

Review: Tissue Engineering in the Nervous System

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The nervous system presents a challenge to the field of tissue engineering because some of its complex neurochemical and neuroanatomical architecture is just beginning to be understood. A combination of advances in molecular neurobiology, gene transfer techniques, and the concomitant advances in the engineering of biomaterials at a molecular level, are making tissue engineering in the nervous system possible. Due to the vast range of fields that this highly interdisciplinary task spans, any review is bound to be somewhat limited. Given that, this review attempts to cover some solutions engineered for: (a) the functional replacement of a missing neuroactive component; (b) the rescue or regeneration of degenerated neural tissue; and (c) the building of intelligent neural cell-based biosensors and simple in vitro neural circuits based on controlled neural cell attachment to electrically relevant substrates. © 1994 John Wiley & Sons, Inc.

Key words: encapsulation • nerve regeneration • cell transplantation • polymers • extracellular matrix

INTRODUCTION

Tissue engineering in the nervous system is the science of designing, creating, and realizing systems where neural cells are organized in a controlled manner, to perform appropriate diagnostic, palliative, and therapeutic tasks in the nervous system. Neural tissue engineering, therefore, involves exploring issues governing the selection and usage of cellular components, and the optimal mode of organization of those cellular components. Cellular organization may include the control of intercellular interactions and the interactions between the cells and their environment.

The function and repair of neuronal cells depends on their intrinsic genetic programs and their extracellular environment. Recent advances in molecular biology and gene transfer techniques have made possible design of cells that express bioactive factors of interest. Strategies involving encapsulation of these cells in a polymeric film with known physicochemical characteristics of molecular-weight cut-off and chemical composition helps control the interactions between the host and the transplanted tissue. Effective immunoisolation of the transplanted tissue may render possible the transplantation of xenogeneic tissue. It has also been shown that the humoral molecules, glial cell

membrane-associated molecules, and extracellular matrix components influence neuronal cell function, attachment, and differentiation by encouraging certain cell phenotypes. Thus, control of the extracellular environment may be another way of controlling and encouraging favorable phenotypes of given cells or tissue.

The convergence of the above-mentioned factors has not only made tissue engineering in the nervous system exciting and challenging but also, more importantly, possible. Currently, tissue engineering in the nervous system has been addressing:

- I. The functional replacement of a missing neuroactive component.
- II. The rescue or regeneration of degenerated neural tissue.
- III. The building of intelligent neural cell-based biosensors and simple in vitro neural circuits.

THE DELIVERY OF A MISSING NEUROACTIVE COMPONENT IN THE NERVOUS SYSTEM

Deficiency of specific neuroactive factors has been implicated in several nervous disorders. These factors maybe neurotransmitters, neurotrophic factors or enzymes. For example, part of the basal ganglia circuitry which plays a role in motor control, consists of striatal neurons receiving dopaminergic input from the substantia nigra. It has been shown that a lack of this striatal dopamine input following the degeneration of the dopaminergic neurons in the substantia nigra is responsible for Parkinson's disease.²⁴ The contribution of tissue engineering in this situation maybe twofold. The first would be to evaluate if the delivery of the missing factor, dopamine in this case, to the target striatal region, would functionally alleviate the symptoms of Parkinson's disease, i.e., tremor, akinesia, and rigidity, and design an effective way of delivering it. The second and perhaps more ambitious task would be to rescue, regenerate, or replace the degenerated tissue, in this case the dopaminergic cells of the substantia nigra and then engineer ways to encourage these cells to project to the striatum and reinnervate the striatal target with dopaminergic inputs. As we shall examine later, attempts have been made to achieve this by stereotactically controlled grafting of neural fetal tissue or by using synthetic polymer nerve guides and extracellular

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matrix elements. It is as yet unclear in the basal ganglia circuit if physical synaptic, dopaminergic reinnervation of the striatum from nigral neurons located in the mesencephalon is necessary for the alleviation of all the symptoms of Parkinson's disease. But it is conceivable that duplication of the original circuit might restore more of the functional aspects of the original circuit including feedback-controlled responses.

Both of the above strategies are not entirely mutually exclusive and have been subjected to investigation. The first approach has resulted in engineered systems by which neuroactive factor(s) may be delivered to the deficient areas. It should be noted at the outset that this approach, in attempting to deliver the missing neuroactive factor(s) to the target area, is only replacing one aspect of the original neuroanatomical and neurochemical circuit of the brain. Its aim does not include the reconstruction of the entire original physical, predisease synaptic state of the circuit. There are several issues that one might wish to consider in designing a delivery system for the neuroactive components. Some of these might be: (a) the factor must have access to the nerve cells; (b) the effect of the factor must be localized; (c) the factor must be stable in its storage environment; and (d) the delivery must be sustained and controlled.

Attempts at fulfilling these requirements have involved using: (a) pumps; (b) slow release polymer systems; and (c) cell transplantation. We shall examine each of these methods, their potential and their shortcomings in succeeding to efficiently deliver neuroactive factors to the nervous system.

