CHAPTER 5

Review of the experimental and clinical literature on nerve tubes for peripheral nerve repair

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INTRODUCTION

At this moment, the gold standard for repair of nerve defects that cannot be directly restored without tension to the nerve ends still is the autologous nerve graft (figure 1A). Most commonly the sural nerve is used, taken from the leg of the patient. Obviously, repair with autografts has several disadvantages, such as the need for an extra incision, limited availability, mismatch in size of the damaged nerve and the donor nerve, and the chance for the development of a painful neuroma. Because of these disadvantages various alternatives have been developed for autograft repair, for instance repair with autogenous venous grafts [1], nerve allografts [2, 3], and nerve tubes, guides or conduits. Practical advantages of nerve tubes are the unlimited right off-the-shelf availability in different sizes that match the damaged nerve (figure 1B). Besides, functional recovery is often reduced after autograft repair compared with direct coaptation repair. Possible explanation for this is that axons need to cross 2 coaptation sites, which might decrease both the number of axons reaching the distal targets and lead to increased misdirection of regenerating axons [4]. An ideal alternative therefore will also lead to improved regeneration and functional results of nerve repair. In this review we give an overview of both the experimental and clinical data present on nerve tubes for peripheral nerve repair. In addition, different modifications to the common hollow or single lumen nerve tube are discussed that may improve the results of regeneration, including collagen/laminin-containing gels, internal frameworks, supportive cells, growth factors, and conductive polymers.

DEVELOPMENT OF NERVE TUBES

The concept of nerve tube repair

The first attempts of nerve tube repair date back to the end of the 19th century (for review see table 1 article by Weiss [5]). The results of these first attempts were disappointing and later viewed by Sunderland as only of historical interest [6]. The concept of the nerve tube was reintroduced in the 1980s, mainly as a tool to investigate the process of regeneration. In the beginning, mostly silicone tubes were used. Later, nerve tubes of also other synthetic non-biodegradable [7-11] and biodegradable materials (including polymers of glycolic acid [12], lactic acid [12, 13] and caprolactone) were developed. These first experiments with silicone nerve tubes by Lundborg et al. demonstrated that axons can successfully regenerate across a 1-cm gap in the rat sciatic nerve model [14]. No regeneration was observed in the absence of the distal nerve stump and across 15-mm defects. This was later explained by the accumulation of neurotrophic factors in the silicone chamber that probably only act over limited distance (neurotropism or chemotaxis). Another explanation might be that the formation of a fibrin matrix (Figure 2), which is essential in the process of regeneration [15], does not occur if the gap is too long [16].

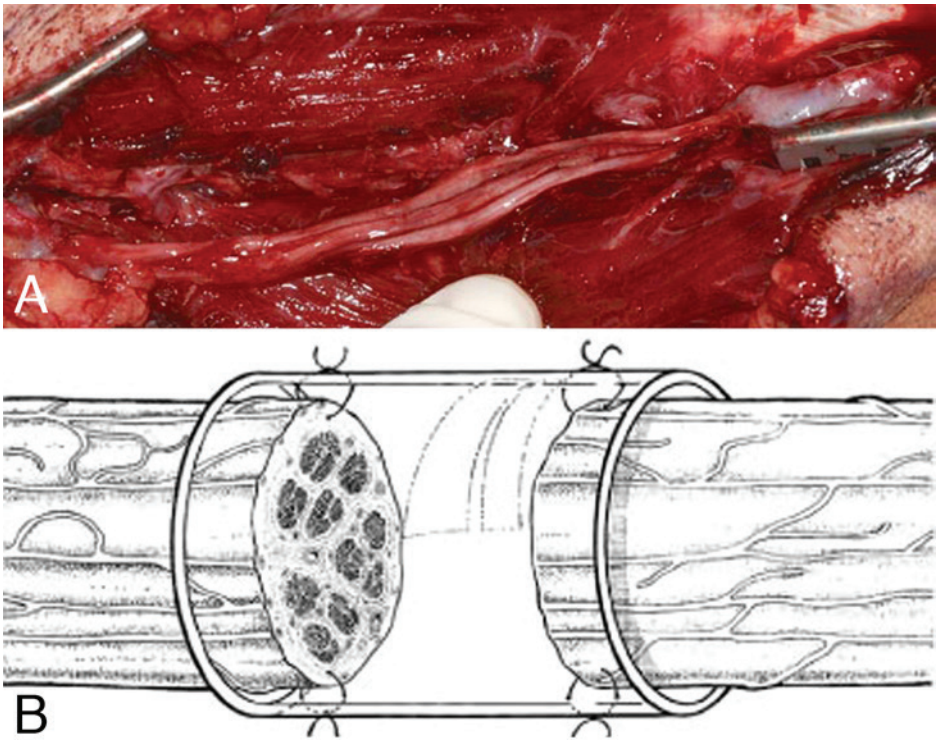

Figure 1

A: Repair of a radial nerve lesion (after a humerus fracture) with autologous sural nerve grafts.

B: Nerve tube repair. *From* Lundborg, G. A 25-year perspective of peripheral nerve surgery: evolving neuroscientific concepts and clinical significance. The Journal of Hand Surgery [Am] 2000; 25 (3): 391-414

Physical characteristics of the nerve tube

Other physical properties, including the dimensions of the nerve tube, prefiling with phosphate buffered saline (PBS) [17], and porosity [16] have also been shown to affect the formation of the fibrin matrix. Jenq and Coggeshall found that the addition of holes to silicone nerve tubes increased both the number of myelinated axons and the length of the gap that could be bridged [18, 19]. Possible explanations were that by adding holes, cells (for example macrophages and leucocytes) and molecules (for example fibrin and fibronectin) involved in the formation of the fibrin matrix could enter the site of regeneration. The importance of the permeability of the nerve tube was later confirmed in other experiments [20-24], although it still remains questionable what exactly is the ideal pore size (microporous or macroporous). Disadvantages of macropores might be that neurotrophic factors can diffuse out of the nerve tube and that the fibrin matrix might be disorganized (orientation perpendicular to the pores instead of longitudinal). It is important to note that permeability not only depends on pore size, but may also be affected by

