsuperscript{43} Moreover, Mani et al.\textsuperscript{44} found a delay in revascularization more than 14 days to occur in 30 mm long, non-vascularized
nerve grafts placed on completely avascular graft beds in rabbit sciatic nerves. These investigators stated, that over a period of 44 weeks, this prolonged ischaemia did not adversely affect nerve regeneration and wondered if early vascularization of nerve grafts was necessary.

**Branching Of Nerves**

Branching between the cell body and the periphery occurs most frequently in the smaller size group of nerves and less in the larger size group. About the extent of branching could be said, that the territory served by a neuron is more extensive than per ultimate branching at the periphery would indicate. In that way widely separated tissues can be brought under the influence of one neuron. Branching influences the spread and concentration of impulses. Cattell and Hoagland\(^45\) reported that the stimulation of an end organ of one cutaneous area alters the receptivity of end organs of a neighboring cutaneous area. Sinclair and co-workers supposed referred pain to be based on branching of sensory fibers carrying pain impulses. From some of these branched fibers one limb runs to the site of origin of the disturbance and the other to the site of pain reference.\(^46,47\) Two mechanisms are suggested; one in which impulses originating from one branch are misinterpreted in the central nervous system as originating from another, and a second in which an axon reflex through such branched axons provokes the liberation of some substance in the area of reference which sets up pain impulses. There is justification for the belief that the territory served by a posterior root ganglion neuron is greater than generally acknowledged.\(^48\)

**Fascicles**

According to Millesi\(^9\) a certain amount of nerve fibers form a fascicle, representing the macroscopic unit of the peripheral nervous system. It contains an endoneurial
frame of connective tissue, built up from tiny collagen fibrils, arranged in a longitudinal and obliquely direction. The endoneurium contains Schwann cells and endoneurial fibroblasts in a 9 to 1 relation.\textsuperscript{49} In the endoneurium are lymph clefts containing lymph. Within the endoneurium an abundant capillary network is present of which the endothelial cells form tight junctions to each other so that a blood-neuron-barrier is formed.\textsuperscript{50-53}

Around the fascicle the perineurium is draped, with an inner layer consisting of a single film of mesothelial cells, separated from the endoneurium by means of a subperineurial space. This layer is responsible for the membrane function, the "diffusion barrier".\textsuperscript{54}

The medial part of the perineurium consists of several smaller layers of flattened perineurial cells with longitudinally formed cell processes and a basal membrane. Between these layers collagen fibers are present with the same diameter as the endoneurial collagen fibers (40 to 65 nm) having a double spiraled orientation.\textsuperscript{27}

The third outmost layer of the perineurium contains collagen fibrils, thicker than those mentioned above. They form a continuous construction with the collagen fibers of the interfascicular epineurium. The components of the perineurial connective tissue layer have different aspects at different locations of the body. The endoneurial capillaries are fed by small vessels protruding through the perineurium into the fascicles. The perineurium, by means of its barrier function, protects against penetration of interfascicular fluids or infectious infiltrations.\textsuperscript{54,55}

Furthermore, the perineurium keeps the tissue pressure higher inside the fascicle than outside. A nerve’s capability to resist compression and traction is chiefly dependent on the qualities of the perineurium mentioned above.\textsuperscript{56} This is also facilitated by the winding course of the nerve trunks.\textsuperscript{27}
Nerve Trunk
Different fascicles are formed into a nerve trunk by means of the epineurium. It contains thicker collagen fibrils (diameter 60 till 110 nm) than the fibrils in the perineurium and endoneurium. The interfascicular epineurium fills the space between the fascicles in such a way that some movement between the fascicles is possible. Pressure in one direction on the nerve trunk is to endure. The epineurium contains blood vessels, lymph vessels and fat tissue. The epineurium just provides some solidity to the nerve trunk. Outside the nerve trunk a loose connective tissue layer, called adventitia or paraneurium, is present. This layer permits motility of the nerve trunk in relation to its environment and so normal motion of the body.

The feeding blood vessels of the nerve are segmental arranged. Superficially arranged vessels run through the paraneurium or the epifascicular epineurium and longitudinal vessels run in the interfascicular space. Vessel trunks for peripheral nerves are different in various body parts, so that several types could be marked.