Pumps

Pumps have been used to deliver opiates epidurally with epidural pumps to relieve pain in long-term cancer patients.¹¹ Pumps have also been used to deliver neurotrophic factors such as NGF intraventricularly as a prelude or supplement to transplantation of chromaffin cells for Parkinson's disease⁷² or as a potential therapy for Alzheimer's disease.⁷³ An ALZET miniosmotic pump has also been used to deliver a solution containing dopamine or dopamine receptor antagonists.^{22,38} But their short life time of 4 weeks may render them clinically impractical along with the associated dangers of having a large reservoir of drug in vivo that may be susceptible to "dumping" of neuroactive substrate. Also, infections and diffusion limitations have been some of the problems associated with this technology. It should also be taken into account that factors such as dopamine are very unstable chemically and have short half-life periods in solutions not containing antioxidative agents and may not be delivered by such devices involving reservoir storage of the compound. This problem may be overcome by some of the delivery techniques described below, for example, the slow release polymer systems.

Slow Release Polymer Systems

It has been reported that administration of the dopamine precursor L-dopa, in conjunction with a decarboxylase inhibitor, is an effective approach for the alleviation of some of the motor symptoms of Parkinson's disease.¹⁶ However, it has been observed that after several years of orally administered L-dopa therapy, the clinical response begins to fluctuate in a manner that could be correlated to the fluctuations of plasma L-dopa levels.^{13,37,69,85} It has been demonstrated that a slow release ethylene vinyl acetate polymer system loaded with L-dopa could sustain steady, elevated plasma levels of L-dopa for at least 225 days when implanted subcutaneously in rats.⁷⁹ Polymeric delivery systems involving the trapping of the molecule of interest inside a polymer matrix that slowly releases it may be successful in achieving some of the goals of the ideal delivery system to the brain.^{41,59,60} The advantages of this technique might be that drugs with a short in vivo half-life period might be protected from degradation, harmful side effects of systemic administration might be avoided, and with proper design of devices, the dose levels might be maintained in a therapeutically desirable range with near zero-order release. Some of the disadvantages of this system, however, are the finite loading amounts and difficulties in shutting off release or adjusting dose levels to individual patients. Also, if long-term release is required, then the device dimensions may become impractical. For such long-term release, there are polymer device geometry constraints that may restrict the possibility of intracerebral implants for more localized delivery of neuroactive factor. The possibility of using stereotactically implanted dopamine-containing liposomes in a rodent model of Parkinson's disease has been explored to overcome this problem.²³ The authors demonstrate a partial amelioration in the rotational behavioral test in the rodent Parkinson's model with this mode of therapy. While liposomes present a very promising approach to delivery of neurotropic factors, this technique is yet to find wide application due to limitations like the relatively short effective time periods. However, active research is underway to make intelligent liposomal membranes which would attach to specific tissue targets and this may present an exciting way of delivering neuroactive components to particular regions of the brain. Therefore, the search for a more ideal neuroactive component delivery system continues and points to cell transplantation as a possible alternative.

Cell Transplantation

Recent advances in molecular biology and gene transfer techniques have given rise to a rich array of cellular sources, diverse in the range of neuroactive compounds they secrete and pathologies they target. These include cells that release neurotransmitters, neurotrophic factors, or enzymes. Thus, transplantation of cells that might functionally replace the

damaged host tissue might be a good way to reconstruct the original host structure. The use of a cellular source for various neurochemicals is a definite advantage in that these cells might undergo self-renewal by cell division (in the case of cell lines); use the host's nutritive compounds like oxygen and glucose, etc.; and manufacture and release the factors of interest continually over a long period of time. This strategy overcomes shortcomings of the earlier modes of delivery described in that there is no storage reservoir of neurochemicals of interest in large amounts, and the supply lasts as long as the transplanted cells survive, and maintain their phenotype and their transgene expression in the case of gene therapy. Some of the tissues transplanted experimentally so far may be classified in the following manner:

Transplantation of Autologous Primary Cells

This technique involves the procurement of primary cells from the host, expanding them if necessary to generate requisite amounts of tissue, engineering them if so required by using gene transfer techniques and then transplanting them back into the "donor" at the appropriate site. For instance, autologous Schwann cells have been isolated and transplanted into the brain and have been shown to enhance retinal nerve regeneration, presumably by the release of factors that influence regeneration.⁶⁸ Schwann cells have been shown to express various neurologically relevant molecules.^{20,70,78} Autologous Schwann cells have also been shown to work as nerve bridges to help reconstruction of rat sciatic nerve after axotomy.³⁵ Primary skin fibroblasts have been genetically engineered to secrete L-dopa, a dopamine precursor molecule, and transplanted intracerebrally into the autologous host's striatum, and behavioral recovery has been shown to be possible in a rat model for Parkinson's disease.²¹ The same group has also reported that nerve growth factor, tyrosine hydroxylase, glutamic acid decarboxylase, and choline acetyltransferase genes may be successfully introduced and expressed in primary fibroblasts. More recently, muscle cells have been engineered to express tyrosine hydroxylase and transplanted successfully in a rat model of Parkinson's disease.⁴⁷

Thus nonneural cells such as fibroblasts or muscle cells may be selected, in part for the ease with which they can be engineered genetically, and made neurologically relevant, guided by the function that one wishes to replace. It might, therefore, be possible to engineer cells to suit particular pathologies as a step toward being able to engineer biomimetic tissues and then place them in appropriate locations and contexts in vivo. However, it may not always be possible to procure autologous tissue. Other sources of tissues have therefore been explored, and these include fetal tissue, usually in conjunction with immunosuppression.

