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Tissue engineered nerve constructs: where do we stand?

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Abstract

Driven by enormous clinical need, interest in peripheral nerve regeneration has become a prime focus of research and area of growth within the field of tissue engineering. While using autologous donor nerves for bridging peripheral defects remains today's gold standard, it remains associated with high donor site morbidity and lack of full recovery. This dictates research towards the development of biomimetic constructs as alternatives. Based on current concepts, this review summarizes various approaches including different extracellular matrices, scaffolds, and growth factors that have been shown to promote migration and proliferation of Schwann cells. Since neither of these concepts in isolation is enough, although each is gaining increased interest to promote nerve regeneration, various combinations will need to be identified to strike a harmonious balance. Additional factors that must be incorporated into tissue engineered nerve constructs are also unknown and warrant further research efforts. It seems that future directions may allow us to determine the "missing link."

Keywords: nerve tissue engineering • Schwann cell cultures • extracellular matrices • growth factors • scaffolds

Introduction

The field of tissue engineering has grown exponentially over the past few decades fueled by the excitement of developing and replacing viable and functional body tissues. Although foundations were laid in 1933 by Bisceglie with implanted mouse tumor cells encased in an immunoshielding biocompatible polymer membrane, [1] it was not until the late 1980's and early 1990's that the field rapidly progressed. One leading growth area centered around the interest of peripheral nerve regeneration. To date, the standard for bridging peripheral nerve defects involves the use of autografts. Although functional, the associated morbidity at the donor site and the lack of full recovery dictates research towards the development of biomimetic constructs as alternatives [2, 3].

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The interdisciplinary field of tissue engineering has changed over the last several years. The once expanding field has decreased with a more realistic approach regarding specific tissue types. Particularly with nerve tissue, initial enthusiasm with promising results obtained *in vitro* soon diminished with the harsh reality that cells and growth factors did not behave as expected in the clinical situation. Furthermore, the ability to bridge small nerve gaps in a rat or mouse model could not be translated directly to the clinical setting where larger (2 to 3 cm) gaps are clinically common. Last, the diversity of the nervous system demands differing approaches in different locations. For example, the central nervous system's support cells (astrocytes) seem to be more inhibitory to axonal proliferation as compared with the peripheral system's Schwann cells [4]. Sensory nerves seem to respond better to regeneration than motor neurons [5]. Consequently, more distal injuries seem to have better long-term results because division of the motor and sensory branches is more delineated with greater distance from the spinal cord.

To understand the complicated interaction of nerve components for tissue engineering, one must first understand the molecular interactions in nerve injury or repair. Although significant progress has been made in this field, full elucidation of the complex interactions of nerve repair falls short. We are in an infancy of an era of tissue engineering.

In the United States, more that 50,000 surgical procedures are performed each year for peripheral nerve repair and the number of injuries is estimated to be even greater [6, 7]. Economically, this translates into a significant amount of lost worker days due to the extent of work-associated injuries to the upper extremity. Tissue engineering in peripheral nerve repair will clearly have increased clinical applicability, but the question remains whether we can develop and use a functional tissue engineered nerve construct. This review will address current developments in peripheral nerve tissue engineering and the impact these will have in the future. To understand current approaches to nerve engineered constructs, several critical components should be considered: scaffolds for mechanical support, support cells, growth factors, and some unidentified material that we term ''extra-cellular matrix.''

Scaffolds

Original attempts at nerve repair date back to 1608, but it was not until the nineteenth century that physicians began to focus considerable effort on nerve restoration [8]. Unfortunately, most early surgical reconstructive techniques for the repair of peripheral nerve defects resulted in poor functional recovery. Alternative materials, including fat sheaths, gauze, bone, and metal tubes were used in an attempt to repair damaged nerves [9]. In the 1960's, Millesi introduced the concept of microsurgery, in which individual nerve fascicles in each nerve stump could be precisely aligned and tension minimized as a means to dramatically improve the success of nerve regeneration [10–12]

There are several key properties that all guidance channels should possess: (**1**) they must be readily formed into a conduit having a desired diameter and wall thickness, (**2**) they should be simple to implant using microsurgical techniques, (**3**) they must be sterilizable, and (**4**) they should be biodegradable [13]. Permanent materials are less desireable because they may pose a more severe risk of infection, tend to provoke connective tissue responses, and can compress nerves or become dislodged. Synthetic and natural materials have been used for guidance channels. Williams, *et al.* demonstrated that when silicone tubes are used, within hours the tubes fill with nerve cables and blood vessels. During the second week, fibroblasts, Schwann cells, macrophages, and endothelial cells permeate the fibrin matrix. Axons sprouting from the proximal nerve stump elongate along the matrix [14, 15] (Fig. 1).