This knowledge is very important in nerve transplantation.

Structure Of The Fascicle In Relation To The Nerve Trunk
According to Millesi, the fascicle is the macroscopic unit of the peripheral nervous system. This arbitrary statement is mainly based on a surgical point of view. Sunderland has shown in his extensive anatomical studies of peripheral nerves that the fascicle composition of a peripheral nerve changes every 15 mm in distal direction. According to his findings human peripheral nerves are in general composed of fascicular structure (or funiculi). As nerves are engaged in repeated division, assembling features and plexus formations, the fascicular pattern is altered rapidly. Dissimilarities in the pattern of the fascicles in the nerve ends after disruption of the nerve reduces the chances of obtaining en-to-end position of the fas-
The fascicular structure within a peripheral nerve alters very frequently along its course, so that a trajectory with the same fascicular structure is not longer than 15 mm. This so-called plexiform arrangement of the fascicles is a problem in nerve reconstruction for the coaptation of one fascicle to another as the fascicles of both nerve stumps do not correspond to each other any more. The fascicular structures also influence the mechanical character of the nerve trunk. In case a nerve trunk consists of many and little fascicles, the nerve trunk can adapt better to stretching and compression than in case the nerve trunk consists of one or a few fascicles. The more fascicles are present in the nerve trunk, the more is the relative part of non-fascicular tissue in the nerve trunk. The result is an increasing danger of coaptation of fascicular tissue with non-fascicular tissue in nerve reconstruction. According to Millesi, three forms of fascicular structure in a nerve segment are recognized: 1. A monofascicular structure, in which the fascicular part contains more than 85% of the diameter of the nerve. 11. An oligofascicular structure. In these nerves the number of fascicles vary between 2 till 12, and, in case of surgery, manipulation of the separated fascicles is mechanically easy. But if the fascicles are too small, this fascicular procedure is not possible. 111. A polyfascicular structure. The fascicular part of the diameter of the nerve contains less than 40% to 60% of the whole diameter of the nerve. In case of a trunk-to-trunk coaptation, it is likely that coaptation between fascicular tissue and non-fascicular tissue will occur easily. Within this polyfascicular structure a distinction between group arrangement and non-group arrangement is possible. In case of group arrangement or detection of group arrangement along the course of the nerve segment, a fascicle procedure is possible. The continuous presence of groups of fascicles over a longer distance of the nerve is proven in anatomical specimens.
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Nerve Degeneration And Regeneration

The studies of Holmes and Young,$^6$ Sanders and Young,$^6$ Simpson and Young,$^6$ and Hammond and Hinsey$^6$ indicated, that in severed nerves the decrease in diameter of endoneurial tubes starts soon after the injury and reaches its maximum at three months. Different phases of the nerve regenerating process in the nerve tube can be recognized in nerve repair, overlapping each other chronologically.$^2,^6^9$ The first phase after nerve trauma shows inflammatory reactions accompanied by cell death, cell repair and phagocytosis of cellular debris. Macrophages play an important role in nerve regeneration. Axonal outgrowth may be increased by external application of macrophages.$^7^0,^7^1$ Probably neurotrophic factors are released from macrophages. In the first week a fibrin clot is formed and connects the proximal and distal nerve stump. This fibrin bridge forms a scaffold for guiding the migration of fibroblasts, Schwann cells, vascular sprouts and longitudinal advancement of axons across the nerve gap. Nerve transsection induces the formation of nerve growth factor (NGF) receptors located on the cell surfaces of the Schwann cells, that form the bands of Büngner.$^7^2$ When regenerating axons grow out along the Schwann cell surface, factors bound to the NGF receptors, are picked up and transferred into the growth cones. These factors are transported retrogradely to the perikarya of nerve cells. In this way a track that regenerating axons can follow is formed on the surface of the Schwann cells. The Schwann cells in the transected nerve produce a range of factors such as ciliary neurotrophic factor (CNTF)$^7^3$ and brain-derived neurotrophic factor (BDNF).$^7^4$ Laminin and fibronectin are important molecules in the basal lamina of Schwann cells promoting axonal elongation.$^7^5,^7^7$ The cell migration is followed by differentiation into neuronal, glial and vascular elements, and directed towards their original interrelationships. So the formation of capillaries, restorations of the origins of axons and Schwann cells and branching of axonal sprouts will take place.$^2$ Misdirected axonal sprouts are deleted when the
proximal nerve stump reconnects the distal stump. Some collateral sprouting leads to pathological abnormalities, like functional deficits and neuroma formation. During this phase compartmentalization of axons into fascicles is clear. Then the phase of growth of regenerating axons will follow. Firstly thin nerve fibers pass through the nerve suture after that the fibers grow thicker and thicker, the number of non-myelinated nerve fibers decreases and the number of myelinated fibers increases. Regeneration goes on into the distal stump until target organs are reached and innervated. The regenerating axons meet distal endoneurial tubes with a permanently reduced diameter. It results in a decreased conduction of impulse in the regenerated fibers.