Fetal Tissue Transplantation

One of the advantages of fetal tissue is its ability to survive and integrate into the host adult brain. Fetal neural

tissue to replace the degenerated nervous tissue may be procured from allogeneic sources. Transplantation of fetal neural tissue allografts might be useful in treating some human neurodegenerative diseases,⁶⁴ including Parkinson's disease.⁶³ While promising results have been reported using this technique,¹⁸ availability of donor tissue and potential ethical issues involving creating a demand for aborted fetuses may be potential shortcomings. Transplantation of xenogeneic fetal tissue with immunosuppression using cyclosporin A has been demonstrated to be feasible⁹³ and this approach might yield more abundant amounts of tissue for transplantation and overcome some of the possible ethical dilemmas in using human fetal tissue. However, immunosuppression may not be sufficient in preventing rejection.

Encapsulated Xenogeneic Tissue Transplantation

Polymeric encapsulation of xenogeneic tissues might be a viable strategy to transplant xenogeneic tissues without systemic immunosuppression.^{2,88} However, no integration of the transplanted tissue with the host with synaptic connections is possible with this technique. Typically, the capsules have pores large enough for nutrients to reach the transplanted tissue and let the neuroactive factors out, but the pores are too small to let the molecules of the immune system reach the transplanted tissue. At the same time, this strategy retains all the advantages of using cells as controlled, local manufacturers of the neuroactive factors. Also, the use of encapsulation eliminates the restriction of having to use postmitotic tissue for transplantation to avoid tumor formation. This allows the use of dividing cell lines for transplantation without the worry of in vivo tumor formation due to the physical restriction of the polymeric capsule preventing escape of the encapsulated tissue. The encapsulation system may also support localized cell renewal within the capsule and might result in long-term survival of encapsulated tissue's functional viability, and also make possible a dependable, self-renewing supply of tissue for xenogeneic transplantation. Should the capsule break, the transplanted cells should be rejected and eliminated by the host immune system.

Some of the tissue engineering issues involved in optimizing the encapsulation technique are (a) the type and configuration of the encapsulating membrane; (b) the various cells to be used for encapsulation; and (c) the matrix in which the cells are embedded.

Type and configuration of the encapsulating membrane. One single overwhelming factor determining the size of the device is the oxygen diffusion and availability to the encapsulated cells. This consideration influences the device design and encourages situations where the distances between the O₂ source, usually a capillary, and the inner core of transplanted tissue are kept as minimal as possible. The size of the device also influences the kinetics of release of the neuroactive molecules, the "response

time" being slower in larger capsules with thicker membrane walls.

The capsule membrane maybe a water-soluble system stabilized by ionic or hydrogen bonds formed between two weak polyelectrolytes—typically an acidic polysaccharide, such as alginic acid or modified cellulose, and a cationic polyaminoacid, such as polylysine or polyornithine.^{30,61,96} Gelation of the charged polyelectrolytes is caused by ionic crosslinking in the presence of di- or multivalent counterions. However, the stability and mechanical strength of these systems is questionable in the physiological ionic environments. The major advantage of using these systems is that it obviates the need of organic solvent use in the making of the capsules and might be less cytotoxic in the manufacturing process.

The capsule membrane may also be a thermoplastic, yielding a more mechanically and chemically stable membrane. This technique involves loading of cells/tissue of interest in a preformed hollow polymer fiber and then sealing the ends either by heat or with an appropriate glue. The hollow fibers are typically fabricated by a dry jet wet spinning technique which involves hollow fiber membrane formation by phase inversion of a polymer solution, i.e., polyacrylonitrile–polyvinylchloride copolymer dissolved in DMSO.⁷ The use of thermoplastic membranes allows the manipulation of membrane structure, porosity, thickness, and permeability by appropriate variation of polymer solution flow rates, viscosity of the polymer solution, the nonsolvent used, etc.

Our laboratory has developed membranes which are biocompatible and stable over long periods of time in vitro and in the brain. With this system, long-term cross-species transplants of dopaminergic xenogeneic tissues, with graft survival and functional efficacy in the brain, have been reported.⁸ One experimental model for Parkinson's disease involves the lesion-induced interruption of dopaminergic nigral input to the striatum. PC12 cells, a catecholaminergic cell line derived from a rat pheochromocytoma, were encapsulated within thermoplastic PAN/PVC capsules and implanted in the striatum of adult guinea pigs. PC12 cells have been shown to synthesize, store, and release dopamine.^{32,33} Both in vitro and in vivo, well-preserved expression of tyrosine hydroxylase-positive PC12 cells was observed for at least 12 weeks. Thus, PC12 cells survived cross-species transplantation and maintained their phenotype in the absence of systemic immunosuppression, demonstrating the feasibility of using polymeric encapsulation for the long-term, cross-species transplantation of xenogeneic tissues.⁸

The choice of cells and tissues for encapsulation. Because long-term cross-species transplantation of xenogeneic tissue has been shown to be feasible using the encapsulation technology, it is possible to envisage the transplantation of various genetically engineered cells for the treatment of several neurological disorders. This technique allows access to an ever-expanding source of tissues which have been engineered to produce the required factor and presents

the opportunity to encapsulate them. For instance, Horellou and Mallet⁴⁴ have retrovirally transferred the human TH cDNA into the mouse anterior pituitary AtT-20 cell line potentially resulting in a plentiful supply of dopamine-secreting cells that can then be transplanted into the striatum.

