Peripheral nerve graft architecture affects regeneration

Vleggeert-Lankamp, Carmen Lia Anne-Marie

CHAPTER 2

The role of evaluation methods in the assessment of peripheral nerve regeneration through synthetic conduits; a systematic review

C.L.A.M. Vleggeert-Lankamp

Neuroregulation group, Department of Neurosurgery, Leiden University Medical Centre (LUMC), P.O. Box 9600, NL-2300 RC Leiden, the Netherlands

The world must be peopled (W. Shakespeare)

Introduction

An adequate evaluation method is essential in order to properly assess regeneration of peripheral nerves after injury. An evaluation method is useful when 1) it clarifies the events occurring in peripheral nerve regeneration, or when 2) it is able to distinguish the properties of nerves that regenerated through distinct (experimental) nerve grafts.

Evaluation methods used to assess the outcome of synthetic nerve grafts that are developed to compete with or even outperform the current gold standard, i.e. the autograft, mainly aim at the second goal mentioned. However, to the best of our knowledge, data regarding the resolving power of the evaluation methods that are presently frequently used is absent. This motivated us to systematically review the evaluation methods used in peripheral nerve regeneration.

This review was limited to synthetic nerve grafting of the rat sciatic nerve bridging a gap of minimally 5 mm. The used evaluation methods were charted and the presence of a statistically significant difference between synthetic nerve grafted and unoperated, auto- or isografted and other synthetic grafted nerves, which were described in the same paper were scored. An evaluation method was adjudged preferable and thus recommendable for future experiments if it demonstrated to have resolving power in a convincing majority of papers describing that evaluation method.

Methods

Search strategy

We systematically searched Pubmed (1975-Dec 2004) for English language papers by entering 'prostheses and implants', 'spinal nerves', and 'rats' as medical subject heading (MeSH) terms and combining them with the textwords 'tube', 'tubes', 'tubal', 'tubular', 'conduit', 'conduits', 'biocompatible material', 'biocompatible materials', 'nerve guide', 'nerve guides', 'bioabsorb* AND tube*', 'bioabsorb* AND conduit*', 'bioabsorb* AND nerve AND guide', 'biodegrad* AND tube', biodegradable nerve guide', biodegrad* conduit*' OR (tissue* AND enigneer* AND scaffold*), 'spinal nerves' or 'sciatic nerve', the combination of textwords ('nerves' or 'nerve' AND 'spine' or 'spinal' or 'sciatic'), 'rats', 'rattus', the combination of textwords 'repair*' AND of 'peripheral nerves' or 'peripheral nerve', 'nerve*' AND 'guide*' AND 'conduit*' AND 'material*', 'nerve*' AND 'regeneration' AND 'bioabsorb*'. We then retrieved all relevant papers and searched the reference lists of retrieved papers to find other potentially relevant papers.

Selection

We assessed titles or abstracts for inclusion. When a title or abstract could not with certainty be rejected, we obtained the full text paper. Only studies concerning sciatic nerve grafting in the rat were considered. The graft had to be a synthetic nerve graft. Papers describing nerve

regeneration through venes, arteries, pieces of muscle, or Schwann cell filled nerve grafts were thus excluded. Likewise, studies in which a systemic treatment was applied to the rat, i.e. systemic administration of drugs or radiation of the rat, were excluded. It was no objective if the synthetic nerve graft was filled with a growth factor of a matrix substance. The paper should describe the results of a control group, i.e. a group with unoperated sciatic nerves or autografted or epineurial sutured ('isografted') sciatic nerves, or the results of two or more synthetic nerve grafts or one type of synthetic nerve graft filled with different growth factors or matrices.

The follow up of the rats should be at least 4 weeks after grafting surgery and the gap to be bridged by the synthetic nerve graft should be at least 5 mm. At least 3 rats should be present in each experimental and control group. The evaluation method had to be described clearly and should concern more than a mere visual inspection of the result without quantification. The statistical method of analysis had to be described properly. In studies that assayed the test parameters at different time points, we considered the outcomes at the latest point available in each study.

Data analysis

All evaluation methods described in each paper meeting the review criteria were collected and all manners in which the evaluation method was performed were summarized. For each evaluation method separately, all papers describing this evaluation method were gathered and the actual quantitative data of unoperated, autografted and synthetic nerve grafted nerves were tabulated. Finally, for each separate paper, it was scored whether a statistically significant difference was observed between unoperated and synthetic grafted nerves, between auto- or isografted nerves and synthetic grafted nerves, and between different synthetic grafted nerves. Based on these scores, the resolving power of each evaluation method was judged.

Results and discussion

The literature search resulted in 69 studies meeting our criteria. Nerve morphology, electrophysiology, muscle morphology and functional recovery were described using 17 different parameters (table 1). The number of papers describing this parameter (evaluation method) was indicated. Table 1 summarizes how many times a statistically significant difference or a non significant difference between grafted nerves was obtained.

Histology

Historically, nerve histology is the most common method to evaluate peripheral nerve regeneration, and up to this day it remains the most frequently used method.

Nerve fibre count

Nerve fibre count - Summary of materials and methods used

To count nerve fibres, nerves were fixated, embedded and transversely sectioned. Light microscopy was used to count myelinated fibres, while electron microscopy was used to evaluate the much smaller unmyelinated fibres. For fixation transcardial *perfusion* with saline, followed by a buffered (para)formaldehyde and/or glutaraldehyde solution or *immersion* in the latter solution was performed. Subsequently tissues were postfixed in an osmium tetroxide buffer and dehydrated. Then tissues were embedded in paraffin or (epoxy)resin, sectioned and stained with a toluidine blue or phenylenediamine solution for light microscopy or with uranyl acetate and aqueous lead citrate for electron microscopy. One micrometer sections could very well be studied at lightmicroscopic level. In the early days nerve fibres were counted by hand, but nowadays digital images are collected and imported into imaging software for evaluation. In most cases not all nerve fibres were counted, but just those in sample areas, after which the total number was computed from the sample area and the total area of the nerve cross section.

In the papers meeting the criteria of this review nerve fibre counts were performed in the middle of nerve grafts or distal to nerve grafts. Sometimes counted nerve fibres were separated in two groups based on their diameter being larger or smaller than 6 µm [10].

Nerve fibre count - Summary of outcome – 31 papers

Twenty eight papers reported on myelinated fibres and 3 reported on myelinated and unmyelinated nerve fibres [23, 42, 79] (table 2). Fifteen papers evaluated the number of fibres at the midgraft level, thirteen papers evaluated the number of fibres distal to the graft, one paper evaluated the number of fibres at midgraft and distal to the graft level, and two papers evaluated the number of fibres at 2 mm intervals along the grafted part.

Nerve fibre count	Synthetic nerve graft was compared to:		
	unoperated	**autograft/epineurial suturing**	**synthetic nerve graft**
sign. difference	5	9	14
no sign. diff.	3	5	9
sign. not mentioned	23	17	8

There was only a limited number of papers that compared synthetic nerve graft data to unoperated data (5 + 3 out of 31). Since five papers described a significant difference, and three described no significant difference, the outcome is not conclusive whether regenerated nerves

demonstrated a different number of nerve fibres compared to unoperated nerves. In the papers that compared the number of nerve fibres in the synthetic nerve grafts to other grafted nerves, the majority demonstrated a significant difference. In order to evaluate whether the site of nerve fibre counting was of any influence to this outcome, the results were additionally separated with respect to counting at the midgraft level or distal to the graft (table 3).

The results in both groups were however comparable, meaning that in both groups differences between unoperated and grafted nerves were not conclusive and that in both groups grafted nerves demonstrated differences in fibre numbers. By comparing the numbers of nerve fibres, which were grouped by diameter size, no differences between the different synthetic nerve grafts could be discerned either [10].

Nerve fibre count - Discussion

Counting the number of nerve fibres is considered to be a good tool to make a distinction between different nerve grafts, but it is a complicated tool to judge what graft is preferable. The significance of the number of fibres in a regenerated nerve is namely not known. A lower number of nerve fibres than an unoperated nerve seems not favorable, since this indicates that not all nerve fibres from the proximal nerve stump did regenerate [6] However, if less nerve fibres with a large diameter and excellent electrophysiological properties would have regenerated, this would be favoured over a larger number of small diameter nerve fibres with poor electrophysiological properties.

An unchanged number of equally sized nerve fibres compared to an unoperated nerve can represent the most favorable situation; namely that in a regenerated nerve all nerve fibres from the proximal nerve stump grew out, did not branch and reached their original target. We do however know that in regenerating nerves, the nerve fibres branch at the site of lesion, and that as a consequence the number of nerve fibres distal to the lesion site will increase [6, 8, 10, 41, 54, 59]. Therefore, a comparable number of nerve fibres to an unoperated nerve rather represents the situation that only a number of nerve fibres from the proximal stump regenerated which subsequently branched.

An increased number of nerve fibres in comparison with an unoperated nerve is likely to represent the outgrowth of a larger number of nerve fibres from the proximal stump that subsequently branched. Thus, an increase in nerve fibres is only favorable if branching is judged as favorable. Branching is favorable from the point of view that the same neuron can simultaneously reach different target organs, and thereby increase the chance that the original target organ is reinnervated. The accuracy of reinnervation may later be improved by means of pruning of mistaken collaterals [31, 37, 82]. However, the drawback of branching is that the neuron has to make a lot of effort to produce and maintain all branches. This effort can better be used to mature a limited number of regenerated nerve fibres, by for instance increasing the ion channel density in the membrane.

The evaluation of the number of nerve fibres in peripheral nerve regeneration should actually aim at the regeneration and targeting of individual nerve fibres. Recently, methods have

been found to visualize the branching arbors of individual neurons [62]. It is also possible to retrogradely label nerve fibres, and by staining the axon back to its origin, the motor or sensory nature of the axon can be identified. Such methods give more insight in the outcome of nerve regeneration than just the counting of nerve fibres.

Nerve fibre diameter

Nerve fibre diameter - Summary of materials and methods used

The diameter of nerve fibres was frequently calculated as the 'idealized diameter' by dividing the perimeter of the myelin sheath by π. More recent papers extract the diameter size from the digitally obtained nerve fibre area (usually including the myelin sheath). The diameter was calculated from the imaginary circle corresponding to the fibre area ($2\sqrt{(\text{fibre area}/\pi)}$).

Fibre diameter frequency distributions could be prepared by distributing the diameters of nerve fibres into a number of classes (for instance 180 classes of 0.1 µm each), and plotting them versus the (percentage of) the number of fibres present in that class. When comparing fibre diameters across studies, it is important to consider the method of fixation. Perfusion fixated nerve fibres tend to be somewhat smaller in diameter (max. 12.5 µm in the rat sciatic nerve and its branches) [66] in comparison to immersion fixed nerve fibres (max. 15.5 µm in the rat sciatic nerve and its branches) [54].

Nerve fibre diameter - Summary of outcome- 26 papers

Twenty-two paperstudies use immersion fixation, four perfusion (table 4).

Nerve fibre diameter	Synthetic nerve graft was compared to:		
	unoperated	**autograft/epineurial suturing**	**synthetic nerve graft**
sign. difference	10	4	5
no sign. diff.	0	7	7
sign. not mentioned	12	11	10

All papers comparing synthetic nerve graft data to unoperated data demonstrated a difference. Nerve fibre diameters were always smaller in regenerated compared to unoperated nerves. In the papers that compared the diameter of nerve fibres in the synthetic nerve grafts to other grafted nerves, the majority was unable to exhibit a significant difference.