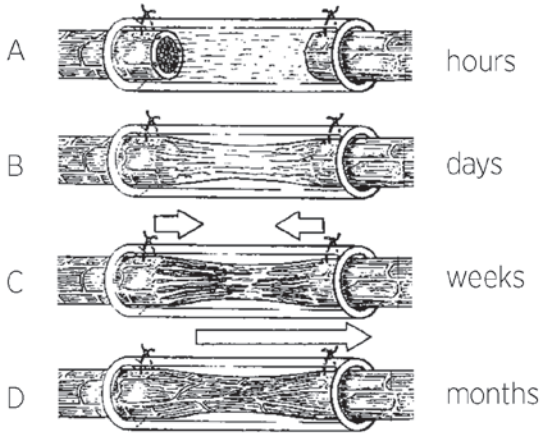

Figure 2
The different phases in the process of regeneration across the nerve tube. A: within hours after implantation the lumen fills with fluid containing neurotrophic factors and various inflammatory cells. B: within days a fibrin matrix is formed between the nerve stumps. C: in weeks Schwann cells, fibroblasts and microvessels migrate along the fibrin matrix from both proximal and distal nerve ends. D: in months axons regenerate from the proximal nerve stump into the matrix. *From* Dahlin and Lundborg. Use of tubes in peripheral nerve repair. Neurosurg Clin N Am 2001; 12 (2): 341 – 352

for example hydrophilic properties of the material. Next to porosity, the surface texture and dimensions of the nerve tube have been found to affect the formation of the fibrin matrix [8]; with smooth surfaces (for example in silicone nerve tubes) the longitudinal matrix coalesces and forms a free floating nerve cable, while with rough surfaces the tissue disperses and completely fills the lumen of the nerve tube [25].

With the potential use of nerve tubes for clinical nerve repair, especially biodegradable nerve tubes, other physical characteristics were also investigated, including swelling and degradation properties. Swelling of a nerve tube might primarily block the lumen for regeneration or secondarily lead to compression of the regenerated nerve. Degradation may cause swelling by the accumulation of degradation products that increase the osmotic value of the nerve tube [26, 27]. Besides, degradation products might be toxic or interfere with the process of regeneration. Degradation may also affect the porosity and tensile properties of the nerve tube. These tensile properties are important because a nerve tube should be flexible for implantation into mobile limbs, but at the same time the nerve tube should be resistant to deformation (elongation, breaking or kinking) and strong enough to hold a suture. Transparency is preferred for suturing and accurate positioning of the nerve stumps. In the end, nerve tubes must be sterilizable without compromising the physical properties mentioned above. In table 1 we have summarized the known physical properties of some of the frequently used nerve tubes. It must be

noted that physical properties of the nerve tube not only depend on the biomaterial, but also on other factors such as the dimensions of the nerve tube and fabrication technique. Not all nerve tubes that are now available for clinical use have been characterized extensively *in vitro* before clinical application.

Evaluation methods and animal models

Different evaluation methods and animal models have been used to investigate the process of regeneration across nerve tubes. Most experiments have been performed in the rat sciatic nerve model. Commonly used evaluation methods in this model include electrophysiology, nerve morphometry, and walking track analysis. The first most important observation however is the percentage successful regeneration across the nerve tube. Failures have been reported due to collaps, swelling, and suture pullout [12, 28-30]. The second most important observation is the quantity of regeneration across the nerve tube. This is mostly determined for the number of axons (myelinated and/or unmyelinated) at the middle part and/or distal to the nerve tube and is then preferably compared to both the numbers in normal nerve and after autograft repair. The numbers of axons that have been reported in the literature however differ [31]. Sometimes only the density of nerve fibers in a specified area is provided [32, 33] (table 1). This area may not be representative of the total cross-sectional area of the nerve. The total number of axons also is not the best parameter to quantify regeneration, because this number is increased early in the process of regeneration due to collateral sprouting or branching, and has been found to decrease later [34]. Different factors may stimulate the sprouting or branching of axons, for example the addition of Schwann cells [35-37] or neurotrophic factors (see part modified nerve tubes). Numbers may increase without an actual increase in the number of motoneurons and dorsal root ganglion cells from which axons have regenerated across the nerve tube. Quantification of regeneration across the nerve tube can therefore best be performed in our opinion with retrograde tracing to determine these numbers [38]. This technique with fluorescent dyes that are retrogradely transported to the motoneuron or dorsal root ganglion can also be used to analyze the accuracy of regeneration across the nerve tube. For example, different tracers can be applied sequentially to the same nerve branch before and after nerve repair to determine the direction of regenerating axons or simultaneously to different nerve branches (for instance the tibial and peroneal nerves) to determine the dispersion of regenerating axons across the nerve tube [39]. Although nerve tube repair is often suggested to lead to an improved orientation of regenerating nerve fibers, only a few studies have actually investigated the accuracy of regeneration across the nerve tube [38, 40-43]. These studies did not show an improved accuracy after nerve tube repair compared with direct coaptation or autograft repair. Brushart et al. found that regenerating axons might disperse across the tube and that this dispersion increases with gap length [44]. This dispersion of regenerating axons might lead to (1) misdirection of regenerating axons or (2) polyinnervation of different targets by axons originating from

