



UNIVERSITÀ DEGLI STUDI DI TORINO

This is an authorversion of the contribution published on:

Questa è la versione dell'autore dell'opera:

Int Rev Neurobiol. 2013;108:35-57. doi: 10.1016/B978-0-12-410499-0.00002-2.

Review.

The definitive version is available at:

La versione definitiva è disponibile alla URL:

<http://www.sciencedirect.com/science/article/pii/B9780124104990000022>

Tissue Engineering and Peripheral Nerve Reconstruction: An Overview

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Abstract

Nerve repair is no longer regarded as merely a matter of microsurgical reconstruction. To define this evolving reconstructive/regenerative approach, the term tissue engineering is being increasingly used since it reflects the search for interdisciplinary and integrated treatment strategies. However, the drawback of this new approach is its intrinsic complexity, which is the result of the variety of scientific disciplines involved. This chapter presents a synthetic overview of the state of the art in peripheral nerve tissue engineering with a look forward at the most promising innovations emerging from basic science investigation. This review is intended to set the stage for the collection of papers in the thematic issue of the International Review of Neurobiology that is focused on the various interdisciplinary approaches in peripheral nerve tissue engineering.

The higher regeneration potential of the peripheral nervous system is at the basis of the usually higher degree of recovery after peripheral nerve trauma provided that the continuity of the nerve is maintained or, if lost, adequately reconstructed (Geuna, Fornaro, Raimondo, & Giacobini-Robecchi, 2010; Geuna et al., 2009; Raimondo et al., 2011). However, complete recovery is only occasionally achieved after a nerve lesion and, in many cases, the clinical outcome is rather unsatisfactory (Battiston, Raimondo, et al., 2009; Siemionow & Brzezicki, 2009). Today, there is a growing consensus that further improvements in peripheral nerve repair and regeneration are no more a matter of developing new microsurgical tools and techniques, but rather one of a multitranslational regenerative medicine approach aimed at reaching a new level of innovation that brings together different scientific disciplines. The aim of this chapter is not to carry out an extensive review of the enormous number of papers published on nerve repair and regeneration, but rather to provide an overview of the state of the art in peripheral nerve tissue engineering with a look forward at the most promising innovations emerging from the recent advancements originating from basic and clinical research in the main scientific disciplines involved: reconstructive microsurgery, transplantation, biomaterial science, physical therapy, and pharmacotherapy.

1. RECONSTRUCTIVE MICROSURGERY

Reconstructive microsurgery is the key discipline among the various ones that have enriched the world of peripheral nerve tissue engineering over the recent years. In fact, the surgeon is the key ring of the chain that brings scientific and technological innovation to the patient's bed. Yet, the surgeon should participate in the design of the basic science experiments in order to optimize the whole process of research and development (Battiston, Papalia, Tos, & Geuna, 2009). Although surgical nerve reconstruction has been attempted since the ancient times (Battiston, Papalia, et

al., 2009), its main improvements have been made over the last few decades (Siemionow & Brzezicki, 2009). Techniques for microsurgical nerve reconstruction include direct suture (end-to-end neurorrhaphy), neurolysis, nerve autografts, and nerve transfers (Siemionow & Brzezicki, 2009). Particularly noteworthy is the latter surgical approach, which has seen widespread application over the very recent years (Teboul, Kakkar, Ameer, Beaulieu, & Oberlin, 2004; Tung & Mackinnon, 2010; Zhang & Gu, 2011) and has widened the surgical options in the repair of very severe nerve traumas, including brachial plexus lesions. Microsurgical techniques for nerve repair have improved very much, making it possible to foresee that further improvement in peripheral nerve tissue engineering would not depend mainly on a further implementation of the single surgical techniques; nonetheless, improvement might still be achieved from technological innovation and the development of new reconstructive procedures. For instance, the use of glue instead of nerve suturing is very promising since experimental studies in animal models have indicated that its performance might be equal, or even superior, to epi/peri-neurial microsuturing (Sameem, Wood, & Bain, 2011; Whitlock et al., 2010). Another area of potential technological advancement is represented by robot-assisted surgical reconstruction (Liverneaux, Nectoux, & Taleb, 2009; Nectoux, Taleb, & Liverneaux, 2009; Zorn et al., 2008) although the use of robots in peripheral nerve reconstruction is still low in comparison to other surgical fields. Results from experimental studies on robotic nerve reconstruction are very encouraging (Latif et al., 2008) and it can be foreseen that robot-assisted technologies will be favored more and more by peripheral nerve surgeons over the next years. Finally, development of innovative microsurgical acts and techniques can also be foreseen and the history of the last decades teaches us that progress can be derived from revisiting and/or modifying an old surgical technique, rather than by a complete innovation. The history of end-to-side neurorrhaphy is an example since this surgical technique had already been described in the eighteenth century (Papalia et al., 2007) and was rediscovered by Viterbo, Trindade, Hoshino, and Mazzoni Neto (1994); today, it represents an interesting innovation in peripheral nerve repair (Geuna, Papalia, & Tos, 2006; Papalia et al., 2003).

2. TRANSPLANTATION

Among the different pillars of tissue engineering, transplantation is definitely the approach that is drawing the most interest in regenerative medicine. While at the beginning transplantation strategies were based on whole organ transplantation, today they are evolving to more sophisticated approaches based on the employment of only parts of an organ (tissue transplantation), or even single-cellular (cell transplantation) or sub-cellular constituents (gene transfer).

2.1. Organ/tissue transplantation

Organ/tissue transplantation for peripheral nerve gap repair is represented by autografts, that is, the transplantation of an autologous nerve segment harvested from the sacrifice of another “less precious nerve.” Nerve autografts were introduced by Millesi (1981) and Millesi, Meissl, and Berger (1972), on the basis of the evidence that suturing the nerve stumps under tension hinders nerve regeneration and represents the “gold standard” for nerve gap bridging (Siemionow & Brzezicki, 2009). However, the harvesting of a healthy nerve represents a clear limitation of this

technique, and therefore alternative nerve conduits have been sought over the last decades. Veins are the most commonly used biological alternative to nerve autografts, in clinical practice as well (Terzis & Karypidis, 2009). This type of tissue autotransplantation had been introduced as early as 1909 by Wrede (1909), who reported functional recovery after reparation of the median nerve by means of a 45-mm-long vein tube. The interest in this surgical technique revived with the clinical studies by Chiu and Strauch (1990) and Walton, Brown, Matory, Borah, and Dolph (1989) who showed that sensory nerve repair by vein autografts may lead to satisfactory return of sensibility comparable to the nerve grafting technique and, since then, vein conduits have seen a discrete spread among nerve surgeons (Chiu, 1999). Another alternative to nerve autografts that has received attention among surgeons is the use of skeletal muscle guides (Fawcett & Keynes, 1986; Keynes, Hopkins, & Huang, 1984; Kong, Zhong, Bo, & Zhu, 1986). This technique, which was first reported in 1940 (Kraus and Reisner, 1940), finds its rationale in the similarities between the muscle basal lamina and the endoneurial tubes of degenerating nerves that guide Schwann cell (SC) migration and axonal regrowth (Fawcett & Keynes, 1986). Various experimental studies showed that both fresh and denatured muscle conduits have the potential for bridging peripheral nerve defects (Meek & Coert, 2002; Mligiliche, Tabata, Endoh, & Ide, 2001), and clinical studies showed that muscle grafts are effective in obtaining some degree of functional recovery in most patients (Fawcett & Keynes, 1986; Norris, Glasby, Gattuso, & Bowden, 1988; Pereira, Bowden, Gattuso, & Norris, 1991; Pereira, Bowden, Narayanakumar, & Gschmeissner, 1996; Pereira, Palande, et al., 1991; Rath, 2002). Since the effectiveness of both vein and muscle grafts is limited to short nerve gap repair, because long vein segments tend to collapse while regenerated axons tend to grow outside long muscle grafts without reaching the distal nerve stump (Battiston, Tos, Cushway, & Geuna, 2000; Battiston, Tos, Geuna, Giacobini-Robecchi, & Guglielmone, 2000), the possibility of combining the two approaches, that is, filling up vein tubes with muscle fibers, has been explored (Brunelli & Brunelli, 1993). This muscle-vein combined technique for nerve reconstruction has been extensively investigated in experimental models (Fornaro, Tos, Geuna, Giacobini-Robecchi, & Battiston, 2001; Geuna, Tos, Battiston, & Giacobini-Robecchi, 2004; Raimondo et al., 2005; Tos et al., 2007) over the last two decades and papers reporting its successful clinical employment in both sensory and mixed nerves (also in the case of gaps longer than 30 mm) have already been published (Battiston, Geuna, Ferrero, & Tos, 2005; Battiston, Tos, Cushway, et al., 2000; Battiston, Tos, Geuna, et al., 2000; Marcoccio & Vigasio, 2010; Tos, Battiston, Ciclamini, Geuna, & Artiaco, 2012). It can thus be expected that its use with patients will increase over the next years. Finally, the use of acellularized nerve allografts is receiving much attention because of the ability of these conduits to bridge large nerve defects (Glaus, Johnson, & Mackinnon, 2011; Rivlin, Sheikh, Isaac, & Beredjiklian, 2010; Stefanescu, Jecan, Badoiu, Enescu, & Lascar, 2012). Very recently (Brooks et al., 2012), the results of a large clinical trial were published showing an excellent functional outcome similar to that of traditional autografts and although the high costs of commercially available processed nerve allografts is a concern, this approach to nerve gap reconstruction holds promise as a successful alternative to traditional nerve autografts.

