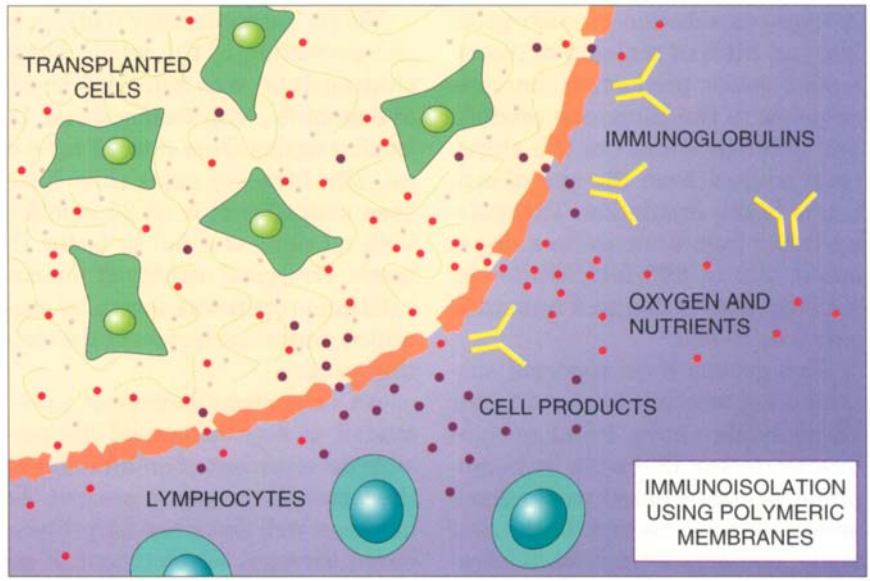
Tissue Engineering

Neural Tissue Engineering

Among the intractable neural pathologies that lie beyond the realm of successful treatment are neurodegenerative disorders such as Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Alzheimer's disease, and severed nerves resulting from acute trauma. The challenge for neural tissue engineers is to devise strategies to either replace the biochemical functions of the compromised neural tissue or functionally reconstruct severed neural pathways.



Transplantation of cells is a promising strategy for the treatment of neurodegenerative diseases. Fetal cells, xenogeneic cells, primary cell lines, or genetically engineered cells may be transplanted. Anders Bjorklund and his collaborators at the University of Lund have conducted extensive studies on transplantation of dopamine-secreting fetal tissue into the brains of patients with Parkinson's disease and have, on occasion, demonstrated marked recovery of motor function for up to two years after transplantation. Fred Gage and his collaborators at the Salk Institute have shown that it is possible to genetically engineer primary (non-neural) cells such as fibroblasts to produce L-dopa in a rat model of Parkinson's disease.

Biomaterials-based approaches such as polymer encapsulation have been devised to facilitate the transplantation of xenogeneic neural tissue into the brain and spinal cord and the subsequent tracking of the transplants. Typically, the polymer capsules have pores that allow small and useful molecules to diffuse out while denying the larger molecules of the immune system access to the transplanted cells. The polymer thus serves as an immunoprotective barrier that makes xenogeneic tissue transplants possible.

Patrick Aebischer and his colleagues in Lausanne have relied on polymer-encapsulation-mediated immunoisolation to transplant xenogeneic neural tissue into rodents, primates, and recently humans. The transplanted tissues have included dopamine-producing cells for Parkinson's disease, cells producing glial-derived and ciliary neurotrophic factors for ALS, and chromaffin cells for treatment of chronic pain.

One of the principal challenges confronting encapsulation technology is to devise a means of prolonging the survival and phenotypic expression of transplanted cells. Often, the central core of the transplanted tissue degenerates within about six months, presumably because of hypoxia.

Some of the strategies currently being explored are (1) designing better capsule geometries, (2) improving the permeability of the polymer membranes to oxygen, (3) reducing protein "fouling" of the pores to allow free diffusion, (4) designing three-dimensional matrices for uniform cell dispersion within the capsules, and (5) genetically engineering cells that are more resistant to hypoxia.

Another possible approach to improving survival of transplanted tissue may be the use of biomaterials designed for drug delivery to release growth factors or neuroprotective agents in combination with cell transplantation. Gage and coworkers have used co-transplantation of cells producing basic fibroblast growth factor and cells producing dopamine in an effort to prolong survival of the dopamine-secreting cells in adult brains.

Developmental neurobiologists have demonstrated that the extracellular environment is critical in neurite extension, axon guidance, and neuronal cell survival in the fetal nervous system. In fact, the superior regenerative capacity of peripheral nerves compared to

"Tissue Engineering" is edited by Jeffrey R. Morgan and Martin L. Yarmush of the Center for Engineering in Medicine, Massachusetts General Hospital and Harvard Medical School.

central nerves in adults is attributed to two differences in the extracellular environment.

