Glaucoma comprises a heterogeneous group of disorders, the final common pathways of which result in the death of retinal ganglion cells (RGC) and subsequent characteristic patterns of visual field loss and excavation of the optic nerve head. Those glaucomas in which elevated intraocular pressure (IOP) predominate are characterized by dysfunction of the trabecular meshwork, resulting in increased resistance to aqueous outflow. Non-pressure-dependent mechanisms remain poorly delineated and may consist of cardiovascular and local ischemic conditions, autoimmune and connective tissue disorders, and genetic predisposition to retinal ganglion cell death.

The lack of a common, unified causal theory for glaucoma has made the development of directed therapeutics difficult. Elevated IOP, a generation ago considered equivalent to the disease itself, is really only a risk factor for it (albeit the most important one). The only available therapeutically proven and marketed drug treatment regimens are those designed to lower IOP to stabilize the progression of RGC death and preserve optic nerve and visual field function, which is tantamount to treating a risk factor but not the disease(s).

The burgeoning field of tissue engineering, and the related discipline of drug delivery, offer tremendous opportunities to improve patient care. Moreover, the future holds exciting potential and presents formidable challenges for the improvement of glaucoma diagnosis, treatment, and, ultimately, prevention. Recent advances in the field of tissue engineering have made it possible to generate de novo tissue through in vitro constructs typically consisting of a biodegradable polymer and stem or progenitor cells derived from the target tissue. This article describes the current state of tissue engineering research, and how this new technology might be applied to the glaucomatous eye.

** BASIC CONCEPT OF TISSUE ENGINEERING **

Tissue engineering, in its prototypical form, involves the combination of a polymer scaffold with a population of stem, progenitor, or precursor cells. This tissue construct is typically cultured under conditions favoring the developmental maturation of the seeded cells and, if the polymer scaffold used is biodegradable, it can result in the formation of structures which are remarkably similar to the normal tissue. Examples of tissue-engineered structures today in the clinic include cartilage\(^1\) and bone,\(^3\) with many other tissues, such as blood vessels,\(^4,5\) cardiac muscle,\(^6\) and liver\(^7\) currently in development. It is becoming increasingly clear that while significant challenges remain, glaucoma would greatly benefit from applying modern tissue engineering strategies to the control of IOP and perhaps the rescue of RGC. The application of tissue engineering techniques for the generation of structures such as the trabecular meshwork or the optic nerve is of course extremely compli-
Biocompatibility of a synthetic device is the property of being biologically compatible by not producing a toxic, injurious, or immunological response in living tissue. The device should not be destroyed by inflammation and should be able to perform with an appropriate host response. Implantable medical devices form a $100 billion business worldwide, and these devices have saved or improved the quality of life of millions of patients.

Biocompatibility studies of materials within the eye were pioneered by clinicians in the early 20th century who attempted to implant various materials, including horsehair, gold, and tantalum, across the aqueous outflow pathway to improve aqueous humor outflow in glaucoma.\(^{6,10}\) In England, Harold Ridley noted that Spitfire pilots in the Royal Air Force often had shards of windscreen material within the globe, apparently without any ill effects. This observation helped lead the way to the development of the first intraocular lens.\(^{11}\) Hip and knee replacements have a limited lifespan, as do vascular grafts and cardiac valves.

In a new approach, the goals for success would depend on the biointegration of the implanted device.\(^{12,13}\) Among the foremost challenges facing researchers in the field is the near-ubiquitous foreign body response seen after implantation of various materials. Foreign body reactions evoke stimulation of giant cells and macrophages that produce cytokines and attract fibroblasts, leading to fibrosis. To avoid this reaction, it is key to pay attention to the surface properties of the biomaterials. One strategy includes the use of “stealth” materials, which prevent detection of the device as foreign by the living tissue. In addition to avoiding fibrosis, its great importance is the development of surfaces that would provide a favorable microenvironment for the proliferation and differentiation of seeded cells. To this effect, much exploration is being done on manipulating the porosity of synthetic structures using cutting-edge technology, such as electrospinning, currently the only method by which fibers of sizes less than 1 µm can be generated. It is hoped that these approaches to enhance biocompatibility by exploiting the interactions that occur between surfaces of engineered devices and the host milieu will achieve normal wound healing and tissue reconstruction.