2.2. Cell transplantation

While enrichment of nerve guides with different cell types has been explored, the most reasonable approach seems to be the use of glial cells because of their key role in axonal regeneration (Geuna et al., 2009). During the regeneration process, glial cells support axonal regrowth not only mechanically, by forming the Büngner bands that guide axons to the distal innervation targets, but also by secreting a number of growth factors and, together with macrophages, removing necrotic tissue and myelin debris (Geuna et al., 2009; Hall, 2001). For these reasons, their absence inside an artificial conduit is likely to be a limiting factor that can be overcome by enriching the conduit with these types of cells or their precursors. It has been shown that SC transplantation inside different types of nerve scaffolds leads to the improvement of both quality and rate of axon regeneration (Goto, Mukozawa, Mori, & Hara, 2010; Hadlock, Sundback, Hunter, Cheney, & Vacanti, 2000; Mosahebi, Woodward, Wiberg, Martin, & Terenghi, 2001). Significantly, this tissue engineering approach has also proved to be effective in bridging long nerve gaps where the use of the vein conduit alone is known to be ineffective (Strauch et al., 2001; Zhang et al., 2002). As an alternative to SCs, several studies have explored the possibility to enrich nerve guides with olfactory ensheathing cells (OECs). Results showed that these glial cells provide trophic/tropic support to regenerating axons (Dombrowski, Sasaki, Lankford, Kocsis, & Radtke, 2006; Guntinas-Lichius et al., 2001; Radtke et al., 2005; Verdu et al., 1999). It has also been shown that OECs can integrate into the host repaired nerve and contribute to the myelination of the regenerated axons (Dombrowski et al., 2006; Radtke & Vogt, 2009). In spite of the promising experimental results, the employment of autologous glial raises some concerns in the perspective of clinical application, especially in case of acute nerve injuries, because of the time required for expanding autologous glial cells in culture and the risk of fibroblast contamination (Moreno-Flores et al., 2006; Mosahebi et al., 2001). Therefore, the use of neuro-glial precursors, which have the potential to differentiate into both neurons and glia (Bithell & Williams, 2005), has been proposed as an alternative to primary glial cell autotransplantation. However, experimental studies carried out so far have led to conflicting results: while some studies have shown that artificial nerve guides enriched with neuro-glial stem cells promote axonal regeneration (Heine, Conant, Griffin, & Hoke, 2004; Murakami et al., 2003), other studies have reported no effects, not even a negative one (Amado et al., 2010, 2008). Another option for cell transplantation in peripheral nerves is the use of mesenchymal stem cells (MSCs) as they can be easily obtained, purified, and expanded in culture, offering a potentially unlimited source of cells for tissue engineering (Caplan & Dennis, 2006; Geuna, 2001; Tohill & Terenghi, 2004). Another advantage of MSCs is that they can be obtained from various adult stem cell niches, such as bone marrow, adipose tissue, tooth pulp, and umbilical cord blood (Alhadlaq & Mao, 2004). MSCs are thought to be able to differentiate into multiple cell lineages including neuron-like and gliallike cells (Alhadlaq & Mao, 2004; Kingham et al., 2007; Mantovani et al., 2010; Raimondo, Penna, Pagliaro, & Geuna, 2006) and it has been shown that human MSCs can be differentiated into neural cells in vitro and transplanted in the injured facial nerve of the guinea pig for improving nerve regeneration (Cho et al., 2010).

2.3. Gene transfer

Gene transfer represents one of the pillars of tissue engineering in various biomedical fields including peripheral nerve regeneration (Haastert & Grothe, 2007; Hoyng, Tannemaat, De Winter,

Verhaagen, & Malessy, 2011; Mason, Tannemaat, Malessy, & Verhaagen, 2011; Zacchigna & Giacca, 2009). Gene therapy has been used to promote nerve regeneration through the local supplying of neurotrophic factors since their systemic administration might lead to side effects that are almost avoided by local delivery. Yet the development of nontoxic, nonimmunogenic viral vectors driving long-term transgene expression makes their use much safer today (Zacchigna & Giacca, 2009). In particular, viral vectors based on the adeno-associated virus (AAV), a nonpathogenic and widespread parvovirus, are attracting much interest because they are incapable of autonomous replication and are able to transduce both dividing and non-dividing cells, showing a specific tropism for post-mitotic cells including neurons. Because these vectors do not contain any viral genes—which are transiently transfected in trans for the packaging process—they elicit virtually no inflammatory or immune response. As a consequence, transgene expression from these vectors persists for several months in a variety of animal tissues in vivo (Monahan & Samulski, 2000). The high effectiveness of skeletal muscle infection by AAVs makes it possible to use them for transferring genes for nerve regeneration either through the infection of the muscles surrounding nerve lesion site, or even by fashioning muscle-vein-combined scaffolds previously potentiated by AAV gene transfer (Fornaro et al., 2001; Geuna et al., 2003; Zacchigna & Giacca, 2009).

3. BIOMATERIAL SCIENCE

Definitely, the search for new peripheral nerve substitutes is one of the issues that has received the most attention in the context of peripheral nerve repair and regeneration research. The considerable progress in material science in recent years (Williams, 2009) has stimulated the design and experimental testing of a considerable number of new nerve guides and it is far beyond the aim of this chapter to review that enormous body of literature in detail (Cunha, Panseri, & Antonini, 2011; Daly, Yao, Zeugolis, Windebank, & Pandit, 2012; de Ruitter, Malessy, Yaszemski, Windebank, & Spinner, 2009; Deumens et al., 2010; Jiang, Lim, Mao, & Chew, 2010; Nectow, Marra, & Kaplan, 2012; Pfister et al., 2011; Siemionow, Bozkurt, & Zor, 2010; Steed, Mukhatyar, Valmikinathan, & Bellamkonda, 2011). Biomaterials for tissue engineering can be classified using various approaches (Pfister et al., 2011; Williams, 2009) and, regarding nerve repair applications, a simple three-category classification can be adopted according to the three generations of biomaterials that have been developed in this area (Geuna, Tos, & Battiston, 2012). The first generation is represented by nonabsorbable materials. The first attempts, which led to rather poor results, were based on the employment of polyethylene, polyvinyl, and rubber tantalum metal cuffs (Campbell, 1970; Ducker & Hayes, 1968; Fields, Le Beau, Longo, & Ellisman, 1989). Other nonabsorbable nerve guides that have been used, also in clinical practice, albeit with contrasting results, are polytetrafluoroethylene (Stanec & Stanec, 1998) and Gore-Tex (Pitta, Wolford, Mehra, & Hopkin, 2001). In more recent years, the use of silicon conduits led to the first clinically positive results (Dahlin, Anagnostaki, & Lundborg, 2001), especially for repairing short nerve gaps (<5 mm), leading to the concept that intentionally leaving a short gap between the two nerve stumps can enhance nerve regeneration by allowing the accumulation of cells and extracellular matrix, which can stimulate correct axonal regrowth (Dahlin & Lundborg, 2001). However, the main concern regarding the clinical employment of nonabsorbable synthetic

material in humans is the occurrence of complications caused by local fibrosis, triggered by the implanted material (Dahlin et al., 2001; Merle, Dellon, Campbell, & Chang, 1989). Therefore, the second generation of nerve guides has been focused on bioabsorbable tubes that have been tested both experimentally and in clinical practice (Dellon & Mackinnon, 1988; Luis et al., 2007; Mackinnon & Dellon, 1990a, 1990b; Meek et al., 1999; Navarro et al., 1996; Nicoli Aldini et al., 1996; Robinson et al., 1991; Tountas et al., 1993; ValeroCabre et al., 2001; Yannas & Hill, 2004; Young, Wiberg, & Terenghi, 2002). Nerve conduits made of polyglycolic acid were shown to be effective for restoring nerve defects (Mackinnon & Dellon, 1990a) and approved by the FDA for use in humans. In a multicentric randomized prospective study on digital nerve reconstruction with this type of nerve guides (Weber, Breidenbach, Brown, Jabaley, & Mass, 2000), it was shown that it provides superior results both for short gaps (<4 mm), in comparison to end-to-end repair, and for longer defects (up to 30 mm), compared to nerve autografts.

Finally, the third generation of nerve guides has been developed, within the absorbable material category, as represented by biomimetic biomaterials, that is, components of the extracellular matrix. Among the most promising biomimetic biomaterials for nerve regeneration, collagen proved to lead to functional recovery similar to nerve autografts in the rat and the primate (Archibald, Shefner, Krarup, & Madison, 1995; Li, Archibald, Krarup, & Madison, 1992). More recently, particular attention has been directed toward chitosan, a derivative of chitin, which has shown notable effectiveness in promoting nerve regeneration in experimental animal models (Amado et al., 2008; Lauto et al., 2008; Matsumoto, Kaneko, Oda, & Watanabe, 2010; Simoes et al., 2010; Yamaguchi, Itoh, Suzuki, Osaka, & Tanaka, 2003). Since living tissues are complex structures, in tissue engineering not only is the type of material important but also its 3D structure and thus the design of the artificial tissue/organ (Cui, Boland, D'Lima, & Lotz, 2012; Yang, Leong, Du, & Chua, 2001). Yet it appears that future progress in nerve tissue engineering will develop from a combination of different approaches rather than the optimization of a single approach (Battiston, Raimondo, et al., 2009). For these reasons, it is expected that their implementation in nerve prosthesis will not emerge only from the introduction of new materials or improvement of the existing ones, but rather from the combined use of other complementary tissue engineering tools. So far, various peripheral nerve prostheses have been translated to the clinical employment. In all cases, artificial nerves are represented by hollow tubes. The first nerve guide that has been introduced to the clinical employment is made of polyglycolic acid (Neurotube®). Other materials that have been used so far include poly-DL-lactide caprolactone (Neurolac®), polyvinyl alcohol hydrogel, in the form of tube (SaluTunnel™) and wrap (Salubridge™), resorbable porcine small intestinal submucosa (AxoGuard™), and resorbable collagen (Neuragen®, NeuroMatrix™, NeuroFlex™, RevolNerv®)(de Ruitter et al., 2009; Kehoe, Zhang, & Boyd, 2012; Meek & Coert, 2008). Although the application of artificial hollow tubes for nerve reconstruction has proven to lead to successful functional recovery in several clinical trials (Lundborg, Rosen, Dahlin, Danielsen, & Holmberg, 1997; Rinker & Liao, 2011; Weber et al., 2000), it appears that surgeons are still waiting for a new generation of nerve guides that may guarantee similar (or even better) results in comparison to traditional nerve autografts.