Nerve fibre diameter - Discussion

The nerve fibre diameters varied between 6.1 – 8.5 µm in unoperated sciatic nerves, and between 2.8 – 4.2 µm in autografted nerves at the midgraft or the distal to the graft level. There were two exceptions: Den Dunnen [13] reported a diameter of 1.7 µm and Whitworth a diameter of 4.5 µm [83]. Whitworth embedded the nerves in Tissuetek® and reported also larger than usual values for unoperated and tube grafted nerves (resp. 8.5 and 4.45 µm). The

small diameter reported by den Dunnen may have been due to the repair technique; only two epineurial stitches were placed at each coaptation site.

Four papers reported a significant difference between the diameter in an autografted and a synthetic nerve grafted nerve [13, 44, 47, 85]. Den Dunnen reported a smaller diameter in the autografted nerve compared to the synthetic grafted nerve, likely to be due to the repair technique. The other three papers demonstrated that the diameter in an autografted nerve was larger than in a synthetic nerve grafted nerve. However, Lee and Maeda demonstrated that this only applied to the comparison of the autografted nerve with an empty or fibrin filled silicone graft.

Five papers reported a significant difference between synthetic nerve grafts [32, 39, 47, 79, 84]. Again, most of these papers reported that a significant difference only could be demonstrated in comparing an autograft with an empty silicone graft [39, 47, 79]. Xu and Harley however, demonstrated an acceptable variation in diameters and found a significant difference [32, 39, 47, 79, 84].

In conclusion, the variations in diameter of regenerated nerves were too small to use the diameter size as a useful tool to compare nerve grafts. Only 9 of 23 papers described a significant difference between grafted nerves, and in 6 of these 9 papers, the difference was only obtained by making the comparison with a nerve graft that was expected to perform poorly.

As may be expected, the diameter size increases in time. This is best documented by Chamberlain [9] who reported a mean fiber diameter for regenerated nerves between 3.2 and 3.8 µm 30 weeks after surgery and between 3.6 and 4.4 µm at 60 weeks after surgery. However, the diameter of the nerve fibres also increases upon maturation of the rat. Den Dunnen reported a 6.1 µm nerve fibre diameter in unoperated rats in a group of rats compared to rats at 10 weeks after surgery [13, 16] and of 8.5 µm in a group of rats that was compared to rats at 2 years after surgery [15]. All those rats weighed approx. 200 gram at the time of surgery and were thus of comparable age.

Ten papers reported diameter frequency distributions [1, 9, 10, 16, 22, 23, 34, 53, 69, 79]. Differences between unoperated and regenerated nerves were demonstrated, but none of them reported a significant difference between autografted and synthetic nerve grafted or between various synthetic nerve grafted nerves. However, it was reported that there was a 'shift in the distribution' towards the large diameter bins comparing the results of autograft and the best performing tube after 30 and 60 weeks [9] and comparing the results of two different synthetic nerve grafts [79]. Whether these changes were significant was not clear. Ahmed [1] reported a bimodal distribution of nerve fibre diameters not only in unoperated, but also in autografted and tube grafted nerves, 6 months after surgery.

We consider the documentation of diameter frequency distributions to be relevant in order to demonstrate differences between regenerated nerves. Moreover, from our own experiments we know [77, 78, chapter 4 to 6 (this thesis)] that fitting the frequency distributions to the sum of two lognormal distributions reveals not only the mean diameter per fibre class,

but also the number of nerve fibres present in that class. That certainly helps in making a difference between experimental paradigms.

Nerve fibre density

The nerve fibre density was calculated as the number of nerve fibres per mm ^{2} or another square measure. Two of 16 papers determined not only the nerve fibre density of myelinated, but also of unmyelinated nerve fibres. In this review we only discuss the densities of myelinated nerve fibre studies.

Nerve fibre density - Summary of outcome - 16 papers

Five of six papers that commented on the difference in density between unoperated and synthetic nerve grafted nerves reported that the density in unoperated nerves was significantly lower (table 5). One paper described that the density in regenerated nerves decreased with time after surgery, in the direction of unoperated nerves [19]. Most of the papers comparing densities in grafted nerves demonstrated significant differences. In general, the density in autografted nerves was lower compared to synthetic nerve grafted nerves.

Nerve fibre density	Synthetic nerve graft was compared to:		
	unoperated	**autograft/epineurial suturing**	**synthetic nerve graft**
sign. difference	5	6	4
no sign. diff.	1	3	3
sign. not mentioned	10	7	9

Nerve fibre density - Discussion

In the two papers that demonstrated that the density was higher in autografted nerves, the density of the autograft was compared to an empty silicone tube [44, 86]. Nine of fifteen papers reported significant differences between experimental nerves, thus it is possible to detect differences between tube grafted nerves based on densities. This makes it relevant to determine this parameter in the future.

G-ratio

The G-ratio was expressed as the ratio of the axon diameter and the myelinated fibre diameter. It was considered to be a parameter to assess the maturity of nerve fibres.

G-ratio - Summary of outcome – 10 papers

Three of five papers that commented on the difference in G-ratio between unoperated and synthetic nerve grafted nerves report significant differences. Two of these demonstrated a smaller G-ratio in unoperated nerves (table 6). In grafted nerves, the majority of papers described no differences between groups.

G-ratio	Synthetic nerve graft was compared to:		
	Unoperated	**autograft/epineurial suturing**	**synthetic nerve graft**
sign. difference	3	0	2
no sign. diff.	2	3	4
sign. not mentioned	5	7	4

G-ratio - Discussion

The finding that the G-ratio in unoperated nerves was smaller compared to regenerated nerve fibres, would indicate that regenerated nerves had a relatively thinner myelin sheath. Den Dunnen demonstrated that G-ratios 2 years after surgery were equal to unoperated values, and this would imply that upon maturation the relation axon/myelin sheath would decrease [15].

The survey of data however demonstrated that literature was not conclusive whether the G-ratio is larger in unoperated or in regenerated nerves (table 6). Considering that even this is difficult to assess, the parameter is not useful in making a distinction between regenerated nerves. We recommend not to include the G-ratio in future experiments.

N-ratio

The N-ratio was calculated as the total myelinated fibre area divided by the total tissue cable area. The N-ratio represented the total mass of the regenerating nerve in the tube, and was a parameter indicative of both the number of sprouting events and the degree of maturation of the regenerating nerve [39]. A low N-ratio was indicative for a relatively large amount of fibrous tissue [13].

N-ratio - Summary of outcome – 9 papers

All papers that commented on the difference in N-ratio between unoperated and synthetic nerve grafted nerves reported that the N-ratio in unoperated nerves was significantly higher. But the N-ratio increased with time after surgery [16]. The majority of papers which compared the N-ratio in grafted nerves exhibited a significant difference (table 7).

N-ratio	Synthetic nerve graft was compared to:		
	unoperated	**autograft/epineurial suturing**	**synthetic nerve graft**
sign. difference	4	3	5
no sign. diff.	0	1	1
sign. not mentioned	5	5	3

N-ratio - Discussion

Two papers demonstrated that the N-ratio in autografted nerves was higher compared to tube grafted nerves, and one paper reported a lower value in autografted nerves [13]. However, the repair technique in that single article (2 epineurial stitches at each coaptation site) was poorly

performed, as stated before. There were significant differences between regenerated nerves in 6 of 8 papers, so we consider the N-ratio a promising parameter to evaluate in the future.

Nerve histological success ratio

The succes ratio is a simple method to evaluate the success of nerve regeneration. The presence of myelinated nerve fibres distal to the graft was evaluated and scored as a 'yes' or 'no'.

Nerve histological success ratio - Summary of outcome – 10 papers

Only in 3 papers the success ratio was used as a tool to statistically differentiate between nerve grafts (table 8).

Success ratio	Synthetic nerve graft was compared to:		
	unoperated	**autograft/epineurial suturing**	**synthetic nerve graft**
sign. difference	0	1	1
no sign. diff.	0	0	1
sign. not mentioned	10	9	8

Nerve histological success ratio - Discussion

Unfortunately, authors did scarcely perform statistical analysis on their success rate data in order to make differences between grafted nerves. However, the data exhibit enough variation to make a statistically relevant difference between grafted nerves likely. We would therefore certainly recommend to score the nerve histological success ratio.

Retrograde labeling

Retrograde labeling - Summary of materials and methods used

Tracers (DiI, Fast Blue, Fluoro Gold, True Blue, HRP) were applied to the nerves by offering tiny crystals of tracer to the transected nerves [76] or by injecting them distal to the implantation site in the nerve [23, 34] or in the muscles [67]. Different tracers could be offered to different nerves to determine the distribution and regeneration capacity of neurons projecting to different targets, like the gastrocnemic, tibial or plantar muscles [76].

Care had to be taken during dissection and tracer application to avoid bleeding and blood diffusion of the fluorescent dye. Animals were allowed to survive for 2 to 8 days to allow accumulation of tracers in the somata of the spinal motoneurons and/or DRGs. Subsequently, fixation by perfusion took place and (parts of) the lumbar segment of the spinal cord and the DRGs were removed. The lumbar spinal cord was cut longitudinally in 25 to 50 µm thick sections, and the DRGs in 14 to 40 µm thick sections and labeled neurons were counted. Single, double or even triple labeling allowed comparison of different experimental paradigms.

Retrograde labeling - Summary of outcome – 5 papers

In four papers the presence of tracer was evaluated in both motoneurons and DRG neurons of the lumbar spinal column and in one paper only the motoneurons in this area were evaluated for tracer presence [76] (table 9). Quantitatively, the number of motoneurons could be compared between experimental paradigms.

Number of motoneurons	Synthetic nerve graft was compared to:		
	unoperated	**autograft/epineurial suturing**	**synthetic nerve graft**
sign. difference	2	1	1
no sign. diff.	1	0	3
sign. not mentioned	2	4	1

Generally, after injury and regeneration the number of motoneurons decreased and the number of DRG neurons decreased or increased.

Retrograde labeling - Discussion

The gathered data on retrograde labeling demonstrated almost no significant differences between unoperated, autografted and tube grafted nerves. But it was obvious that the data were very illustrative for the growth patterns of nerve fibres that existed during nerve regeneration. We would therefore strongly recommend to include evaluation of retrograde tracing in future experiments.

Electrophysiology

Compound Muscle Action Potential (CMAP)

CMAP - Summary of materials and methods used

The evoked Compound Muscle Action Potential (CMAP) was measured after electrical stimulation. Stimulation was performed by placing bipolar hooked platinum stimulating electrodes proximal to the nerve graft or by placing skin electrodes and stimulating the sciatic nerve percutaneously. In order to measure the muscle action potential mono- or bi-polar intramuscular electrodes were placed in the belly of each muscle. Frequently used target muscles were the gastrocnemic, tibial or plantar muscles. Temperature influences the conduction velocity of the electrical signal over the nerve (doubles on an each increase of 10 degrees Celcius [58]) and it was therefore relevant to be informed about it.

Some authors measured the latency of the signal, some the amplitude of the signal. The latency was often expressed as the ratio of the latency at the experimental side and the latency at the contralateral unoperated side. The amplitude was often expressed as the amplitude at the experimental side divided by the amplitude at the contralateral unoperated side (or the preoperative value) x 100%. Either the amplitude of the Hoffman reflex (H-reflex wave) or the

M-wave was measured. The amplitude of the Hoffman reflex (H-reflex) provides a measure of the motoneuron excitation achieved by a controlled amount of Ia-afferent activation and is therefore dependent on both motoneuron excitability and presynaptic inhibition of Ia-afferents. When the plantar flexor muscles are activated isometrically, the H-reflex of the soleus muscle has been shown, in some studies, to increase with increased neural activation, whereas others have shown unchanged or slightly lower H-reflexes in an active as compared to a passive muscle [57].