Another promising area for the use of engineering techniques is in the treatment of some neurodegenerative disorders where a lack of neurotrophic factors is believed to be part of the pathophysiology of various neurodegenerative disorders. Neurotrophic factors are soluble proteins which are required for the survival of neurons. These factors often exert a trophic effect, i.e., they have the capability of attracting growing axons. The "target" hypothesis describes the dependence of connected neurons on a trophic factor which is retrogradely transported along the axons after release from the target neurons.

In the absence of trophic factors, the neurons shrink and die, presumably to avoid potential misconnections. Experimental data suggests that local delivery of nerve growth factor (NGF)^{39,95} and/or brain-derived neurotrophic factor (BDNF)^{14,54} may be useful in Alzheimer's disease. Local delivery of brain-derived nerve growth factor (BDNF)^{45,54} and/or glial-derived nerve growth factor (GDNF) may be beneficial in Parkinson's disease,⁶² whereas ciliary neurotrophic factor (CNTF)^{74,84} and/or BDNF,⁷⁵ the neurotrophins NT-3 and NT-4/5,⁴⁹ could positively impact amyotrophic lateral sclerosis (ALS; or Lou Gehrig's disease).

Fibroblast lines have been used in CNS transplantation studies because of their convenience for gene transfer techniques.²⁹ The transplantation of encapsulated genetically engineered fibroblasts to produce NGF has been shown to prevent lesion-induced loss of septal ChAT expression following a fimbria-fornix lesion.⁴² This model is important because it is characterized by deficits in learning and memory, resembling those of Alzheimer's disease.

Another potential application of the encapsulation technique is in the control of pain. The release of analgesic substances such as enkephalins, endorphins, catecholamines, neuropeptide Y, neurotensin, and somatostatin in the cerebrospinal fluid of brain structures is involved in pain control.¹² Chromaffin cells isolated from the adrenal medulla have been shown to release these various substances. Allografts of the adrenal chromaffin cells have been shown to alleviate pain when transplanted in the subarachnoid space in terminal cancer patients.⁸⁰ Transplantation of encapsulated xenogeneic chromaffin cells may provide a long-term source of pain-reducing neuroactive substances.⁸¹ Bovine chromaffin cells survive and maintain their phenotype when encapsulated in PAN/PVC polymeric capsules in culture (Fig. 1) and when transplanted into adult sheep (unpublished results). This may circumvent the problem of the limited availability of human adrenal tissue in grafting procedures of chromaffin tissue.

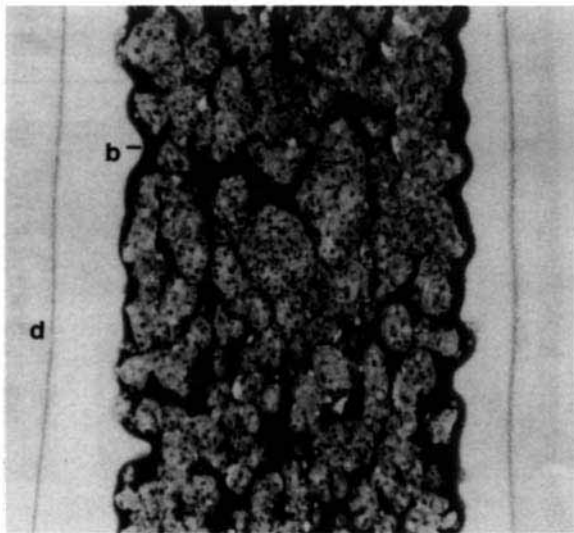


Figure 1. Bovine chromaffin cells encapsulated in PAN/PVC polymeric capsules embedded in an ionically cross-linked alginate matrix at 4 weeks in culture. The picture shows the polymeric membrane (p), the toluidine blue-stained alginate (q), and viable chromaffin cells (r).

A deficiency of the expression of biogenic amines, especially serotonin and norepinephrine, has been implicated in depression.¹⁹ Neurotransmitters such as substance P may also protect nervous tissue from excitotoxicity-mediated neurological disorders.⁵⁵ It is possible that appropriate selection of cells secreting the above factors can alleviate some of the symptoms in the above disorders.

Matrices for encapsulation. The physical, chemical, and biological properties and characteristics of the matrix in which the cells have been immobilized may play an important role in determining the transplanted cell's state and function. Broadly, matrices can be classified into the following types: cross-linked polyelectrolytes; collagen in solution or as porous beads; naturally occurring extracellular matrix derivatives such as Matrigel; and fibrin clots and biosynthetic hydrogels with appropriate biological cues bound to them to elicit a specific response from the cells of interest. The gel matrix has several functions: it can prevent the formation of large cell aggregates that lead to the development of central necrosis as a consequence of insufficient oxygen and nutrient access; it may allow anchorage-dependent cells to attach and spread on the matrix substrate; and it may induce differentiation of a cell line, and therefore slow down or stop its division rate.

Negatively charged polyelectrolytes such as alginate have been successfully used for the immobilization of adrenal chromaffin cells.¹⁰ Positively charged substrates, such as those provided by the amine groups of chitosan, allow attachment and spreading of fibroblasts.⁹⁹ Biologically derived Matrigel induces differentiation of various cell lines such as Chinese hamster ovary (CHO) cells, astrocyte lines, or fibroblast lines (unpublished observations). Spongy collagen matrices, as well as fibrin matrices, seem to possess similar qualities. Our laboratory is currently evaluating the

use of biosynthetic hydrogel matrices with biologically relevant peptides covalently bound to the polymer backbone. It is hypothesized that these matrices may elicit a specifically designed response from the encapsulated cells.