4. PHYSICAL THERAPY

The usefulness of physical therapy for functional rehabilitation to prevent muscle atrophy and drive cortical remodeling after nerve injury and repair is widely acknowledged (Lundborg, 2003). Much less consensus exists about the possibility to use physical therapy to directly improve the effectiveness of nerve tissue engineering. Actually, clinical application of physical therapy approaches for improving nerve regeneration is anecdotal and much of the research is still at the experimental/preclinical level. Electrical stimulation has been widely experimentally investigated as a therapeutic strategy in addition to microsurgery to improve functional recovery (Gordon, Brushart, & Chan, 2008; Haastert-Talini, 2014; Wang et al., 2009). Electrical stimulation has been shown to speed up axonal growth, increase the number of regrowing axons through the graft (Gordon et al., 2008), and enhance SC proliferation (Huang et al., 2010) and neurotrophic factor levels (Wang et al., 2009). Different electrical stimulation techniques have been successfully used to stimulate denervated muscles or proximal nerve stumps such as transcutaneous electrical stimulation (Gigo-Benato et al., 2010), percutaneous stimulation (Chen et al., 2001), direct low-frequency electrical stimulation (Gordon, Sulaiman, & Ladak, 2009), and electrical stimulation via synthetic nerve guidance channels (Ghasemi-Mobarakeh et al., 2011). Also, in clinical trials, electrical stimulation resulted in an improvement in functional recovery (Gordon et al., 2009; Goto et al., 2010). Another physical therapy approach that is receiving increasing attention is phototherapy. The first experimental data showing that light can exert a positive effect on axonal regrowth and nerve regeneration are old, and it is only over the last few years that an increasing number of papers have begun providing a body of evidence in support of the effectiveness of phototherapy in improving peripheral nerve regeneration (Anders, Geuna, & Rochkind, 2004; Gigo-Benato, Geuna, & Rochkind, 2005; Rochkind, Geuna, & Shainberg, 2009). The possibility of combining phototherapy with other nerve tissue engineering strategies is very promising (Hsieh et al., 2012; Jin, Prabhakaran, Liao, & Ramakrishna, 2011) and, although clinical studies are still limited (Chow, Johnson, Lopes-Martins, & Bjordal, 2009), it appears that the time has come for larger clinical trials. Another promising approach for improving the outcome of nerve tissue engineering is physical exercise. Various studies have shown that active exercise improves nerve regeneration and enhances functional recovery (Armada da Silva, Pereira, Amado, & Veloso, 2014; Asensio-Pinilla, Udina, Jaramillo, & Navarro, 2009; English, Cucoranu, Mulligan, & Sabatier, 2009; Malysz et al., 2010; Marqueste, Alliez, Alluin, Jammes, & Decherchi, 2004; Sabatier, Redmon, Schwartz, & English, 2008; van Meeteren, Brakkee, Helders, & Gispen, 1998). The motorized walking or running treadmill test, a technique used to exercise rodents following injury, demonstrates that active exercise enhances axonal elongation (Sabatier et al., 2008), increases the number of regeneration axons (English et al., 2009), and improves the functional outcome (Ilha et al., 2008; van Meeteren, Brakkee, Hamers, Helders, & Gispen, 1997; van Meeteren et al., 1998). Passive exercise, commonly used in rehabilitation, has been reported to stimulate nerve regeneration and functional recovery (Ilha et al., 2008; Udina, Puigdemasa, & Navarro, 2011). Other interesting approaches include stimulation by magnetic fields (Wang & Zhao, 2010), shockwaves (Hausner & No 'gra 'di, 2014), manual stimulation (Bischoff et al., 2009), and neural interfaces (del Valle & Navarro, 2014; Herrera-Rincon, Toret, Sanchez-Jimenez, Avendano, & Panetsos, 2012).

5. PHARMACOTHERAPY

In spite of the great progresses of pharmacology in many other fields of medicine and surgery, there is still not any established drug treatment protocol for specifically improving nerve regeneration after trauma and reconstruction. Although, of course, these patients may be given various medicaments along with the postoperative, with the aim of treating concurrent conditions (e.g., antibiotics for infections) and sometimes as alimentary integrators (such as acetyl-carnitine), no dedicated drug is usually administered after nerve surgery with the goal of improving the degree of nerve regeneration and maturation. While no specific nerve regeneration-promoting drug has still entered the clinics, on the experimental side, many studies have suggested that various pharmacological approaches may have a positive effect on this complex healing process. It is far beyond the goal of this chapter to revisit all the drugs that can have a potential effect on nerve regeneration. Just to mention some of the most promising molecules, particular interest is being given to immunosuppressants (Yan, Sun, Hunter, Mackinnon, & Johnson, 2012) and various hormones, such as melatonin (Odaci & Kaplan, 2009) and erythropoietin (Yin, Zhang, Bo, & Gao, 2010). Evidence has also been provided recently that corticosteroids exert a positive effect on nerve regeneration (Mohammadi, Amini, & Eskafian, 2013). Other drugs that have shown positive effects on peripheral nerve regeneration are etifoxine, a ligand of the translocator protein (18 kDa), which modulates inflammatory responses (Girard et al., 2008); flunarizine, a calcium channel antagonist and vasodilator (Patro, Chattopadhyay, & Patro, 1999); cilostan, an antiplatelet and vasodilatation agent (Yamamoto, Yasuda, Kimura, & Komiya, 1998); and GM1 gangliosides (Lopez et al., 2010; Silva-Neto, Vasconcelos, Silva-Junior, & Beder-Ribeiro, 2009). It appears thus that the time is also ripe for clinical trials with candidate nerve regeneration-promoting drugs.

6. CONCLUDING REMARKS: COMBINING THE DIFFERENT TISSUE ENGINEERING APPROACHES, THE MAIN CHALLENGE FOR IMPROVING NERVE REPAIR OUTCOME

An emerging consensus among basic and clinical scientists is that in order to optimize the strategy for tissue engineering of the peripheral nerve, a new level of innovation is needed that brings together in a multitranslational approach the different pillars of tissue engineering. Figure 2.1 illustrates this concept. Reconstructive microsurgery is definitely the key element in this web, not only because it represents the link between innovative research and the patient but also because surgeons must interact with all scientists from other cultural backgrounds. In particular, interaction with biologists and biotechnologists is very important especially when transplantation approaches are concerned since transplantation is progressively evolving from whole organ transplantation to more sophisticated forms of tissue engineering based on the employment of only parts (tissue transplantation), or even single-cellular (cell transplantation), or sub-cellular constituents (gene transfer), of an organ. Moreover, surgeons must interact with engineers and materials scientists in the light of the recent enormous advances in nanotechnology that makes it possible to develop and design very complex synthetic scaffolds to repair neural defects. Finally, surgeons must interact with pharmacologists and physical therapists in order to define combined therapeutic strategies that are more and more effective in improving the outcome of nerve tissue engineering.

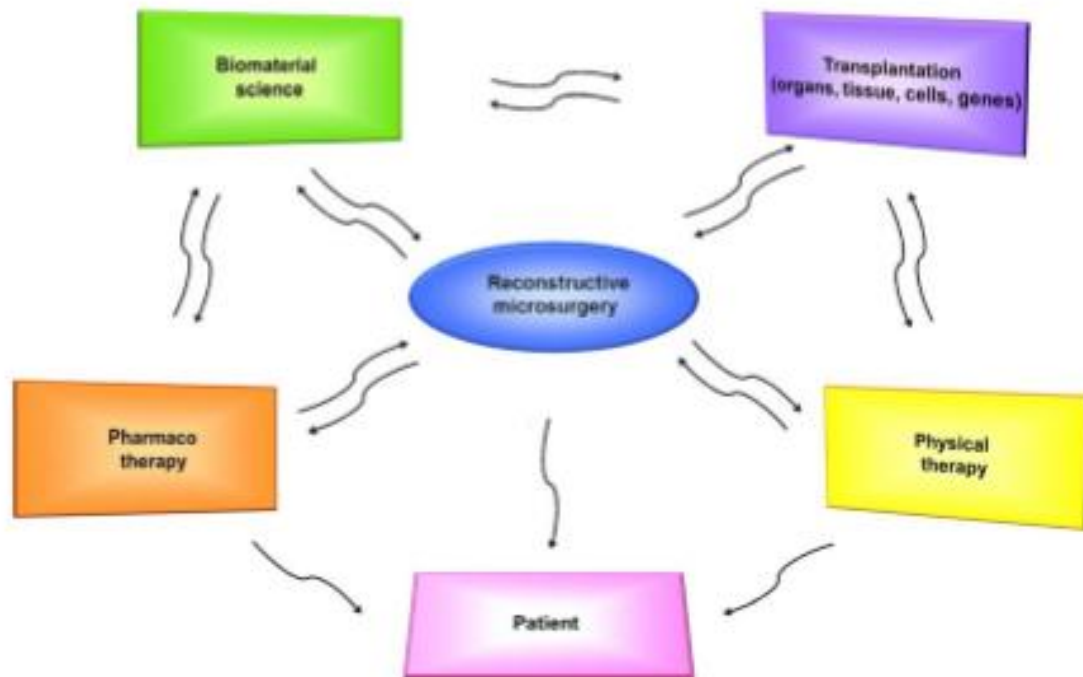


Figure 2.1 Schematic drawing depicting the various disciplines involved in tissue engineering of the peripheral nerve.