Considerable focus has been placed on the use of natural materials for nerve scaffolds. Various autologous tissues have been demonstrated to increase regeneration competence in rats including epineurial sheaths, tendon, vein [16] and basal laminae extracted from decellularized muscle [4]. These autologous tissues have also been impregnated with growth factors or coated with collagens for further stimulation of axonal outgrowth and Schwann cell migration [16] Raimondo *et al.* recently demonstrated a vein filled with skeletal muscle as a suitable means of sustaining Schwann cell migration and proliferation [17]. The advantage of biocompatibility decreases the potential toxic effect of using inert or synthetic material. The hurdle that remains is the

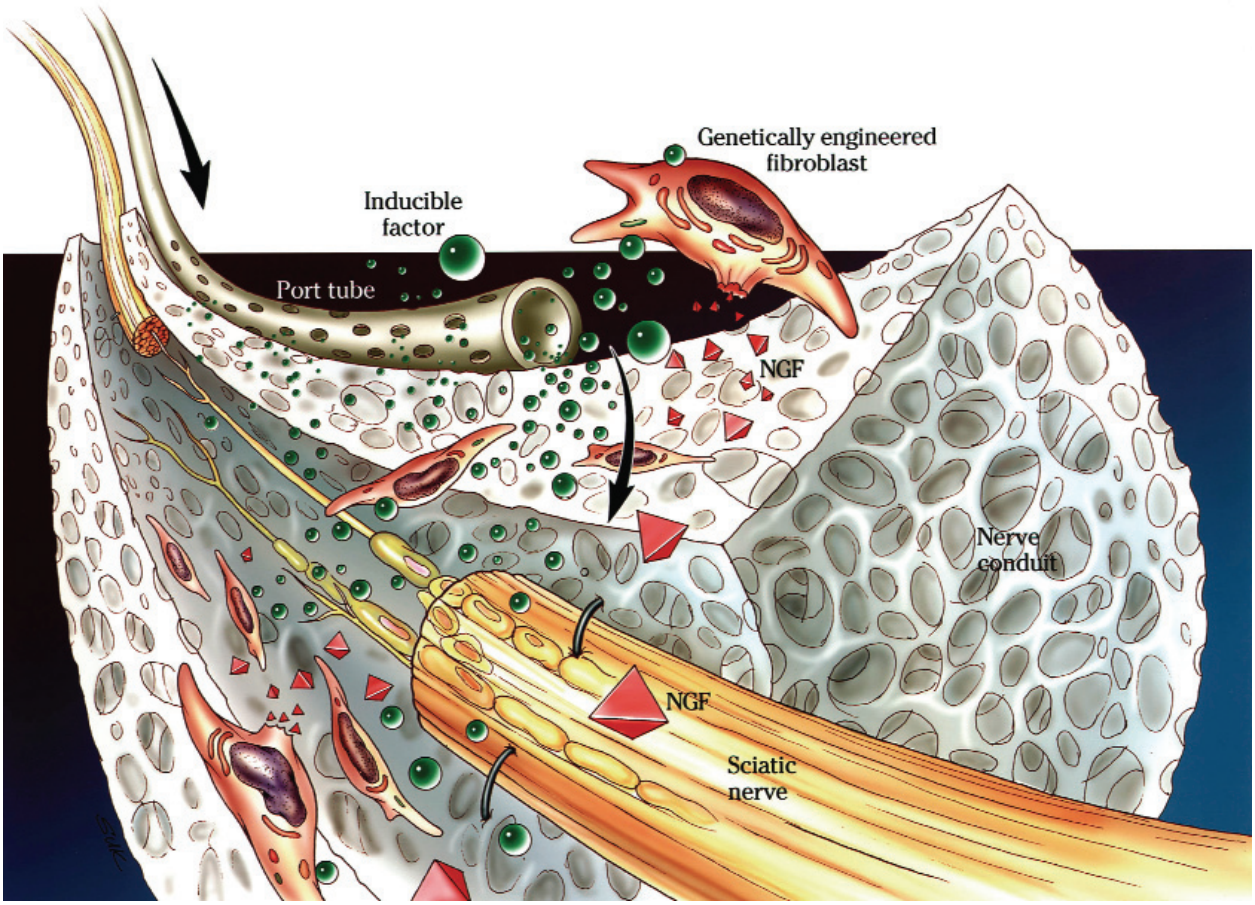

Fig. 1 Schematic Model of Nerve Tissue Engineering utilizing nerve conduits and highlighting the role of nerve growth factors, cultured cells, and genetically modified cells

undesirable immune response depending on the patient or the source of the material [13, 18]. Allografts and xenografts have not been effective clinically because of issues with host response. Acellular grafts, although non-immunogenic, tend to result in a significant degree of inflammation when implanted, mainly because the decellularization fails to completely extract cellular remnants or leaves damaged extracellular matrices. However, due to the current literature there have been a number of studies which have demonstrated the reconstruction of relatively large nerve gaps with acellular nerve grafts repopulated with autogenous "support cells" or enhanced with growth factors [4]. In addition, these allografts have been modified so they are less immunogenic and have also been repopulated with autogenous schwann cells to allow reconstruction of large gaps. This is indeed a tissue engineering approach for developing alternative peripheral nerve constructs. Recent approaches with chemical detergents have proven more effective in preserving structural integrity [19]. The potential use of cadaveric products through specialized processing may eliminate the cells responsible for this immunologic reaction, leaving the backbone structure for axonal growth. Further, recent work has demonstrated that low-dose immunosuppression with FK 506 has yielded improved regeneration with the use of allografts [20–21].