**SCAFFOLDS**

Scaffolds are highly important in tissue engineering, drug release, and regenerative medicine. Although some scaffolds occur naturally, most are synthetic. Composite scaffolds have been created that contain both natural and synthetic compounds. In “tunable” scaffolds, the chemistry, mechanics, 3-dimensional architecture, and degrada-

tion rate can all be controlled during scaffold construction.\(^{14}\) Any modification of these factors can greatly alter the body’s response to the scaffold. Thus, when cardiomyocytes were co-cultured and engineered with polyurethane scaffolds, the new structure was able to generate synchronous beating, yet a minor change in the spacing led to a change in beating.\(^{4}\) Interestingly, this type of change did not occur when stem cells rather than cardiomyocytes were used, suggesting that both the scaffold and the seeded cell population play key roles in the generation of composite structures. A cellular bladder matrix is another example in which cell culture techniques, as well as 3-dimensional architecture, greatly alter the resultant tissue.\(^{15}\)

One family of scaffolds that may someday be exploited by glaucoma specialists is recombinant human elastin polypeptides.\(^{16}\) These polymers are able to self-assemble and to form structures that resemble the trabecular meshwork. As with other scaffolds, the 3-dimensional architecture of these scaffolds can be manipulated, perhaps allowing for a controlled porosity and the generation of a bioengineered trabecular meshwork in the future.

**SCAFFOLDS USED TODAY IN TISSUE REGENERATION**

**Bone and Central Nervous System**

One of the tissues that can be most successfully generated from scaffolds is bone. Osteofoam (BoneTec, Toronto, Ontario) is a scaffold with geometry much like that seen in natural bone, which can be seeded with bone-marrow cells. When the tissue construct was implanted into a bone defect site in rabbits, new bone tissue was formed, effectively joining the damaged bone segments.\(^{17}\) This constitutes an example in which merely providing a permissive substrate enables and directs the natural regenerative capacity of the damaged tissue.\(^{3}\)

The same approach can also be exploited to aid systems in which regeneration is normally minimal or absent, such as the central nervous system (CNS). Although since the studies of Ramon y Cajal,\(^{18}\) it had been thought that the CNS is unable to regenerate once damaged, it is becoming increasingly clear that this lack of self-repair is not solely an intrinsic defect of the injured neurons, but also a consequence of their microenvironment. Extracellular matrices, astrocyte and oligodendrocyte responses, as well as cytokines and growth factors have all been implicated in the general phenomenon of regenerative inhibition. Thus, the field of CNS tissue engineering has sought to provide permissive environments to allow regeneration of axons from brain, spinal cord, and retina. One strategy uses tubes with modified surface peptides (to provide cell-substrate interactions) that would serve as nerve guidance channels and first attract, then direct axons. This approach has shown great promise, but thus far has had a limited capacity for functional regeneration. The future of this field, highly relevant to optic nerve disease, hinges on generating 3-dimensional constructs that better resemble the environment of the developing nervous system, where the exquisitely intricate patterned growth forms the mature organ.\(^{19}\)
Spinal Cord

Studies on regeneration of damaged spinal cord have used 3-dimensional constructs to both promote and guide regeneration of damaged spinal cord axons, and to inhibit the growth of glial scar formation. These constructs have 10- to 15-μm-diameter porous channels to allow for diffusion of nutrients and are seeded with neural stem cells. When implanted into the hemisectioned spinal cord of adult rats, they allow regenerating axons to bridge the injured cord. Animals with hemisectioned cords walk dragging 1 leg, while those with the injury plus the implanted engineered construct recover locomotion and show significantly improved Basso-Beattie-Bresnahan scores, the standard by which locomotion is measured in rodent models of spinal cord injury.20 The mechanism of behavioral recovery in these experiments likely represents a combination of stem cell–induced regeneration, directed regrowth of severed axons by the scaffold, and an improved local microenvironment. This approach, in which polymer scaffolds are combined with stem or progenitor cells in culture, and then grafted into the injured CNS, is one way in which tissue engineering could be applied to optic nerve disease.