In conclusion, it clearly appears that future progress in regenerative medicine will not develop from the improvement of a single strategy, but rather from the optimized combination of many different approaches. The multilevel and interdisciplinary approach thus appears to be the main challenge for peripheral nerve tissue engineering since different competences and expertise need to be brought together. Though challenging, this approach represents an exciting opportunity for researchers to explore new scientific fields with the hope that it will allow us to make significant clinical advances in the forthcoming years.

ACKNOWLEDGMENT The research leading to this chapter has received funding from the European Community's Seventh Framework Programme (FP7-HEALTH-2011) under grant agreement no. 278612 (BIOHYBRID).

REFERENCES Alhadlaq, A., & Mao, J. J. (2004). Mesenchymal stem cells: Isolation and therapeutics. *Stem Cells and Development*, 13(4), 436–448. <http://dx.doi.org/10.1089/1547328041797552>. Amado, S., Rodrigues, J. M., Luis, A. L., Armada-da-Silva, P. A., Vieira, M., Gartner, A., et al. (2010). Effects of collagen membranes enriched with in vitro-differentiated N1E115 cells on rat sciatic nerve regeneration after end-to-end repair. *Journal of Neuroengineering and Rehabilitation*, 7, 7. <http://dx.doi.org/10.1186/1743-0003-7-7>. Amado, S., Simoes, M. J., Armada da Silva, P. A., Luis, A. L., Shirotsaki, Y., Lopes, M. A., et al. (2008). Use of hybrid chitosan membranes and N1E-115 cells for promoting nerve regeneration in an axonotmesis rat model. *Biomaterials*, 29(33), 4409–4419. <http://dx.doi.org/10.1016/j.biomaterials.2008.07.043>. Anders, J. J., Geuna, S., & Rochkind, S. (2004). Phototherapy promotes regeneration and functional recovery of injured peripheral nerve. *Neurological Research*, 26(2), 233–239. <http://dx.doi.org/10.1179/016164104225013914>. Archibald, S. J., Shefner, J., Krarup, C., & Madison, R. D. (1995). Monkey median nerve repaired by

nerve graft or collagen nerve guide tube. *Journal of Neuroscience*, 15(5 Pt 2), 4109–4123. Armada-da-Silva, P. A. S., Pereira, C., Amado, S., & Veloso, A. P. (2014). Role of physical exercise for improving posttraumatic nerve regeneration. *International Review of Neurobiology*, 109, 125–149.

Asensio-Pinilla, E., Udina, E., Jaramillo, J., & Navarro, X. (2009). Electrical stimulation combined with exercise increase axonal regeneration after peripheral nerve injury. *Experimental Neurology*, 219(1), 258–265. <http://dx.doi.org/10.1016/j.expneurol.2009.05.034>.

Battiston, B., Geuna, S., Ferrero, M., & Tos, P. (2005). Nerve repair by means of tubulization: Literature review and personal clinical experience comparing biological and synthetic conduits for sensory nerve repair. *Microsurgery*, 25(4), 258–267. <http://dx.doi.org/10.1002/micr.20127>.

Battiston, B., Papalia, I., Tos, P., & Geuna, S. (2009). Chapter 1: Peripheral nerve repair and regeneration research: A historical note. *International Review of Neurobiology*, 87, 1–7. [http://dx.doi.org/10.1016/S0074-7742\(09\)87001-3](http://dx.doi.org/10.1016/S0074-7742(09)87001-3).

Battiston, B., Raimondo, S., Tos, P., Gaidano, V., Audisio, C., Scevola, A., et al. (2009). Chapter 11: Tissue engineering of peripheral nerves. *International Review of Neurobiology*, 87, 227–249. [http://dx.doi.org/10.1016/S0074-7742\(09\)87011-6](http://dx.doi.org/10.1016/S0074-7742(09)87011-6).

Battiston, B., Tos, P., Cushway, T. R., & Geuna, S. (2000). Nerve repair by means of vein filled with muscle grafts I. Clinical results. *Microsurgery*, 20(1), 32–36.

Battiston, B., Tos, P., Geuna, S., Giacobini-Robecchi, M. G., & Guglielmone, R. (2000). Nerve repair by means of vein filled with muscle grafts. II. Morphological analysis of regeneration. *Microsurgery*, 20(1), 37–41.

Bischoff, A., Grosheva, M., Irintchev, A., Skouras, E., Kaidoglou, K., Michael, J., et al. (2009). Manual stimulation of the orbicularis oculi muscle improves eyelid closure after facial nerve injury in adult rats. *Muscle & Nerve*, 39(2), 197–205. <http://dx.doi.org/10.1002/mus.21126>.

Bithell, A., & Williams, B. P. (2005). Neural stem cells and cell replacement therapy: Making the right cells. *Clinical Science (London)*, 108(1), 13–22. <http://dx.doi.org/10.1042/CS20040276>.

Brooks, D. N., Weber, R. V., Chao, J. D., Rinker, B. D., Zoldos, J., Robichaux, M. R., et al. (2012). Processed nerve allografts for peripheral nerve reconstruction: A multicenter study of utilization and outcomes in sensory, mixed, and motor nerve reconstructions. *Microsurgery*, 32(1), 1–14. <http://dx.doi.org/10.1002/micr.20975>.

Brunelli, G. A., & Brunelli, G. R. (1993). Direct muscle neurotization. *Journal of Reconstructive Microsurgery*, 9(2), 81–90. <http://dx.doi.org/10.1055/s-2007-1006656>, discussion 89–90.

Campbell, J. B. (1970). Peripheral nerve repair. *Clinical Neurosurgery*, 17, 77–98.

Caplan, A. I., & Dennis, J. E. (2006). Mesenchymal stem cells as trophic mediators. *Journal of Cellular Biochemistry*, 98(5), 1076–1084. <http://dx.doi.org/10.1002/jcb.20886>.

Chen, Y. S., Hu, C. L., Hsieh, C. L., Lin, J. G., Tsai, C. C., Chen, T. H., et al. (2001). Effects of percutaneous electrical stimulation on peripheral nerve regeneration using silicone rubber chambers. *Journal of Biomedical Materials Research*, 57(4), 541–549.

Chiu, D. T. (1999). Autogenous venous nerve conduits. A review. *Hand Clinics*, 15(4), 667–671, ix.

Chiu, D. T., & Strauch, B. (1990). A prospective clinical evaluation of autogenous vein grafts used as a nerve conduit for distal sensory nerve defects of 3 cm or less. *Plastic and Reconstructive Surgery*, 86(5), 928–934.

Cho, H. H., Jang, S., Lee, S. C., Jeong, H. S., Park, J. S., Han, J. Y., et al. (2010). Effect of neural-induced mesenchymal stem cells and platelet-rich plasma on facial nerve regeneration in an acute nerve injury model. *Laryngoscope*, 120(5), 907–913. <http://dx.doi.org/10.1002/lary.20860>.

Chow, R. T., Johnson, M. I., Lopes-Martins, R. A., & Bjordal, J. M. (2009). Efficacy of lowlevel laser therapy in the management of neck pain: A systematic review and