Table 1
Experimental data

Nerve tube					Model			Evaluation		
Material	Permeability	Flexibility	Degradation	Swelling	Animal	Nerve	gap size	methods	control	follow-up
Natural										
collagen[1-3]	diffusion of molecules up to 215 Å			3x dry weight	monkeys	median	4, 5 mm	electrophysiology, number of axons	reversed autograft	up to 760 ds
					rats	sciatic	4 mm	electrophysiology	and normal, direct coaptation, and negative controls	4 and 12 wks
Synthetic										
Nonbiodegradable										
silicone [4]					rat	sciatic	6, 10, 15, 20 mm	nerve histology	absence distal nerve stump	1 mo
Biodegradable										
polyglycolic acid[5]					monkeys	ulnar	3 cm	electrophysiology, nerve fiber density	sural nerve grafts	1 yr
poly(L-lactic acid) [6]	83.5%, 12.1μm *1	*2 80 MPa, 1.0 MPa, 0.02 mm/mm	Mn 43% at 8 weeks		rats	sciatic	1 cm	SFI, gastrocnemius muscle weight, nerve fiber density	reversed isograft	16 wks
poly(DL-lactic co-glyclic acid) [7], 75:25	83%, 20μm *1	*2 8 MPa, 0.95 MPa, 0.02 mm/mm	Mn 38% at 8 weeks							

poly(L-lactide-co-6-caprolactone) [8, 9], 50:50	low and high *3	*4 3 MPa, 25 MPa, 490%	Mn 50% at 10 months		mice	sciatic	6 mm	electrophysiology, sweating tests, percentages successful reinnervation, number of axons	silicone Teflon, collagen, polysulfone	4, 5 mo
					rats	sciatic	8 mm	electrophysiology, simultaneous retrograde tracing, SFI	normal, autograft, silicone	90 ds
poly(DL-lactide-ε-caprolactone)[10-12], 50:50 (DL 85:15)		*5 2.5MPa	45% mass loss at 8 mo	300% vol increase at 3 mo	rats	sciatic	1 cm [11] 15mm [12]	nerve fiber density electrophysiology, video analysis	reversed autograft	10 wks 5 mo

* 1 porosity and mean pore size, measured by mercury porosimetry 7
* 2 modulus, tensile strength, and tensile strain 7, also tested during degradation in phosphate buffered saline (PBS) at 37ºC for up to 8 weeks
* 3 low prepared with fine powder of amylose (<10μm), high with glucose of around 10μm, permeability tested with UV spectrscopy
* 4 elasticity modulus, tensile strength, and percent elongation
* 5 tensile strength, rapid loss after 3 weeks 10

Abbreviations: ds =days, Mn = average molecular weight, mo = months, SFI = sciatic function index from walking track analysis (paper-paint or ink method), vol = volume, wks = weeks, yr = year

the same neuron. This compared to autograft repair that contains more regenerating branched axons inside the basal lamina tubes [45].

Functional analysis eventually is the most important method for translating results of nerve tube repair into patients. This type of analysis has not been frequently included in the evaluation of nerve tube repair. The reason for this might be that the most commonly used method, the sciatic function index (SFI), that is based on footprint analysis [46, 47], lacks sensitivity. This might be caused by contractures [48] and autotomy [49], but also because the SFI evaluates the distal foot muscles that often don't recover because of the prolonged time of denervation [50]. We developed a novel evaluation method, called 2D motion analysis, that can be used to measure recovery of more proximally located muscles from the ankle angles of maximum plantar and dorsiflexion during the stance and swing phases (**Chapter 3**). This method is more sensitive than the SFI and is currently being used by our laboratory for the functional analysis after different nerve repair techniques. Advantage of functional analysis in comparison to other evaluation methods also is that animals can be evaluated at multiple time points. Combined with electrophysiology this can provide insight in the time to reinnervation and recovery.

Electrophysiology is frequently included in the evaluation of results after nerve tube repair. Mostly compound muscle action potentials (CMAPs) are recorded and analyzed for the amplitude, area under the curve, or latency [31]. This method is not as time-consuming as most other evaluation methods, but it is important to note that it should not be used instead of functional evaluation. CMAP recovery after nerve repair may be relatively better than functional recovery due to distal sprouting that results in larger motor units, and due to misdirected axons that contribute to the CMAP, but probably not to recovery of function [51].

Different animal models have also been used for the analysis of nerve tube repair, including mice, rabbits, and monkeys (table 1). Disadvantage of this use of larger animal models is that it makes it difficult to compare the results between studies, especially for the extrapolation of the size of the nerve gap [52]. Obvious advantage of larger animals is the closer to human comparison, especially for the primate model. Both the polyglycolic acid (PGA) and collagen nerve tube (that are now available for clinical use, see below) have first been investigated experimentally in monkeys [33, 53, 54]. Dellon and Mackinnon in 1988 published the first study in which they compared repair of a 3-cm gap in the ulnar nerve (proximal to the elbow) in adult male *Macca cynomolgus* monkeys with sural nerve grafts, solid and mesh PGA tubes (8 repairs per group) [33]. After 1-year of follow-up nerve fiber densities did not differ from normal after the different repair techniques. Unfortunately, absolute numbers were not provided. Electromyography demonstrated recovery in 19 out of 28 (68%) of the intrinsic muscles studied in the solid and mesh tube groups (2 muscles per repair, 7 repairs per group). Recovery after autograft repair

was not reported because the Martin-Gruber anastomosis in this group had not been divided. Electromyography results were reported for 7 tube repairs, because in one case of solid tube repair there was no continuity (the reason for exclusion of one of the mesh tubes was not reported). In 3 out of 7 solid and 4 out of 8 mesh tubes some scar tissue was observed in the center of the tube. Later, the same authors published another study performed in monkeys, in which regeneration across 2 and 5-cm nerve gaps in radial sensory and ulnar nerves was compared for crimped and mesh glycolide trimethylene carbonate (Maxon) and collagen nerve tubes [55]. Poor regeneration was found across 5-cm nerve gaps.
Archibald et al. compared repair of 4 mm gaps with collagen nerve tubes and autografts (reversed segments) in rats (sciatic nerve) and *Macaca fasicularis* monkeys (median nerve, 2cm above the wrist) [53]. This study showed that collagen nerve tube repair was as effective as autograft repair in terms of physiological responses from target muscle and sensory nerves. Later they reported a second study on collagen nerve tube repair of 5-mm median nerve lesions (again 2cm above the wrist) in monkeys, which included 3 years of electrophysiologic assessment and nerve morphometry [54]. In this study a significantly increased number of axons distal to the repair site (1.2-2x) was found after both collagen nerve tube and autograft repair.