Most of the above-described techniques are attempts at identifying the missing components of various neuropathologies; finding an appropriate source for those components; and, if necessary, designing a cellular source via genetic engineering and then designing an optimal mode of delivery of the components be it chemical or cellular. This approach, however, falls short of replacing the physical neuroanatomical synaptic circuits in the brain which in turn may play an important role in the physiological feedback regulation mechanisms of the system. Attempts to duplicate in vivo predisease neuronal structure have been reported. For instance, the bridging of the nigrostriatal pathway, or the septohippocampal pathway, has attracted some attention. Victorin et al.⁹³ have reported long-distance-directed axonal growth from human dopaminergic mesencephalic neuroblasts implanted along the nigrostriatal pathway in 6-hydroxydopamine-lesioned rats. Other studies^{17,27} have shown reinnervation of deinnervated striatum by substantia nigral implants. In the next section, we shall also see that the use of synthetic guidance channels and extracellular matrix cues may also be employed to guide axons to their appropriate targets.⁴³ Thus, a combination of all of these techniques may render the complete physical and synaptic reconstruction of a degenerated pathway feasible.

POLYMERS, GROWTH FACTORS AND NERVE REGENERATION

The promotion of nerve regeneration is an important candidate task for tissue reconstruction in the nervous system. Synthetic nerve guidance channels (NGCs) have been used to study the underlying mechanisms of mammalian peripheral nerve regeneration after nerve injury and enhance nerve regeneration. Guidance channels may simplify end-to-end repair and may be useful in repairing long nerve gaps. The guidance channel reduces tension at the suture line, protects the regenerating nerve from infiltrating scar tissue, and directs the sprouting axons toward their distal targets. The properties of the guidance channel can be modified to optimize the regeneration process. The nerve guidance channel may also be used to create a controlled environment in the regenerating site. The channel properties, the matrix filling the NGC, the cells seeded within the channel lumen, and polymer-induced welding of axons can all be strategies used to optimize and enhance nerve regeneration and effect nervous tissue reconstruction. Table I lists some of the kinds of nerve guidance channels used so far.

The Active Use of Channel Properties

In the past, biocompatibility of a biomaterial was evaluated by the degree of its passivity or lack of "reaction" when

Table I. Nerve guidance channels.

	Reference
I. The channel wall	
1. <i>Passive polymeric channels</i>	
Silicone elastomer	Lundborg et al. ⁶⁵
Polyvinyl chloride	Scaravalli ⁸²
Polyethylene	Madison et al. ⁶⁶
Polytetrafluoroethylene	Valentini et al. ⁹²
2. <i>Selectively permeable polymer channels</i>	
Acrylonitrile vinylchloride copolymer	Uzman et al. ⁸⁹
Collagen	Archibald et al. ¹⁵
Expanded polytetrafluoroethylene	Young et al. ⁹⁸
3. <i>Resorbable polymer channels</i>	
Polyglycolic acid	Molander ⁶⁷
Poly-L-lactic acid	Nyilas ⁷¹
Polyesters	Henry ⁴⁰
Collagen	Archibald ¹⁵
4. <i>Electrically charged polymer channels</i>	
Silicone channels with electrode cuffs	Kern et al. ^{51,52}
Polyvinylidene fluoride (piezoelectric)	Aebischer et al. ¹
Polyvinylidene fluoride-trifluoroethylene copolymer (piezoelectric)	Fine et al. ²⁶
Polytetrafluoroethylene (electret)	Valentini et al. ⁹²
5. <i>Polymer channels releasing trophic factors</i>	
Ethylene-vinylacetate copolymer	Aebischer et al., ⁴ Guénard et al. ³⁴
II. Intrachannel, luminal matrices	
1. Fibrin matrix	Williams et al. ⁹⁴
2. Collagen-glycosaminoglycan template	Yannas et al. ⁹⁷
3. Matrigel	Valentini et al. ⁹¹
III. Cell seeded lumens for trophic support	
1. Schwann cell seeded lumens (PNS)	Aebischer et al., ⁴ Guénard et al. ³⁵
2. Schwann cell seeded lumens (CNS)	Kromer and Cornbrooks, ^{57,58} Smith and Stevenson, ⁸⁶ Hoffman and Aebischer (to appear)

implanted into the body. However, the recognition that the response of the host tissue is related to the mechanical, chemical, and structural properties of the implanted biomaterial has led to the design of materials that might promote and encourage an active, beneficial response to manipulation of its microstructural properties, permeability, electrical properties, and the loading of its channel wall with neuroactive components that might then be released locally into the regenerating environment, etc. The strategy here is to engineer a tailored response from the host and take advantage of the natural repair processes.

Surface Microgeometry

The morphology of regenerating peripheral nerves is modulated by the surface microgeometry of polymeric guidance channels.⁶ Channels with a smooth inner wall give rise to organized, longitudinal fibrin matrices, resulting in discrete free-floating nerve cables with numerous myelinated axons. The rough inner surface channels, however, give rise to an unorganized fibrin matrix with few regenerating axons. Thus, the physical textural properties and porosity of the channel can influence nervous tissue behavior (Fig. 2) and

may be used to elicit a desirable reaction from the host tissue.

