Transplantation of autologous Schwann cells for the repair of segmental peripheral nerve defects

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Peripheral nerve injuries are a source of chronic disability. Incomplete recovery from such injuries results in motor and sensory dysfunction and the potential for the development of chronic pain. The repair of human peripheral nerve injuries with traditional surgical techniques has limited success, particularly when a damaged nerve segment needs to be replaced. An injury to a long segment of peripheral nerve is often repaired using autologous grafting of “noncritical” sensory nerve. Although extensive axonal regeneration can be observed extending into these grafts, recovery of function may be absent or incomplete if the axons fail to reach their intended target. The goal of this review was to summarize the progress that has occurred in developing an artificial neural prosthesis consisting of autologous Schwann cells (SCs), and to detail future directions required in translating this promising therapy to the clinic.

In the authors’ laboratory, methods are being explored to combine autologous SCs isolated using cell culture techniques with axon guidance channel (AGC) technology to develop the potential to repair critical gap length lesions within the peripheral nervous system. To test the clinical efficacy of such constructs, it is critically important to characterize the fate of the transplanted SCs with regard to cell survival, migration, differentiation, and myelin production. The authors sought to determine whether the use of SC-filled channels is superior or equivalent to strategies that are currently used clinically (for example, autologous nerve grafts). Finally, although many nerve repair paradigms demonstrate evidence of regeneration within the AGC, the authors further sought to determine if the regeneration observed was physiologically relevant by including electrophysiological, behavioral, and pain assessments. If successful, the development of this reparative approach will bring together techniques that are readily available for clinical use and should rapidly accelerate the process of bringing an effective nerve repair strategy to patients with peripheral nerve injury prior to the development of pain and chronic disability. (DOI: 10.3171/FOC.2009.26.2.E4)

KEY WORDS • axon guidance channel • peripheral nerve injury • Schwann cell

TRAUMATIC human peripheral nerve injuries are an extremely heterogeneous group of disorders. Consequently, a thorough knowledge of the natural history and indications for surgical repair is essential for the physician treating patients with such an injury. There exist a number of peripheral nerve injuries in humans that cannot be satisfactorily treated by currently available techniques. Patients may have a segmental loss of neural tissue either at the time of injury or at the time of initial evaluation, when neural scar tissue has formed. If a neural scar develops, this tissue must be resected prior to surgical repair of the damaged nerve, because studies have demonstrated that the best results occur when there is no

cross-sectional area of inert scar to block regenerating axons arising from the proximal stump.⁴³ The removal of the scar tissue from within the nerve will often entail resecting scarred nerve segments back until normal nerve anatomy can be recognized. A gap will be created within the nerve as a result of this maneuver. In either the acute or secondary situation, coaptation of the proximal and distal nerve ends after a nerve gap exists will produce undue tension at the site of repair and thereby inhibit axonal regeneration and promote formation of an intraneural neuroma.^{81,85}

Functional recovery is also limited by the degree of axonal regeneration, nerve gap length, specificity of reinnervation of target organs, condition of the end organ (particularly the motor endplate) and availability of sufficient donor nerve.^{6,10,27} The current clinical standard in managing a peripheral nerve injury with a gap is to transplant a segment of “noncritical” autologous nerve between the proximal and distal stumps of the injured

Abbreviations used in this paper: AGC = axon guidance channel; ECM = extracellular matrix; FDA = Food and Drug Administration; GFP = green fluorescent protein; PAN-PVC = polyacrylonitrile–polyvinyl chloride; PNS = peripheral nervous system; SC = Schwann cell.

nerve. The donor nerve for autografts is almost always a sensory nerve such as the sural nerve. It should be emphasized that harvesting such a donor nerve will itself create a new neurological deficit, because the patient will sacrifice an area of sensation in the hope of restoring more important motor and/or sensory function in the arm or leg. Other potential complications include disfiguring scars, painful neuromas, or even the possibility of a hematoma or infection at the harvest site. Despite these potential complications, the use of a peripheral nerve autograft to repair gaps within the PNS currently remains the gold standard with which to compare other forms of treatment.²⁵ The donor graft that is routinely used is the sural nerve, which is a purely sensory nerve composed of multiple small fascicles that are often branched and embedded in extensive connective tissue.