Sunderland studied regeneration and functional end result in patients with delayed repair and patients where repair was undertaken immediately or shortly after severance. He found that the distal stump retains, for at least 12 months, the capacity to transmit axons to the periphery in a manner that does not differ significantly in the two studied groups. Furthermore, muscle function can be fully restored following reinnervation when the distal stump has been denervated for the same period.

He concluded that despite endoneurial tube atrophy functionally efficient pathways were present at the end of the observation period. If this function repair represents the repair of original fiber diameters then the endoneurial shrinkage, which is maximal at 3 months, is not irreversible. So endoneurial shrinkage is present, but doesn't hamper a total repair of function, notwithstanding the presence of fibers with a decreased or normal diameter.

The diameter of a motor axon supplying a muscle can be decreased experimentally without affecting the characteristic response of the muscle to nerve stimulation. Besides these findings it is shown, that under these experimental conditions and in disorganized nerve fibers, the conduction velocity across the affected seg-
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ment is slowed but remains normal above and below that section.\(^{81,83}\)

**Remarkable Features In Healing And Regeneration After Nerve Injuries**

Motor function recovers better and faster than sensory function and the results of nerve reconstruction are better in children than in adults.\(^{84}\) After a nerve trauma with total loss of the morphological continuity of the nerve fiber, proximal to the lesion the fiber may degenerate up to the next node of Ranvier or up to the neuron cell body.\(^{27}\) Distal to the lesion a Wallerian degeneration develops, with axonolysis and degeneration of the myelin. At the nerve stump transudate with fibrin is formed. Proliferation of fibroblasts at the site of the fascicle stump end will develop and even more inside the epi-and interfascicular epineurium. Axon sprouting will be formed at the terminal or lateral side of the remaining healthy axons.\(^{85}\) One axon is able to form 50 axon sprouts maximally. In case of axon damage and large retrograde degeneration, axon sprouting will take place relatively more proximally in the nerve stump. The sprouts have to grow over a longer segment to arrive at the cut surface of the stump. The outgrowth of the axon sprouts is dependent on the contact with Schwann cells.\(^{85}\) Minifascicles are formed. In a normal situation this regeneration ends, but in special circumstances regeneration continues as a neuroma growing over a longer trajec.\(^{86}\) In case of prolonged regeneration such neuromas may give rise to the well known pain syndrome.

Normal and optimal circumstances in the internal milieu in the endoneurial space are maintained by joined action of a delicate barrier system, constituted by the capillary endothelium and the perineurium.\(^{54}\) In total nerve lesions with interruption of continuity these barriers are destroyed for a long time. Proliferating cells in the space between two nerve stumps originate from the different nerve layers as well as the surrounding tissue. Within the first weeks this zone is filled with proliferating fibroblasts, Schwann cells, collagen fibers and capillaries.\(^{85,87}\)
In 1981, Lundborg and Hansson et al.\textsuperscript{85} studied the influence of the distal nerve stump on the direction of outgrowing nerve fibers from the proximal nerve stump. They used a mesothelial compartment as medium at the site of the nerve gap and showed a well-organized growth of nerve fibers and fascicles. The axons were arranged in minifascicles surrounded by newly formed perineurium. The fascicles in turn were grouped together to form a new nerve-like structure surrounded by an epineurial sheath. The vascular architecture showed characteristics of the normal intraneurial system and, as in normal peripheral nerves, mast cells were scattered along the interneurial vessels.