CMAP - Summary of outcome- 14 papers latency, 9 papers amplitude

Almost all papers, but one, that commented on the difference in CMAP between unoperated and synthetic nerve grafted nerve rats reported that the latency of the CMAP in unoperated rats was shorter and/or the amplitude of the CMAP was higher. Most of the papers that compared the CMAP in grafted nerve rats demonstrated a significant difference (table 10).

CMAP latency	Synthetic nerve graft was compared to:		
	unoperated	**autograft/epineurial suturing**	**synthetic nerve graft**
sign. difference	7	6	5
no sign. diff.	0	3	5
sign. not mentioned	7	5	4

CMAP amplitude	Synthetic nerve graft was compared to:		
	unoperated	**autograft/epineurial suturing**	**synthetic nerve graft**
sign. difference	5	3	4
no sign. diff.	1	3	1
sign. not mentioned	3	3	4

CMAP latency and amplitude combined	Synthetic nerve graft was compared to:		
	unoperated	**autograft/epineurial suturing**	**synthetic nerve graft**
sign. difference	12	9	9
no sign. diff.	1	6	6
sign. not mentioned	11	9	9

Compound Muscle Action Potential - Discussion

The uniformity of the observations concerning the latency and the amplitude qualifies the CMAP as a parameter that is easy to interprete. Since the majority of papers also conveyed a significant difference between regenerated nerves, we would certainly recommend to score the CMAP in future experiments. The results did not demonstrate that there was a preference towards determining the CMAP latency or the CMAP amplitude, or towards comparing a ratio or the actual determined value in milliseconds or milliVolts.

Mean Conduction Velocity (MCV)

MCV - Summary of materials and methods used

To measure the Mean Conduction Velocity (MCV) the nerve had to be electrically stimulated at some point and the electrical activity had to be measured at a distinct point. Bipolar hooked platinum stimulating and recording electrodes were regulary used to serve this purpose and were placed resp. before and after the area of interest (i.e. the nerve graft). The MCV was calculated as the ratio of the conducting distance and the latency time to the peak of the maximal action current.

The MCV is strongly dependent on temperature [58] and it is therefore relevant to be informed about it. In *in vivo* experiments, a heating lamp was often used to keep the body temperature at approximately 37 degrees Celcius.

MCV - Summary of outcome – 11 papers

In the papers that compared synthetic nerve graft data to unoperated data, the outcome was not conclusive whether regenerated nerves demonstrated a different MCV compared to unoperated nerves (table 11). If there was a difference, the MCV in regenerated nerves was always lower. In the papers that compared the MCV in the synthetic nerve grafts to other grafted nerves, only in a limited number of cases a significant difference was demonstrated.

MCV	Synthetic nerve graft was compared to:		
	unoperated	**autograft/epineurial suturing**	**synthetic nerve graft**
sign. difference	4	1	3
no sign. diff.	3	6	3
sign. not mentioned	4	4	5

Mean Conduction Velocity - Discussion

The MCV is mainly dictated by the diameter of the conducting nerve fibres [36] and the internodal distance [11]. In the histology part it was already demonstrated that the diameter of nerve fibres decreased in regenerated nerves, but that there were hardly any differences between the diameters of autografted and synthetic nerve grafted nerves. This same pattern was observed here.

Three papers reported a significant difference between synthetic nerve grafts. Two of these solely reported this difference because they included an (empty) silicone graft, with a renown poor performance. In the other paper six synthetic nerve grafts were compared and only one of them exhibited a significant difference with the other grafts.

Literature describes conduction velocities in A-fibres (larger than 6 µm) ranging from 30-120 m/s [70], in B-fibres (1.3-3 µm) ranging from 3-15 m/s [3, 70], and in C-fibres (<1.3 µm) ranging from 0.1-2 m/s [70]. The conduction velocity is determined by the fastest fibres present, i.e. the A-fibres. The conduction velocities presented in this summary are consistent with these data.

It is possible that the diameter of regenerating nerve fibres increases in time and that the MCV will likewise increase. Moreover, in regenerated nerves the internodal distance is shorter, and this distance may increase in time, which would increase conduction velocity. Most authors evaluated the rats 3-4 months after surgery. Chamberlain waited 14 months and is one of the two authors that reported a significant difference in MCV between regenerated nerves.

In future experiments it is only relevant to measure MCV when long survival times are included, with respect to an expected increase in diameter and internodal distance in time. Furthermore, when the MCV is measured, we recommend to unoperated temperature during the experiments thoroughly, since the MCVs demonstrated a tendency to vary less in experiments carried out at a controlled 37 degrees Celcius.

ElectroMyoGraphy (EMG)

Two authors reported on an electromyographic evaluation that cannot be qualified as a CMAP [10, 51]. Meek implanted EMG electrodes in the midbelly of the gastrocnemic and tibial muscles of both hindlegs (one experimental, one unoperated) [51]. The animals were allowed to walk freely and the recording electrodes were connected to an amplifier system. Representative EMG data were colleced from each rat during 10 stepcycles and rated on the occurrence of abnormalities such as slow increase of burst amplitudes, irregularity of burst activity and abnormal EMG activity during specific phases of the stepcycle. In the results a description was given of the EMG data in the unoperated, autografted and tube grafted rats. Sometimes ratings were mentioned and it was concluded that there were no statistical differences between the rats with autografts and tube grafts. There was no comment on the statistical difference between unoperated and tube grafted rats, but it was stated that the EMG patterns in the gastrocnemius and tibialis anterior muscles in the grafted rats were highly abnormal.

Chamberlain stimulated the sciatic nerve proximal to the lesion site with bipolar stimulating electrodes and with single stimuli [10]. The animal was monitored for the appearance of gastrocnemius muscle twitch and toe flexion. In all but the empty silicone tube grafted rats the gastrocnemius muscle and the plantar muscles of the foot responded with visible movement to electrical stimulation of the sciatic nerve. The qualitative intensity of the muscular response in rats with regenerated nerves did not differ significantly from the response observed in the unoperated rats and the stimulation parameters for reading twitch threshold did not differ between unoperated and regenerated nerves.

ElectroMyoGraphy - Discussion

The method of Meek is difficult to reproduce. Moreover, no statistical differences between the groups were obtained. Likewise, no statistical differences between the groups were obtained by Chamberlain. Neither of the two methods are recommended to perform in the future.

Somatosensory Evoked Potentials (SEP)

One author recorded *in vivo* somatosensory-evoked potentials (SEP) [26]. Electrical stimulation was directly applied using a needle electrode to the sciatic nerve distal to the graft. SEPs were recorded epidurally using screw electrodes placed on the left cerebral sensorimotor cortex. SEPs were evaluated based on the peak-to-peak amplitude and latency. Three different synthetic nerve grafts were tested. One of the tubes did not demonstrate a SEP after 6 mos, while the other 2 grafts demonstrated a significant difference compared to each other regarding both latency and amplitude. However, this difference disappeared at the end of the 12 months evalution period.

Somotosensory Evoked Potential - Discussion

If the evaluation period is not too long, the results may deliver additional information about choosing between synthetic nerve grafts. In order to be informed about the regeneration of the sensory nerve fibres this method is preferred over the pinching tests mentioned below. However, the performance of a SEP is rather complex, which will surely cause that it will not be performed on a large scale.

Muscle morphology

Muscle weight

Muscle weight - Summary of materials and methods used

The muscle weight of muscles distal to a regenerated sciatic nerve was claimed to be proportional to the degree of sciatic nerve innervation and thus a parameter for functional recovery [86]. Mostly, the gastrocnemic muscle was considered. It was possible to weigh the (wet) muscle from the experimental group and compare it to (wet) muscles of unoperated rats. Usually however, the relative muscle weight was determined, defined as the ratio of the muscle weight from the experimental side to that of the unoperated side, or as a ratio to the rats' body weight.

Muscle weight - Summary of outcome – 11 papers

All papers that commented on the difference in muscle weight between unoperated and synthetic nerve grafted nerves reported a significant higher weight in unoperated rats (table 12). Half of the papers comparing muscle weight in grafted nerves exhibited a significant difference, and half of the papers did not.

Muscle Weight	Synthetic nerve graft was compared to:		
	unoperated	**autograft/epineurial suturing**	**synthetic nerve graft**
sign. difference	5	1	5
no sign. diff.	0	4	2
sign. not mentioned	6	6	4

Muscle weight - Discussion

The uniformity of the observations on muscle weight qualifies muscle weight as a parameter that is easy to interprete. However, in only half of the cases the muscle weight differentiated between grafted nerves, which makes it doubtful whether this parameter is useful as an evaluation tool. On the other hand, the test is easy to perform and it is informative about the expected function of the muscle distal to the grafted nerve.

Muscle circumference

One author [26] evaluated muscle mass using a circumferential measurement technique. The circumference of both the unoperated muscle and the muscle distal to the grafted nerve was measured at distances 20 and 30 mm proximal to the heel. All animals that exhibited a tissue bridge through the synthetic nerve guide had muscle circumferences that were not significantly different from those of unoperated animals. If however, the synthetic nerve graft was empty, the muscle circumference was significantly smaller.

Muscle circumference - Discussion

The presence of a tissue cable through the synthetic nerve graft correlated very well with the presence of a significant difference in muscle circumference. Since it is much easier to check whether a tissue cable traversed the graft than to measure the muscle circumference this evaluation method is not recommended. However, this conclusion is drawn based on only a single paper.

Functional tests

Automutilation

Automutilation was only quantified in two papers [14, 50] Den Dunnen defined automutila - tion as the presence of exposed bone or loss of a part of the foot or toes [14]. There was 22% percent automutilation in autografted and 15% automutilation in synthetic nerve grafted rats. This difference was not significant. Meek scored automutilation as moderate if there were superficial wounds restricted to the nails or the cutaneous part, and as severe if there was exposed bone or loss of a part of the foot or toes [50]. In the tube grafted group 75% demonstrated no automutilation and 25% severe automutilation. A statistical analysis was not performed.

Automutilation - Discussion

It is not te be expected that subtle differences between synthetic nerve grafted nerves will be detected by this evaluation method. We would not recommend to perform it in the future.

Muscle tetanic force

Muscle tetanic force - Summary of materials and methods used

To measure muscle tetanic force, the long trajectory from sciatic nerve to the innervated muscle and the tendon up towards its origin had to be exposed. The tendon was cut and fixed with a ligature. The joints were transfixed and the tendon ligature was connected to a force transducer. The nerve was supramaximally stimulated proximal to the regenerated segment with (platinum) stimulating wire electrodes. The transducer signal was led through an amplifier and the muscle tetanic force was determined.

Muscle tetanic force - Summary of outcome – 7 papers

The one paper that commented on the difference in muscle tetanic force between unoperated and synthetic nerve grafted nerves reported that the force in unoperated muscles was significantly higher (table 13). Two papers compared the muscle tetanic force in autografted and tube grafted nerves. In the paper that demonstrated a significant difference, this difference was obtained by comparing the best performing graft with a very poorly performing graft. Five papers compared different synthetic nerve grafts, and only one of these reported a significant difference. This difference was obtained by comparing to an empty silicone tube.