Molecular Weight Cut-Off

The molecular weight cut-off of the NGCs has been shown to influence peripheral nerve regeneration in rodent models.⁵ The molecular weight cut-off may influence nerve regeneration possibly by controlling the exchange of molecules between the channel lumen and the external wound-healing environment. This may be important, because the external environment secretes humoral factors that can either promote or inhibit nerve regeneration, and this might be controlled by the use of guidance channels with appropriate molecular weight cut-offs.

Electrical Properties

In vivo regeneration following transection injury in the peripheral nervous system has been reported to be enhanced by galvanotropic currents produced in silicone channels fitted with electrode cuffs.^{51,52} Statically charged "electret" polytetrafluoroethylene (PTFE) tubes show more myelinated axons compared with uncharged tubes in peripheral

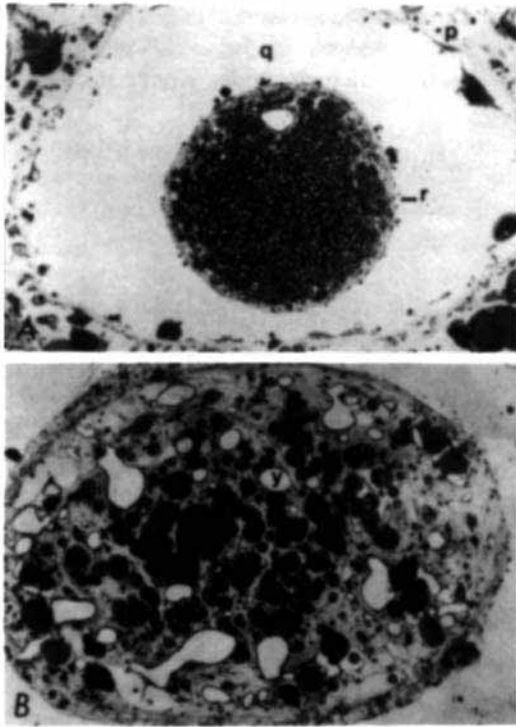


Figure 2. Transverse section at the midpoint of (A) PAN/PVC semipermeable polymeric guidance channel (p), and (B) expanded polytetrafluoroethylene polymeric guidance channel at 4 weeks postsciatic nerve lesion. Notice the influence of surface microgeometry of the channel in creating a looser fascicular nerve bundle (x) in (B) compared with (r) in (A) with capillary ingrowth (y). (q) denotes the lumen of the semipermeable polymer guidance channels. Both (A) and (B) are at the same degree of magnification.

nerves.⁹² Dynamically active piezoelectric polymer channels have also been shown to enhance nerve regeneration in the sciatic nerves of adult rats.²⁶

Release of Bioactive Factors from Channel Wall

The polymer guidance channel can be loaded with various factors to study and enhance nerve regeneration. Basic fibroblast growth factor released from an ethylene–vinyl acetate copolymer guidance channel facilitates peripheral nerve regeneration across long nerve gaps after a rat sciatic nerve lesion.⁴ The possible influence of interleukin-1 (IL-1) on nerve regeneration was studied by the release of IL-1 receptor antagonist (IL-1ra) from the wall of EVA copolymer channel.³⁴ The authors conclude that a naturally occurring antagonist of IL-1 receptor impedes peripheral nerve regeneration, suggesting that activated macrophages, which normally release IL-1 after axotomy, play an essential role in controlling peripheral nerve regeneration through the release of stimulatory and/or inhibitory molecules. It is conceivable that the release of appropriate neurotrophic factors may specifically enhance subsets of axons, i.e., motor neurons or sensory neurons.

Intraluminal Matrices for Optimal Organization of Regeneration Microenvironment

The physical support structure of the regenerating environment may play an important role in determining the extent of regeneration. An oriented fibrin matrix placed in the lumen of silicone guidance channels accelerated the early phases of peripheral nerve regeneration.⁹⁴

Silicone channels filled with a collagen–glycosaminoglycan template bridged a 15-mm nerve gap, whereas no regeneration was observed in unfilled tubes.⁹⁷ However, even matrices known to promote neuritic sprouting in vitro may impede peripheral nerve regeneration in semipermeable guidance channels if the optimal physical conditions are not ensured.⁹¹ Therefore, the structural, chemical, and biological aspects of the matrix design may all play a role in determining the fate of the regenerating nerve. The importance of the effect of the physical environment on regeneration, mediated by its influence on fibroblast and Schwann cell behavior, has been demonstrated in several studies and has been reviewed by Schwartz⁸³ and Fawcett et al.²⁵ Thus, the choice of a hydrogel with structural, physical, chemical, and biological cues conducive to nerve regeneration may enhance nerve regeneration, and this strategy is currently being explored in our laboratory. Preliminary results show that the presence of biosynthetic hydrogel inside the lumen of a synthetic guidance channel may help bridge long nerve gaps in the spinal ventral root model of rats.