In neuroma formation following severe nerve injuries without approximation of the nerve stumps, the nerve trunk shows formation of small fascicles growing in a disorganized pattern in a connective tissue mass. Formation of small fascicles is a phenomenon observed in association with nerve injuries involving disruption of axonal continuity in the nerve segments close to the level of the lesion as if the endoneurial content is extracted from an opened fascicle.\textsuperscript{88,89} The minifasciculation or compartmentalization may occur by enveloping the regenerating axons within a perineurial sheath very early after nerve injury.

The perineurium is of importance for the maintenance of an optimal endoneurial environment which is believed to be essential for normal function of axons.\textsuperscript{11,90} In case of a nerve lesion with disruption of perineurial continuity all protection barriers are broken, and the axons will be growing into the environment of a healing wound characterized by changes in pH, pO\textsubscript{2}, and pCO\textsubscript{2}. The normal membrane function of premature axons may well alter by these biochemical changes. A broken perineurial barrier may interfere with axonal transport systems under certain conditions.\textsuperscript{91} Therefore, restitution of the perineurial barrier is logical and necessary at the site of nerve lesion. Lundborg and Hansson\textsuperscript{85} observed in tissue culture experiments that regenerating nerve fibers have the tendency to grow together.
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in bundles, called ‘fasciculation’. The underlying mechanisms remain to be explained. Levi-Montalcini and Hamburger et al.\textsuperscript{92} were the first to describe what we now call a neurotrophic factor. These neurotrophic factors play an important role in nerve regeneration. Survival of perikarya of nerve cells after axotomy is facilitated by many neurotrophic factors from multiple sources. They are usually classified into three major groups: neurotrophins, neuroepoietic cytokines, and fibroblast growth factors. Moreover, there are additional groups of other neurotrophic factors.\textsuperscript{2} The cellular and molecular basis for survival of nerve cell bodies and the outgrowth of axons after injury is very complex, but there has been a substantial development in this field.\textsuperscript{93,97} These neurotrophic factors have often been applied in the tube model for investigating nerve regeneration.\textsuperscript{98,104}

Classification Of Nerve Injuries

Two classification systems of nerve injuries can be distinguished. The anatomical classification of Sunderland\textsuperscript{27} and a surgical classification.\textsuperscript{60} An important difference between these two systems is, that the surgical classification system gives a better insight in the spontaneous regeneration of nerve lesions.

Classification Of Sunderland\textsuperscript{27}

**First-degree injury.** Interruption of conduction has occurred at the site of injury, with preservation of anatomical continuity of all components comprising the nerve trunk, including the axon. There is no Wallerian degeneration. In case of severe injury myelin can be damaged in a segmental part of the nerve. If there is no compression from outside or formation of fibrosis inside the nerve, regeneration will start within a relatively short time.
**Second-degree injury.** The injury has caused a total discontinuity of the axons. Distal to the lesion axonolysis and Wallerian degeneration will be formed. There is complete loss of motor, sensory and sympathetic functions in the autonomous distribution of the injured nerve. Disintegration of the axon has developed accompanied by a breakdown of its myelin sheath and atrophy of the affected muscles. The endoneurial structures and the basic membrane of the nerve are not damaged. If there is no compression from outside the nerve or formation of fibrosis inside the total delay between injury and onset of recovery will take much more time than after the first-degree injury, although regeneration will eventually be completely.

**Third-degree injury.** There is a total disruption of the axon and damage of the endoneurial tissue, but the integrity of the perineurium will be preserved. There will be axonolysis and Wallerian degeneration. Spontaneous regeneration will start, but the restoration will never be totally. The sprouts of the regenerating axon remain within their original fascicle. Forming of fibrosis in the nerve will hamper regeneration, as compression from outside the nerve will do.

**Fourth-degree injury.** This injury even causes disruption of perineurium. As a result there is loss of fascicle structure. A big part of the continuity of the nerve will be formed by connective tissue. In these circumstances spontaneous regeneration hardly occurs.