Muscle tetanic force	Synthetic nerve graft was compared to:		
	unoperated	**autograft/epineurial suturing**	**synthetic nerve graft**
sign. difference	1	1	1
no sign. diff.	0	1	4
sign. not mentioned	6	5	2

Muscle tetanic force - Discussion

It was to be expected that there were differences between unoperated and experimental muscle tetanic forces. Nevertheless, this was actually demonstrated only once. This evaluation method does give insight in the functional recovery of the target organ after grafting the innervating nerve. Therefore it might still be an interesting evaluation method to perform. The decrease in tetanic force is less than the decrease in the muscle cross sectional area because the cross sectional area is highly related to the daily contractions of the muscle [17]. Tetanic force may therefore be a parameter that better expresses the function of the muscle than the cross sectional area or the muscle weight. Although the experimental set up is rather elaborate, we do as yet recommend to perform this evaluation method in the future.

Walking track analysis

Walking track analysis - Summary of materials and methods used

Walking track analysis is a fairly common method to evaluate functional peripheral nerve regeneration. The characteristics of the hindpaw prints are measured and an overall value is represented as the Sciatic Functional Index (SFI). The prints of the hindpaw(s) are evaluated for print length (PL), toe spread (TS; distance from toe 1 to 5) and intermediate toe spread (IT; distance from toe 2 to 4). PL is dependent on gastrocnemic muscle activation, and TS and IT are mainly influenced by paw extensor and paw intrinsic muscles contraction during the stance phase of walking. Sciatic nerve injury causes an increase in PL and a decrease in TS and IT. With these parameters the sciatic functional index is calculated using a formula developed by De Medinaceli [48] and modified by Bain [4]. The SFI is 0 for non injured animals and -100 after complete transection of the sciatic nerve.

In order to visualize the print of the hindpaw there are several methods. (1) The hindpaw can be painted with a dye and the rat walks in a box over white paper (8 papers), (2) the hindpaw can be painted with film developer and the rat walks in the dark over photo-sensitive paper (6 papers), (3) the hindpaw can be wet and the rat walks in a box over pH-sensitive or watersensitive bromphenol blue impregnated paper (3 papers).

Recently a much more refined method has been developed to evaluate functional recovery by studying the walking pattern of the rat by a video assessment over a runway [51]. The lateral and ventral view of the animal are visualized and the walking movements of the rat were recorded with a video camera. Two different methods were described. Meek described that the videotape was then replayed frame-by-frame and several aspects of the walking pattern of the rats were scored: toe spread during stance phase, foot placement, the occurrence of dragging of the hindpaw, exorotation of the foot, the swing phase, and the regularity and fluency of walking. Normality was scored by 'yes' and abnormality by 'no'. The positively scored parameters per rat were summarized and the percentages per group calculated. De Ruiter marked three points on the rat hind paw and studied the movement of those three points and subsequently related these three point to each other [61]. Gamez [26] studied dorsiflexion and locomotor function of the operated limb. The rats were observed and the locomotor function and dorsiflexion were described but not quantitatively described.

Walking track analysis - Summary of outcome – 17 papers

In the limited number of papers that compared SFI data in synthetic grafted nerves to unoperated data, the majority demonstrated a significant difference (table 14). From the papers that compared the SFI in the synthetic nerve grafted rats to the SFI in other nerve grafted rats, the majority was unable to demonstrate a significant difference.

Walking track analysis	Synthetic nerve graft was compared to:		
	unoperated	**autograft/epineurial suturing**	**synthetic nerve graft**
sign. difference	4	3	2
no sign. diff.	1	7	5
sign. not mentioned	12	7	10

One of the papers that reported a significant difference used a paired t-test [33]. Since the out - come in the two groups is not related in pairs, such analysis is not allowed and the outcome is thus scored as 'no comment', instead of a 'yes'. Another paper that reported a significant difference did not clearly mention whether the SFI at the end of the evaluation period was significantly different (which would make the outcome relevant) or whether the mean of SFIs at all timepoints was significantly different (which would make the outcome irrelevant) [1].

Meek [51] demonstrated a significant difference between autografted and tube grafted nerve rats considering fluency of walking, foot placement and swing phase.

Walking track - Discussion

The results of three of four papers that reported a significant difference between synthetic nerve grafted rats and other grafted rats are questionable. In one of the papers the timepoint on which significance is reached is not clear [1], and in another paper a difference was solely reported when comparing with an empty silicone graft, with a notorious performance. Evans reported a significant difference between grafted nerves while the values varied between after an evaluation period of 18 weeks [20], but apparently this difference disappeared after 35 weeks [19].

Moreover, in two papers no difference in SFI could be obtained, while the other evaluation results would certainly imply this [47, 85]. The first paper namely described success rates of the compared nerves of 0 and 100% respectively. In the second paper an autograft and a synthetic nerve graft were compared and it was reported that no tissue bridge crossed the synthetic nerve graft.

There was only one paper that compared the SFI in grafted nerves after a long evaluation period (ca. 1 yr) [10]. The SFI was measured at 30 and 60 weeks, and no significant differences between the two timepoints was reported. They state that the SFI improved significantly between 1 and 12 weeks, and then remained relatively constant for the remainder of the experiment. They claim that this is due to the development of toe contractures, which took place in 80% of the experimental population. All of the toe contractures developed between 6 and 18 weeks. Contractures were earliest developed in the autograft group, and latest by the empty silicone tube group [10].

Based on the current information we would not recommend to use the SFI as an evaluation method in the future. The occurrence of contractures in the hind paw of the sciatic nerve grafted rat is likely to interfere with the outcome of the SFI and diminishes the resolving power of the evaluation method. However, if the problem of the contraction of the hind paw can be

overcome, evaluation of the walking pattern of the rat can be one of the most important evaluation methods, since it represents a functional outcome. Therefore, the methods used by de Ruiter and Meek [51, 61], which take the full motion of the rat hind leg into consideration are promising.

Sensory tests

Sensory tests - Summary of materials and methods used

Three methods were described to evaluate sensory recovery after grafting peripheral nerves. The first method described was to stimulate (1.0 mA) 3 different points along the lateral side of the foot. A reflex was found positive when the paw was withdrawn and/or the toes were spread. When a positive reflex was found, the current was further decreased by steps of 0.1 mA and this was repeated in order to find the threshold. The second method described was to pinch the nerve distal or proximal to the nerve graft with a pair of forceps. Contraction of muscles on the back or retraction of the leg indicated the presence of regenerating sensory fibers in the pinching segment, while no response was taken as an indication of the absence of such fibers. The third method involved the pinching of the foot pad and detecting a withdrawal response.

Sensory tests - Summary of outcome – 10 papers

Statistics were hardly performed on the sensory tests data.

Sensory tests	Synthetic nerve graft was compared to:		
	unoperated	**autograft/epineurial suturing**	**synthetic nerve graft**
sign. difference	1	0	0
no sign. diff.	1	1	0
sign. not mentioned	8	9	10

Sensory tests - Discussion

The scarce available data on statistics on sensory tests were not very promising to expect that subtle differences between synthetic nerve grafted nerves can be revealed by using this evaluation method. The test is however indicative whether nerve fibres did regenerate through the graft. It is a rather blunt test and the method to quantify the results introduced by the Groningen group (first method) is appealing. However, these papers also lacked an extended description of statistical comparison.

Conclusion

Nerve fibre diameter distribution and retrograde labeling are two methods that can attribute to the insight in processes playing a role in nerve regeneration. Methods that have been demonstrated to have resolving power are nerve fibre count, nerve fibre density, N-ratio, and nerve histological success ratio. Electrophysiological evaluation, and particularly the CMAP, of the regenerated nerve is also considered as a discrimative tool. Determining the MCV is not valuable, certainly not for short term experiments. Studying muscle morphology may be useful; muscle weight and muscle Cross Sectional Area (not discussed) are useful tools in discriminating between grafted nerves.

From the functional tests the SFI is outdated, and automutilation and sensory tests seem not discriminative enough. Muscle tetanic force may be discriminative. The new full motion walking track indices are very promising because they are discriminative and informative.

The evaluation methods described each assess only a part of the outcome of the regenera - tive process. A proper combination of evaluation methods is therefore preferred to properly evaluate nerve regeneration.

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Table 1. Overview

The gathered evaluation methods are presented, followed by the number of papers describing this method. It is indicated how many times a statistically significant difference between grafted nerves was present or not.

	Number of papers		Number of papers	Comparison of grafted nerves	
				NOT sign	sign
Nerve morphology	38	**Nerve fibre count**	31	13	**23**
		Diameter	26	14	9
		Density	15	6	**10**
		G-ratio	9	7	2
		N-ratio	9	2	**8**
		Success ratio	10	1	**2**
Retrograde labeling	5			3	2
Electrophysiology	29	**CMAP**	15	12	**18**
		MCV	11	9	4
		EMG	2	2	0
		SEP	1	0	1
Muscle morphology	11	Muscle weight	11	6	6
		Muscle circumference	1	0	1
Functional recovery	29	Automutilation	2	1	0
		Muscle tetanic force	7	5	2
		Walking track	17	12	5
		Sensory tests	10	1	0

Table 2. Number of nerve fibres (see pp. 46-47)

The numbers indicated are of myelinated nerve fibres. In the second column the type of synthetic nerve graft that is evaluated is indicated, followed by the number of different types of nerve grafts between brackets. The weight of the rat is indicated in grams and the survival period is indicated in weeks. The nerves in which the number of nerve fibres is measured are abbreviated as follows: 'sciatic' for sciatic nerve, 'tib' for tibial nerve, and 'per' for peroneal nerve. In the 'sign to unoperated' column it is indicated whether the number of myelinated nerve fibres in one of the synthetic nerve graft groups is significantly different from unoperated nerves. In the 'sign to auto' column the difference between autografted and synthetic nerve grafted nerves is indicated and in the 'sign to other tube' column the difference between different synthetic nerve guides is indicated. * only significant compared to the silicone tube (empty or filled with PBS). n.i. not indicated.

Table 3. Number of nerve fibres at midgraft compared to distal to the graft level

Evaluation of significance between synthetic nerve grafts and unoperated, autografted and other synthetic nerve grafted nerves. The results are separated for the evaluation of the number of nerve fibres counted at the midgraft level and the number of nerve fibres distal to the graft.

Midgraft nerve fibre count	Synthetic nerve graft was compared to:		
	unoperated	**autograft/epineurial suturing**	**synthetic nerve graft**
sign. difference	2	6	6
no sign. diff.	2	2	4
sign. not mentioned	11	7	5

Distal to the graft nerve fibre count	Synthetic nerve graft was compared to:		
	unoperated	**autograft/epineurial suturing**	**synthetic nerve graft**
sign. difference	2	4	6
no sign. diff.	1	3	3
sign. not mentioned	10	6	4

Table 4. Diameter of nerve fibres (see pp. 48-49)
The diameter of the nerve fibres is indicated in µm. The diameter indicated is the mean diameter of myelinated nerve fibres including the myelin sheath, unless indicated differently. In the second column the type of synthetic nerve graft that is evaluated is indicated, followed by the number of different types of nerve grafts between brackets. The weight of the rat is indicated in grams and the survival period is indicated in weeks. In the 'sign to unoperated' column it is indicated whether the diameter of myelinated nerve fibres in one of the synthetic nerve graft groups is significantly different from unoperated nerves. In the 'sign to auto' column the difference between autografted and synthetic nerve grafted nerves is indicated and in the 'sign to other tube' column the difference between different synthetic nerve guides is indicated. * only significant compared to the silicone tube (empty or filled with PBS). n.i. not indicated.