metaanalysis of randomised placebo or active-treatment controlled trials. *Lancet*, 374(9705), 1897–1908. [http://dx.doi.org/10.1016/S0140-6736\(09\)61522-1](http://dx.doi.org/10.1016/S0140-6736(09)61522-1). Cui, X., Boland, T., D’Lima, D. D., & Lotz, M. K. (2012). Thermal inkjet printing in tissue engineering and regenerative medicine. *Recent Patents on Drug Delivery & Formulation*, 6(2), 149–155. Cunha, C., Panseri, S., & Antonini, S. (2011). Emerging nanotechnology approaches in tissue engineering for peripheral nerve regeneration. *Nanomedicine*, 7(1), 50–59. <http://dx.doi.org/10.1016/j.nano.2010.07.004>. Dahlin, L. B., Anagnostaki, L., & Lundborg, G. (2001). Tissue response to silicone tubes used to repair human median and ulnar nerves. *Scandinavian Journal of Plastic and Reconstructive Surgery and Hand Surgery*, 35(1), 29–34. Dahlin, L. B., & Lundborg, G. (2001). Use of tubes in peripheral nerve repair. *Neurosurgery Clinics of North America*, 12(2), 341–352. Daly, W., Yao, L., Zeugolis, D., Windebank, A., & Pandit, A. (2012). A biomaterials approach to peripheral nerve regeneration: Bridging the peripheral nerve gap and enhancing functional recovery. *Journal of the Royal Society, Interface*, 9(67), 202–221. <http://dx.doi.org/10.1098/rsif.2011.0438>. deRuiter, G. C., Malessy, M. J., Yaszemski, M. J., Windebank, A. J., & Spinner, R. J. (2009). Designing ideal conduits for peripheral nerve repair. *Neurosurgical Focus*, 26(2), E5. <http://dx.doi.org/10.3171/FOC.2009.26.2.E5>. del Valle, J., & Navarro, X. (2014). Interfaces with the peripheral nerve for the control of neuroprostheses. *International Review of Neurobiology*, 109, 63–83. Dellon, A. L., & Mackinnon, S. E. (1988). An alternative to the classical nerve graft for the management of the short nerve gap. *Plastic and Reconstructive Surgery*, 82(5), 849–856. Deumens, R., Bozkurt, A., Meek, M. F., Marcus, M. A., Joosten, E. A., Weis, J., et al. (2010). Repairing injured peripheral nerves: Bridging the gap. *Progress in Neurobiology*, 92(3), 245–276. <http://dx.doi.org/10.1016/j.pneurobio.2010.10.002>. Dombrowski, M. A., Sasaki, M., Lankford, K. L., Kocsis, J. D., & Radtke, C. (2006). Myelination and nodal formation of regenerated peripheral nerve fibers following transplantation of acutely prepared olfactory ensheathing cells. *Brain Research*, 1125(1), 1–8. <http://dx.doi.org/10.1016/j.brainres.2006.09.089>. Ducker, T. B., & Hayes, G. J. (1968). Experimental improvements in the use of Silastic cuff for peripheral nerve repair. *Journal of Neurosurgery*, 28(6), 582–587. <http://dx.doi.org/10.3171/jns.1968.28.6.0582>. English, A. W., Cucoranu, D., Mulligan, A., & Sabatier, M. (2009). Treadmill training enhances axon regeneration in injured mouse peripheral nerves without increased loss of topographic specificity. *Journal of Comparative Neurology*, 517(2), 245–255. <http://dx.doi.org/10.1002/cne.22149>. Fawcett, J. W., & Keynes, R. J. (1986). Muscle basal lamina: A new graft material for peripheral nerve repair. *Journal of Neurosurgery*, 65(3), 354–363. <http://dx.doi.org/10.3171/jns.1986.65.3.0354>. Fields, R. D., Le Beau, J. M., Longo, F. M., & Ellisman, M. H. (1989). Nerve regeneration through artificial tubular implants. *Progress in Neurobiology*, 33(2), 87–134. Fornaro, M., Tos, P., Geuna, S., Giacobini-Robecchi, M. G., & Battiston, B. (2001). Confocal imaging of Schwann-cell migration along muscle-vein combined grafts used to bridge nerve defects in the rat. *Microsurgery*, 21(4), 153–155. Geuna, S. (2001). Embryonic cell grafting for the treatment of peripheral nervous system diseases. *Neuroreport*, 12(17), A101–A102. Geuna, S., Fornaro, M., Raimondo, S., & Giacobini-Robecchi, M. G. (2010). Plasticity and regeneration in the peripheral nervous system. *Italian Journal of Anatomy and Embryology*, 115(1–2), 91–94. Geuna, S., Papalia, I., & Tos, P. (2006). End-to-side (terminolateral) nerve regeneration: A challenge for neuroscientists coming from an intriguing nerve repair concept. *Brain Research Reviews*, 52(2), 381–388.

<http://dx.doi.org/10.1016/j.brainresrev.2006.05.002>. Geuna, S., Raimondo, S., Nicolino, S., Boux, E., Fornaro, M., Tos, P., et al. (2003). Schwann-cell proliferation in muscle-vein combined conduits for bridging rat sciatic nerve defects. *Journal of Reconstructive Microsurgery*, 19(2), 119–123. <http://dx.doi.org/10.1055/s-2003-37818>, discussion 124. Geuna, S., Raimondo, S., Ronchi, G., Di Scipio, F., Tos, P., Czaja, K., et al. (2009). Chapter 3: Histology of the peripheral nerve and changes occurring during nerve regeneration. *International Review of Neurobiology*, 87, 27–46. [http://dx.doi.org/10.1016/S0074-7742\(09\)87003-7](http://dx.doi.org/10.1016/S0074-7742(09)87003-7). Geuna, S., Tos, P., & Battiston, B. (2012). Emerging issues in peripheral nerve repair. *Neural Regeneration Research*, 7(29), 2267–2272. Geuna, S., Tos, P., Battiston, B., & Giacobini-Robecchi, M. G. (2004). Bridging peripheral nerve defects with muscle-vein combined guides. *Neurological Research*, 26(2), 139–144. <http://dx.doi.org/10.1179/016164104225013752>. Ghasemi-Mobarakeh, L., Prabhakaran, M. P., Morshed, M., Nasr-Esfahani, M. H., Baharvand, H., Kiani, S., et al. (2011). Application of conductive polymers, scaffolds and electrical stimulation for nerve tissue engineering. *Journal of Tissue Engineering and Regenerative Medicine*, 5(4), e17–e35. <http://dx.doi.org/10.1002/term.383>. Gigo-Benato, D., Geuna, S., & Rochkind, S. (2005). Phototherapy for enhancing peripheral nerve repair: A review of the literature. *Muscle & Nerve*, 31(6), 694–701. <http://dx.doi.org/10.1002/mus.20305>. Gigo-Benato, D., Russo, T. L., Geuna, S., Domingues, N. R., Salvini, T. F., & Parizotto, N. A. (2010). Electrical stimulation impairs early functional recovery and accentuates skeletal muscle atrophy after sciatic nerve crush injury in rats. *Muscle & Nerve*, 41(5), 685–693. <http://dx.doi.org/10.1002/mus.21549>. Girard, C., Liu, S., Cadepond, F., Adams, D., Lacroix, C., Verleye, M., et al. (2008). Etifoxine improves peripheral nerve regeneration and functional recovery. *Proceedings of the National Academy of Sciences of the United States of America*, 105(51), 20505–20510. <http://dx.doi.org/10.1073/pnas.0811201106>. Glaus, S. W., Johnson, P. J., & Mackinnon, S. E. (2011). Clinical strategies to enhance nerve regeneration in composite tissue allotransplantation. *Hand Clinics*, 27(4), 495–509. <http://dx.doi.org/10.1016/j.hcl.2011.07.002ix>. Gordon, T., Brushart, T. M., & Chan, K. M. (2008). Augmenting nerve regeneration with electrical stimulation. *Neurological Research*, 30(10), 1012–1022. <http://dx.doi.org/10.1179/174313208X362488>. Gordon, T., Sulaiman, O. A., & Ladak, A. (2009). Chapter 24: Electrical stimulation for improving nerve regeneration: Where do we stand? *International Review of Neurobiology*, 87, 433–444. [http://dx.doi.org/10.1016/S0074-7742\(09\)87024-4](http://dx.doi.org/10.1016/S0074-7742(09)87024-4). Goto, E., Mukozawa, M., Mori, H., & Hara, M. (2010). A rolled sheet of collagen gel with cultured Schwann cells: Model of nerve conduit to enhance neurite growth. *Journal of Bioscience and Bioengineering*, 109(5), 512–518. <http://dx.doi.org/10.1016/j.jbiosc.2009.11.002>.

Guntinas-Lichius, O., Angelov, D. N., Tomov, T. L., Dramiga, J., Neiss, W. F., & Wewetzer, K. (2001). Transplantation of olfactory ensheathing cells stimulates the collateral sprouting from axotomized adult rat facial motoneurons. *Experimental Neurology*, 172(1), 70–80. <http://dx.doi.org/10.1006/exnr.2001.7774>. Haastert, K., & Grothe, C. (2007). Gene therapy in peripheral nerve reconstruction approaches. *Current Gene Therapy*, 7(3), 221–228. Haastert-Talini, K., & Grothe, C. (2014). Electrical stimulation for promoting peripheral nerve regeneration. *International Review of Neurobiology*, 109, 111–124. Hadlock, T., Sundback, C., Hunter, D., Cheney, M., & Vacanti, J. P. (2000). A polymer foam conduit seeded with Schwann cells promotes

guided peripheral nerve regeneration. *Tissue Engineering*, 6(2), 119–127. <http://dx.doi.org/10.1089/107632700320748>. Hall, S. (2001). Nerve repair: A neurobiologist's view. *Journal of Hand Surgery (Edinburgh, Scotland)*, 26(2), 129–136. <http://dx.doi.org/10.1054/jhsb.2000.0497>. Hausner, T., & No 'gra 'di, A. (2014). The use of shock waves in peripheral nerve regeneration: New perspectives? *International Review of Neurobiology*, 109, 85–98. Heine, W., Conant, K., Griffin, J. W., & Hoke, A. (2004). Transplanted neural stem cells promote axonal regeneration through chronically denervated peripheral nerves. *Experimental Neurology*, 189(2), 231–240. <http://dx.doi.org/10.1016/j.expneurol.2004.06.014>. Herrera-Rincon, C., Toretts, C., Sanchez-Jimenez, A., Avendano, C., & Panetsos, F. (2012). Chronic electrical stimulation of transected peripheral nerves preserves anatomy and function in the primary somatosensory cortex. *European Journal of Neuroscience*, 36(12), 3679–3690. <http://dx.doi.org/10.1111/ejn.12000>. Hoyng, S. A., Tannemaat, M. R., De Winter, F., Verhaagen, J., & Malessy, M. J. (2011). Nerve surgery and gene therapy: A neurobiological and clinical perspective. *Journal of Hand Surgery (European Volume)*, 36(9), 735–746. <http://dx.doi.org/10.1177/1753193411420348>. Hsieh, Y. L., Chou, L. W., Chang, P. L., Yang, C. C., Kao, M. J., & Hong, C. Z. (2012). Low-level laser therapy alleviates neuropathic pain and promotes function recovery in rats with chronic constriction injury: Possible involvements in hypoxia-inducible factor 1alpha (HIF-1alpha). *Journal of Comparative Neurology*, 520(13), 2903–2916. <http://dx.doi.org/10.1002/cne.23072>. Huang, J., Hu, X., Lu, L., Ye, Z., Zhang, Q., & Luo, Z. (2010). Electrical regulation of Schwann cells using conductive polypyrrole/chitosan polymers. *Journal of Biomedical Materials Research Part A*, 93(1), 164–174. <http://dx.doi.org/10.1002/jbm.a.32511>. Ilha, J., Araujo, R. T., Malysz, T., Hermel, E. E., Rigon, P., Xavier, L. L., et al. (2008). Endurance and resistance exercise training programs elicit specific effects on sciatic nerve regeneration after experimental traumatic lesion in rats. *Neurorehabilitation and Neural Repair*, 22(4), 355–366. <http://dx.doi.org/10.1177/1545968307313502>. Jiang, X., Lim, S. H., Mao, H. Q., & Chew, S. Y. (2010). Current applications and future perspectives of artificial nerve conduits. *Experimental Neurology*, 223(1), 86–101. <http://dx.doi.org/10.1016/j.expneurol.2009.09.009>. Jin, G., Prabhakaran, M. P., Liao, S., & Ramakrishna, S. (2011). Photosensitive materials and potential of photocurrent mediated tissue regeneration. *Journal of Photochemistry and Photobiology. B*, 102(2), 93–101. <http://dx.doi.org/10.1016/j.jphotobiol.2010.09.010>. Kehoe, S., Zhang, X. F., & Boyd, D. (2012). FDA approved guidance conduits and wraps for peripheral nerve injury: A review of materials and efficacy. *Injury*, 43(5), 553–572. <http://dx.doi.org/10.1016/j.injury.2010.12.030>. Keynes, R. J., Hopkins, W. G., & Huang, L. H. (1984). Regeneration of mouse peripheral nerves in degenerating skeletal muscle: Guidance by residual muscle fibre basement membrane. *Brain Research*, 295(2), 275–281. Kingham, P. J., Kalbermatten, D. F., Mahay, D., Armstrong, S. J., Wiberg, M., & Terenghi, G. (2007). Adipose-derived stem cells differentiate into a Schwann cell phenotype and promote neurite outgrowth in vitro. *Experimental Neurology*, 207(2), 267–274. <http://dx.doi.org/10.1016/j.expneurol.2007.06.029>. Kong, J. M., Zhong, S. Z., Bo, S., & Zhu, S. X. (1986). Experimental study of bridging the peripheral nerve gap with skeletal muscle. *Microsurgery*, 7(4), 183–189. Kraus, H., & Reisner, H. (1940). Behandlungsergebnisse von Verletzungen pripherer Nerven mit besonderer Berucksichtigung der Schussverletzungen del Jahre 1919, 1927, und 1934. *Arch Klin Chir*, 199, 318–336. Latif, M. J., Afthinos, J. N., Connery, C. P.,