**Fifth-degree injury.** There is loss of continuity of the nerve trunk, resulting in complete loss of motor, sensory and sympathetic functions in the autonomous distribution of the severed nerve. The nerve ends may remain separated, or they may become joined by an attenuated strand of tissue, composed of a fibroblastic and Schwann cell framework transmitting regenerating axons. The latter phenomen-
Chapte rr  1 eno nn  wil l onl y  happe n  afte r  a  sufficientl y  lon g  perio d  i n  whic h  neural , Schwann
cell and fibroblastic activity is enabled to bridge the gap. The amount of scar tissue
formed between the nerve stumps may vary from a small connecting strand to an
extensive tissue mass, completely burying the nerve stumps.

**Surgical Classification**

**Fibrosis of the epifascicular epineurium (A).** This term signifies fibrosis of the
circumferential layer of the epineurium: the epifascicular epineurium. Shrinkage
induces compression at the whole nerve trunk like a too narrow panty. Such fibrosis
can be met in a first, second or third degree nerve injury in Sunderland’s classifica-
tion, in that case the degrees are called 1A, 2A and 3A.

**Interfascicular fibrosis (B).** The fibrosis continues into the interfascicular con-
nective tissue, more or less extensively. Also these lesions can be named according
to Sunderland’s classification, using the terms 1B, 2B or 3B.

**Intrafascicular fibrosis (C).** This injury only corresponds with Sunderland’s
third degree injury, so just 3C is used. Fibrosis extends in the endoneurial space as
result of severe trauma or as result of long delay. Spontaneous regeneration is not
possible anymore. In this case, fibrosis resection is the preferred procedure.

**Nerve Transplantation**

According to Phillipieux and Vulpian, the first autotransplants of nerves were
established in 1870 and the first homoiotransplants in 1880. Although auto-
transplants often gave positive results homoiotransplants did not. Several investiga-
gators tried to suppress the antigenetic properties of the homoiotransplants by
radiation, however without constant success. Autotransplantation was suc-
cessful when several thin nerves, forming a cable, were used. The time necessary for ingrowth of this transplant depended on the size of the transplant. If the transplanted nerve segment is too long, it will disappear before neurofibrils are able to grow through. As a donor nerve, the sural nerve, the medial antibrachial cutaneous nerve or the lateral femoral cutaneous nerve are used. Experiments based on freezing or chemical treatment of donor nerve to prevent early degeneration of the protein were not successful, nor were treatments with antimetabolites or corticosteroids to influence the acceptor’s tolerance. The results of investigations of Horch and Lisney in 1981 showed smaller diameters and smaller myelin sheaths of the nerve central in and distal to the transplantation site. A *restitutio ad integrum* could not be achieved at all.

**Tubulization**

In the cell biology of neural regeneration important factors are: the interaction of the Schwann cell and the axon, the dynamics of axoplasmatic transport, the neurophysiology of sensory receptor, and motor end plate function. These factors have played an important role in the development of tubulization in nerve reconstruction. The concept of neurotrop(h)ism and the contact guidance was also of importance in determining regeneration across a nerve gap. According to Mackinnon and Dellon neurotrophism implies an ability to influence maturation of the nerve, neurotropism to influence direction of nerve regeneration. The results of experimental studies on neurotrop(h)ism have influenced the experimental design of peripheral nerve reconstruction, especially of tubulization. Since the last decades of the nineteenth century, many experiments were performed with tubulization of the nerve lesion. The main reason was to prevent growth of connective tissue penetrating into the regenerating nerve and neuroma formation in the lesion. Tubes of material like bone, veins, arteries, silicone were tested, but
not found successful because of hampering the nutrition of the nerves.