Table 6. G-ratio of nerve fibres (see pp. 52-53)
The G-ratio is calculated as the axon diameter/outer myelin sheath diameter. In the second column the type of synthetic nerve graft that is evaluated is indicated, followed by the number of different types of nerve grafts between brackets. The weight of the rat is indicated in grams and the survival period is indicated in weeks. In the 'sign to unoperated' column it is indicated whether the G-ratio in one of the synthetic nerve graft groups is significantly different from unoperated nerves. In the 'sign to auto' column the difference between autografted and synthetic nerve grafted nerves is indicated and in the 'sign to other tube' column the difference between different synthetic nerve guides is indicated. * only significant compared to the silicone tube (empty or filled with PBS). n.i. not indicated.

Table 7. N-ratio of nerve fibres (see pp. 52-53)
The N-ratio is calculated as the total myelinated fibre area divided by the total tissue cable area. In the second column the type of synthetic nerve graft that is evaluated is indicated, followed by the number of different types of nerve grafts between brackets. The weight of the rat is indicated in grams and the survival period is indicated in weeks. The nerves in which the number of nerve fibres is measured are abbreviated as follows: 'sciatic' for sciatic nerve, 'tib' for tibial nerve, and 'per' for peroneal nerve. In the 'sign to unoperated' column it is indicated whether the N-ratio in one of the synthetic nerve graft groups is significantly different from unoperated nerves. In the 'sign to auto' column the difference between autografted and synthetic nerve grafted nerves is indicated and in the 'sign to other tube' column the difference between different synthetic nerve guides is indicated. * only significant compared to the silicone tube (empty or filled with PBS). n.i. not indicated.

Table 8. Nerve histological success ratio (see pp. 54-55)
The success ratio is expressed as the ratio of rats with a particular nerve guide scored with a 'yes' and the total number of rats which had this particular nerve guide implanted. In the second column the type of synthetic nerve graft that is evaluated is indicated, followed by the number of different types of nerve grafts between brackets. The weight of the rat is indicated in grams and the survival period is indicated in weeks. The succes ratio in autografted and synthetic grafted nerves is expressed as a percentage. In the 'sign to unoperated' column it is indicated whether the N-ratio in one of the synthetic nerve graft groups is significantly different from unoperated nerves. In the 'sign to auto' column the difference between autografted and synthetic nerve grafted nerves is indicated and in the 'sign to other tube' column the difference between different synthetic nerve guides is indicated. * only significant compared to the silicone tube (empty or filled with PBS). n.i. not indicated.

Table 9. Retrograde labeling (see pp. 56-57)
After retrograde labeling of nerve fibres, several parameters can be observed: the number of motoneurons, the number of DRG neurons, the percentage of multiple labeled neurons and the routing of the nerve fibres. The results of these four parameters were scored and summarized in this table.
In the second column the type of synthetic nerve graft that is evaluated is indicated, followed by the number of different types of nerve grafts between brackets. The weight of the rat is indicated in grams and the survival period is indicated in

weeks. The nerves in which the number of nerve fibres is measured are abbreviated as follows: 'sciatic' for sciatic nerve, 'tib' for tibial nerve, and 'per' for peroneal nerve. The muscles in which the tracer is injected or to which the injected nerve projects are abbreviated as follows: 'gas' for gastrocnemic muscle, 'tib' for anterior tibial muscle, 'plant' for plantar muscles, and 'per long' for the peroneus longus muscle.

The site from which the sections were taken is indicated and the section thickness is indicated between brackets. The numbers of motoneurons and DRG neurons are indicated. The labeled DRG number is by one author indicated as a percentage of the total number of cells present in that section. In the 'sign to unoperated' column it is indicated whether the parameter indicated in one of the synthetic nerve graft groups is significantly different from unoperated nerves. In the 'sign to auto' column the difference between autografted and synthetic nerve grafted nerves is indicated and in the 'sign to other tube' column the difference between different synthetic nerve guides is indicated. n.i. not indicated.

Table 10. Compound Muscle Action Potential (see pp. 58-59)

The upper part of the table summarizes the papers that report on the latency of the signal, and the lower part summarizes the papers that report on the amplitude of the signal. In the second column the type of synthetic nerve graft that is evaluated is indicated, followed by the number of different types of nerve grafts between brackets. The weight of the rat is indicated in grams and the survival period is indicated in weeks. The temperatured ('temp') are indicated as 'vivo' to indicate that the CMAP was measured *in vivo* or as the temperature in degrees Celcius. Stimulation is either 'bipolar' with bipolar hooked platinum stimulating electrodes placed proximal to the nerve graft or the sciatic nerve is percutaneously stimulated ('percut'). The muscles in which the CMAP is measured are abbreviated as follows: 'gas' for gastrocnemic muscle, 'tib' for anterior tibial muscle, 'plant' for plantar muscle, and 'interosseus' for interosseus muscle. The latency is often expressed as the 'ratio' of the latency at the experimental side and the latency at the contralateral unoperated side. It can also be expressed as the 'time' (in milliseconds 'ms') needed from stimulation of the nerve to achieving a signal in the muscle, or as nerve mean conduction velocity (NMCV) in metres per second ('m/s'). The amplitude is often expressed as the amplitude at the experimental side divided by the amplitude at the contralateral unoperated side (or the preoperative value) x 100%. It can also be expressed as a 'voltage' in milliVolt (mV).

In the 'sign to unoperated' column it is indicated whether the CMAP in one of the synthetic nerve graft groups is significantly different from unoperated nerves. In the 'sign to auto' column the difference between autografted and synthetic nerve grafted nerves is indicated and in the 'sign to other tube' column the difference between different synthetic nerve guides is indicated. n.i. not indicated.

Table 11. Mean Conduction Velocity (see pp. 60-61)

The MCV is calculated as the ratio of the conducting distance and the latency time to the peak of the maximal action current. In the second column the type of synthetic nerve graft that is evaluated is indicated, followed by the number of different types of nerve grafts between brackets. The weight of the rat is indicated in grams and the survival period is indicated in weeks. The temperature ('temp') is indicated as 'vivo' to indicate that the MCV was measured *in vivo* or as the temperature in degrees Celcius.

The distance between the electrodes is indicated as 'interelectrode distance'. The MCV is expressed in metres per second. In the 'sign to unoperated' column it is indicated whether the MCV in one of the synthetic nerve graft groups is significantly different from unoperated nerves. In the 'sign to auto' column the difference between autografted and synthetic nerve grafted nerves is indicated and in the 'sign to other tube' column the difference between different synthetic nerve guides is indicated. * only significant compared to the silicone tube (empty or filled with PBS). n.i. not indicated.

Table 12. Muscle Weight (see pp. 60-61)

The muscle weight was determined while the muscle was still wet. In the second column the type of synthetic nerve graft that is evaluated is indicated, followed by the number of different types of nerve grafts between brackets. The weight of the rat is indicated in grams and the survival period is indicated in weeks. The weight is indicated in grams, or the ratio of the muscle weight at the experimental side and the muscle weight at the contralateral unoperated side. In the 'sign to unoperated' column it is indicated whether the muscle weight in one of the synthetic nerve graft groups is significantly different from unoperated nerves. In the 'sign to auto' column the difference between autografted and synthetic nerve grafted nerves is indicated and in the 'sign to other tube' column the difference between different synthetic nerve guides is indicated. * only significant compared to the silicone tube (empty or filled with PBS). n.i. not indicated.

Table 13. Muscle tetanic force (see pp. 62-63)

In the second column the type of synthetic nerve graft that is evaluated is indicated, followed by the number of different types of nerve grafts between brackets. The weight of the rat is indicated in grams and the survival period is indicated in weeks. The muscle tetanic force is determined in milliNewton (mN), Newton per cm2, percentage of unoperated value. In

one paper the unoperated value is expressed in grams. Considering that Newton is calculated in weight per accelaration due to gravity (being approximately 10), 219 gram is comparable to 2190 mN. In the 'sign to unoperated' column it is indicated whether the muscle tetanic force in one of the synthetic nerve graft groups is significantly different from unoperated nerves. In the 'sign to auto' column the difference between autografted and synthetic nerve grafted nerves is indicated and in the 'sign to other tube' column the difference between different synthetic nerve guides is indicated. * only significant compared to a very poorly performing paradigm. n.i. not indicated.

Table 14. Walking track analysis (see pp. 62-63)
All papers used the Sciatic Functional Index. The contralateral paw was in all cases unoperated. In the second column the type of synthetic nerve graft that is evaluated is indicated, followed by the number of different types of nerve grafts between brackets. The weight of the rat is indicated in grams and the survival period is indicated in weeks. In the 'Dye' column the dye which is put on the paws is indicated and in the 'material' column the material is indicated over which the rats are directed to walk. Some authors take 100 as a unoperated value and 0 as the lowest value possible. Others take 0 as a unoperated value and –100 as the lowest value possible. Since the original method of Medicanelli used values between –100 and 0, we consider these values as correct, and transform incorrect values of others if necessary.. However, for reasons of simplicity we leave out all minus marks. In the 'sign to unoperated' column it is indicated whether the SFI in one of the synthetic nerve graft groups is significantly different from unoperated nerves. In the 'sign to auto' column the difference between autografted and synthetic nerve grafted nerves is indicated and in the 'sign to other tube' column the difference between different synthetic nerve guides is indicated. * only significant compared to a very poorly performing paradigm. # statistics incorrect. n.i. not indicated.

Table 15. Sensory tests (see pp. 64-65)
In the second column the type of synthetic nerve graft that is evaluated is indicated, followed by the number of different types of nerve grafts between brackets. The weight of the rat is indicated in grams and the survival period is indicated in weeks. In the 'stimulation' column it is indicated how the stimulation of the paw is performed. In the 'evaluation' column the way to evaluate the reaction of the rat is indicated. In the results columns a percentage means the percentage of rats that actually demonstrated the behaviour indicated under 'evaluation'. In the 'sign to unoperated' column it is indicated whether the sensory test in one of the synthetic nerve graft groups is significantly different from unoperated nerves. In the 'sign to auto' column the difference between autografted and synthetic nerve grafted nerves is indicated and in the 'sign to other tube' column the difference between different synthetic nerve guides is indicated. * only significant compared to a very poorly performing paradigm. n.i. not indicated.