Cell Seeded Lumens for Trophic Support

Cells secreting various growth factors may play an important role in organizing the regeneration environments, for instance Schwann cells in the peripheral and central nervous system. It has been reported that regenerating axons do not elongate through acellular nerve grafts if Schwann cell migration is impeded.³⁶ Syngeneic Schwann cell derived from adult nerves and seeded in semipermeable guidance channels enhance peripheral nerve regeneration.³⁵ Schwann cells may also be effective in inducing regeneration in the central nervous system.^{43,57,58,86}

CNS glial cells have a secretory capacity which can modulate neuronal function. Astrocytes release proteins which enhance neuronal survival and induce neuronal growth and differentiation. These effects can be blocked by antagonists of voltage-dependent calcium channels and may be partly mimicked by Bay K 8644, a calcium channel agonist.⁹⁰ Two of these neurotrophic proteins may, on the basis of their physical properties and effects on ciliary ganglion neurons, be ciliary neurotrophic factor (CNTF) and basic fibroblast growth factor (bFGF). Also, when a silicone channel was seeded with astrocytes of different ages, ranging from P9 to P69 (postnatal), it was observed that, although P9 astrocytes did not interfere with peripheral nerve regeneration, adult astrocytes downregulated axonal growth.⁴⁸ Thus, the cellular environment in the site of

injury may play an important role in determining the extent of regeneration. Knowledge of these factors may also be employed in designing optimal environments for nerve regeneration.

Polyethyleneglycol-Induced Axon Fusion

Rapid morphological fusion of severed myelinated axons may be achieved by the application of polyethylene glycol (PEG) to the closely apposed ends of an invertebrate myelinated axon.⁵⁶ Appropriate choice of PEG concentration and molecular mass, tight apposition, and careful alignment of the cut ends of the nerve may result in the direct fusion of axons. However, this technique is only applicable when the two ends of the severed nerve are closely apposed to each other, before the onset of Wallerian degeneration.

CNS Regeneration

Most of the above studies have been conducted in the peripheral nervous system (PNS). In the CNS, however, endogenous components do not support axonal elongation. But regeneration may occur with supporting substrates. Entubulation with semipermeable acrylic copolymer tubes have been shown to allow bridging of transected rabbit optic nerve with a cable containing myelinated axons.³ Cholinergic nerve regeneration into basal lamina tubes containing Schwann cells, demonstrating the influence of Schwann cells in promoting CNS axonal regeneration, has been reported.⁵⁸ In another study, polymer nerve guidance channels were seeded with adult Schwann cells suspended in an ECM-containing gel and implanted as a bridge in Fisher rats to effect regeneration after a fimbria-fornix lesion in the septo-hippocampal model system. As a result, acetylcholinesterase-positive axons enter the channel lumen after 2 weeks postimplantation. Transmission electron microscopy studies reveal the intimate interactions of Schwann cell with the host axons.⁴³

Thus, polymer guidance channels, appropriate matrices, and growth factors, together creating the right environmental cues, may be effective in inducing regeneration in the CNS. Therefore, both in the PNS and CNS, manipulation of the natural regenerative capacities of the host either by guidance factors, or stimulation by electrical or trophic factors or structural components of the regenerating microenvironment, can significantly enhance regeneration and help the reconstruction of severed or damaged neural tissue.

NEURONAL CIRCUITS AND BIOSENSORS

The electrochemical and chemoelectrical transduction properties of neuronal cells can form the basis of a cell-based biosensing unit. Also, the unique information processing capabilities of neuronal cells through synaptic modulation may form the basis of designing simple neuronal circuits *in vitro*. For both of the above applications, controlled neuronal cell attachment, closely and tightly coupled to

the substrate and the sensitivity of the substrate to pick up or monitor changes in the cells' electrical activity with a sensitive microelectrode, would have to be put in place.

The use of bioactive material systems, tailored to control neuronal cell attachment on the surface and still amenable to the incorporation of electrical sensing elements, like a field effect transistor (FET), would be one way of actualizing a feasible system. Therefore, composite material systems, which might incorporate on their surface covalently patterned bioactive peptides to control cell attachment and neurite extension, may be a step toward the fulfillment of the above goal. Oligopeptides derived from larger extracellular proteins like laminin have been shown to mediate specific cell attachment via cell surface receptors.^{31,46,53} Cell culture on polymeric membranes modified with the above bioactive peptidic components may give rise to a system in which neuronal cell attachment and neuritic process outgrowth may be controlled. This control may help in designing microelectronic leads to complete the cell-electronic junction. Preliminary recordings from a FET-based neuron-silicon junction using leech Retzius cells²⁸ have been reported. Though there are many problems, such as attaining optimal coupling, this could form the basis of a "neural chip." A neural chip could potentially link neurons to external electronics for applications in neuronal cell-based biosensors, neural circuits, and limb prostheses. To achieve this, polymer surface modification and intelligent use of extracellular matrix components through selective binding have to be used.

Studies in our laboratory have been aimed at understanding the underlying mechanisms involving protein adsorption onto polymeric substrates and their role in influencing and controlling nerve cell attachment.⁷⁶ Controlled neuronal cell attachment within a tolerance range of 20 microns may be achieved either nonspecifically via monoamine surfaces or specifically via oligopeptides derived from ECM proteins like laminin and fibronectin (Fig. 3), mediated by integrin cell surface receptors (unpublished results). Molecular control of neuronal cell attachment and interfacing neuronal cells with electrodes may have applications in the design and fabrication of high-sensitivity neuron-based biosensors with applications in detection of low level neurotransmitters.