In 1956, Campbell and Basset used Millipore, an artificial membrane with pores of 0.45 μm. This procedure was used in nerve lesions repaired by means of nerve transplantations. After approximately 6 months the tubes had to be removed to prevent calcium accumulation in the membrane with consequent loss of permeability. Tubulization for nerve repair became popular as an interesting experimental model to investigate nerve regeneration. 102, 104, 130, 131 Silicone tubes appeared to be successful to study the mechanisms of nerve regeneration. 132 The introduction of tubes was based on the concept that regeneration of nerves after nerve reconstruction would be favored. Several items were involved. Firstly, minimization of surgical trauma, secondly, a short gap between the nerve stumps inside the tube would increase the possibilities for actions of neurotrophic and neurotropic substances, and thirdly, a closed tube would allow accumulation of those factors that are normally synthesized in a nerve after trauma. Implantation of silicon tubes to bridge gaps in the rat sciatic nerve model resulted in spontaneous formation of a new nerve. However, quality of the new nerve structure was directly related to the gap length. With gaps exceeding 10 to 15 mm, bridging of the gap was not successful in the rat sciatic nerve model. 133, 134 The tube model was therefore found to be a useful tool in studying axonal regeneration in which effects of various factors and substances on nerve growth could be easily tested. 98, 104 Silicone rubber tubes filled with various types of factors, materials or cells are often used to improve regeneration. 135-142 Lundborg described that silicone rubber tubes cannot be used as an alternative to nerve grafts over extended lengths, because of their impermeability. 2 Rudolph et al. 143 described myofibroblasts around silicone breast implants, already in 1987. Chamberlain et al. 144 reported for the first time contractile capsules around the silicone tubes. As silicone rubber tubes are always encapsulated by fibrous tissue and this causes constriction of the nerve another surgical interven-
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tion is necessary to remove the tube. Chamberlain et al. inserted a highly porous collagen-glycosaminoglycan (CG) matrix in silicone tubes and in collagen tubes. They found an increase of the number of axons per nerve, of the number of large diameter axons, and of the mean fiber diameter compared to unfilled tubes. Madison and co-workers found significant positive effects on nerve regeneration after applying laminin-containing gel as a matrix in non-toxic biodegradable nerve guides. Nerve reconstruction with openings into the perineurium at the site of lesion gives rise to regenerating nerve filaments from those openings forming neuromas and hampering normal function of the nerve.

The presence of the fibrin clot in the first four days after nerve reconstruction with tubulization turned out to be essential for the bridging during the period of early traction and pulling of the tissue and the nerve. After that period and before the suture of the reconstructed nerve could stand normal mechanical pulling, the fibrin clot must disappear because otherwise fibrosis occurs in the nerve stumps. The fibrin clot should be brought around and between the nerve stumps. The clot was invaded by capillaries and cellular elements from the proximal and distal nerve stumps. This matrix served as growth terrain for axons migrating from the proximal nerve stump. Fibronectin and laminin were demonstrated in this matrix. The amount of regenerating neurofilaments increased with the extent of coaptation. The fibrin-clotting factor XIII had a positive influence on nerve healing.

Various modifications in the tube concept have been used for studying the physiology of nerve regeneration. The tubes have been filled with dialyzed plasma, laminin, testosterone, gangliosides, matrigel, laminin and collagen, and hyaluron. Other developments were tubes filled with cultured Schwann cells, and the introduction of various biodegradable tubes. In his thesis, Den Dunnen described the development of an artificial nerve guide. This nerve
guide was constructed from a biodegradable copolymer of lactic acid and ε-caprolactone. A disadvantage of this nerve guide was, that 2 years after implantation fragments of the nerve guide could still be observed in the fibrous tissue, surrounding the regenerated nerve. These fragments caused a chronic foreign body reaction with scar tissue formation, which may result in constriction of the nerve, in turn leading to secondary nerve impairment. Furthermore, he described that nerve guides should stay intact for the period of 16 weeks in which regenerating nerve fibers are crossing the nerve gap and should degrade thereafter. The biomaterial degraded within 1 year and the material was non-cytotoxic. Within the first 3 months, degradation was characterized by swelling of the biomaterial up to 300%. Since such a swelling will have a negative influence on the regenerating nerve, the author tested nerve guides with a variety of internal diameters. The nerve guide with an internal diameter of 150% of the severed nerve diameter led to nerve regeneration in which the first myelinated nerve fibers had crossed the 1-cm nerve gap in the nerve guide within 3 weeks. He also described faster and qualitatively better results of regeneration through the nerve guide compared to nerve reconstruction using an autologous nerve graft. He evaluated the phenomenon of fibrin coating inside a nerve guide on the speed and the quality of nerve regeneration. He concluded that Schwann cells and fibroblasts migrated over a fibrin-bridge between the nerve stumps inside the nerve guide and that outgrowing axons followed. This dense network of fibrin slowed down regeneration and caused a severe inflammatory response during the replacement of the fibrin bridge. Den Dunnen and Meek et al. used various other biodegradable tubes. Collagen tubes have been proven useful to bridge nerve gaps in experimental animals and primates.