Table 2. Number of nerve fibres

	Graft biomaterial (number of types evaluated)	Distance bridged	Weight of rat	Survival period
Harley [32]	Crosslinked collagen (5)	15	175-200	9
Scherman [65]	Polyglactin sutures (2)	7	200	12
Liu [45]	Filled silicone (3)	10	300-350	6
Gamez [26]	Filled gelatin (3)	10	300-350	24
Cai [7]	Filled silicone tube packed with Poly-lactic acid microfilaments (4)	14	n.i.	10
Lee [44]	Filled silicone (7)	13	n.i.	6
Ahmed [1]	Impregnated collagen (2)	10	250	26
Midha [53]	Filled crosslinked PHEMA hydrogel (6)	10	250-275	8
Yu [86]	Filled polysulfone (6)	10	200-300	9
Ngo [56]	Silicone tube packed with Poly-lactic acid microfilaments (4)	10, 14, 18	250-300	10
Xu [84]	Filled silicone or polyphosphoester (7)	10	200	14
Yoshii [85]	Collagen tube packed with collagen filaments (2)	20	250	8
Fine [23]	Filled ethylene vinyl acetate (3)	15	325-350	1,5
Scherman [64]	Sutures	7, 15	220	12
Wang [81]	Poly(phosphoester) (2)	10	200-250	14
Nicoli Aldini [55]	Poly-L-lactide-co-6-caprolactone or Poly-[organo]-phosphazene tubes	10	350	26
Arai [2]	Silicone packed with filaments (6)	15	200	14
Scherman [63]	Sutures (2)	7	200	14
Chamberlain [10]	Filled silicone or collagen (4)	10	175-200	60
Terris [72]	Filled silicone (5)	5	250	13
Chamberlain [9]	Silicone or porous collagen packed with collagen filaments (6)	10	175-200	60
Voinesco [79]	Filled silicone (2)	8	250-300	8
Tham [73]	Filled silicone (3)	10	200-250	12
Sterne [69]	Impregnated fibronectin mats (2)	10	n.i.	35
Giardino [28]	poly(L-lactide-co-6-caprolactone)	10	300	26
Seckel [67]	Injectable nerve guide (2)	10	n.i.	12
Maeda [47]	Silicone with autograft (3)	18	225-250	16
Evans [18]	Silicone	5	250-300	14
Robinson [60]	filled poly(DL-lactide-ε-caprolactone) (3)	8	250-350	16
Hollowell [34]	Filled silicone (2)	8	250-300	10
Jenq [42]	Porous silicone (2)	8	n.i.	8

Midgraft/ Distal to graft	Fixation	Myelinated nerve fibre count unoperated	Myelinated nerve fibre count auto	Myelinated nerve fibre count tube	Sign to unoperated	Sign to auto	Sign to other tube
midgraft	immersion	n.i.	n.i.	4500 - 8000			no
distal tibial	immersion	n.i.	n.i.	midgraft 2931-3534, tib 2718-3286			no
midgraft	immersion	n.i.	n.i.	5129-13400			yes*
2 mm sections	immersion	ca 7700	n.i.	500-16000	yes		yes
distal sciatic	perfusion	n.i.	n.i.	500-2000			yes
midgraft, distal sciatic	immersion	n.i.	mid 9920, dist 5315	mid 0-6462, dist 0-4094		yes	
midgraft	perfusion	6338	7953	5518-7764	yes	yes	yes
distal sciatic	immersion	n.i.	2271	220-2534		yes	yes
midgraft	perfusion	8638	5743	800-3200	yes	yes	yes
distal sciatic	perfusion	n.i.	n.i.	1300-4200			yes
n.i.	immersion	n.i.	n.i.	5024-10830			yes
midgraft	immersion	n.i.	4837	0-5491		yes	
midgraft	perfusion	n.i.	n.i.	5-4942			yes
distal tibial	immersion	n.i.	4700	300-3600		yes	
midgraft	immersion	7991	16975	6684-8080	no	yes	
distal sciatic	immersion	n.i.	2290	2275-2359		no	
midgraft	immersion	n.i.	n.i.	0-6230			no
midgraft	immersion	n.i.	n.i.	1170-1370			no
distal tib and per	immersion	4900 (tib) 1700 (per)	4200 (tib) 2900 (per)	4500-5000 (tib) 2100-3000 (per)	yes	no	yes
distal sciatic	immersion	n.i.	n.i.	17210-30577			no
midgraft	immersion	6200	13800	200-16000		yes	yes
2 mm sections	perfusion	n.i.	n.i.	1779-7804			yes
midgraft	immersion	n.i.	n.i.	4932-10338			
distal sciatic	immersion	6859	n.i.	4932-7003	no		yes
midgraft	immersion	n.i.	2290	2350-7500		no	
n.i.	immersion	n.i.	n.i.	6000-7500			no
distal sciatic	immersion	8500	3200	0-4500	yes	yes	no
distal sciatic	immersion	n.i.	epi: 13923	11772		no	
midgraft	immersion	8000	8200	6500-7000	no	no	yes
distal sciatic	perfusion	7800	n.i.	6894-7567			no
midgraft	perfusion	7905	n.i.	6317-7183			no

Table 4. Diameter of nerve fibres

	Graft biomaterial (number of types evaluated)	Distance bridged	Weight of rat	Survival period	Midgraft/ Distal to graft
Harley [32]	Crosslinked collagen (5)	15	175-200	9	midgraft
Itoh [38]	Filled silicone (5)	14	180-200	12	distal tibial
Lee 19	Filled silicone (7)	13	n.i.	6	midgraft, distal sciatic
Ahmed [1]	Impregnated collagen (2)	10	250	26	midgraft
Midha [53]	Filled crosslinked PHEMA hydrogel (6)	10	250-275	8	midgraft, distal sciatic
Francel [25]	Silicone or Lactosorb with piece of autograft (4)	20	250	16	distal sciatic
Xu [84]	Filled silicone or polyphosphoester (7)	10	200	14	n.i.
Yoshii [85]	Collagen tube packed with collagen filaments (2)	20	250	8	midgraft
Fine [23]	Filled ethylene vinyl acetate (3)	15	325-350	1,5	midgraft
Wang [81]	Poly(phosphoester)(2)	10	200-250	14	midgraft
Itoh [39]	Silicone or collagen packed with collagen fibres (7)	10	200	8	midgraft
Terris [72]	Filled silicone (5)	5	250	13	distal sciatic
Chamberlain [9]	Silicone or porous collagen packed with collagen filaments (6)	10	175-200	60	midgraft
Voinesco [79]	Filled silicone (2)	8	250-300	8	2 mm pieces
Francel [24]	Silicone filled with autograft (2)	13	225-250	16	midgraft, distal sciatic
Tham [73]	Filled silicone (3)	10	200-250	12	midgraft
Sterne [69]	Impregnated fibronectin mats (2)	10	n.i.	35	distal sciatic
Den Dunnen [13]	Poly(DL-lactide-ε-caprolactone)	10	200	10	1 mm prox and 1 mm distal to graft
Den Dunnen [16]	Poly(DL-lactide-ε-caprolactone)	10	200	10	1 mm prox and 1 mm distal to graft
Whitworth [83]	Fibronectin mats	10	200-250	9	n.i.
Maeda [47]	Silicone with autograft (3)	18	225-250	16	distal sciatic
Den Dunnen [15]	Poly(L-lactide-ε-caprolactone)	10	200	104	prox, midgraft, dist sciatic
Evans [18]	Silicone	5	250-300	14	distal sciatic
Robinson [60]	Filled poly(DL-lactide-ε-caprolactone) (3)	8	250-350	16	midgraft
Hollowell [34]	Filled silicone (2)	8	250-300	10	distal sciatic
Le Beau [43]	Silicone	10	180-200	78	n.i.

Fixation	Diameter unoperated	Diameter auto	Diameter tube	Sign to unoperated	Sign to auto	Sign to other tube
immersion	n.i.	n.i.	1.8-3.1			yes
n.i.	n.i.	n.i.	3.05-3.43			no
immersion	n.i.	mid 2.93, dist 2.82	mid 0.64-3.01, dist 0.45-1.71		yes*	
perfusion	small 2.8, large 8.3	small 3.0, large 8.1	small 2.8-2.9, large 8-8.2	No	no	no
immersion	n.i.	dist 3.58	mid 3.73-4.00, dist 3.13-3.33		no	no
immersion	7.2	3.2	3.4-3.6	Yes	no	
immersion	n.i.	n.i.	3.2-4.1			yes
immersion	n.i.	3.3	2.3		yes	
perfusion	n.i.	n.i.	1.42-1.61 (axon)			no
immersion	7.41	3.75	3.48-3.71	Yes	no	
immersion	n.i.	n.i.	2.2 - 6 (axon)			yes*
immersion	n.i.	n.i.	2.9-3.5			no
immersion	8.5	n.i.	3.6-4.4			
perfusion	n.i.	n.i.	3.07-3.8			yes*
immersion	6.5	dist 3.1	dist/mid 3.0			
immersion	n.i.	n.i.	3-3.4			
immersion	6,76	n.i.	4.61-4.77	Yes		no
immersion	6.1	1.7	prox 2.9, dist 2.8	Yes	yes	
immersion	6.1	n.i.	prox 2.9, dist 2.8	Yes		
immersion	n.i.	4.5	4.45		no	
immersion	6	4.2	2.5-3.5	Yes	yes*	yes*
immersion	8.5	n.i.	prox 5, mid 4.5, dist 4	Yes		
immersion	n.i.	epi 3.89	3.72		no	
immersion	7	3.5	4	Yes	no	no
perfusion	6	n.i.	2.8-2.9			no
immersion	5.13	n.i.	2.39	Yes		

Table 5. Density of nerve fibres

	Graft biomaterial (number of types evaluated)	Distance bridged	Weight of rat	Survival period	Midgraft/Distal to graft
Itoh [38]	Filled silicone (5)	14	180-200	12	distal tibial
Lee [44]	Filled silicone (7)	13	n.i.	6	midgraft, distal sciatic
Francel [25]	Silicone or Lactosorb with piece of autograft (4)	20	250	16	distal sciatic
Yu [86]	Filled polysulfone (6)	10	200-300	9	midgraft
Xu [84]	Filled silicone or polyphosphoester (7)	10	200	14	n.i.
Fine [23]	Ethylene vinyl acetate copolymer filled with BSA	15	325-350	47 days	midgraft
	Ethylene vinyl acetate copolymer, with a polymer rod that releases NGF				
	Ethylene vinyl acetate copolymer, with a polymer rod that releases GDNF				
Wang [80]	Filled Poly-DL-lactide (2)	15	200	18	midgraft, distal sciatic
Wang [81]	Poly(phosphoester) (2)	10	200-250	14	midgraft
Itoh [39]	Silicone or collagen packed with collagen fibres (4)	10	200	8	midgraft
Evans [19]	Poly-L-lactide	12	200-250	35	midgraft, distal sciatic
Evans [20]	Poly-L-lactide	10	250	16	midgraft, distal sciatic
Francel [24]	Silicone filled with autograft (2)	13	225-250	16	midgraft, distal sciatic
Den Dunnen [13]	Poly-(DL-lactide-ε-caprolactone)	10	200	10	1 mm prox and 1 mm distal to graft
Den Dunnen [16]	Poly-(DL-lactide-ε-caprolactone)	10	200	10	1 mm prox and 1 mm distal to graft
Whitworth [83]	Fibronectin mats	10	200-250	9	n.i.
Den Dunnen [15]	Poly-(L-lactide-ε-caprolactone)	10	200	104	prox, midgraft, dist sciatic
Robinson [60]	Filled poly(DL-lactide-ε-caprolactone)(3)	8	250-350	16	midgraft

Fixation	Density unoperated	Density auto	Density tube	Sign to unoperated	Sign to auto	Sign to other tube
n.i.	n.i.	n.i.	6700-12000			yes
immersion	n.i.	mid 19122, dist 10731	mid 1404-28678, dist 80-10389		yes*	
immersion	7404	7211	7026-8865	no	no	
perfusion	17118	16983	5000-17425	yes	yes*	yes
immersion	n.i.	n.i.	10530-21712			yes
perfusion	2-4/100 µm2 (Den Dunnen)	n.i.	n.i.			
			32.8/100 µm2 unmyel			
			21.9/100 µm2 unmyel			no
immersion	n.i.	n.i.	mid 11720-34802, dist 11146-12993			no
immersion	14225	38104	34388-41042	yes	no	
immersion	n.i.	n.i.	1000 - 15000			yes
immersion	n.i.	mid 26000, distal 22000	mid 33000, distal 20000		mid yes, distal no	no
immersion	n.i.	mid 17000, distal 14000	mid 22000, distal 10000		yes	
immersion	13000	22000	26000			
immersion	75/1500µm2	28/1500 µm2	prox 174/1500µm2, dist 165/1500µm2	yes	yes	
immersion	25/area		prox 58/area, dist 55/area	yes		
immersion		438/frame	413/frame		no	
immersion	120/area		prox 300/area, mid 350/area, dist 300/area			
immersion	20000	18000	50000-60000	yes	yes	no

