Tissue engineering and peripheral nerve reconstruction: an overview.

Tissue Engineering and Peripheral Nerve Reconstruction: An Overview

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Abstract

Nerverepairisnomoreregardedasmerelyamatterofmicrosurgicalreconstruction.To define this evolving reconstructive/regenerative approach, the term tissue engineering is being increasingly usedsince it reflects the search for interdisciplinary andintegrated 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 presentsa syntheticoverview ofthe state of the art inperipheral nerve tissue engineeringwithalookforwardatthemostpromisinginnovationsemergingfrombasicscience 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 onlyoccasionallyachievedafteranerve lesionand,inmany cases,theclinical 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 (endto-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, Ameur, 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-assistedtechnologieswill befavored moreandmoreby 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). Anotheralternative to nerve autografts that has receivedattention 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-veincombined 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 Bu ¨ngner 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; GuntinasLichius 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 Ruiter, 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, a 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 Ruiter 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 & Liau, 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),manualstimulation(Bischoffetal.,2009),andneuralinterfaces(delValle&Navarro,2014; Herrera-Rincon, Torets, 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 acetil-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. Itisfarbeyond the goalofthis chapter torevisitallthe 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 modulatesinflammatoryresponses(Girardetal.,2008);flunarizine,acalcium channel antagonist and vasodilatator (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 DIFFERENTTISSUEENGINEERINGAPPROACHES,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,surgeonsmustinteractwithengineersandmaterialscientistsin the light of the recent enormous advances in nanotechnology that makes it possible to develop and design very complex synthetic scaffolds to repair neuraldefects.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.

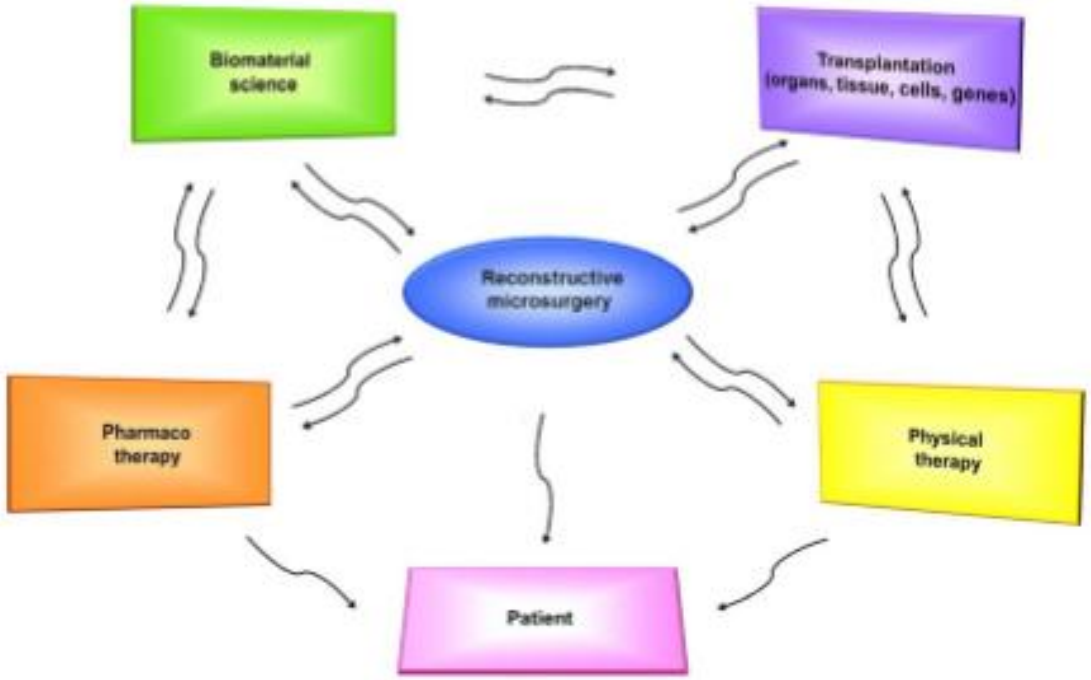

Figure 2.1 Schematic drawing depicting the various disciplines involved in tissue engineering of the peripheral nerve.

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.

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