The functional result after suture of transected nerves depends on initial findings, surgical technique and the type and intensity of follow-up treatment.
Furthermore, the final success of nerve reconstruction depends on the experience and emotions around the loss of sensory or motor qualities, which shows an individual variability. Mackinnon\(^1\) described important advances in the field of microsurgery, which improved the treatment of injured peripheral nerves. Technical operative skills are required to deal with these injuries. She generated a sequence of eight basic principles or axioms that form the basis of the present management of the nerve-injured patient:

1. Quantitative preoperative and postoperative clinical assessment is required for both the motor and sensory system. Assessment of muscle wasting and strength (e.g. pinch and grip measurements) can be provided for evaluation of motor function. Measurement of threshold (vibration or pressure stimulus) and innervation density (two-point discrimination) as well as light-touch perception (protective sensation) will assess the sensory system.

2. Microsurgical technique, including magnification and microsurgical instruments and sutures, should be used.

3. Nerve repair must be "tension-free".

4. When tension-free repair is not possible, an interposition or interfascicular nerve graft should be performed.

5. Postural positioning of the extremity to facilitate an end-to-end suturing is discouraged. Both nerve repair and nerve graft should be carried out with the extremity in a neutral position without any tension at the site of repair.

6. When the clinical and surgical condition permits, primary nerve repair should be performed.

7. When the intraneurial topography of the peripheral nerve permits, group fascicular repair should be performed. When the function of the fascicles is primarily mixed sensory and motor without well-defined groups of fascicles, epineurial repair should be performed.
8. A course of postoperative motor and sensory re-education will maximize the potential surgical result.

In 1998, Sparmann\textsuperscript{120} described in his publication "Nervenprothetik" the immunological capacity of the outgrowing axons to react with a cellular defense to nerve grafts, hampering the outgrowth of axons.

Rats are generally used in experimental peripheral nerve reconstruction research. They possess excellent regenerative capacities.\textsuperscript{120} However, in the rabbit nerve regeneration is comparable to the situation in man. We thought it important to test nerve regeneration in various reconstructive procedures in a rabbit model. The results of experiments in rabbits have more clinical relevance than those of experimental studies in rats. The rabbit saphenous nerve model has hardly been used in reconstructive sensory nerve reconstruction. The saphenous nerves of rabbits were investigated by Becker et al.\textsuperscript{178,179} and Kienecker et al.\textsuperscript{180} These investigators anastomosed cut saphenous nerves in the rabbit by primary microsurgical suture and studied the effect of locally applied factors on degeneration and regeneration. The results showed that after local application of glucocorticoids the formation of scar tissue and neuromata was decreased. In another study they used cold lesion of the nerve to cause secondary regeneration and systematically treated the lesioned nerves by systematic administration of a combination of vitamins B\textsubscript{1}, B\textsubscript{6} and B\textsubscript{12}.\textsuperscript{180} The morphological results showed that the number of regenerating axons was higher than in the control group, treated with saline solution. Because sensory nerves regenerate slower than motoric nerves, we thought it important to investigate regeneration of sensory nerves. We chose the saphenous nerve in rabbits as a model for our studies. To evaluate the results of biodegradable nerve guides, collagen was chosen, since this material has a biological origin. Besides the general requirements of a nerve guide, like guiding the regenerating nerve fibers towards the distal nerve stump, preventing neuroma-formation and preventing
ingrowth of fibrous tissue, the nerve guide and the reconstruction procedures should be in accordance with the aforementioned seven basic principles of Mackinnon (no. 2 - 8). Moreover, morphometry used for counting the number of axons during nerve regeneration is important but a time-consuming procedure. In our experimental animal investigations, concerning peripheral nerve reconstruction, we needed a method for staining, visualization, and counting of axons to provide optimal and fast information about architecture and total amount of axons, their diameter, and their portion of the fascicle in the nerve. We therefore developed a new method realizing these characteristics. This method is based on immunohistochemical staining of transverse sections. Quantification of nerve fibers was performed by using a confocal laser scanning microscope and by storing the images digitally.