Several biodegradable synthetic materials have been demonstrated to support nerve regeneration. Polyesters such as polylactic acid, polyglycolic acid, and poly(lactic-co-glycolic acid) (PLGA) were early choices for investigation because of their availability and FDA approval [13]. Although a variety of additional materials have been developed, the approval of PLGA for clinical use has encouraged its use over other materials. Physical properties,

such as conduit dimensions, wall porosity, surface texture, and inherent electrical properties, also dramatically influence the effects of nerve regeneration [13]. These alterations in physical properties directly effect the diffusion of nutrient materials, allow the acceleration of axonal growth for certain characteristics such as smooth surfaces, and may inhibit detrimental cells such as fibroblasts, which may cause further scar formation [5].

The exact nature of the ideal conduit is unknown. A combination of synthetic and natural materials may be the ideal solution. The ability of axons to traverse an autograft through preset channels created by Schwann cell alignment has not been duplicated with the materials or designs currently in use. The use of a round tube may not be the most ideal construct for larger nerve gaps. New innovations such as electrical poling have also been applied to bioengineered conduits and have increased the ability of injured nerves to reach their target organs through enhanced axonal regeneration and improved conduction rates [22]. As our ability to design and modify scaffold material to appropriate sizes and dimensions improves, the correct combination will be found.

Support cells

The inclusion of neuronal support cells is critical for axonal proliferation. Although the exact underlying mechanisms regulating dynamic axon/Schwann cell apposition are unknown, experiments support the concept that Schwann cells offer a highly preferred substrate for axon migration and release of bioactive factors that further enhance nerve migration [20]. In the normal nerve, Schwann cells seem to be quiescent; however, upon nerve injury, Schwann cells in the distal nerve undergo extensive change concomitant with axonal degeneration. Within hours, Schwann cells begin to express MAC-2, a galactose-specific lectin, which targets the galactolipid-rich myelin in nonimmune opsonin-independent lectinphagocytosis and mediates the bulk of phagocytosis within the first few hours of injury. This phagocytosis is also supported by the migration of macrophages [23–33]. Thus, Schwann cells play an important role in peripheral nerve regeneration probably through several mechanisms. These include the release of growth factors and perhaps a mechanical role by bridging the gap between axonal growth cone migration and the basement membrane. Despite the role that Schwann cells play, studies that have demonstrated improved axonal growth by placing Schwann cells within a scaffold have not solved all of the problems of axonal regeneration [34–39]. Questions regarding immunogenicity remain. How long can these Schwann cells survive? Can these cells be pre-placed within a conduit and remain viable for transportation and implantation? Further, the use of autogenous Schwann cells would still require the harvest of peripheral sensory nerves for culture and expansion before implantation.

Alternative sources of cells must be explored. Data from our laboratory has demonstrated the ability of fibroblasts to act like Schwann cells, releasing nerve growth factors (NGF) for axonal proliferation [40]. The use of genetically engineered dermal fibroblast cells allows ease of harvest (through skin biopsy), ease of expansion and growth, and more resilience for implantation. However, the concerns of excessive scar formation have limited our initial enthusiasm for these cells. Alternative cells (HEK-293) have recently been used and may offer similar efficacy without the elevated risk of scar formation [41–43]. Apart from inducing a limited immunologic response, this cell line is human and embryonic, making it a promising delivery system for inducible expression of growth factors [41–43].

With the growing applicability of stem cells in all avenues of medicine, increased opportunities become available to allow the use of a variety of cell lines for nerve differentiation such as embryonic stem cells, bone marrow-derived stem cells, somatic pluripotent stem cells, adipose-derived stem cells, mesenchymal stem cells, and progenitor cells [44–45]. The advantage of using these cells lines is that the potential differentiation may be stimulated by the advancing axons. Furthermore, it is postulated that Schwann cells are the primary support cells for axonal migration peripherally. Additional cells, though, may be responsible for this axonal growth. Pluripotent stem cells may allow multiple differentiation paths, creating an environment that is ripe for the support of axon regeneration. The majority of this work to date has been focused in the central nervous system, although there have been recent developments peripherally. Murakami *et al.* transplanted neuronal progenitor cells derived from the fetal hippocampus in the rat model and demonstrated