Modifications of the surgical technique used for nerve grafts have reached a plateau in the ability to improve outcomes, and the testing of a cellular/tissue bioengineering strategy is desirable. Studies have shown that the regenerating axons in peripheral nerve grafts are usually observed in association with columns of SCs contained in the fascicular compartment of the nerve. In contrast, acellular nerve grafts support limited regeneration.⁴⁰ These observations suggest the hypothesis that regeneration is critically dependent on the presence of SCs, and that the degree of regeneration and direction of growth may depend on the precise arrangement of the SCs in the graft. If this hypothesis is true, the provision of artificial nerve grafts consisting of nervelike conduits containing columns of SCs will provide a unique opportunity to augment regeneration above and beyond that observed with sural nerve grafts, and this would provide a major breakthrough in a clinical area. Finally, because large numbers of SCs can be obtained by growing the cells in culture with mitogens, the construction of a neural prosthesis with specific widths and lengths could be designed, which will improve the prognosis for patients who have no therapeutic alternatives. It is our goal to repair a critical-sized nerve defect in an animal model by using autologous SCs contained within an AGC, resulting in increased axonal regrowth into the distal stump of the peripheral nerve as well as improved functional recovery. Investigators have performed a series of experiments, and these will be reviewed to determine the extent to which PNS regeneration can be enhanced by the use of artificial nerve grafts containing SCs.

Historical and Modern Materials and Concepts

Use of AGCs as Nerve Guides: Tubes to Repair Peripheral Nerve Injuries With Gaps

Historically, Glück (1880) and Vanlair (1882) were the first to try tubes to repair peripheral nerves with short defects by using hollow cylinders of bone in experimental animals (see Fields et al.).²⁸ Other biological conduits include veins, arteries, and muscle,^{18,19} which have had variable success for the repair of short nerve defects. One potential problem of these techniques is the collapse of the biological nerve guide from the pressure of the sur-

rounding tissues. Numerous other synthetic biologically compatible materials (acrylic copolymer, cellulose filter, collagen, hydrogel, polygalactin, rubber, silicone)⁵⁴ have been used as tubular constructs.^{5,28,43,54,69,79,83} Repairing gaps in the PNS with an AGC can also provide a vehicle for administering adjuncts (factors and/or cells) to study further and to promote peripheral nerve regeneration. The addition of trophic factors,^{20,21,72,73} ECM components,^{55,86,87} and support cells^{33,34,47,74} provides modifications of the tube environment that have been shown to enhance regeneration in the PNS.⁸⁹

Although nerve grafts are the standard of care for the management of nerve gaps, the use of AGCs to repair gaps in the PNS offers several potential advantages compared with sural nerve grafts. First, no donor nerve graft needs to be harvested, thus preventing donor site morbidity. Second, the repair is technically relatively easier to perform. Third, the internal diameter of the channels can be tailored precisely to fit the cut nerve ends. Finally, agents known to promote peripheral nerve regeneration can be administered. The addition of trophic factors, ECM components, and support cells modifies the tube environment in ways that can potentially enhance regeneration in the PNS. Other potential advantages of nerve guides compared with nerve graft repair include the following: 1) rapid and effective tensionless nerve repair; 2) directed tissue repair through the nerve guide, which prevents axonal escape at the repair site and potentially reduces neuroma formation; and 3) reducing the invasion of connective tissue cells from the surrounding tissues, which otherwise may lead to scarring.

An example of an AGC is the NeuraGen nerve guide device (Integra LifeSciences Corp.). This device is an FDA-approved, absorbable collagen tube designed to be an interface between the nerve and the surrounding tissue. It creates a flexible, robust conduit for axonal growth across a nerve gap. The level of functional recovery achieved with the NeuraGen nerve guide is equivalent to direct suture repair in several experimental paradigms tested in both rats and primates³ for short, segment defects.²⁴ Some of the most important factors in the selection of this channel for the studies proposed, as opposed to other nerve guides such as PAN-PVC, include the fact that it has been FDA approved for use in humans since 2001, and has been implanted as a saline-filled nerve guide in > 15,000 patients for short nerve defects, mainly in the distribution of the digital nerve.