The aim of this thesis was to investigate the applicability of a biodegradable nerve guide “processed porcine collagen” for the reconstruction of peripheral nerves with or without a gap. The results of these experiments were compared with the results of similar experiments with autologous veins, silicone rubber tubes and epineurial suturing, methods already used in clinical practice.
In the first chapter an overview of the literature is presented.

In the second chapter a new method for morphometric analysis of axons in experimental peripheral nerve reconstruction is described.

In the third chapter the results of experimental nerve reconstruction by using vein graft conduits in the rabbit saphenous nerve are presented.

In the fourth chapter the results of experimental nerve reconstruction by using processed porcine collagen conduits in the rabbit saphenous nerve are presented.

In the fifth chapter the results of experimental nerve reconstruction by using silicone rubber conduits in the rabbit saphenous nerve are presented.

In the sixth chapter the results of experimental nerve reconstruction by using epineurial suturing, vein graft conduits, processed porcine collagen conduits, and silicone rubber conduits in the rabbit saphenous nerve are compared and discussed.
References

13. Gasser HS. Discussion of Frankenhäuser B: The hypothesis of saltatory con-
Chapte rr 1


14. Gasser HS. Properties of dorsal root in medullated fibers on the two sides of

15. Gasser HS. Comparison of the structure, as revealed with the elecron micro-
scope, and the physiology of the inmedullated fibers in the skin nerves and

16. Geren BB, Raskind J. Development of the fine structure of the myelin sheath

17. Hess A, Lansing AI. The fine structure of peripheral nerve fibers. Anat Rec
117: 175, 1953.

18. Geren BB. The formation from the Schwann cell surface of myelin in the

19. Causey G, Hoffman H. The relation between the Schwann cell and the axon in

20. Hess A. The fine structure and morphological organization of non-myelin-

21. Terry RD, Harkin JS. Regenerating peripheral nerve sheaths following


24. Robertson JD. The unit membrane of cells and mechanisms of myelin forma-

25. Porter KR, Bonneville MA. An introduction to the fine structure of cells and

26. Glees P. Observations on structure of connective tissue sheaths of cutaneous

27. Sunderland S, Roche AF. Axon-myelin relationships in peripheral nerve fibers.


34. Eccles JC, Sherrington CS. Numbers and contraction-values of individual motor-units examined in some muscles of the limbs. Proc Roy Soc B 106: 326, 1930.


40. Lillie RS. Factors affecting transmission and recovery in the passive iron nerve model. J Gen Physiol 7: 473, 1925.


64. Williams HB. Peripheral nerve injuries in children. In: Kernahan DA,
Chapte rr  1


66. Sanders FK, Young JZ. The role of the peripheral stump in the control of the fiber diameter in regenerating nerves. J Physiol (Lond ) 103: 119, 1944.


40


107. Campbell JB, Bassett CAL. The surgical application of monomolecular filters (Millipore) to bridge gaps in peripheral nerves and to prevent neuroma formation. Surg Forum 7: 570, 1956.


General introduction

Chir 25: 606, 1880.


135. Williams LR, Varon S. Modification of fibrin matrix formation in situ enhan-


155. Zeng L, Worseg A, Albrecht G. Bridging of peripheral nerve defects with exo-


163. Den Dunnen WF, Meek MF, Grijpma DW, Robinson PH, Schakenraad JM. In vivo and vitro degradation of poly[50/(50)(85)/(15)(L)/(D)LA/epsilon-CL],


173. Archibald SJ, Karup C, Shefner J, Li ST, Madison RD. A collagen-based nerve


175. Mackinnon SE, Dellon AL. A study of nerve regeneration across synthetic (Maxon) and biologic (collagen) nerve conduits for nerve gaps up to 5 cm in the primate. J Reconstr Microsurg 6: 117, 1990.