Studies are also currently in progress involving polymeric hydrogels and controlling neuronal cell behavior in three-dimensional (3-D) tissue culture environs as a step toward building 3D neuronal tissues. The choice of an appropriate hydrogel chemistry and structure, combined with the possibility of the gel serving as a carrier for ECM proteins or their peptidic analogs, may enable one to enhance regeneration when seeded in a nerve guidance channel. Also, the use of appropriate hydrogel chemistries in combination with laser-directed photochemistry-mediated chemical modification of the polymer backbone may be feasible in controlling the direction and differentiation of neuronal cells in three dimensions. Covalent binding of bioactive components like

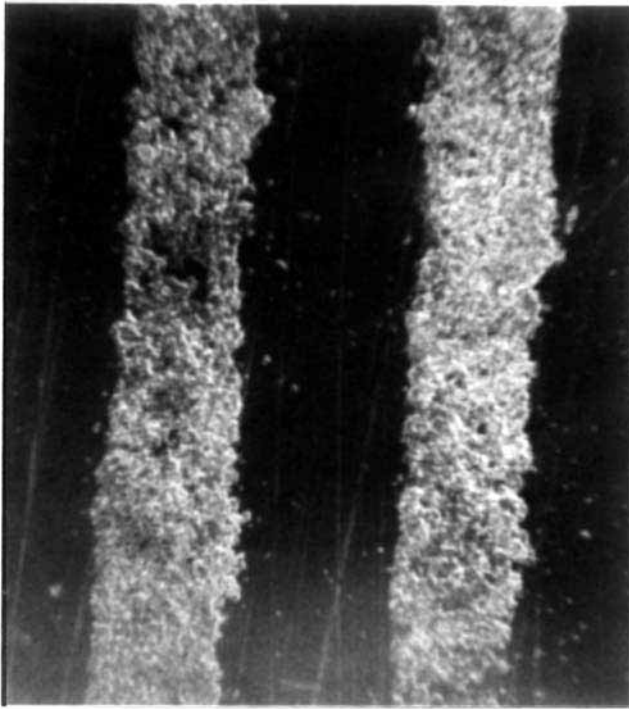


Figure 3. Stripes of confluent PC12 cells attached to regions of Teflon-FEP modified with laminin oligopeptide (SIKVAV).

the laminin oligopeptides to the hydrogel backbone may give a specific character to the gel so that it may elicit specific responses from anchorage-dependent neuronal cells (Fig. 4). Such a system could be useful in organizing nerves in 3D either for bridging different regions of the brain with nerve cables or for the 3D organization of nerves for optimal coupling with external electronics in the design of artificial limb prosthesis. Either way, development of such systems presents an interesting challenge for tissue engineering.

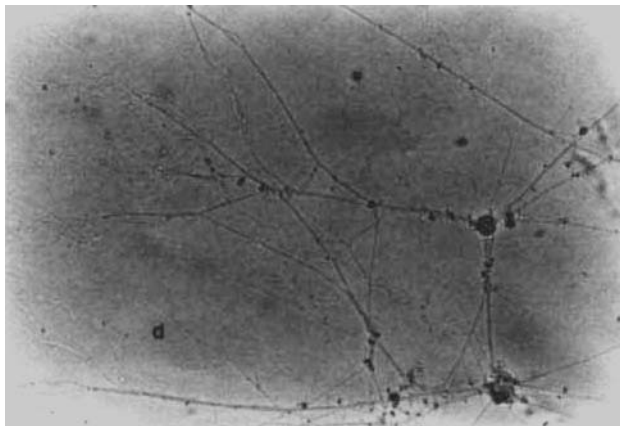


Figure 4. Neurites from a rat embryonic day 9 superior cervical ganglion in a 3D agarose gel derivatized with a laminin oligopeptide (YIGSR). Notice (p) the neurite disappearing into another plane demonstrating the 3D nature of neurite extension.

CONCLUSION

Advances in gene transfer techniques and molecular and cell biology offer potent tools in the functional replacement of various tissues of the nervous system. Each of these cell's functions can be optimized with the design and selection of its optimal extracellular environment. Substrates that support neuronal differentiation in two and three dimensions may play an important role in taking advantage of the advances in molecular and cell biology. Thus research aimed at tailoring extracellular matrices with the appropriate physical, chemical, and biological cues may be important in optimizing the function of transplanted cells, inducing nerve regeneration, or in the construction of neuronal tissues in two and three dimensions in a controlled fashion. Controlled design and fabrication of polymer hydrogels and polymer scaffolds on a scale that is relevant for single cells may also be important. This would presumably control the degree and the molecular location of permissive, attractive, and repulsive regions of the substrate and, in turn, control cellular and tissue response both *in vitro* and *in vivo*.

Biological molecules like laminin, collagen, fibronectin, and tenascin may provide attractive and permissive pathways for axons to grow. On the other hand, some sulfated proteoglycans have been shown to inhibit or repulse neurites.⁸⁷ The use of these molecules coupled with a clearer understanding of protein-mediated material–cell interaction may pave the way for neural tissue engineering, molecule by molecule, in three dimensions. Thus, the possibility of tailoring the genetic material of a cell to make it neurologically relevant, and the possibility of controlling its expression by optimizing its extracellular environment, makes tissue engineering in the nervous system a challenging endeavor.

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