their differentiation into Schwann cells that helped promote axonal regeneration through a nerve defect [46]. Neural stem cells have also been effective at restoring motor function in the setting of chronically denervated peripheral nerves [47]. Neural stem cells have been identified in the adult central nervous system and have successfully been propagated *in vitro*, but the source of donor tissue and expansion difficulty of such cells could pose a significant barrier [48]. Olfactory ensheathing cells (OEC's) have been described as the progenitor cells of the peripheral nervous system [49]. They are a glial-cell type and continually regenerate to myelinate the olfactory nerves. OEC's have stimulated axonal regeneration in the spinal cord; their potential in the peripheral nervous system is still debatable [4, 49]. Hair follicle stem cells, which may be easier to access, have been transdifferentiated into Schwann cells and indicate promise in enhancing rate of restoration of nerve function [50]. Bone marrow stromal cells, or mesenchymal stem cells (MSC), have a similar advantage in their accessibility, and demonstrate comparable results in supporting peripheral nerve regeneration, mainly by exhibiting characteristics similar to Schwann cells [51–54]. MSC's can be differentiated to glial-type cells *via* induction with various mitogens *in vitro*. Tohill and Torenghi have demonstrated that these cells may confer some beneficial effect on Schwann cell growth indicating both stimulatory and supportive roles [49]. Given the apparent plasticity of such stem cells and their presence in adult tissues, it is certain that stem cells will be integral components in neural tissue engineering.

Growth factors

Soluble neurotropic and neurotrophic factors can be incorporated directly into nerve guidance conduits. Some of these factors include NGF, brain-derived neurotrophic factor (BDNF), insulin-like growth factor (IGF-1, IGF-2), platelet-derived growth factors, fibroblast growth factor, and ciliary neurotrophic factor. Although a variety of family compounds (*e.g.*, neurotrophins, cytokines, and insulin derivatives) stimulate peripheral nerve migration directly or indirectly, NGF seems to have the greatest effect [55–69]. There is increasing evidence that many neurotrophic molecules (NGF, BDNF, NT-3, NT-4/5) act directly to promote survival and indirectly on regenerating axons *via* non-neuronal cells. NGF has been demonstrated to prevent the death of axotomized sensory neurons completely after exogenous administration [55–69].

Growth factors can be delivered through a variety of mechanisms. Traditionally, growth factors were delivered exogenously through the application of tubes or injections. This delivery system allows a one-time ''spike'' that occurs after the application. Further, the delivery of growth factors in the desired concentration at the axonal/Schwann cell interface (location specific) cannot be regulated. Alternative methods for delivery have been explored, including the use of microsphere technology [4]. The problem with microsphere delivery of growth factors is that degradation can be variable. Degradation begins immediately upon delivery, although recent work demonstrates that growth factor delivery duration can be sustained for an extended period of time [70]. Growth factor levels may require alternative delivery times and sequences. The same holds true for the application and incorporation of growth factors within synthetic scaffolds. Thus, protocols for the use of growth factors must address the sequence, timing, dosage, delivery vector, and duration of therapy [71–73]. In an attempt to incorporate these issues in growth factor delivery, focus has turned to plasmid or viral vectors as modes of controlled gene expression of growth factors. Retrovirus, adeno-associated virus, adenovirus, and herpes virus have all been utilized as vehicles for gene therapy, with the latter two being more common in neural application. Transfection of delivery cells with Muristerone A, or similar derivatives, or tetracycline-responsive promoters has also proven to be a successful means of molecular control [4]. Our laboratory has successfully regulated NGF secretion *in vitro* and *in vivo*, allowing dosing, timing, and continued release through the transfection of HEK-293 cells [41–43]. We believe that this application offers a more attractive delivery mechanism than what has previously been used.

Extracellular matrix

The additional factors that must be incorporated into tissue engineered nerve constructs are

unknown. We have tentatively labeled this as ''extracellular matrix.'' If support cells, scaffolds, and growth factors were all that was needed, we should have been able to produce a tissue engineered construct. There seems, however, to be some additional material or a combination of materials that is missing. Insoluble extracellular matrix molecules, including laminin, fibronectin, and some forms of collagen, have been demonstrated to promote axonal extension. They have been demonstrated to improve axonal regeneration when incorporated into the lumen of nerve guidance channels [74–77]. Chitosan, a polysaccharide derivative of chitin, possesses antitumor and antibacterial properties and has been shown to promote migration and proliferation of Schwann cells, thereby gaining increased interest to promote nerve regeneration [78]. Combinations of natural materials have also gained approval. For example, a chitosan/peptide blend conduit has been found to improve adhesion and differentiation of PC12 cells when compared to chitosan or composite material alone [7]. Poly-3-hydroxybuturate, a natural polymer used as a bacterial storage product, has also demonstrated utility as an alternative for a conduit in bridging nerve gaps in rabbits [79]. The incorporation of extracellular matrix materials alone, however, is not enough. Future directions may allow us to determine the ''missing link.''