Table 6. G-ratio of nerve fibres

	Graft biomaterial (number of types evaluated)	Distance bridged	Weight of rat	Survival period	Midgraft/Distal to graft
Midha [53]	Filled crosslinked PHEMA hydrogel (6)	10	250-275	8	midgraft, distal sciatic
Xu [84]	Filled silicone or polyphosphoester (7)	10	200	14	n.i.
Wang [81]	Poly(phosphoester) (2)	10	200-250	14	midgraft
Scherman [63]	Polyamide or polyglactin sutures (2)	7	200	14	midgraft
Voinesco [79]	Filled silicone (2)	8	250-300	8	2 mm pieces
Sterne [69]	Impregnated fibronectin mats (2)	10	n.i.	35	distal sciatic
Den Dunnen [13]	Poly(DL-lactide-ε-caprolactone)	10	200	10	1 mm prox and 1 mm distal to graft
Den Dunnen [16]	Poly(DL-lactide-ε-caprolactone)	10	200	10	1 mm prox and 1 mm distal to graft
Den Dunnen [15]	Poly(L-lactide-ε-caprolactone)	10	200	104	prox, midgraft, dist sciatic
Robinson [60]	Filled poly(DL-lactide-ε-caprolactone)(3)	8	250-350	16	midgraft

Table 7. N-ratio of nerve fibres

	Graft biomaterial (number of types evaluated)	Distance bridged	Weight of rat	Survival period	Midgraft/ Distal to graft
Harley [32]	Crosslinked collagen (5)	15	175-200	9	midgraft
Francel [25]	Silicone or Lactosorb with piece of autograft (4)	20	250	16	distal sciatic
Suzuki [71]	Tendon chitin (4)	15	180-200	12	midgraft
Itoh [40]	Treated collagen (4)	10	180-200	12	midgraft
Itoh [39]	Silicone or collagen packed with collagen fibres (4)	10	200	8	midgraft
Scherman [63]	Polyamide or polyglactin sutures (2)	7	200	14	midgraft
Chamberlain [10]	Filled silicone or porous collagen packed with collagen filaments (6)	10	175-200	60	prox and distal sciatic, tib and per
Den Dunnen [13]	Poly(DL-lactide-ε-caprolactone)	10	200	10	1 mm prox and 1 mm distal to graft
Den Dunnen [16]	Poly(DL-lactide-ε-caprolactone)	10	200	10	1 mm prox and 1 mm distal to graft

Fixation	G-ratio unoperated	G-ratio auto	G-ratio tube	Sign to unoperated	Sign to auto	Sign to other tube
immersion	n.i.	dist 0.36	mid 0.65, dist 0.34		no	no
immersion	n.i.	n.i.	0.7-0.81			yes
immersion	0.44	0.68	0.64-0.67	yes	no	
immersion	n.i.	n.i.	0.46-0.67			no
perfusion	n.i.	n.i.	0.58-0.71			yes*
immersion	0.59	n.i.	0.50	yes		no
immersion	0.65	0.15-0.30	0.15-0.30			
immersion	0.65		0.74	yes		
immersion	0.6		prox, mid, dist 0.6	no		
immersion	0.4	0.5	0.5-0.55	no	no	no

Fixation	N-ratio unoperated	N-ratio autograft	N-ratio tube	Sign to unoperated	Sign to auto	Sign to other tube
immersion	n.i.	n.i.	0.07-0.32			yes
immersion	0.55	0.13	0.13-0.19	yes	no	
immersion	n.i.	0.44	0.29-0.41		yes	yes
immersion	n.i.	0.40	0.05-0.35		yes	yes
immersion	n.i.	n.i.	0.04-0.15			yes
immersion	n.i.	n.i.	0.07-0.11			no
immersion	tib 0.25, per 0.07	tib 0.09, per 0.05	tib 0-0.15, per 0.06	yes		yes
immersion	0.46	prox 0.17, dist 0.16	prox 0.3, dist 0.29	yes	yes	
immersion	0.46		0.3	yes		

Table 8. Nerve histological success ratio

	Graft biomaterial (number of types evaluated)	Distance bridged	Weight of rat	Survival period
Liu [45]	Filled silicone (3)	10	300-350	6
Cai [7]	Filled silicone tube packed with poly-lactic acid microfilaments (4)	14	n.i.	10
Lee [44]	Filled silicone (7)	13	n.i.	6
Midha [53]	Filled crosslinked PHEMA hydrogel (6)	10	250-275	8
Francel [25]	Silicone or Lactosorb with piece of autograft (4)	20	250	16
Yu [86]	Filled polysulfone (6)	10	200-300	9
Ngo [56]	Silicone tube packed with poly-lactic acid microfilaments (4)	18	250-300	10
Itoh [40]	Treated collagen (4)	10	180-200	12
Francel [24]	Silicone filled with autograft (2)	13	225-250	16
Jenq [42]	Porous silicone (2)	8	n.i.	8

Midgraft/ Distal to graft	Success rate auto	Success rate tube	Sign to unoperated	Sign to auto	Sign to other tube
midgraft	n.i.	30			
distal sciatic	n.i.	75-100			
midgraft, distal sciatic	n.i.	0-66			
distal sciatic	100	50-100			
distal sciatic	100	0-100			
midgraft	100	0-100			
midgraft, distal sciatic	n.i.	100			no
midgraft	n.i.	66-100		yes	
midgraft, distal sciatic	100	0-92			
distal		45-100			yes

Table 9. Retrograde labeling

	Graft biomaterial (number of different nerve guides tested)	Distance bridged	Survival period	Tracer application	Tracer
Valero-Cabre [76]	Poly-L-lactide-ε-caprolactone or silicone (2)	10	14	per, tib (branch to gas+plantar)	Dil, Fast Blue, Fluoro Gold
Fine [23]	Filled ethylene vinyl acetate copolymer (3)	15	7	sciatic 3-5 mm distal to implant	Fluoro Gold
	Ethylene vinyl acetate copolymer, with a polymer rod that releases NGF				
	Ethylene vinyl acetate copolymer, with a polymer rod that releases GDNF				
Seckel [67]	Filled injectable nerve guide (2)	10	12	gas and per long	True Blue
Hollowell [34]	Filled silicone (2)	8	10	5 mm distal to graft	HRP
	Silicone filled with NGF				
Madison [46]	Filled silicone (3)	15-20	16	5 mm distal to graft	HRP
	Silicone filled with laminin				
	Silicone filled with collagen gel				

	Graft biomaterial (number of different nerve guides tested)	Distance bridged	Survival period in weeks	Tracer application	Tracer
Fine [23]	Filled ethylene vinyl acetate copolymer (3)	15	7	sciatic 3-5 mm distal to implant	Fluoro Gold
Seckel [67]	Filled injectable nerve guide (2)	10	12	gas and per long	True Blue
Hollowell [34]	Filled silicone (2)	8	10	5 mm distal to graft	HRP
Madison [46]	Filled silicone (3)	15-20	16	5 mm distal to graft	HRP

	Graft biomaterial (number of different nerve guides tested)	Distance bridged	Survival period in weeks	Tracer application	Tracer
Valero-Cabre [76]	Poly-L-lactide-ε-caprolactone or silicone (2)	10	14	per, tib (branch to gas+plantar)	Dil, Fast Blue, Fluoro gold resp.

	Graft biomaterial (number of different nerve guides testes)	Distance bridged	Survival period in weeks	Tracer application	Tracer
Valero-Cabre [76]	Poly-L-lactide-ε-caprolactone or silicone (2)	10	14	per, tib (branch to gas+plantar)	Dil, Fast Blue, Fluoro gold resp.

Survival with tracer (days)	Sections (um)	Number motoneur unoperated	Number motoneur auto	Number motoneur tube	Sign to contr	Sign to auto	Sign to other tube
8	L1-L6 (50)	1238	1186	802-935	yes	yes	no
5	L3-L6 (25)	448	n.i.	0-98			yes
				20			yes
				98.1			yes
7	lumbar	n.i.	n.i.	95-100			no
2	L3-L6 (40)	2027	n.i.	1724-1813	no		no
				1724			no
3	lumbar	n.i.	n.i.	221-242			
				221			not indicated
				242			not indicated

Survival with tracer (days)	Sections DRG (um)	Number DRG neurons unoperated	Number DRG neurons auto	Number DRG neurons tube	Sign to contr	Sign to auto	Sign to other tube
5	L4-L5 (14)	64%	n.i.	0.8-22.7%			yes
7	L4-L6 (32)	n.i.	n.i.	245-250			no
2	L3-L6 (40)	8809	n.i.	9608-10246	no		no
3	L3-5 (40)	n.i.	n.i.	5-46			

Survival with tracer (days)	Sections (um)		% multiple labeling auto	% multiple labeling tube	Sign to contr	Sign to auto	Sign to other tube
8	L1-L6 (50)		5.8	6-10.1	yes	yes	no

Survival with tracer (days)	Sections (um)	Routing unoperated	Routing autograft	Routing tube	Sign to contr	Sign to auto	Sign to other tube
8	L1-L6 (50)	Gas 23%, Tib 45%, Plantar 30%	Gas 11%, Tib 45%, Plantar 37%	Gas 15-18%, Tib 33-48%, Plantar 30-38%	yes	yes	yes

Table 10. Compound Muscle Action Potential

	Graft biomaterial (number of different nerve guides tested)	Temp	Survival period	Stimu-lation	Muscle	What is measured
CMAP latency						
Itoh [38]	Filled silicone (5)	vivo	12	bipolar	gas	ratio
Ahmed [1]	Impregnated collagen (2)	37	26	bipolar	plant	ratio
Suzuki [71]	Tendon chitin (4)	n.i.	14	bipolar	gas	ratio
Valero Cabre [74]	Silicone, poly-L-lactate-ε-caprolactone, dual silicone (3)	vivo	14	bipolar	tib, gas, plant	ratio
Itoh [40]	Treated collagen (4)	n.i.	14	bipolar	gas	ratio
Wang [80]	Filled Poly-DL-lactide (2)	vivo	18	n.i.	n.i.	time
						NMCV
Valero Cabre [75]	Silicone	34-36	14	percut	gas, plant	ratio
Valero Cabre [76]	Silicone or poly-L-lactide-ε-caprolactone (2)	34-36	14	percut	tib, gas, plant	ratio
Seckel [67]	Filled injectable nerve guide (2)	n.i.	12	bipolar	gas	NMCV
Gibson [30]	Silicone, polyglactin, polypropylene (3)	vivo	14	percut	gas	time
Zetti [87]	Polytetrafluoro ethylene	30	39	percut	inter osseus	time
Robinson [60]	Filled poly(DL-lactide-ε-caprolactone) (3)	25	16	bipolar	gas, tib	time
Gibson [29]	Silicone	n.i.	14	percut	gas	time
Cuadros [12]	Polytetrafluoro ethylene	n.i.	18	bipolar	forefoot ext muscles	NMCV
CMAP amplitude						
Zhang [88]	Filled silicone (2)	vivo	18	bipolar	tib	voltage
Wang [80]	Filled Poly-DL-lactide (2)	vivo	18	n.i.	n.i.	voltage
Valero Cabre [75]	Silicone	34-36	14	percut	gas, plant	ratio
Valero Cabre [74]	Silicone, poly-L-lactate-ε-caprolactone, dual silicone (3)	vivo	14	bipolar	tib, gas, plant	ratio
Valero Cabre [76]	Silicone or poly-L-lactide-ε-caprolactone (2)	34-36	14	percut	tib, gas, plant	ratio (M-wave)
						ratio (H-wave)
Nicoli Aldini [55]	Poly-L-lactide-co-6-caprolactone and poly-[organo]-phosphazene (2)	vivo	26	n.i.	flexor dig. sup.	voltage
Giardino [27]	Poly-L-lactide-co-6-caprolactone and poly-DL-lactide (2)	n.i.	26	bipolar	flexor dig. sup.	voltage
Evans [18]	Silicone	37	14	bipolar	gas, tib	ratio
Robinson [60]	Filled poly(DL-lactide-ε-caprolactone) (3)	25	16	bipolar	gas, tib	voltage