Perin, N., Bhora, F. Y., Chwajol, M., et al. (2008). Robotic intercostal nerve graft for reversal of thoracic sympathectomy: A large animal feasibility model. *International Journal of Medical Robotics and Computer Assisted Surgery*, 4(3), 258–262. <http://dx.doi.org/10.1002/rcs.205>. Lauto, A., Foster, L. J., Avolio, A., Sampson, D., Raston, C., Sarris, M., et al. (2008). Sutureless nerve repair with laser-activated chitosan adhesive: A pilot in vivo study. *Photomedicine and Laser Surgery*, 26(3), 227–234. <http://dx.doi.org/10.1089/pho.2007.2131>. Li, S. T., Archibald, S. J., Krarup, C., & Madison, R. D. (1992). Peripheral nerve repair with collagen conduits. *Clinical Materials*, 9(3–4), 195–200. Liverneaux, P., Nectoux, E., & Taleb, C. (2009). The future of robotics in hand surgery. *Chirurgie de la Main*, 28(5), 278–285. <http://dx.doi.org/10.1016/j.main.2009.08.002>. Lopez, P. H., Zhang, G., Zhang, J., Lehmann, H. C., Griffin, J. W., Schnaar, R. L., et al. (2010). Passive transfer of IgG anti-GM1 antibodies impairs peripheral nerve repair. *Journal of Neuroscience*, 30(28), 9533–9541. <http://dx.doi.org/10.1523/JNEUROSCI.228110.2010>. Luis, A. L., Rodrigues, J. M., Amado, S., Veloso, A. P., Armada-Da-Silva, P. A., Raimondo, S., et al. (2007). PLGA 90/10 and caprolactone biodegradable nerve guides for the reconstruction of the rat sciatic nerve. *Microsurgery*, 27(2), 125–137. <http://dx.doi.org/10.1002/micr.20317>. Lundborg, G. (2003). Richard P. Bunge memorial lecture. Nerve injury and repair—A challenge to the plastic brain. *Journal of the Peripheral Nervous System*, 8(4), 209–226. Lundborg, G., Rosen, B., Dahlin, L., Danielsen, N., & Holmberg, J. (1997). Tubular versus conventional repair of median and ulnar nerves in the human forearm: Early results from a prospective, randomized, clinical study. *The Journal of Hand Surgery*, 22(1), 99–106. [http://dx.doi.org/10.1016/S0363-5023\(05\)80188-1](http://dx.doi.org/10.1016/S0363-5023(05)80188-1). Mackinnon, S. E., & Dellon, A. L. (1990a). Clinical nerve reconstruction with a bioabsorbable polyglycolic acid tube. *Plastic and Reconstructive Surgery*, 85(3), 419–424. Mackinnon, S. E., & Dellon, A. L. (1990b). A study of nerve regeneration across synthetic (Maxon) and biologic (collagen) nerve conduits for nerve gaps up to 5 cm in the primate. *Journal of Reconstructive Microsurgery*, 6(2), 117–121. <http://dx.doi.org/10.1055/s-20071006810>. Malysz, T., Ilha, J., Nascimento, P. S., De Angelis, K., Schaan, B. D., & Achaval, M. (2010). Beneficial effects of treadmill training in experimental diabetic nerve regeneration. *Clinics (São Paulo, Brazil)*, 65(12), 1329–1337. Mantovani, C., Mahay, D., Kingham, M., Terenghi, G., Shawcross, S. G., & Wiberg, M. (2010). Bone marrow- and adipose-derived stem cells show expression of myelin mRNAs and proteins. *Regenerative Medicine*, 5(3), 403–410. <http://dx.doi.org/10.2217/rme.10.15>. Marcoccio, I., & Vigasio, A. (2010). Muscle-in-vein nerve guide for secondary reconstruction in digital nerve lesions. *The Journal of Hand Surgery*, 35(9), 1418–1426. <http://dx.doi.org/10.1016/j.jhsa.2010.05.019>. Marqueste, T., Alliez, J. R., Alluin, O., Jammes, Y., & Decherchi, P. (2004). Neuromuscular rehabilitation by treadmill running or electrical stimulation after peripheral nerve injury and repair. *Journal of Applied Physiology*, 96(5), 1988–1995. <http://dx.doi.org/10.1152/jappphysiol.00775.2003>. Mason, M. R., Tannemaat, M. R., Malessy, M. J., & Verhaagen, J. (2011). Gene therapy for the peripheral nervous system: A strategy to repair the injured nerve? *Current Gene Therapy*, 11(2), 75–89. Matsumoto, I., Kaneko, M., Oda, M., & Watanabe, G. (2010). Repair of intra-thoracic autonomic nerves using chitosan tubes. *Interactive Cardiovascular and Thoracic Surgery*, 10(4), 498–501. <http://dx.doi.org/10.1510/icvts.2009.227744>. Meek, M. F., & Coert, J. H. (2002). Clinical use of nerve conduits in peripheral-nerve repair: Review of the literature. *Journal of Reconstructive Microsurgery*, 18(2), 97–109. <http://dx.doi.org/10.1055/s-2002-19889>. Meek, M. F., & Coert, J. H.