Future directions

Primary neurorrhaphy and use of nerve autografts remain the gold standards for nerve gap repair. Significant advances have been made in devising alternatives to these options, including use of bioengineered conduits, manipulation of growth factor expression through genetic engineering, and induction of stem cell differentiation. With respect to incorporation of many of these applications clinically, we find ourselves at a stage of infancy with many barriers remaining to be overcome. It is evident that different cell types often require unique culture environments, making it difficult to design a multilayer tissue engineered construct. Ahead lies the challenge to create a scaffold capable of supporting a variety of cell types through histioconductive processes. There remains much to learn regarding the use of growth factors. The appropriate dosing or the complex cascade and coordinated sequences of their elaborations are not completely understood. Many growth factors have pleiotropic effects on cells. Defining precisely the appropriate expression sequence, dosing, and duration for growth factors is critical. Genetic engineering may hold the key to future development of tissue engineered nerve constructs. With the ability to identify and perhaps delete specific genetic sequences, we might be able to identify, modify and/ or manipulate genes critical to neuronal development. Embryonic stem cells and pleuripotent cells will likely have a strong impact on advances toward finding a suitable alternative to autologous nerve grafts. Perhaps the answer to the ideal bioengineered nerve graft will come as a mélange of all these factors. In any instance, similar to other developments in different fields of Tissue Engineering [80–84], we are headed for a future filled with exciting new developments.

References

1. **Bisceglie V.** Uber die antineoplastische Immunitat; heterologe Einpflanzung von Tumoten in Huhner-Embryonen. *Ztschr Krebsforsch* 1933; 40: 122–40.
2. **Beazley WC, Milek MA, Reiss BH.** Results of nerve grafting in severe soft tissue injuries. *Clin Orthop Relat Res.* 1984; 188: 208–12.
3. **Dellon AL, MacKinnon SE.** An alternative to classical nerve graft for the management of the short nerve gap. *Plast Reconstr Surg.* 1988; 82: 849–56.
4. **Schmidt C, Leach JB.** Neural tissue engineering: strategies for repair and regeneration. *Annu Rev Biomed Eng.* 2003; 5: 293–347.
5. **Evans GRD.** Approaches to tissue engineered peripheral nerve. *Clin Plastic Surg.* 2003; 30: 559–63.
6. **Lee AC, Yu VM, Lowe JB 3rd, Brenner MJ, Hunter DA, Mackinnon SE, Sakiyama-Elbert SE.** Controlled release of nerve growth factor enhances sciatic nerve regeneration. *Exp Neurol.* 2003; 184: 295–303.
7. **Ciardelli G, Chiono V.** Materials for peripheral nerve regeneration. *Macromol Biosci*. 2006; 6: 13–26.
8. **Ferrara G.** Nuova selva di chirurgia divisiain tre parti. Venice: S Combi; 1608.
9. **Glück T.** Ueber neuroplastik auf dem wege der trans-plantation. *Arch Klin Chir.* 1880; 25: 606–16.
10. **Millesi H, Ganglberger J, Berger A.** Erfharungen mit der mikrochirurgie peripherer nerven. *Chirurgica Plastica.* 1967; 34: 47.