CLINICAL USE OF NERVE TUBES FOR PERIPHERAL NERVE REPAIR

Currently, various nerve tubes are available for clinical nerve repair: *Neurotube* (polyglycolic acid), *Neuragen* (collagen), *Neurolac* (polycaprolactone), *NeuroMatrix* and *Neuroflex* (both collagen), and *SaluBridge* (hydrogel, non-biodegradable) [57]. These nerve tubes are mainly used in the repair of small nerve gaps (<3cm) in small sensory nerves, such as digital nerve lesions, but they are also increasingly used in lesions of larger nerves [13]. In addition, recently a processed allograft (*Avance* from *AxoGen*) has become available for clinical use. Below we only discuss the results of the large series and randomized studies that have been reported on the clinical use of the silicone, polyglycolic acid (PGA), and poly(DL-lactide-e-caprolactone) (PLC) nerve tubes (summarized in table 2). In addition, series have been reported on the use of non-biodegradable polytetrafluoroethylene (PTFE) nerve tubes (Gore-Tex or Teflon) for median and ulnar nerve [56] and inferior alveolar/lingual nerve lesions[57, 58], a small series on the use of collagen (*Neuragen*) nerve tubes in the repair of obstetrical brachial plexus injuries [59], and a number of cases on the use of PGA nerve tubes (for the repair of the inferior alveolar nerve [60], medial plantar nerve [61], zygomatic and buccinatory branches of the facial nerve[62], the spinal accessory nerve [63], for nerve reconstruction after a hallux-to-thumb transfer [64], and for interfascicular median nerve repair with multiple PGA tubes [65]). Combinations of PGA tubes with collagen sponges [66, 67] and an interposed nerve segment [68] have also been used in patients, and a chitosan tube with internal oriented filaments of PGA [69] (see part modifications to the common hollow nerve tube).

Table 2
Clinical series and randomized controlled trials (RCT)

Nerve tube							Evaluation			
Material	First author (year)	Study type	Number(s)	Patient age (yrs)	Nerve(s), location	Gap size	Methods	Control	Interval	Follow-up period
Synthetic										
Nonbiodegradable										
Silicone	Lundborg (1997)	RCT	11 patients, 8 controls	12 – 72, mean 29	median and ulnar, <10cm proximal to wrist	3 – 4 mm	tactilometry for perception vibration, Semmes-Weinsteins monofilaments, s2PD and m2PD (Moberg's method), neuroma/ hyperesthesia,/ coldintolerance strength abduction dig I or II with intrinsicmeter	direct repair	no	1 year
	Lundborg (2004)	RCT	17 patients, 13 controls	12 – 72, mean 32		3 – 5 mm	Model Instrument for Outcome after Nerve Repair, neurophysiology			5 years

Biodegradable										
Polyglycolic acid	Mackinnon (1990)	series	15 patients, 16 repairs	30.5 (SD 7.6)	digital	0.5 – 3.0 cm, mean 1.7	s2PD and m2PD: excellent **: ≤6mm and ≤3mm good: 7-15mm and 4-7mm poor: ≥15mm and ≥7mm	no	none placed acutely	11 – 32 mo, mean 22.4
	Weber (2000)	RCMT	62 repairs, 74 controls	17 – 65, mean 35	digital, distal to wrist	7.0 mm *, control 4.3		gap <8 mm direct repair gap >8 mm nerve graft	50 <72 hrs 5 4-20 days 7 > 20 days	mean 9.4 mo, control 8.1
	Battiston (2005)	series	19	15 – 67, mean 40	digital	1.0 – 4.0 cm, mean 2.0	s2PD and m2PD, MRC, quick-DASH	muscle-vein combined conduits	primary - 16 months	6 – 74 mo, mean 30
poly(DL lactide-ε-caprolactone)	Bertleff (2005	RCMT	21, 13 controls	mean 43	digital, distal to wrist	up to 2.0 cm	s2PD and m2PD	direct repair	not provided	1 year

* a gap of 5 mm was left intentionally, even in defects of 0 - 4 mm

** in Weber study outcome was defined for the lower of the s2PD or m2PD, as defined in the study by Mackinnon and Dellon, except that excellent outcome was defined for m2PD ≤ 4 mm.

Abbreviations: s2PD and m2PD = static and moving two-point discrimination , MRC = strength for British Medical Research Council Scale, RCMT = randomized controlled multicenter trial, , quick-DASH (disabilities of the arm, shoulder, and hand), mo = months, EP = electrophysiology

Silicone nerve tubes

In 1997 Lundborg et al. published their first results with 1-year follow-up of a prospective randomized study, in which small defects (3-4 mm) after fresh and complete clean-cut transection of the ulnar and median nerves proximal to the wrist (up to 10 cm) were repaired with silicone nerve tubes (11 patients) or conventional microsurgical direct coaptation repair (8 patients) [70]. A number of tests were used to evaluate the results (table 2). In general, no significant differences were found between the two types of repair. Also for the 5-year follow-up (2004) no significant difference in outcome was found, except that there was significantly less cold intolerance after silicone nerve tube repair [71]. The use of silicone nerve tubes however has been heavily criticized [72, 73], mainly because of the potential late compression of the nerve by the non-biodegradable tube. Critics often refer to a study by Merle et al. [74], in which silicone tube (1 patient) and sheath repair (2 patients) resulted in chronic nerve compression. Later also a study by Braga-Silva was reported on silicone nerve tube repair of median and ulnar nerve lesions (up to 3cm) in which 7 out of 26 patients requested removal of the nerve tube because of local discomfort [75]. Dahlin and Lundborg themselves performed a re-exploration surgery in 7 patients, as an ethically permitted part of their prospective study (4 patients complained of local discomfort), but found no signs of neuroma and only a mild microscopic foreign body reaction in 2 cases [76]. After removal of the silicone nerve tube, there was no new impairment of nerve function. They emphasized that in their studies silicone nerve tubes were used with a diameter exceeding the diameter of the nerve by at least 30%. Nevertheless, they acknowledged that a biodegradable nerve tube would be better, provided that it degrades with minimal tissue reaction and without impairment of nerve regeneration [77].