	Auto			Tube					
Unoperated	gas	tib	plant	gas	tib	plant	Sign to unoperated	Sign to auto	Sign to other tube
n.i.	n.i.			1.32-1.49					no
n.i.			1.12			1.12-1.34	yes	no	no
n.i.	1.24			3.4-5.1				yes	no
0.93	1.08	1.3	1.37	1.55-1.87	1.5-1.9	1.55-1.95	yes	yes	yes
n.i.	1.2			1.3-4.5				yes	yes
2.92			n.i.			3.22-4.60			yes
47.1						24-32			yes
0.9	1.1		1.35	1.55		1.5	yes	yes*	
1.00	1.73	1.7	1.63	1.6-1.75	1.95	1.92	yes	no	no
n.i.	n.i.			9.0-16.0					yes
n.i.	4.5			5.0-6.0				yes	
1.76			n.i.			2.66	yes		
tib 0.65, gas 0.6	0.9	1.1		0.8-1	1-1.1		yes	no	no
n.i.	4			5.5				yes*	
50.6			n.i.			21.7	yes		
n.i.		n.i.			5.3-9.5				yes*
16.4			n.i.			8.6-11.7			yes
1.15	0.62		0.22	0.57		0.16	yes	no	
1.13	0.69	0.57	0.22	0.43-0.56	0.23-0.46	0.11-0.25	yes	yes	yes
1.0	0.39	0.42	0.23	0.29-0.37	0.3-0.36	0.15-0.25	yes	yes	yes
1.0	0.58	0.62	0.27	0.53-0.56	0.49-0.54	0.26-0.28	yes	no	no
n.i.			2.8			3.1-3.6		no	
2.8			n.i.			3.6	no		
tib 0.79, gas 0.88	0.82	0.74		0.88	0.37		yes	yes*	
gas 15, tib 10	5	4		8.0-9.0	3.0-4.0		yes	no	no

Table 11. Mean Conduction Velocity

	Graft biomaterial (number of different nerve guides tested)	Temp	Survival period	Inter electrode distance
Zhang [88]	Filled silicone (2)	vivo	18	20
Francel [25]	Silicone and Lactosorb with piece of autograft (4)	37	16	ca. 26
Chamberlain [9]	Silicone or porous collagen packed with collagen filaments (6)	n.i.	60	20
Meyer [52]	Silicone	35	18	20
Ho [33]	Filled collagen (4)	vivo	13	16
Francel [24]	Silicone filled with autograft (2)	37	18	20
Tham [73]	Filled silicone (3)	26-27	12	n.i.
Maeda [47]	Silicone filled with autograft (3)	37	18	25
Robinson [60]	Filled poly(DL-lactide-ε-caprolactone) (3)	25	16	15
Hoppen [35]	Poly(DL-lactide-ε-caprolactone)	vivo	9	n.i.
Fields [21]	Silicone	37	10	n.i.

Table 12. Muscle Weight

	Graft biomaterial (number of different nerve guides tested)	Weight of rat	Survival period	What is measured
Gastrocnemic muscle				
Scherman [65]	Coated polyglactin sutures (2)	200	12	weight
Yu [86]	Filled polysulfone (6)	200-300	9	ratio
Brown [5]	Filled silicone (2)	200-250	14	ratio
Evans [19]	Poly-L-lactide	200-250	35	weight
Evans [20]	Poly-L-lactide	250	16	weight
Tham [73]	Filled silicone (3)	200-250	12	ratio
Cuadros [12]	Polytetrafluoroethylene	250-350	18	ratio to rat body weight
Soleus muscle				
Meyer [52]	Silicone	243	32	ratio
Simon [68]	Filled fibronectin (4)	n.i.	17	weight
Extensor digitorum longus muscle				
Simon [68]	Filled fibronectin (4)	n.i.	17	weight
Gastrocnemic and soleus muscle together				
Terris [72]	Filled silicone (5)	250	13	ratio

Unoperated	Auto	Tube	Sign to unoperated	Sign to auto	Sign to other tube
n.i.	n.i.	18-33			yes*
41	37	0-55	no	no	no
67	47	0-50	yes	yes	yes
81	40	35	yes	no	
n.i.	n.i.	43-52			no
n.i.	n.i.	n.i.	no	no	
30	n.i.	10.0-22.0			yes*
59	43	0-29	yes	no	
70	60	55-60	yes	no	no
n.i.	48	50	no	no	
80	n.i.	35-40			

Unoperated	Auto	Tube	Sign to unoperated	Sign to auto	Sign to other tube
n.i.	n.i.	0.7-0.84			no
1.1	0.45	0.27-0.44	yes	yes	yes
n.i.	n.i.	0.25-0.39			yes*
n.i.	1.4	2.1		no	
n.i.	1.15	0.9		no	
n.i.	n.i.	0.32-0.52			yes*
0.94	0.45	0.39	yes	no	
1.39	0.83	0.78	yes	no	
0.016	n.i.	0.006-0.013	yes		yes
0.013	n.i.	0.004-0.010	yes		yes
n.i.	n.i.	0.51-0.58			no

Table 13. Muscle tetanic force

	Graft biomaterial (number of different nerve guides tested)	Weight of rat	Survival period
Gastrocnemic muscle			
Scherman [65]	Polyglactin sutures (2)	200	12
Brown [5]	Filled silicone (2)	200-250	4
Scherman [64]	Sutures with piece of autograft (3)	220	12
Arai [2]	Filled silicone (6)	200	14
Tham [73]	Filled silicone (3)	200-250	12
Tibial muscle			
Arai [2]	Filled silicone (6)	200	14
Soleus muscle			
Meyer [52]	Silicone	243	32

Table 14. Walking track analysis

	Graft biomaterial (number of different nerve guides tested)	Weight of rat	Survival period	Dye
Ahmed [1]	Impregnated collagen (2)	250	26	India ink
Yu [86]	Filled polysulfone (6)	200-300	9	Trypan blue
Valero Cabre [74]	Silicone, poly-L-lactate-ε-caprolactone, dual silicone (3)	260	13	Acrylic paint
Yoshii [85]	Collagen tube packed with collagen filaments (2)	250	12	India ink
Valero Cabre [75]	Silicone	260	13	Acrylic paint
Evans [19]	Poly-L-lactide	200-250	35	Water soluble ink
Chamberlain [10]	Filled silicone or collagen (4)	175-200	60	Water
Meek [50]	Poly-(DL-lactide-ε-caprolactone)	200	52	Film developer
Evans [20]	Poly-L-lactide	250	18	Water soluble ink
Terris [72]	Filled silicone (5)	250	14	Water
Ho [33]	Filled collagen (4)	n.i.	14	Water
Francel [24]	Silicone with piece of autograft (2)	225-250	16	Film developer
Meek [49]	Poly-(DL-lactide-ε-caprolactone)	200	15	Film developer
Seckel [67]	Filled injectable nerve guide (2)	n.i.	8	Methylene blue
Maeda [47]	Silicone with piece of autograft (3)	225-250	16	Film developer
Evans [18]	Silicone	250-300	14	Film developer
Gibson [30]	Silicone, polyglactin, polypropylene (3)	250	14	Film developer

What is measured	Unoperated	Auto	Tube	Sign to unoperated	Sign to auto	Sign to other tube
force (mN)	n.i.	n.i.	4812-6722			no
force per area (N/cm2)	n.i.	n.i.	14-19			no
force (mN)	14500	8300	200-5000		yes*	
percentage of unoperated	n.i.	n.i.	19-43			no
force (mN)	8000	n.i.	2000-5000			yes
percentage of unoperated	n.i.	n.i.	6-35			no
percentage of unoperated		70	65	yes	no	

Material	Unoperated	Auto	Tube	Sign to unoperated	Sign to auto	Sign to other tube
White paper	7	70	55-70			yes
White paper	n.i.	82	84-104		yes	yes
White paper	3	72	70-73	yes	no	
White paper	n.i.	71	84-106		no	
White paper	n.i.	70	70-80		no	
White paper	n.i.	92-114	104-114		no	
pH sens paper	9	83-87	83-87		yes*	
Photographic paper	0	n.i.	50	yes		
White paper	n.i.	70	90		yes	
Bromphenol blue impregnated paper	n.i.	n.i.	77-89			no
Bromphenol blue impregnated paper	n.i.	n.i.	70-80			yes#
Photographic paper	n.i.	67	n.i.			no
Photographic paper	0	n.i.	30	yes		
White paper	n.i.	n.i.	75-95			no
Photographic paper	0	80	85-95	yes	no	no
Photographic paper	n.i.	n.i.	47		no	
Photographic paper	n.i.	n.i.	n.i.	no	no	no

Table 15. Sensory tests

	Graft biomaterial (number of different nerve guides tested)	Weight of rat	Survival period	Stimulation
Electro-stimulation				
Den Dunnen [14]	Poly(DL-lactide-caprolactone)	200	12	3 points along lateral side paw
Meek [50]	Poly(DL-lactide-ε-caprolactone)	200	52	3 points along lateral side paw
Meek [49]	Poly(DL-lactide-ε-caprolactone)	200	15	3 points along lateral side paw
pinching of the nerve				
Xu [84]	Filled silicone or poly(phosphoester) (7)	200	14	pinch the sciatic nerve distal to the tube
Wang [81]	Poly(phosphoester)(2)	200-250	14	pinch the sciatic nerve distal to the tube
Arai [2]	Silicone packed with filaments (6)	200	14	pinch the sciatic nerve distal to the tube
Scherman [63]	Polyamide or polyglactin sutures	200	12	pinch the sciatic nerve distal to the tube
pinching of the foot				
Chamberlain [10]	Filled silicone or collagen (4)	175-200	60	pinch the toes
Den Dunnen [13]	Poly(DL-lactide-ε-caprolactone)	200	10	plantar side of paw
Cuadros [12]	Polytetrafluoroethylene	250-350	18	pinch the footpad

Evaluation	Unoperated	Auto	Tube	Sign to unoperated	Sign to auto	Sign to other tube
Paw withdrawal / spread of toes	0.2 to 0.3 mA	decrease of current	decrease of current			
Paw withdrawal / spread of toes	0.2 mA	n.i.	1mA dropped to 0.2 mA			
Paw withdrawal / spread of toes	0.2 mA	n.i.	1mA dropped to 0.25 mA	yes		
Contraction of muscle on the back or retraction of the leg	n.i.	n.i.	36-70%			
Contraction of muscle on the back or retraction of the leg	n.i.	n.i.	40-92%			
Contraction of muscle on the back or retraction of the leg	n.i.	n.i.	0-100%			
Contraction of muscle on the back or retraction of the leg	n.i.	n.i.	88-100%			
vocalization, withdrawal paw, prox. muscle flexion	100%	100%	0-100%			
Paw withdrawal	n.i.	100%	100%			
Paw withdrawal	n.i.	100%	100%	no	no	