First, in the adult central nervous system, the extracellular environment is composed of several myelin inhibitory molecules. Second, the response of the adult CNS to trauma involves an up-regulation of negatively charged proteoglycans such as chondroitin sulfates. The challenge for neural tissue engineers is to overcome the inhibitory environmental influences at the site of injury.

In either the peripheral or the central nervous system, there are two general conditions that are necessary to facilitate nerve regeneration: the neuron should "want" to extend its processes, and the extracellular substrate should be conducive to supporting or stimulating process extension.

In the peripheral nervous system, Schwann cells extend trophic and tropic support by secreting neurotrophic factors in response to injury. They also play a role in formation of the "fibrin cable" that forms the first bridge between the severed ends and facilitates peripheral nerve regeneration by secreting adhesion proteins such as laminin. However, across larger gaps, the rate and extent of regeneration become inadequate because of substrate inadequacies. Current clinical practice is to use autologous peripheral nerve grafts to bridge such gaps, but multiple grafts are often needed, and harvesting autologous tissue results in loss of function at other sites.

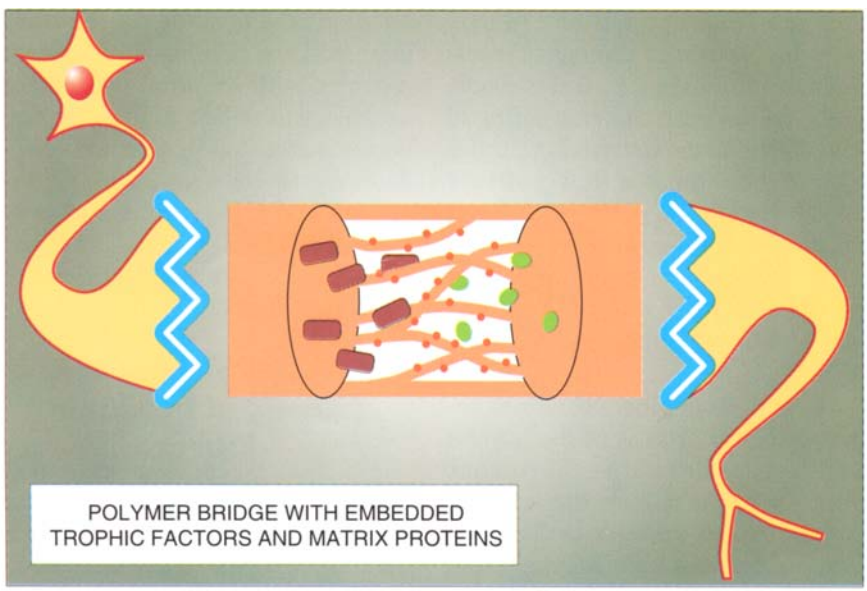
On the other hand, the absence of any trophic support and the presence of an inhibitory environment, combined with glial scar formation, severely limits regeneration in the central nervous system, where there are no Schwann cells. The cutting edge in traditional biological strategies to elicit CNS regeneration is to use Schwann cell transplants. However, the density of autologous Schwann cells needed to elicit minimal CNS regeneration is approximately 80 to 120 million cells per milliliter, so that it would take at least two months to culture Schwann cells in sufficient numbers. Over that time, wallerian degeneration of the injured nerve is extremely likely.

Tissue engineering approaches currently being explored combine local delivery of neurotrophic factors to sustain and stimulate neurite extension with bioactive polymer matrices that are conducive to neurite extension and can be used as bridges across severed nerves. Synthetic hydrogels such as agarose gels can be chemically modified to present potent extracellular matrix proteins to the site of nerve injury. The hypothesis is that the matrix proteins that stimulate and guide axons in the fetus have the greatest likelihood of supporting neurite extension in the adult.

In addition, if micro-scale drug delivery vehicles can be embedded in these gels without physically impeding the growth of axons through the gels, then trophic and tropic support can be provided to the nerve cell body. My colleagues and I have been exploring the use of polysaccharide-based polymeric gels made of hydroxyethylated agarose as bridge materials. We have demonstrated that low-concentration agarose hydrogels possess a physicochemical structure that supports neurite extension in three dimensions from various peripheral and central neurons.

A number of problems remain to be solved. First, the rules that govern neurite extension in a three-dimensional substrate are not known. That is, the mechanical, diffusion, and charge characteristics an "ideal" substrate ought to have are largely unknown. Second, the conformation and role of specific growth factors and extracellular matrix proteins in promoting neurite extension need to be investigated more thoroughly. Third, the neural cell modulation of cytoskeletal and second messenger systems in response to substrates remains to be studied in detail.

Some of the issues mentioned here are "frontier" research questions, common to any tissue engineering effort that involves organizing a cell response in three-dimensional scaffolds. However, given the dire need for novel therapeutic strategies in neurodegenerative diseases and in bridging severed central nerves, neural tissue engineering offers both great promise and stiff intellectual challenge.



RAVI V. BELLAMKONDA

Department of Biomedical Engineering
Case Western Reserve University

Sci & Med 6(3):6-7, 1999.