Polyglycolic acid (PGA) nerve tubes

In 2000 Weber et al presented the results of the first multicenter randomized study on the repair of digital nerves with gaps up to 3cm using glycolic acid (PGA) nerve tubes. Ten years before that Mackinnon and Dellon had already presented a series of 15 patients in which they had also used polyglycolic acid (PGA) nerve tubes to repair digital nerve defects up to 3 cm [78]. In that study excellent results were reported for 5 patients (33%), good results for 8 patients (53%) and poor results for 2 patients (14%). In the randomized study by Weber et al., PGA nerve tube repair was compared with standard repair (direct coaptation for gaps <8 mm and nerve graft repair for gaps >8 mm). The overall results at 1-year follow-up showed no significant difference between the two groups with excellent and good outcome in respectively 44% and 30% of the repairs with PGA nerve tubes compared to 43% of both excellent and good outcome after standard repairs. The authors subsequently performed a subgroup analysis for different gap lengths (≤ 4 mm, 5 to 7 mm, and 8 mm to 3 cm) that demonstrated excellent results for gaps ≤ 4 mm for moving 2-point discrimination (m2PD) in 91% of PGA nerve tube repairs compared to 49% of standard repairs (p=0.02). As commented by Lund-

borg in the discussion on this article, the statistics of this study are difficult to interpret because of the heterogeneous data (for example different levels of injury and mechanisms of injury were included). Also, the numbers per group of PGA nerve tube and standard repair for subgroup analysis were not provided. It is not clear also why separate subgroup analysis was performed for gaps ≤4 mm. Although the authors mention that it is generally accepted that 4 mm is the maximum gap length for digital nerves to be repaired with minimal tension by the end-to-end method, in the standard repair group all gaps of 5-7mm were repaired by direct coaptation. In the 5-7mm gap group excellent results were obtained in only 17% of the PGA nerve tube repairs and 57% of the standard repairs (p=0.06). Noteworthy, the technique that was used to measure two-point discrimination that was not based on the Moberg approach [79] with application of very light pressure (just enough to blanch the skin), but with increasing pressure until the stimulus was perceived by the patient (see discussion by Lundborg).

Another large series on PGA nerve tube repairs of 19 digital nerves in 17 patients with gaps up to 4 cm was published in 2005 by Battiston et al.[80]. In this study very good results (S3+ and S4, defined for static 2-point discrimination (s2PD) up to 15 mm, were reported for 13 patients (76.5%) and good results in 3 patients (17.7%). Analysis of the data however shows that in only 2 patients S4 (s2PD 2–6 mm) was obtained and that there were no excellent results for m2PD (≤ 3 mm, by the definition used in the studies by Mackinnon [78] and Weber [81], see table 2), and good results were obtained (m2PD 4 – 7 mm) in only 4 out of 19 repairs.

In conclusion, PGA nerve tubes might lead to comparable results as conventional nerve repair in the repair of small gaps in digital nerve lesions, but care should be taken with the interpretation of the data and the wide application to the repair of other nerve lesions based on these results.

Poly(DL-lactide-ε-caprolactone) (PLC) nerve tubes

In 2003 Bertleff et al. presented the results of a multicenter trial in which digital nerve repair for gaps up to 2 cm was compared for polylactide caprolactone (PLC) nerve tube and standard repair, which were all direct coaptation repairs (with the finger flexed to reduce tension) [82]. Randomization was performed separately for gaps ≤ 4 mm, 4–8 mms, and 8–20 mms. Sensory recovery was evaluated at 3, 6, 9 and 12 months for the s2PD and m2PD measured with the Pressure-Specified Sensory Device [83]. There were no significant differences in two-point discrimination for PLC and direct coaptation repair of gaps up to 2 cm, but unfortunately results for subgroup analysis were not provided. The pressure, which was applied (to feel the stimulus), seemed larger in the PLC nerve tube repair group than in the direct repair group (figure 6, no statistics provided). More wound healing problems were observed after PLC nerve tube repair than after direct coaptation. In a recent review Meek et al. also commented that small fragments of biomaterial in

experiments with PLC nerve tubes were still found 24 months after implantation and that PLC nerve tubes are normally stiff and only flexible after putting in warm saline before implantation [84]. A more extensive report on the use of PLC nerve tubes (according to the authors) will soon be published [84]. So far there is ample evidence to support the clinical use of PLC tubes.

In conclusion, in our opinion at this moment care should be taken with the wide use of tubes in peripheral nerve repair, not only because of the concerns that are mentioned above, but also because of the following reasons. First, little is still known about the accuracy of regeneration across nerve tubes. In the repair of larger mixed or motor nerves dispersion of regenerating axons across the nerve tube may lead to misdirection and polyinnervation (see part on development of nerve tubes) and result in impaired functional recovery due to for example co-contraction or synkinesis. It must be noted also that in most experimental studies on nerve tube repair accuracy of regeneration and functional analysis were not included. Finally, it must be noted that not all nerve tubes that are now available for clinical use have been characterized extensively *in vitro* and that long-term effects of biodegradable nerve tubes have not (yet) been reported (table 2, follow-up studies 1-2 years).

MODIFICATIONS TO THE SINGLE LUMEN NERVE TUBE

Different modifications to the common hollow or single lumen nerve tube have been investigated to enhance regeneration and extend the gap that can be bridged (figure 3 on page 77). Pre-filling of the nerve tubes with phosphate buffered saline (PBS) and the addition of pores have already been mentioned in the section on the development of nerve tubes. Below we discuss the addition of different extracellular molecules (collagen and laminin), internal frameworks, supportive cells, and nerve growth factors.