This technique has been used in the eye to attempt repairing a diseased retina.21 Retinal stem cells, derived from neonatal green fluorescent protein mice, were seeded onto poly-DL-lactic-co-glycolic acid copolymers and cultured for up to 14 days. These stem cell/polymer constructs were then grafted into the subretinal space of mice and pig recipients. The expression of green fluorescent protein in the stem cells, which allows in vivo monitoring of graft survival, was excellent in mouse allografts, but limited to 5 weeks in porcine xenografts. These experiments were directed towards repair of the photoreceptor layer, in an attempt to treat retinal degenerations such as retinitis pigmentosa and age-related macular degeneration. In the model, the constructs’ main function is to encourage the appropriate migration and differentiation of the grafted stem cells into photoreceptor neurons (rods and cones). These cells must form a local synapse with resident bipolar neurons in the outer plexiform layer, as well as elaborate the inner and outer segments necessary for efficient phototransduction. While these goals now seem approachable using tissue engineering techniques, it is worth noting that neither has yet been achieved.

A new type of scaffold which uses self-assembly of peptides has been developed.22 These peptides (16 amino acids and 5 nm in length), with alternating hydrophilic and hydrophobic surfaces, undergo self-assembly to form nanofibers, which further form interwoven matrices and a scaffold. Cells on the scaffold peptide can migrate on the fiber but not in between, because they are the same size. These polymers, first shown to exist in the yeast protein zuotin, can be used as a sort of “molecular cement” to induce either cell attachment or nerve regeneration.23 In vivo studies have shown that injection of a solution of self-assembling peptide nanofibers into a lesioned brachium would act in some way to close the severed brain. It is not clear how this closure occurs, but is likely to reflect a modification of the normal CNS response to injury. The use of novel biomaterials, in this case through molecular self-assembly, has a great deal of promise in regenerative medicine, and particularly repair of the damaged CNS. It is clear that new biomaterials will be needed before one can contemplate replacing the atrophic optic nerve with a synthetic construct, and the new structures being generated by Zhang and colleagues may lead the way to functional nerve replacement.22,24,25

Sources of Stem Cells for Tissue Engineering

Stem Cells for CNS

The use of stem cells is an intrinsic part of tissue engineering and plays a key role in the reconstruction of implantable tissues. Pluripotent, self-renewing stem cells can be obtained from human embryos—the now famous human embryonic stem cells.26,27 Using these cells, researchers have been successful in generating cells of ectodermal, endodermal, and mesodermal lineages. However, although a few cell lineages, such as fat, bone, and perhaps dopaminergic neurons, can be obtained by a combination of embryonic stem cell culture paradigms, other specific cell types have proven more difficult to generate.

The derivation of stem cells from human embryos is fraught with difficulties, both ethical and regulatory. This barrier has encouraged researchers to search for other sources of stem cells, especially cells that can generate CNS cell types. It has recently been demonstrated that a number of tissues, including those derived from adults, contain stem or progenitor cells that can, under specific conditions, differentiate into cells of the tissue from which the cells were derived. This list of tissues includes muscle, skin, cornea, liver, retina and brain. The list is not exhaustive, and it continues to grow at a rapid pace. Interestingly, it appears that a number of tissues outside the traditional CNS compartments may contain cells capable of generating neural stem cells.

Enteric glia may be a source for CNS cells for spinal cord regeneration.28 The gut contains a broad spectrum of peripheral nervous system neurons, particularly axon-supporting glial cells, which can be readily isolated. To test the use of enteric glial cells, Rathbone’s group29 has used a reanastomosis model of the spinal cord. In this model, the spinal dorsal root (the posterior nerve fibers of a spinal nerve which carry sensory information to the CNS) is cut. However, when enteric glial cells are injected into the zone of the dorsal root cut, larger numbers of nerve fibers now grow into the lesioned zone.29 These investigators evaluated function by a muscle reflex system (a pinch), and found that the response was improved in up to 80% of animals receiving enteric glial transplants at the time of injury. When other cell types were injected, regeneration of fibers or recovery of the response did not occur. These results demonstrate both functional and anatomical regeneration. The mechanism underlying this recovery of function is thought to be the modification of the “inhibitory” CNS microenvironment, in this case by replacing the CNS cells with peripheral nervous system–derived cells. This nerve regeneration approach might one day have a role to play in optic nerve repair.
Stem Cells for Vascular Regeneration