11. **Millesi H.** Peripheral nerve surgery today: turning point or continuous development? *J Hand Surg [Br]*. 1990; 15: 28.
12. **Millesi H.** Progress in peripheral nerve reconstruction. *World J Surg.* 1990; 14: 733–47.
13. **Hudson R, Evans GRD, Schmidt C.** Engineering strategies for peripheral nerve repair. *Clin Plast Surg.* 1999; 4: 617– 28. (Reprinted in *Orthop Clin N Am.* 2000; 3 1: 485–97.)
14. **Williams LR, Longo FM, Powell HC.** Spatial-temporal progress of peripheral nerve regeneration within a silicone chamber: parameters for a bioassay. *J Comp Neurol.* 1983; 218: 460–70.
15. **Williams LR, Varon S.** Modification of fibrin matrix formation in situ enhances nerve regeneration in silicone chambers. *J Comp Neurol.* 1985; 231: 209–20.
16. **Lundborg G.** Alternatives to autologous nerve grafts. *Handchir Mikrochir Plast Chir.* 2004; 36: 1–7.
17. **Raimondo S, Nicolino S, Tos P, Battiston B, Giacobini-Robecchi MG, Perroteau I, Geuna S.** Schwann cell behavior after nerve repair by means of tissue-engineered muscle-vein combined guides. *J Comp Neurol.* 2005; 489: 249–59.
18. **DeLustro F, Dasch J, Keefe J.** Immune responses to allogeneic and xenogeneic implants of collagen and collagen derivatives. *Clin Orthop.* 1990; 260: 263–79.
19. **Hudson TW, Liu S, Schmidt CE.** Engineering an improved acellular nerve graft *via* optimized chemical processing. *Tissue Eng* 2004; 10: 1346–58.
20. **Sobol JB, Lowe III JB, Yang RK, Sen SK, Hunter DA, Mackinnon SE.** Effects of delaying FK506 administration on neuroregeneration in a rodent model. *J Reconstr Microsurg.* 2003; 19: 113–8.
21. **Myckatyn T, MacKinnon SE, McDonald JW.** Stem cell transplantation and other novel techniques for promoting recovery from spinal cord injury. *Transpl Immunol.* 2004; 12: 343–58.
22. **Bryan DJ, Tang JB, Doherty SA, Hile DD, Trantolo DJ, Wise DL, Summerhayes IC.** Enhanced peripheral nerve regeneration through a poled bioresorbable poly(lactic-co-glycolic acid) guidance channel. *J Neural Eng.* 2004; 1: 91–8.
23. **Evans GRD.** Peripheral nerve injury: a review and approach to tissue engineered constructs. *Anat Rec.* 2001; 263: 396–404.
24. **Martini R.** Expression and functional roles of neural cell surface molecules and extracellular matrix components during development and regeneration of peripheral nerve. *J Neurocytol.* 1994; 23: 1–28.
25. **Tonge DA, Golding JP.** Regeneration and repair of the peripheral nervous system. *Semin Neurosci.* 1993; 5: 385–90.
26. **Woerly S, Plant GW, Harvey AR.** Neural tissue engineering: from polymer to biohybrid organs. *Biomaterials* 1996; 17: 301–10.
27. **Beier JP, Kneser U, Stern-Strater J, Stark GB, Bach AD.** Y chromosome detection of three-dimensional tissue-engineered skeletal muscle constructs in a syngeneic rat animal model. *Cell Transplant.* 2004; 13: 45–53.
28. **Madison RD.** Point sources of Schwann cells result in growth into nerve entubulation repair site in the absence of axons: effects of freeze-thawing. *Exp Neurol*. 1994; 128: 266–75.
29. **De Vries GH.** Schwann cell proliferation. In: Dyck PJ, Thomas PK, editors. Peripheral neuropathy. Philadelphia: W.B. Saunders; 1993. p. 290–8.
30. **Rath EM, Kelly D, Bouldin TW, Popko B.** Impaired peripheral nerve regeneration in a mutant strain of mice (Enr) with a Schwann cell defect. *J Neurosci.* 1995; 15: 7228–37.
31. **Hadlock T, Elisseeff J, Langer R, Vacanti J, Cheney M.** A tissue-engineered conduit for peripheral nerve repair. *Arch Otolaryngol Head Neck Surg.* 1998; 124: 1081–6.
32. **Zhang Y, Campbell G, Anderson PN, Martini R, Schachner M, Lieberman AR.** Molecular basis of interactions between regenerating adult rat thalamic axons and Schwann cells in peripheral nerve grafts II: tenascin-c. *J CompNeurol.* 1995; 361: 210–24.
33. **Zhang Y, Campbell G, Anderson PN, Martini R, Schachner M, Lieberman AR.** Molecular basis of interactions between regenerating adult rat thalamic axons and Schwann cells in peripheral nerve grafts I: neural cell adhesion molecules. *J Comp Neurol.* 1995; 361: 193–209.
34. **Terenghi G.** Peripheral nerve injury and regeneration. *Histol Histopathol.* 1995; 10: 709–18.
35. **Brown RE, Erdmann D, Lyons SF, Suchy H.** The use of cultured Schwann cells in nerve repair in a rabbit hindlimb model. *J Reconstr Microsurg.* 1996; 12: 149–52.
36. **Levi AD, Guenard V, Aebischer P, Bunge RP.** The functional characteristics of Schwann cells cultured from human peripheral nerve after transplantation into a gap within the rat sciatic nerve. *J Neurosci.* 1994; 14: 1309–19.
37. **Guenard V, Kleitman N, Morrissey TK, Bunge RP, Aebischer P.** Syngeneic Schwann cells derived from adult nerves seeded in semipermeable guidance channels enhance peripheral nerve regeneration. *J Neurosci.* 1992; 12: 3310–20.
38. **Osawa T, Tohyama K, Ide C.** Allogeneic nerve grafts in the rat, with special reference to the role of Schwann cells basal laminae in nerve regeneration. *J Neurocytol.* 1990; 19: 833–49.
39. **Evans GR, Brandt K, Katz S, Chauvin P, Otto L, Bogle M, Wang B, Meszlenyi RK, Lu L, Mikos AG, Patrick CW Jr.** Bioactive poly(L-lactic acid) conduits seeded with Schwann cells for peripheral nerve regeneration. *Biomaterials* 2002; 23: 841–8.
40. **Patrick CW, Zheng B, Schmidt M, Herman PS, Chauvin BP, Fan Z, Stark B, Evans GR.** Dermal fibroblasts genetically engineered to release NGF. *Ann Plast Surg.* 2001; 47: 660–5.
41. **Jimenez JC, Tyson DR, Dhar S, Nguyen T, Hamai Y, Bradshaw RA, Evans GR.** Human embryonic kidney cells (HEK-293): characterization and dose response for modulated release of NGF for nerve regeneration. *Plast Reconstr Surg.* 2004; 113: 605–10.
42. **McConnell MP, Dhar S, Nguyen T, Naran S, Calvert JW, Sundine MJ, Bradshaw RA, Evans GR.** Nerve growth factor expression response to induction agent booster dosing in transfected human embryonic kidney cells. *Plast Reconstr Surg.* 2005; 115: 506–14.