(2008). US Food and Drug Administration/Conformit Europeapproved absorbable nerve conduits for clinical repair of peripheral and cranial nerves. *Annals of Plastic Surgery*, 60(4), 466–472. Meek, M. F., Dijkstra, J. R., Den Dunnen, W. F., Ijkema-Paassen, J., Schakenraad, J. M., Gramsbergen, A., et al. (1999). Functional assessment of sciatic nerve reconstruction: Biodegradable poly (DLA-epsilon-CL) nerve guides versus autologous nerve grafts. *Microsurgery*, 19(8), 381–388. Merle, M., Dellon, A. L., Campbell, J. N., & Chang, P. S. (1989). Complications from silicon-polymer intubulation of nerves. *Microsurgery*, 10(2), 130–133. Millesi, H. (1981). Interfascicular nerve grafting. *Orthopedic Clinics of North America*, 12(2), 287–301. Millesi, H., Meissl, G., & Berger, A. (1972). The interfascicular nerve-grafting of the median and ulnar nerves. *The Journal of Bone and Joint Surgery. American Volume*, 54(4), 727–750. Mligiliche, N., Tabata, Y., Endoh, K., & Ide, C. (2001). Peripheral nerve regeneration through a long detergent-denatured muscle autografts in rabbits. *Neuroreport*, 12(8), 1719–1722. Mohammadi, R., Amini, K., & Eskafian, H. (2013). Betamethasone-enhanced vein graft conduit accelerates functional recovery in the rat sciatic nerve gap. *Journal of Oral and Maxillofacial Surgery*, 71(4), 786–792. <http://dx.doi.org/10.1016/j.joms.2012.08.009>. Monahan, P. E., & Samulski, R. J. (2000). Adeno-associated virus vectors for gene therapy: More pros than cons? *Molecular Medicine Today*, 6(11), 433–440. Moreno-Flores, M. T., Bradbury, E. J., Martin-Bermejo, M. J., Agudo, M., Lim, F., Pastrana, E., et al. (2006). A clonal cell line from immortalized olfactory ensheathing glia promotes functional recovery in the injured spinal cord. *Molecular Therapy*, 13(3), 598–608. <http://dx.doi.org/10.1016/j.ymthe.2005.11.014>. Mosahebi, A., Woodward, B., Wiberg, M., Martin, R., & Terenghi, G. (2001). Retroviral labeling of Schwann cells: In vitro characterization and in vivo transplantation to improve peripheral nerve regeneration. *Glia*, 34(1), 8–17. Murakami, T., Fujimoto, Y., Yasunaga, Y., Ishida, O., Tanaka, N., Ikuta, Y., et al. (2003). Transplanted neuronal progenitor cells in a peripheral nerve gap promote nerve repair. *Brain Research*, 974(1–2), 17–24. Navarro, X., Rodriguez, F. J., Labrador, R. O., Buti, M., Ceballos, D., Gomez, N., et al. (1996). Peripheral nerve regeneration through bioresorbable and durable nerve guides. *Journal of the Peripheral Nervous System*, 1(1), 53–64. Nectoux, E., Taleb, C., & Liverneaux, P. (2009). Nerve repair in telemicrosurgery: An experimental study. *Journal of Reconstructive Microsurgery*, 25(4), 261–265. <http://dx.doi.org/10.1055/s-0028-1104562>. Nectow, A. R., Marra, K. G., & Kaplan, D. L. (2012). Biomaterials for the development of peripheral nerve guidance conduits. *Tissue Engineering. Part B, Reviews*, 18(1), 40–50. <http://dx.doi.org/10.1089/ten.TEB.2011.0240>. Nicoli Aldini, N., Perego, G., Cella, G. D., Maltarello, M. C., Fini, M., Rocca, M., et al. (1996). Effectiveness of a bioabsorbable conduit in the repair of peripheral nerves. *Biomaterials*, 17(10), 959–962. Norris, R. W., Glasby, M. A., Gattuso, J. M., & Bowden, R. E. (1988). Peripheral nerve repair in humans using muscle autografts. A new technique. *The Journal of Bone and Joint Surgery. British Volume*, 70(4), 530–533. Odaci, E., & Kaplan, S. (2009). Chapter 16: Melatonin and nerve regeneration. *International Review of Neurobiology*, 87, 317–335. [http://dx.doi.org/10.1016/S0074-7742\(09\)87016-5](http://dx.doi.org/10.1016/S0074-7742(09)87016-5). Papalia, I., Cardaci, A., d'Alcontres, F. S., Lee, J. M., Tos, P., & Geuna, S. (2007). Selection of the donor nerve for end-to-side neurorrhaphy. *Journal of Neurosurgery*, 107(2), 378–382. <http://dx.doi.org/10.3171/JNS-07/08/0378>. Papalia, I., Geuna, S., Tos, P. L., Boux, E., Battiston, B., & Stagno D'Alcontres, F. (2003). Morphologic and functional study of rat median nerve repair by terminolateral neurorrhaphy of the ulnar nerve.

Journal of Reconstructive Microsurgery, 19(4), 257–264. <http://dx.doi.org/10.1055/s-2003-40582>.

Patro, I. K., Chattopadhyay, M., & Patro, N. (1999). Flunarizine enhances functional recovery following sciatic nerve crush lesion in rats. *Neuroscience Letters*, 263(2–3), 97–100.

Pereira, J. H., Bowden, R. E., Gattuso, J. M., & Norris, R. W. (1991). Comparison of results of repair of digital nerves by denatured muscle grafts and end-to-end sutures. *Journal of Hand Surgery (Edinburgh, Scotland)*, 16(5), 519–523.

Pereira, J. H., Bowden, R. E., Narayanakumar, T. S., & Gschmeissner, S. E. (1996). Peripheral nerve reconstruction using denatured muscle autografts for restoring protective sensation in hands and feet of leprosy patients. *Indian Journal of Leprosy*, 68(1), 83–91.

Pereira, J. H., Palande, D. D., Subramanian, A., Narayanakumar, T. S., Curtis, J., & Turk, J. L. (1991). Denatured autologous muscle graft in leprosy. *Lancet*, 338(8777), 1239–1240.

Pfister, B. J., Gordon, T., Loverde, J. R., Kochar, A. S., Mackinnon, S. E., & Cullen, D. K. (2011). Biomedical engineering strategies for peripheral nerve repair: Surgical applications, state of the art, and future challenges. *Critical Reviews in Biomedical Engineering*, 39(2), 81–124.

Pitta, M. C., Wolford, L. M., Mehra, P., & Hopkin, J. (2001). Use of Gore-Tex tubing as a conduit for inferior alveolar and lingual nerve repair: Experience with 6 cases. *Journal of Oral and Maxillofacial Surgery*, 59(5), 493–496. <http://dx.doi.org/10.1053/joms.2001.22671>, discussion 497.

Radtke, C., Akiyama, Y., Lankford, K. L., Vogt, P. M., Krause, D. S., & Kocsis, J. D. (2005). Integration of engrafted Schwann cells into injured peripheral nerve: Axonal association and nodal formation on regenerated axons. *Neuroscience Letters*, 387(2), 85–89. <http://dx.doi.org/10.1016/j.neulet.2005.06.073>.

Radtke, C., & Vogt, P. M. (2009). Peripheral nerve regeneration: A current perspective. *Eplasty*, 9, e47.

Raimondo, S., Fornaro, M., Tos, P., Battiston, B., Giacobini-Robecchi, M. G., & Geuna, S. (2011). Perspectives in regeneration and tissue engineering of peripheral nerves. *Annals of Anatomy*, 193(4), 334–340. <http://dx.doi.org/10.1016/j.aanat.2011.03.001>.

Raimondo, S., Nicolino, S., Tos, P., Battiston, B., Giacobini-Robecchi, M. G., Perroteau, I., et al. (2005). Schwann cell behavior after nerve repair by means of tissue-engineered muscle-vein combined guides. *Journal of Comparative Neurology*, 489(2), 249–259. <http://dx.doi.org/10.1002/cne.20625>.

Raimondo, S., Penna, C., Pagliaro, P., & Geuna, S. (2006). Morphological characterization of GFP stably transfected adult mesenchymal bone marrow stem cells. *Journal of Anatomy*, 208(1), 3–12. <http://dx.doi.org/10.1111/j.1469-7580.2006.00511.x>.

Rath, E. M. (2002). Skeletal muscle autograft for repair of the human inferior alveolar nerve: A case report. *Journal of Oral and Maxillofacial Surgery*, 60(3), 330–334.

Rinker, B., & Liao, J. Y. (2011). A prospective randomized study comparing woven polyglycolic acid and autogenous vein conduits for reconstruction of digital nerve gaps. *The Journal of Hand Surgery*, 36(5), 775–781. <http://dx.doi.org/10.1016/j.jhssa.2011.01.030>.

Rivlin, M., Sheikh, E., Isaac, R., & Beredjiklian, P. K. (2010). The role of nerve allografts and conduits for nerve injuries. *Hand Clinics*, 26(3), 435–446. <http://dx.doi.org/10.1016/j.hcl.2010.04.010>, viii.

Robinson, P. H., van der Lei, B., Hoppen, H. J., Leenslag, J. W., Pennings, A. J., & Nieuwenhuis, P. (1991). Nerve regeneration through a two-ply biodegradable nerve guide in the rat and the influence of ACTH4-9 nerve growth factor. *Microsurgery*, 12(6), 412–419.

Rochkind, S., Geuna, S., & Shainberg, A. (2009). Chapter 25: Phototherapy in peripheral nerve injury: Effects on muscle preservation and nerve regeneration. *International Review of Neurobiology*, 87, 445–464. [http://dx.doi.org/10.1016/S0074-7742\(09\)87025-6](http://dx.doi.org/10.1016/S0074-7742(09)87025-6).

Sabatier, M. J., Redmon, N., Schwartz, G., & English, A. W. (2008). Treadmill training promotes axon regeneration in injured peripheral nerves.

Experimental Neurology, 211(2), 489–493. <http://dx.doi.org/10.1016/j.expneurol.2008.02.013>.

Sameem, M., Wood, T. J., & Bain, J. R. (2011). A systematic review on the use of fibrin glue for peripheral nerve repair. *Plastic and Reconstructive Surgery*, 127(6), 2381–2390. <http://dx.doi.org/10.1097/PRS.0b013e3182131cf5>.

Siemionow, M., Bozkurt, M., & Zor, F. (2010). Regeneration and repair of peripheral nerves with different biomaterials: Review. *Microsurgery*, 30(7), 574–588. <http://dx.doi.org/10.1002/micr.20799>.

Siemionow, M., & Brzezicki, G. (2009). Chapter 8: Current techniques and concepts in peripheral nerve repair. *International Review of Neurobiology*, 87, 141–172. [http://dx.doi.org/10.1016/S0074-7742\(09\)87008-6](http://dx.doi.org/10.1016/S0074-7742(09)87008-6).

Silva-Neto, J. C., Vasconcelos, B. C., Silva-Junior, V. A., & Beder-Ribeiro, C. M. (2009). Functional histopathological and morphometric study of the use of gangliosides in nerve regeneration in rats after axotomy. *International Journal of Oral and Maxillofacial Surgery*, 38(6), 682–688. <http://dx.doi.org/10.1016/j.ijom.2009.03.723>.

Simoes, M. J., Amado, S., Gartner, A., Armada-Da-Silva, P. A., Raimondo, S., Vieira, M., et al. (2010). Use of chitosan scaffolds for repairing rat sciatic nerve defects. *Italian Journal of Anatomy and Embryology*, 115(3), 190–210.

Stanec, S., & Stanec, Z. (1998). Reconstruction of upper-extremity peripheral-nerve injuries with ePTFE conduits. *Journal of Reconstructive Microsurgery*, 14(4), 227–232. <http://dx.doi.org/10.1055/s-2007-1000173>.