Embryonic stem cell differentiation can be induced to generate endothelial cells, with the goal of reconstructing functional blood vessels. Levenberg and colleagues\textsuperscript{30,31} started with embryonic stem cells, isolated side populations expressing markers of endothelial cell precursors, and treated them with a cocktail of growth factors known to induce differentiation of endothelial cells. They then combined these embryonic stem-derived cells with biodegradable polymer scaffolds. When implanted into severe combined immunodeficient mice, the cells were able to organize into vessel-like structures, some of which contained a lumen, and were perfused with host blood cells. One can imagine a host of applications for this work, such as cell therapy for heart disease, or the generation of blood vessels for bypass or other surgical procedures.

Stem Cells in Eye Niches

In addition to using exogenous stem cells to treat eye diseases such as glaucoma, quiescent endogenous stem cells could also be activated to perform self-repairing function. Stem cells reside in specialized microenvironments or “niches.” These niches provide the signals that at any given time are able to induce the resident stem cells to self-renew, differentiate and replenish other worn out cells. Xie and Spradling\textsuperscript{32} had initially discovered a niche maintaining germ line stem cells in the drosophila ovary. This laboratory has discovered the presence of several growth factors in the ciliary margin zone, including those belonging to the family of the bone morphogenetic proteins (BMP). In other systems, two members of this family, BMP2 and BMP4, have been implicated in differentiation of stem cells and overexpression of BMP2 prevented the differentiation.\textsuperscript{33} Investigators in the laboratory of Ernst Tamm, using bromodeoxyuridine labeling in living monokeys, have presented preliminary evidence of resident stem cells in the trabecular meshwork.\textsuperscript{34} Because the trabecular meshwork loses cells with age and in glaucoma,\textsuperscript{35,36} the potential of activating or repressing factors with could manipulate stem cells differentiation could have great potential in glaucoma therapy.

Sources of Grafts and Organoids for Tissue Engineering

Tissue Grafts for the Optic Nerve

The use of peripheral nervous system tissue to modify or eliminate the inhibitory signals present in the CNS has a long history, including the work of Ramon y Cajal.\textsuperscript{18} Recently, investigators have begun an approach in which the optic nerve is “replaced” by a peripheral nerve (most often the sciatic nerve). Gerald Schneider, PhD, and his group (unpublished information, oral communication September 2003) have reported on the functional reconstruction of the optic pathway using peripheral nerve bridges to restore function in several optic nerve lesion models. Using peripheral nerve grafts as bridges, they demonstrated functional recovery in hamster models of optic nerve section, as well as lesions of the brachium of the superior colliculus (unpublished information, oral communication September 2003). They have more recently begun using the self-assembling peptide nanofibers described earlier as a means of altering the inhibitory signals facing regenerating optic axons.

Organoids for Gastrointestinal Reconstruction

A slightly different approach is illustrated in techniques for tissue regeneration of the gastrointestinal system, including esophagus, stomach, and small and large intestine.\textsuperscript{37,38} To fabricate a tissue-engineered esophagus, the entire tissue of neonatal or adult rats is first harvested. After it has been mechanically disrupted and chemically digested, about 100,000 resulting organoid units (mesenchymal cores surrounded by epithelial cells) are placed on a biodegradable polymer and the construction implanted in a syngeneic host. Four weeks later, the tissue-engineered esophagus will have grown and its histology and immunohistochemistry resembles native esophagus. It exhibits a complete esophageal wall, maintains the esophagus architecture, and the animal carrying the transplant will gain normal weight.