43. **McConnell MP, Dhar S, Naran S, Nguyen T, Bradshaw RA, Evans GR.** *In vivo* induction and delivery of nerve growth factor, using HEK-293 cells. *Tissue Eng.* 2004; 10: 1492–501.
44. **Knight M, Evans GRD.** Tissue engineering: progress and challenges. *Plast Reconstr Surg.* 2004; 114: 26E–37E.
45. **Dezawa M.** Future views and challenges to the peripheral nerve regeneration by cell based therapy. *Rinsho Shinkeigaku* 2005; 45: 877–9.
46. **Murakami T, Fujimoto Y, Yasunaga Y, Ishida O, Tanaka N, Ikuta Y, Ochi M.** Transplanted neuronal progenitor cells in a peripheral nerve gap promote nerve repair. *Brain Res.* 2003; 974: 17–24.
47. **Heine W, Conant K, Griffin JW, Hoke A.** Transplanted neural stem cells promote axonal regeneration through chronically denervated peripheral nerves. *Exp Neurol.* 2004; 189: 231–40.
48. **Morrison SJ, White PM, Zock C, Anderson DJ.** Prospective identification, isolation by flow cytometry, and *in vivo* self-renewal of multipotent mammalian neural crest stem cells. *Cell* 1999; 96: 737–49.
49. **Tohill M, Terenghi G.** Stem cell plasticity and therapy for injuries of the peripheral nervous system. *Biotechnol Appl Biochem.* 2004; 40: 17–24.
50. **Amoh Y, Li L, Campillo R, Kawahara K, Katsuoka K, Penman S, Hoffman RM.** Implanted hair follicle stem cells form Schwann cells that support repair of severed peripheral nerves. *Proc Natl Acad Sci USA.* 2005; 102: 17734–8.
51. **Cuevas P, Carceller F, Dujovny M, Garcia-Gomez I, Cuevas B, Gonzalez-Corrochano R, Diaz-Gonzalez D, Reimers D.** Peripheral nerve regeneration by bone marrow stromal cells. *Neurol Res.* 2002; 24: 634–8.
52. **Cuevas P, Carceller F, Garcia-Gomez I, Yan M, Dujovny M.** Bone marrow stromal cell implantation for peripheral nerve repair. *Neurol Res.* 2004; 26: 230–2.
53. **Dezawa M, Takahashi I, Esaki M, Takano M, Sawada H.** Sciatic nerve regeneration in rats induced by transplantation of in vitro differentiated bone-marrow stromal cells. *Eur J Neurosci.* 2001; 14: 1771–6.
54. **Choi BH, Zhu SJ, Kim BY, Huh JY, Lee SH, Jung JH.** Transplantation of cultured bone marrow stromal cells to improve peripheral nerve regeneration. *Oral Maxillofac Surg.* 2005; 34: 537–42.
55. **Fujimoto E, Mizoguchi A, Hanada K, Yajima M, Ide C.** Basic fibroblast growth factor promotes extension of regenerating axons of peripheral nerve: *in vivo* experiments using a Schwann cell basal lamina tube model. *J Neurocytol.* 1997; 26: 511–28.
56. **Sterne GD, Coulton GR, Brown RA, Green CJ, Terenghi G.** Neurotrophin-3 delivered locally *via* fibronectin mats enhances peripheral nerve regeneration. *Eur J Neurosci.* 1997; 9: 1388–96.
57. **Reichert F, Levitzky R, Rotzhenker S.** Interleukin 6 in intact and injured mouse peripheral nerves. *Eur J Neurosci.* 1996; 8: 530–5.
58. **Fu SY, Gordon T.** The cellular and molecular basis of peripheral nerve regeneration. *Mol Neurobiol.* 1997; 14: 67–116.
59. **Sjoberg J, Kanje M.** Insulin-like growth factor (IGF-I) as a stimulator of regeneration in the freeze-injured rat sciatic nerve. *Brain Res.* 1989; 485: 102–8.
60. **DiStefano PS, Friedman B, Radziejewski C, Alexander C, Boland P, Schick CM, Lindsay RM, Wiegand SJ.** The neurotrophins BDNF, NT-3, and NGF display distinct patterns of retrograde axonal transport in peripheral and central neurons. *Neuron* 1992; 8: 983–93.
61. **Griffin CC, Letourneau PC.** Rapid retraction of neurites by sensory neurons in response to increased concentrations of nerve growth factor. *J Cell Biol.* 1980; 86: 156–61.
62. **Kaechi K, Ikegami R, Nakamura N, Nakajima M, Furukawa Y, Furukawa S.** 4-Methylcatechol, an inducer of nerve growth factor synthesis, enhances peripheral nerve regeneration across nerve gaps. *J Pharmacol Exp Ther.* 1995; 272: 1300–4.
63. **Yip HK, Rich KM, Lampe PA, Johnson EM Jr.** The effects of nerve growth factor and its antiserum on the postnatal development and survival after injury of sensory neurons in the rat dorsal root ganglia. *J Neurosci*. 1984; 4: 2986–92.
64. **Otto D, Unsicker K, Grothe C.** Pharmacological effects of nerve growth factor and fibroblast growth factor applied to the transectioned sciatic nerve on neuron death in adult dorsal root ganglia. *Neurosci Lett.* 1987; 83: 156–60.
65. **Rich KM, Luszczynski JR, Osborne PA, Johnson EM Jr.** Nerve growth factor protects adult sensory neurons from cell death and atrophy caused by nerve injury. *J Neurocytol.* 1987; 16: 261–8.
66. **Melville S, Sherburn TE, Coggeshall RE.** Preservation of sensory cells by placing stumps of transected nerve in an impermeable tube. *Exp Neurol.* 1989; 105: 311–5.
67. **Anton ES, Weskamp G, Reichardt LF, Matthew WD.** Nerve growth factor and its low-affinity receptor promote Schwann cell migration. *Proc Natl Acad Sci USA.* 1994; 91: 2795–9.
68. **Friedlander DR, Grumet M, Edleman GM.** Nerve growth factor enhances expression of neuron-glia cell adhesion molecule in PC 12 cells. *J Cell Biol.* 1986; 102: 413–9.
69. **Bampton ETW, Taylor JSH.** Effects of Schwann cell secreted factors on PC12 cell neuritogenesis and survival. *J Neurobiol.* 2005; 63: 29–48.
70. **Piotrowicz, A, Shoichet MS.** Nerve guidance channels as drug delivery vehicles. *Biomaterials* 2006;27: 2018–27.
71. **Whitaker MJ, Quirk RA, Howdle SM, Shakesheff KM.** Growth factor release from tissue engineering scaffolds. *J Pharm Pharmacol* 2001;53: 1427–37.
72. **Rose FRA, Oreffo ROC.** Bone tissue engineering: hope *vs.* hype. *Biochem Biophys Res Commun.* 2002; 292: 1–7.
73. **Howdle SM, Watson MS, Whitaker MJ, et al.** Supercritical fluid mixing: preparation of thermally sensitive polymer composites containing bioactive materials. *Chem Commun* 2001; 109–10.
74. **Madison RD, Da Silva CF, Dikkes P.** Increased rate of peripheral nerve regeneration using bioresorbable nerve guides and a laminin-containing gel. *Exp Neurol.* 1985; 88: 767–72.