Steed, M. B., Mukhatyar, V., Valmikinathan, C., & Bellamkonda, R. V. (2011). Advances in bioengineered conduits for peripheral nerve regeneration. *Atlas of the Oral and Maxillofacial Surgery Clinics of North America*, 19(1), 119–130. <http://dx.doi.org/10.1016/j.cxom.2010.11.007>.

Stefanescu, O., Jecan, R., Badoiu, S., Enescu, D. M., & Lascar, I. (2012). Peripheral nerve allograft, a reconstructive solution: Outcomes and benefits. *Chirurgia (Bucur)*, 107(4), 438–441.

Strauch, B., Rodriguez, D. M., Diaz, J., Yu, H. L., Kaplan, G., & Weinstein, D. E. (2001). Autologous Schwann cells drive regeneration through a 6-cm autogenous venous nerve conduit. *Journal of Reconstructive Microsurgery*, 17(8), 589–595. <http://dx.doi.org/10.1055/s-2001-18812>, discussion 596–587.

Teboul, F., Kakkar, R., Ameer, N., Beaulieu, J. Y., & Oberlin, C. (2004). Transfer of fascicles from the ulnar nerve to the nerve to the biceps in the treatment of upper brachial plexus palsy. *The Journal of Bone and Joint Surgery. American Volume*, 86-A(7), 1485–1490.

Terzis, J. K., & Karypidis, D. (2009). Outcomes of direct muscle neurotization in pediatric patients with facial paralysis. *Plastic and Reconstructive Surgery*, 124(5), 1486–1498. <http://dx.doi.org/10.1097/PRS.0b013e3181b98924>.

Tohill, M., & Terenghi, G. (2004). Stem-cell plasticity and therapy for injuries of the peripheral nervous system. *Biotechnology and Applied Biochemistry*, 40(Pt1), 17–24. <http://dx.doi.org/10.1042/BA20030173>.

Tos, P., Battiston, B., Ciclamini, D., Geuna, S., & Artiacco, S. (2012). Primary repair of crush nerve injuries by means of biological tubulization with muscle-vein-combined grafts. *Microsurgery*, 32(5), 358–363. <http://dx.doi.org/10.1002/micr.21957>.

Tos, P., Battiston, B., Nicolino, S., Raimondo, S., Fornaro, M., Lee, J. M., et al. (2007). Comparison of fresh and predegenerated muscle-vein-combined guides for the repair of rat median nerve. *Microsurgery*, 27(1), 48–55. <http://dx.doi.org/10.1002/micr.20306>.

Tountas, C. P., Bergman, R. A., Lewis, T. W., Stone, H. E., Pyrek, J. D., & Mendenhall, H. V. (1993). A comparison of peripheral nerve repair using an absorbable tubulization device and conventional suture in primates. *Journal of Applied Biomaterials*, 4(3), 261–268. <http://dx.doi.org/10.1002/jab.770040308>.

Tung, T. H., &

Mackinnon, S. E. (2010). Nerve transfers: Indications, techniques, and outcomes. *The Journal of Hand Surgery*, 35(2), 332–341. <http://dx.doi.org/10.1016/j.jhsa.2009.12.002>. Udina, E., Puigdemasa, A., & Navarro, X. (2011). Passive and active exercise improve regeneration and muscle reinnervation after peripheral nerve injury in the rat. *Muscle & Nerve*, 43(4), 500–509. <http://dx.doi.org/10.1002/mus.21912>. Valero-Cabre, A., Tsironis, K., Skouras, E., Perego, G., Navarro, X., & Neiss, W. F. (2001). Superior muscle reinnervation after autologous nerve graft or poly-L-lactide-epsilon-caprolactone (PLC) tube implantation in comparison to silicone tube repair. *Journal of Neuroscience Research*, 63(2), 214–223. van Meeteren, N. L., Brakkee, J. H., Hamers, F. P., Helders, P. J., & Gispen, W. H. (1997). Exercise training improves functional recovery and motor nerve conduction velocity after sciatic nerve crush lesion in the rat. *Archives of Physical Medicine and Rehabilitation*, 78(1), 70–77. van Meeteren, N. L., Brakkee, J. H., Helders, P. J., & Gispen, W. H. (1998). The effect of exercise training on functional recovery after sciatic nerve crush in the rat. *Journal of the Peripheral Nervous System*, 3(4), 277–282. Verdu, E., Navarro, X., Gudino-Cabrera, G., Rodriguez, F. J., Ceballos, D., Valero, A., et al. (1999). Olfactory bulb ensheathing cells enhance peripheral nerve regeneration. *Neuroreport*, 10(5), 1097–1101. Viterbo, F., Trindade, J. C., Hoshino, K., & Mazzoni Neto, A. (1994). End-to-side neurotomy with removal of the epineurial sheath: An experimental study in rats. *Plastic and Reconstructive Surgery*, 94(7), 1038–1047. Walton, R. L., Brown, R. E., Matory, W. E., Jr., Borah, G. L., & Dolph, J. L. (1989). Autogenous vein graft repair of digital nerve defects in the finger: A retrospective clinical study. *Plastic and Reconstructive Surgery*, 84(6), 944–949, discussion 950–942. Wang, E. T., & Zhao, M. (2010). Regulation of tissue repair and regeneration by electric fields. *Chinese Journal of Traumatology*, 13(1), 55–61. Wang, W. J., Zhu, H., Li, F., Wan, L. D., Li, H. C., & Ding, W. L. (2009). Electrical stimulation promotes motor nerve regeneration selectivity regardless of end-organ connection. *Journal of Neurotrauma*, 26(4), 641–649. <http://dx.doi.org/10.1089/neu.2008.0758>. Weber, R. A., Breidenbach, W. C., Brown, R. E., Jabaley, M. E., & Mass, D. P. (2000). A randomized prospective study of polyglycolic acid conduits for digital nerve reconstruction in humans. *Plastic and Reconstructive Surgery*, 106(5), 1036–1045, discussion 1046–1038. Whitlock, E. L., Kasukurthi, R., Yan, Y., Tung, T. H., Hunter, D. A., & Mackinnon, S. E. (2010). Fibrin glue mitigates the learning curve of microneurosurgical repair. *Microsurgery*, 30(3), 218–222. <http://dx.doi.org/10.1002/micr.20754>.

Williams, D. F. (2009). On the nature of biomaterials. *Biomaterials*, 30(30), 5897–5909. <http://dx.doi.org/10.1016/j.biomaterials.2009.07.027>. Wrede, L. (1909). Überbrückung eines nervendefektes mittels seidennaht und leben venenstueckes. *Deutsches Medizin Wochenschrift*, 35, 1125–1160. Yamaguchi, I., Itoh, S., Suzuki, M., Osaka, A., & Tanaka, J. (2003). The chitosan prepared from crab tendons: II. The chitosan/apatite composites and their application to nerve regeneration. *Biomaterials*, 24(19), 3285–3292. Yamamoto, Y., Yasuda, Y., Kimura, Y., & Komiya, Y. (1998). Effects of cilostazol, an antiplatelet agent, on axonal regeneration following nerve injury in diabetic rats. *European Journal of Pharmacology*, 352(2–3), 171–178. Yan, Y., Sun, H. H., Hunter, D. A., Mackinnon, S. E., & Johnson, P. J. (2012). Efficacy of short-term FK506 administration on accelerating nerve regeneration. *Neurorehabilitation and Neural Repair*, 26(6), 570–580. <http://dx.doi.org/10.1177/1545968311431965>. Yang, S., Leong, K. F., Du, Z., & Chua, C. K. (2001).

The design of scaffolds for use in tissue engineering. Part I. Traditional factors. *Tissue Engineering*, 7(6), 679–689. <http://dx.doi.org/10.1089/107632701753337645>. Yannas, I. V., & Hill, B. J. (2004). Selection of biomaterials for peripheral nerve regeneration using data from the nerve chamber model. *Biomaterials*, 25(9), 1593–1600. Yin, Z. S., Zhang, H., Bo, W., & Gao, W. (2010). Erythropoietin promotes functional recovery and enhances nerve regeneration after peripheral nerve injury in rats. *American Journal of Neuroradiology*, 31(3), 509–515. <http://dx.doi.org/10.3174/ajnr.A1820>. Young, R. C., Wiberg, M., & Terenghi, G. (2002). Poly-3-hydroxybutyrate (PHB): A resorbable conduit for long-gap repair in peripheral nerves. *British Journal of Plastic Surgery*, 55(3), 235–240. <http://dx.doi.org/10.1054/bjps.2002.3798>. Zacchigna, S., & Giacca, M. (2009). Chapter 20: Gene therapy perspectives for nerve repair. *International Review of Neurobiology*, 87, 381–392. [http://dx.doi.org/10.1016/S00747742\(09\)87020-7](http://dx.doi.org/10.1016/S00747742(09)87020-7). Zhang, F., Blain, B., Beck, J., Zhang, J., Chen, Z., Chen, Z. W., et al. (2002). Autogenous venous graft with one-stage prepared Schwann cells as a conduit for repair of long segmental nerve defects. *Journal of Reconstructive Microsurgery*, 18(4), 295–300. <http://dx.doi.org/10.1055/s-2002-30186>. Zhang, C. G., & Gu, Y. D. (2011). Contralateral C7 nerve transfer – Our experiences over past 25 years. *Journal of Brachial Plexus and Peripheral Nerve Injury*, 6(1), 10. <http://dx.doi.org/10.1186/1749-7221-6-10>. Zorn, K. C., Bernstein, A. J., Gofrit, O.N., Shikanov, S.A., Mikhail, A. A., Song, D.H., et al. (2008). Long-term functional and oncological outcomes of patients undergoing sural nerve interposition grafting during robot-assisted laparoscopic radical prostatectomy. *Journal of Endourology*, 22(5), 1005–1012. <http://dx.doi.org/10.1089/end.2007.0381>.