Collagen and laminin containing gels

Collagen and laminin are involved in the process of regeneration by forming a substrate for the migration of nonneuronal cells. Filling of silicone nerve tubes with collagen and laminin-containing gels has been shown to increase both the rate of regeneration [11] and the gap that can be bridged (up to 15-20 mm) [85]. This effect however depends on several factors including the concentration [86] and the permeability of the nerve tube [87]. Alignment of the collagen (gravitational or magnetically) may also further enhance regeneration [88]. Currently, different collagen and laminin containing gels (for example BD Matrigel ™) are being used for the incorporation of supportive cells and growth factors [37, 89, 90]. Also, oligopeptides derived from lamini-integrin active sites (such as YIGSR, IKVAV and RGD) are being investigated for potential role in guidance of regenerating axons [91].

Internal framework

An internal framework may also enhance regeneration and increase the gap that can be bridged due to stabilization of the fibrin matrix that is formed inside the nerve tube. Different internal structures have been investigated including polyamide filaments [92], laminin-coated fibers [93], PGA filaments [94] and collagen sponges [93, 95]. The combinations PGA tube - collagen sponge and chitosan tube - PGA filaments have already been used clinically, although there is little information on the effect of these internal structures on the accuracy of regeneration. Different tissues have also been added to the nerve tube, for example interposed nerve segments [96] (the stepping-stone procedure) and denatured muscle [97]. In addition, nerve tubes with a modified microarchitecture have been developed. Yoshii et al. developed a scaffold of longitudinally orientated collagen filaments that has been shown to lead to successful regeneration across gaps of 20 mm [98] and even 30 mm in rats [99]. Another example of a modification to the common single lumen nerve tube structure is the multichannel nerve tube structure [27, 100-103]. This structure has several advantages: it provides more surface area for cell attachment and controlled-release of incorporated growth factors, and may reduce dispersion by containment of axonal branches as in the autografts consisting of multiple basal lamina tubes [39].

Supportive cells

The addition of Schwann cells to the nerve tube has also been found to enhance regeneration in small gaps [36, 37, 89] and to extend the gap that can be bridged to about 2 cm [35, 90], although remarkably, autograft repair in most of these studies still was found to be superior [37, 89, 90, 104, 105]. Schwann cells possibly stimulate regeneration by the production of a range of growth factors, extracellular molecules (laminin), and may play a mechanical role by forming a cable bridging the gap [37]. Schwann cells can also be genetically modified to overexpress certain growth factors and selectively guide different types of axons. A disadvantage of the addition of Schwann is that it still requires the explantation of a donor nerve, to isolate autologous Schwann cells weeks before reconstruction. This may be overcome in the future by the differentiation of for example bone marrow stem cells into Schwann cells [106].

Growth factors

The addition of different growth factors to the nerve tube, including nerve growth factor (NGF), glial cell derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), and fibroblast growth factor (FGF), has also been shown to enhance regeneration and increase the nerve gap that can be bridged (to 15 mm). Growth factors can be added directly to the tube (into a solution) [107] or can be released after absorption to fibronectin mats [108, 109], collagen matrices [30], bovine serum albumin or from delivery systems such as subcutaneous minipumps[110] or microspheres that are incorporated during the fabrication pro-

cess of the nerve tube [111, 112]. The advantage of growth factors in comparison to Schwann cells is that no extra procedure is needed. The advantage of delivery of growth factors from microspheres is the potential for controlled release over an extended period of time without leakage from the tube.

Conductive polymers

Finally, conductive polymers may also enhance regeneration across the nerve tube. Aebischer et al. found significantly increased numbers of myelinated axons after repair with poled versus unpoled polyvinylidene fluoride (PVDF) tubes [113] possibly by accelerated axonal elongation on the charged surface. Schmidt et al. found an almost twofold neurite outgrowth *in vitro* on conductive polypyrrole films after electrical stimulation [114].

CONCLUSION

In this review we provided an overview of the experimental and clinical data currently available on nerve tubes for peripheral nerve repair. At present there is no sound scientific proof of the superiority of the empty hollow biodegradable nerve tubes that are now clinically used as compared to direct coaptation or autograft repair. The repair of all sorts of nerve lesions may lead to unnecessary failures and again a discontinuation of interest in the concept of the nerve tube. The extensions of the applications, especially in the repair of larger mixed or motor nerves, should be carefully evaluated. Also, although the autologous nerve graft has several practical disadvantages, it is important to realize that it still has a number of advantages, such as the presence of Schwann cells that secrete growth factors and basal lamina tubes that contain regenerating axons, besides the favorable properties of natural strength and flexibility of the nerve, and the fact that it is immunocompatible. Eventually, different modifications to the single lumen nerve tube might lead to a nerve tube that is a better alternative than autologous nerve graft repair.

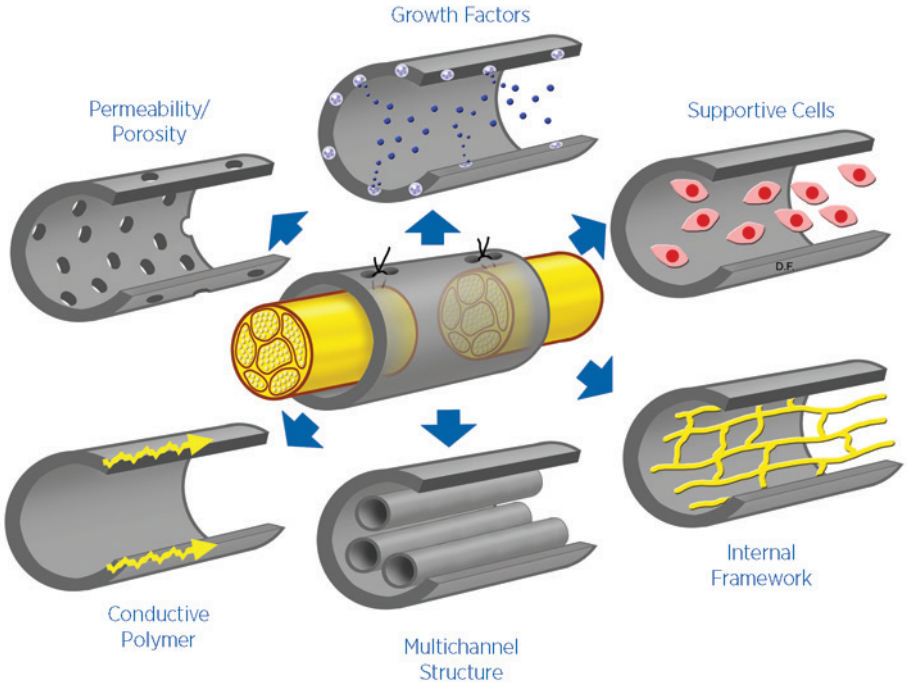

Figure 3

Modifications to the single lumen nerve tube. *Modified from* Hudson TW, Evans, GR, Schmidt, CE. Engineering strategies for peripheral nerve repair. Clin Plast Surg 1999; 26: 617 – 62