75. **Madison RD, da Silva C, Dikkes P, Sidman RL, Chiu TH.** Peripheral nerve regeneration with entubulation repair: comparison of biodegradable nerve guides versus polyethylene tubes and the effects of a laminin-containing gel. *Exp Neurol.* 1987; 95: 378–90.
76. **Rosen JM, Padilla JA, Nguyen KD, Siedman J, Pham HN.** Artificial nerve graft using collagen as an extracellular matrix for nerve repair compared with sutured autograft in a rat model. *Ann Plast Surg.* 1990; 25: 375–87.
77. **Satou T, Nishida S, Hiruma S, Tanji K, Takahashi M, Fujita S, Mizuhara Y, Akai F, Hashimoto S.** A morphological study on the effects of collagen gel matrix on regeneration of severed rat sciatic nerve in silicone tubes. *Acta Pathol Jpn.* 1986; 36: 199–208.
78. **Yuan Y, Zhang P, Yang Y, Wang X, Gu X.** The interaction of Schwann cells with chitosan membranes and fibers *in vitro*. Biomaterials 2004; 25: 4273–8.
79. **Young RC, Wiberg M, Terenghi G.** Poly-3-hydroxybutyrate (PHB): a resorbable conduit for long-gap repair in peripheral nerves. *Br J Plast Surg.* 2002; 55: 235–40.
80. **Horch RE, Kopp J, Kneser U, Beier J, Bach AD**. Tissue engineering of cultured skin substitutes. *J Cell Mol Med.* 2005; 9: 592–608
81. **Horch RE.** Future perspectives in tissue engineering. *J Cell Mol Med.* 2006; 10: 4–6.
82. **Stern-Straeter J, Bach AD, Stangenberg L, Foerster VT, Horch RE, Stark GB, Beier JP.** Impact of electrical stimulation on three-dimensional myoblast cultures - a realtime RT-PCR-study. *J Cell Mol Med.* 2005; 9: 883–92.
83. **Kneser U, Schaefer DJ, Polykandriotis E, Horch RE.** Tissue engineering of bone: the reconstructive surgeon's point of view. *J Cell Mol Med.* 2006; 10: 7–19.
84. **Bach AD, Beier JP, Sternstaetter J, Horch RE.** Skeletal muscle tissue engineering. *J Cell Mol Med.* 2004; 8: 413–22.