An important advantage of adding SCs to tubes is that many of the "factors" that had been previously added in isolation are synthesized and/or presented by the SCs. These include several well-characterized neurotrophic factors;^{1,30,37,38} cell adhesion molecules;^{8,22} and basement membrane components.⁸ The construction of cellular prostheses consisting of human SCs could conceivably have several therapeutic applications in the repair of neural injuries, including human peripheral nerve injuries, spinal cord injuries,¹⁴ and in the repair of demyelinating injuries of the CNS. In addition, the SCs are available to remyelinate regenerating axons.^{27,47,49} Although the addition of SCs is certainly a part of the answer for peripheral nerve regeneration within tubes, the key is the presen-

Transplantation of SCs for the repair of nerve defects

TABLE 1: Literature review on repair of rat sciatic nerve with various gap lengths*

Authors & Year	Graft Biomaterial	Gap (mm)	Survival (wks)
positive results			
Evans et al., 1991	silicone	5	14
Terris et al., 1999	Fr silicone tubing	6	12
Jenq & Coggeshall, 1985	porous silicone	8	8
Williams & Varon, 1985	silicone chambers	10	3
Chamberlain et al., 1998	collagen tube	10	4, 8
Chamberlain et al., 2000	collagen tube	10	30 or 60
Wang et al., 2001	biodegradable poly(phosphoester) conduit	10	12
Yu & Bellamkonda, 2003	polysulfone guidance channels	10	8
Xu et al., 2003†	biodegradable poly(phosphoester) conduit	10	12
Midha et al., 2003	porous poly(2-hydroxyethyl methacrylate-co-methyl methacrylate)	10	8
Rafiuddin Ahmed & Jayakumar, 2003	collagen tube	10	4
negative results			
Seckel et al., 1984	poly(D,L-lactic acid)	10	12
Chamberlain et al., 1998	silicone tube	10	4, 8
Chamberlain et al., 2000	silicone tube	10	30 or 60
Francel et al., 1997	silicone	13	16
Lee et al., 2003	silicone	13	6
Ngo et al., 2003	poly(L-lactic acid) microfilaments	14, 18	10
Lundborg et al., 1982	silicone	15, 20	4

* All tubes were "empty," but the graft biomaterial and survival time varied. A critical distance for sciatic nerve repair without the addition of exogenous factor or cells appears to be ~ 13 mm.

† Five of 14 saline-treated animals showed regeneration at the distal stump.

tation of the cells within the appropriate bioengineered platform.⁶⁶

Concept of Critical Nerve Gap Length After Peripheral Nerve Injury and Repair With Nerve Tubes

In experimental peripheral nerve repair with conventional nerve tubes or AGCs, there is a definite length limit or critical gap length between the proximal and distal stumps of a cut nerve in which nerve regeneration will not occur. Small gaps within peripheral nerves can be reconstructed with a variety of channels, with excellent results;^{4,83} however, there exists a critical, species-specific distance in which a simple tube will not suffice unless extrinsic factors are added. In the rat sciatic nerve it measures > 1 cm,^{29,52,53,84–86} and in the primate ulnar nerve it measures > 3 cm, for which a simple tube will not suffice unless extrinsic factors are added.^{36,52,56} A review of the literature (Table 1) in which empty or saline-filled tubes of various compositions were used to repair segmental defects of the rat sciatic nerve supports the concept of a critical distance of 13 mm. In the rat, a 13-mm gap can be successfully bridged using silicone chambers only when exogenous fibrin matrix precursors^{52,53,84–86} or a small segment of degenerated peripheral nerve are added.²⁹ Specifically, these authors demonstrated no evidence of regeneration within a 15-mm silicone tube (13-mm gap) at 16 weeks, but the addition of a short, interposed 2-mm nerve segment resulted in significant regeneration similar

to that of an autograft. In the monkey, a 3-cm gap can be repaired with a tube made of bioabsorbable polyglycolic acid.²⁵ Modification of the channel composition is one of many ways to influence the environment of regenerating axons. For example, impermeable tubes do not engender regeneration from a cut proximal stump when the distal stump is absent,^{76,87} whereas semipermeable channels with a 50-kD MW cutoff do promote regeneration within the channel, even in the absence of a distal stump.² It is clearly of interest to determine whether the addition of SCs will expand the limits for which tubes can be used to repair gaps in the PNS successfully.