Table 3

Modifications to the single lumen nerve tube

Collagen and laminin-containing gels/ solutions										
	First author (yr)	Nerve tube	Gel/ solution	Animal	Nerve	Gap size (mm)	Methods	Groups/ controls	Follow-up	Most important result(s)
laminin	Madison (1985)	poly-D,L-lactates	Matrigel *	mice	sciatic	4 to 5	HRP-labeling	empty tubes	2 weeks	labeled neurons in all animals with laminin containing gels, none in empty
collagen, laminin	Valentini (1987)	semiperme-able PVC		mice	mouse	4	NM	semipermeable tubes with saline	12 weeks	fewer axons in gel-filled semipermeable tubes
collagen	Madison (1988)	silicone	Vitrogen *, laminin-gel,	rat	sciatic	15, 20	HRP-labeling, distance regen-eration	empty tubes	16 weeks	2x increase in maximum distance of axonal elongation
collagen, laminin	Labrador (1998)	silicone	Vitrogen *, Matrigel **	mouse	sciatic	4, 6	sweating test, pinprick, CMAP, CNAP	diff con, PBS, hyaluronate gel, plasma	up to 4 months	higher levels of target reinnervation with diluted gels
aligned colla-gen, laminin	Verdu (2000)	silicone	Vitrogen *, Matrigel **	mouse	sciatic	6	sweating test, pinprick, CMAP, CNAP	horizontal/ vertical polymerization, magnetic***	up to 4 months	higher number of MF for magnetically aligned gels
laminin-fibronectin coated fibers	Tong (1994)	collagen	collagen fibers	rat	sciatic	10	NM, NAP	uncoated collagen fibers	2 months	more MF and UMF in laminin-fibronectin coated fiber tubes

* Matrigel: solubilized basement membrane preparation containing laminin, type IV collagen, heparane sulfate proteoglycans, entactin, nidogen, and trace amounts of growth factors (12mg/ml), obtained from EHS sarcoma

* Vitrogen: solution of purified bovine dermal collagen, containing 95-98% type I collagen,

*** magnetic induction by placement of filled tubes with longitudinal axis perpendicular to a 9 Tesla magnetic field for 2 hours at 37 ^{0}C

Abbreviation: CMAP = compound muscle action potentials, CNAP = compound nerve action potentials, conc = concentrations, diff = different, HRP = horseradish peroxidase, NAP = nerve action potential, NM = nerve morphometry, MAP = muscle action potential, MF = myelinated fibers, no = number, PBS = phosphate buffered saline, PGA = polyglycolic acid, PLA = poly-D,L-lactic acid, PVC = polyvinylchloride acrylic copolymer

Intrinsic frameworks										
	First author (yr)	Nerve tube	material frame-work, number	Animal	Nerve	Gap size (mm)	Methods	Groups/ controls	Follow-up	Most important result(s)
filaments	Lundborg (1996, 1997)	silicone tubes	polyamide, 8	rat	sciatic	10, 15	NM, NF staining, pinch reflex	empty tubes, no repair	4 weeks	response to pinch and positive staining for NF distal to tube in all cases filaments, none in empty tube
filaments	Yoshii (2003)	no tube	collagen, 2000, 4000	rat	sciatic	20, 30	NM, presence ankle flexion	autograft, collagen tube	8 and 12 weeks	regeneration across 20 and 30mm gaps, more MF for 4000 filaments
sponge	Nakamura (2004)	PGA tube	collagen sponge	dog	peroneal	15	MEP, CNAP	autograft	up to 6 months	shorter latency, larger peak voltage and larger MF for PGA-collagen tubes
muscle tissue	Meek (2001)	PLC	denatured muscle	rat	sciatic	15	NM	non-operated side	up to 12 weeks	regeneration across 15mm gap in all cases
multichannel	de Ruiter (2008)	75:25 PLGA	7	rat	sciatic	10	NM, CMAP, muscle fiber size and type, sim and seq tracing	single lumen tubes, autograft, normal	8 and 12 weeks	tendency to reduce dispersion no MN and MF not significantly different from single lumen nerve tubes

Abbreviations: CMAP = compound muscle action potentials, CNAP = compound nerve action potentials, MEP = muscle-evoked potentials, MF = myelinated fibers, MN = motoneuron, NF = neurofilament, no = number, PGA = polyglycolic acid, PLC = poly(DL-lactide-ε-caprolactone, PLGA = poly(lactic co-glycolic acid), sim = simultaneous, seq = sequential

Schwann cells (SC) *										
	First author (yr)	Nerve tube	Suspension	Animal	Nerve	Gap size (mm)	Methods	Groups/ controls	Follow-up	Most important result(s)
syngeneic (F), heterologous (CD) adult	Guénard (1992)	PAN/PVC, permeable	Matrigel	rat	sciatic	8	NM	different dens: (F) 40, 80, 120 x10^6, (CD) 80 x 10^6, Matrigel, empty, autografts	3 wks	more MF for high dens SC more MF for syngeneic than for heterologous SC
syngeneic adult	Ansselin (1997)	collagen	RPMI 1640 **	rat	sciatic	18	NM, SFI, CMAP	different densities: >or< 5 x 10^5, PBS	6 months	more MF for high dens SC
Fluoro-gold labeled, syngeneic	Kim (1994)	collagen	collagen gel	rat	sciatic	10	NAP	collagen gel alone, sural nerve grafts	60 and 120 days	NAP and no MF equal for SC and sural grafts, but lower for collagen alone
syngeneic, isogeneic, autologous adult	Rodriguez (2000)	PLC, permeable	Matrigel	mouse	sciatic	6	NM, CMAP, CNAP, sweating, pinprick	syngeneic, isogeneic, autologous SC, autograft	4 months	best results for autologous SC