Experimental Matrices

The best biomaterial in which to suspend SCs prior to their transplantation within a nerve channel is still unknown. Investigations into the use of matrices consisting of the following materials: collagen; laminin and fibrin; methylcellulose; and alginate with or without fibronectin^{3,42,51,65,68,69,80} have provided mixed results. The 3D orientation of the matrix and the SCs is as important as the biomaterial itself, because a linear orientation of SCs in the form of a scaffold is needed to obtain organized regeneration. Previous studies conducted within the PNS in which semipermeable guidance channels were used have demonstrated that laminin-containing gels are worse substrates for axonal regeneration than saline-filled tubes.^{33,80} Matrigel (Collaborative Research, Inc.) is a sol-

FIG. 1. Schematic diagram demonstrating how SCs isolated from the sural nerve can be used to potentially repair an extensive brachial plexus nerve injury.

ubulized basement membrane preparation extracted from Engelbreth-Holm-Swarm mouse sarcoma, a tumor rich in ECM proteins. Its major component is laminin, followed by collagen IV, heparin sulfate proteoglycans, and entactin.⁵⁷ At room temperature, the matrix polymerizes to produce biologically active matrix material resembling the mammalian cellular basement membrane.

There is extensive experience with Matrigel to suspend SCs in a variety of transplantation paradigms within the PNS and CNS after injury.^{33,35,47,49,90,91} Although considerable experience exists with this matrix, approval of a suspension matrix derived from xenogeneic tumor cell line is fraught with problems in the realm of human SC transplantation and the FDA. The deposition of fibrin clots *in vivo* occurs after injury in the PNS, and their removal correlates with nerve regeneration. Fibrin clots provide a provisional matrix for invading cells, induce wound healing, and become proteolytically removed by regenerating tissue. In the current study we suspended the autologous SCs within autologous serum and tried to establish the feasibility and safety of this approach, which would greatly facilitate bringing forth such studies to the clinic.

Autologous SC Transplantation

Schwann cells are the principal support cells throughout the PNS and have a remarkable capacity to promote nerve fiber regeneration in both the PNS^{23,33} and the CNS.^{13,23,33,68} The development of an alternative therapeutic approach (Fig. 1) to manage peripheral nerve injuries would include a peripheral nerve biopsy, isolation of the resident SCs and expanding their number with mitogens by using cell culture techniques, construction of a cellu-

lar prosthesis within a guidance channel (nerve tube), and reimplantation of such a prosthesis into a gap to bridge the injured peripheral nerve. The use of cell culture techniques to obtain an abundant source of autologous graft material from a small biopsy sample has been an approach which has already met with clinical success in providing human epidermal cells to cover extensive burns.^{31,32}

Many of the basic steps described above have already been established. An article describing the isolation of SCs from adult rat, primate, and human nerve in the early 1990s was one of the first critical steps.⁶³ Two important steps were as follows: 1) the demonstration that the human SCs could divide in response to exogenous mitogens (heregulin, forskolin, and cholera toxin) and that their numbers could be easily expanded to construct a cellular prosthesis from a small nerve biopsy sample;^{47,50} and 2) that the SCs isolated from the human nerve retained their functional capacity, including the formation of myelin.^{47,49} The mitogen expansion protocol for human SCs has since been simplified and the cholera toxin has been eliminated.¹⁵ Substantial work has been done to elucidate further how adenosine 3',5'-cyclic monophosphate exerts its effects on SC function by regulating intracellular signaling initiated by growth factors.⁶¹ In this work by Monje et al., the interactions between intracellular adenosine 3',5'-cyclic monophosphate and the neuregulin-dependent proliferation of cultured SCs is explored. As the mechanisms of SC proliferation are elucidated, more refined techniques for culture expansion of these cells will be possible.

We have demonstrated that one can create a "simple" artificial peripheral nerve³³ by placing SCs, and even primate and human SCs, within a tube or channel to promote the regeneration of axons across a small nerve gap in primates and immune-deficient rodents (human

FIG. 2. a: Fischer rat SCs prelabeled with GFP. b: Fischer rat SCs stained with S100 protein. c: Nuclear staining with Hoechst 33342. The SC purity was > 99% prior to transplantation of the cells into a guidance channel. Original magnification $\times 20$.

SCs).^{47,49,50} Most of the work to date concerning the use of SCs ($0.8\text{--}2 \times 10^5$ cells/ μl) and guidance channels to repair gaps within the nervous system involves suspension of the cells within a Matrigel matrix and then injecting the cell-matrix suspension within a PAN-PVC chan-

nel.^{33,35,45,47,90,91} The PAN-PVC tubes are somewhat fragile and have certain unfavorable mechanical properties,⁴⁶ including collapse of the tube in larger animals. The current use of the nerve tubes avoids these problems. Experiments performed in rabbits in which autologous SCs, a venous conduit, and Matrigel were used have provided no quantitative or statistical data, but support the use of SCs to enhance long-distance regeneration in this animal model.⁷⁷ The use of vein grafts as a conduit is highly attractive because of their autologous nature, low cost, and ready availability. Notably, surgical experience with the technique has demonstrated significant limitations with vein collapse and regenerative failure. Additional cell-seeded scaffolds include cold-preserved nerve allografts injected with autologous SCs.³⁶ We have found that the use of tubes in combination with cultured autologous SCs could clearly expand the clinical potential of the nerve tube, permitting the repair of a larger array of peripheral nerve injuries, even those with a lengthy gap.

We have demonstrated that autologous, mitogen-expanded, GFP-labeled SCs (Fig. 2) mixed with serum can be used to fill a nerve guide to repair a 13-mm gap within the rat sciatic nerve (Fig. 3). The GFP activity (green staining) is distributed along the length of the NeuraGen nerve guide 4 weeks after surgical repair, suggesting survival of the transplanted SCs. Regenerating axons (Fig. 4) from the sciatic nerve are immunolabeled with antineurofilament antibody (red staining) and are juxtaposed with the transplanted GFP-labeled SCs (green staining).

Future Directions

A growing body of literature addresses the role of motor and sensory pathways as determinants of axonal guidance and end-organ reinnervation.^{9–12,44,57,72} Preferential motor reinnervation is thought to be due to collaboration between the regenerating axons and the appropriate pathway type including the SC.⁵⁸ This process of preferential reinnervation is beneficial to the regenerating nerve in that it increases the specificity of axonal regrowth to the correct target organ and minimizes the amount of neuronal resources wasted on erroneous, nonfunctional connections. Although primarily studied in rodents,^{9,10} preferential motor reinnervation has been shown to occur in primates as well, which suggests that this trait is conserved in humans.⁵⁹ The reverse transcriptase polymerase chain reaction data validate that SCs derived from sensory (cutaneous) nerves and motor (ventral) roots respond differently to denervation and reinnervation, particularly with respect to nerve growth factor, brain-derived neurotrophic factor, vascular endothelial growth factor, human growth factor, and insulin-like growth factor-I.³⁹ Cellular nerve channels can be engineered specifically to contain SCs derived from pure motor or sensory nerves.³⁹

Although previous studies of rat SCs have shown that they can be expanded in number extensively with neuregulin and forskolin, with excellent retention of their capacity to myelinate axons,⁷⁰ there is some evidence that the function of human SCs is changed by extensive growth in culture.^{47,49,62,64} In a single study, to test the ef-

fect of growth in culture on the capacity of human SCs to form myelin after transplantation, the number of myelin

FIG. 3. a: Exposure of the right sciatic nerve before creation of injury with a gap. b: Right sciatic nerve repaired with the NeuraGen nerve guide filled with autologous GFP-labeled SCs. c: Schematic diagram demonstrating filling of the AGC with serum containing GFP-labeled SCs, followed by nerve repair.

sheaths produced by the transplanted SCs decreased after 5 population doublings.^{47,49} The mitogen cocktail at that time consisted of heregulin, forskolin, and cholera toxin (an irreversible inhibitor of adenyl cyclase). In future work, testing the myelination capacity of human SCs prepared exactly as they will be prepared for clinical transplantation (with mitogens consisting only of heregulin and forskolin) will be extremely important. There may be a finite number of population doublings that human SCs can undergo and still maintain their ability to myelinate regenerating axons.

Conclusions

The ideal method to treat segmental nerve defects after injury remains an unanswered question for clinicians. Autologous SC transplantation within an axonal guidance channel can potentially improve functional outcomes after peripheral nerve injuries, with increased axonal regeneration and myelination distal to the site of injury relative to current techniques. A novel but simple artificial neural prosthesis for peripheral nerve repair couples SCs isolated in cell culture with biomaterials and surgery for peripheral nerve repair. The previously published experiments reviewed here are some of the critical steps that would bring autologous SC transplantation to humans.

Disclaimer

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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GFP-SCs
Anti-neurofilament H

Fig. 4. Fluorescent image of regenerating axons immunolabeled with antineurofilament H (red) aligned along with bundles of GFP-labeled (green) SCs in a longitudinal section of the right sciatic nerve repaired with the Nerve Guide filled with GFP-labeled rat SCs at the midpoint region, 4 weeks after transplantation. Original magnification $\times 40$.

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