allogeneic neonatal	Evans (2002)	PLLA, permeable	collagen (Vitrogen)	rat	sciatic	10	NM, SFI, gastroc muscle weight	diff dens: 10^4, 10^6, isograft, collagen, silicone	4 months	MF density in distal nerve in all groups lower than for isografts
syngeneic/ isogeneic neonatal	Sinis (2005)	TMC/CL, permeable	Matrigel	rat	median	20	grasping test, FDS muscle weight, CMAP	normal, autograft, empty	9 months	regeneration only after repair with autograft or nerve tube with SC
isogeneic	Sinis (2007)	TMC/CL, permeable	Matrigel	rat	median (cross-chest)	40	Idem above	non-operated, autograft	12 months	no regeneration across nerve tubes with SC

* syngeneic = from the same strain, isogeneic = from the same litter, allogeneic = same species, but genetically different (although in study by Evans et al Schwann cells were harvested from Sprague-Dwaley rats and implanted into Lewis rats), heterologous = from different species, autologous = from the same animal

** RPMI 1640 medium containing 1.25 U Dispase/ml, 0.05% (wt/vol) collagenase, and 0.1% hyaluronidase

Abbreviations: CD = outbred Sprague Dawley rats, CMAP = compound muscle action potential, dens = density (SC/ml), F = inbred Fisher rats, FDS = flexor digitorum superficialis, gastroc = gastrocnemius, MF = myelinated fibers, NAP = nerve action potential, NM = nerve morphometry, no = number, PAN/PVC = acrylonitrile vinylchloride, PLC = poly(lactide-co-ε-caprolactone), PLGA = poly(lactic co-glycolic acid), PLLA = poly(L-lactic acid), subcut = subcutaneous, SC = Schwann cells, TMC/CL = trimethylenecarbonate-co-epsilon-caprolactone

Growth factors										
	First author (yr)	Nerve tube	carrier/ delivery system	Animal	Nerve	Gap size (mm)	Methods	Groups/ controls	Follow-up	Most important result(s)
NGF	Hollowell (1990)	silicone	saline solution	rat	sciatic	8	NM, HRP-labeling	solution cyt C	10 weeks	no MN and DRG not significantly different
	Derby (1993)	silicone and semiperme-able polysulfone	solution	rat	sciatic	7-8 and 12-13	NM (MF and UMF), behavorial tests	solution cyt C	10mm: 3, 4, 8 wks 15mm: 6 months	'head start': at 3 wks 3x more MF, at 4 wks no difference
	Whitworth (1996)	no tube, rolling of mat	fibronectin mats	rat	sciatic	10	immunostaining, NM	plain mats	up to 60 days	increased penetration distance, slightly greater no MF in distal nerve at 60 days
	Santos (1998)		subcutaneous minipump							
	Lee (2003)	silicone	fibrin-based	rat	sciatic	13	NM	diff conc (5, 20, 50ng/ml), empty, fibrin, isograft	6 weeks	no MF mid and distal to tube not sign diff from isograft for 20 and 50 ng/ml
	Xu (2003)	PPE	microspheres	rat	sciatic	10	NM, reflex response	silicone, saline, BSA microspheres	3 months	more MF in the distal nerve compared to all repair groups
GDNF and NGF	Fine (2002)	EVA	BSA	rat	sciatic	15	NM, FG labeling	BSA alone	47 days, 42 days	no MF: GDNF 4942, NGF 1199, BSA 5 no MN: GDNF 98.1, NGF 20.0, BSA 0 no DRG: GDNF 22.7, NGF 3.3, BSA 0

GDNF and NT-3	Barras (2002)	EVA	BSA	rat	facial	8	NM, FG labeling	BSA alone	6 weeks	no MN: GDNF 981, NT-3 53, BSA 0
BDNF, NGF, and NT-3	Bloch (2001)	EVA	BSA	rat	dorsal root	4	NM (MF and UMF)	BSA alone	4 weeks	no MF (mid tube): BDNF 863, NGF 1843, NT-3 1495, control BSA 293
BDNF and NT-4	Simon (2003)	no tube, rolling of mat	fibronectin mats	rat	sciatic	10	soleus and EDL muscle weight and fiber type	plain mats	120 days	NT-4 reversed soleus mass loss by restoring type I muscle fiber proportion and diameters
NT-3	Sterne (1997)	no tube, rolling of mat	fibronectin mats	rat	sciatic	10	immunostaining, NM	plain mats	up to 8 months	max effect at 15 days with increased penetration distance, greater no MF in distal nerve at 8 months
FGF	Midha (2003)	PHEMA-MMA	collagen gel*	rat	sciatic	10	NM	autograft, collagen gel, empty	8 weeks	no MF in distal nerve comparable to autograft and higher than other groups

* Vitrogel (see collagen and laminin-containing gels)

Abbreviations: BSA = bovine serum albumin, conc = concentrations, Cyt = cytochrome, diff= different, DRG = dorsal root ganglion cells, EDL = extensor digitorum longus, EVA = ethylene-vinyl acetate copolymer, FG = fluorogold, MF = myelinated fibers, MN = motoneurons, PHEMA-MMA = poly(2-hydroxyethyl methacrylate-co-methyl methacrylate), sign = significantly, UMF = unmyelinated fibers

Conductive polymers									
	First author (yr)	Nerve tube	Animal	Nerve	Gap size (mm)	Methods	Groups/ controls	Follow-up	Most important result(s)
PVDF	Aebischer (1987)	poled PVDF	mouse	sciatic	4	NM	unpoled	4, 12 weeks	more MF in poled vs unpoled tubes

Abbreviation: MF = myelinated fibers PDVF = polyvinylidene fluoride

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