For the first production of a tissue-engineered colon, the investigators used organoid units derived from sigmoid colon of Lewis rats and implanted them on a polymer scaffold into the omentum of syngeneic hosts.\textsuperscript{37} A tissue-engineered colon of identical architecture to native colon was generated in 100% of the animals. In addition, the engineered tissue maintained in vitro some of the physiological properties of mature colonocytes. It is very encouraging that a tissue that has been partially disrupted is able to recreate the original architecture once it is implanted back into a living animal.\textsuperscript{39}

Important Considerations for Retinal Regeneration

Axon Guidance

To succeed in regenerating functional RGC, axons of newly formed or implanted cells need to find their target. During development and regeneration after injury, retinal axons follow guidance cues that direct them toward the optic nerve, chiasm, and finally, the midbrain tectum.\textsuperscript{40} Following the correct pathway is complex and is in part governed by the repulsion of RGC axons at multiple decision cues. There is a gradient of cues extending from the motile tip of a developing axon all the way to the brain. Using a new tool that allows the knockdown of a specific protein function in situ (chromophore-assisted laser inactivation or CALI), Wang and Jay\textsuperscript{41} are identifying some of these cues. In a chick embryo optic nerve-crushing model, CALI of myelin-associated protein permitted significant regrowth of retinal axons. This powerful technology has allowed the testing of another protein, Ephrin-A5, which has a direct role in guiding the axons to their proper targets during growth.\textsuperscript{42} The use of CALI on Ephrin-A5 at early stages increased axon outgrowth. Such studies will be able to provide the molecular basis needed for successful regeneration of damaged optic nerve.
Retinal Prosthesis

Another approach to treat retinal degeneration is the development of a microfluidic silicon chip, which could be implanted under the retina and could be activated by light rather than by an electrode (Raymond Iezzi, MD, unpublished data, oral communication September 2003). This photocell device can package a number of produgs including DNA, peptides, hormones, and neurotransmitters, which then are released on the retinal surface on photoactivation. Several molecules are being tested and "caged" in these devices, which consequently could be "uncaged" by a laser pulse and thus would allow controlled drug delivery. Such a controlled delivery system coupled to findings identifying critical times and molecules during the regeneration process could play a major role in tissue engineering.

Survival of Retinal Transplant

Ng et al\(^43\) studied what occurs after transplanting neonatal neuronal retina into the subretinal space of adult mice, an immunoprivileged tissue transplanted into an immunoprivileged site. In order to have continuous observation, they used green fluorescent protein "green" mice and followed the fate of the green graft with an in vivo imaging system. By the 35th day, 50% of the grafts were rejected with by atypical, "silent" rejection, in which the grafts contain high numbers of activated microglia. Interestingly, they discovered that microglia normally residing in the inner retinal layers are greatly induced and migrate to the subretinal space following exposure to bright light.\(^44\) The relevance of these activated microglia in the graft rejection was demonstrated by performing grafts in mice that had spent 2 weeks under bright light conditions. Eighty percent of these grafts disappeared on the 35th day and were completely gone by 180 days. This discovery is of great importance for the future success of retinal transplantation and tissue engineering where rejection of the foreign tissue is a formidable obstacle.

PROGRESS OF TISSUE ENGINEERING IN THE CORNEA

Corneal regeneration and bioengineered corneas are poised to become a very active area of research. Ideally, an artificial cornea should be porous in the periphery, should not induce inflammation, and should have a posterior surface that prevents cell adhesion.\(^45\) A new generation of keratoprosthesis uses a hydrophilic polymer with a central optic region surrounded by a concentric macroporous skirt, which can be biointegrated through host tissue ingrowth.\(^46\) Clinical trials seem to indicate a lower level of complications than with the previous generation of keratoprosthesis, but there are still concerns with optic depositions and brownish discoloration associated with environmental factors. These bioengineered corneas may someday serve as substitutes for human donor tissues, and could fill an important clinical need in cases of high-risk recipients.

It is important to develop ways to produce sufficient numbers of undifferentiated progenitor cells in vitro. Tseng et al\(^47-49\) have used amniotic membrane as a natural scaffold. This ideal matrix was used for the ex vivo expansion of limbal epithelial stem cells and corneal keratocytes, key cell types for tissue engineering of the cornea. The keratocytes maintained their morphology and in addition were able to turn off the fibrotic signal of transforming growth factor β. The amniotic membrane could be very useful in the generation of other types of stem cells. The same group has already extended this approach for the growth of retinal pigment epithelial cells for future retinal transplantation.\(^50\)

FUTURE APPLICATIONS

Definite possibilities exist for applying tissue engineering approaches to the glaucomatous eye. Important potential considerations in the designing of scaffolds for specific uses are the ease of manufacturing various engineered tissues, the cost of applying them clinically, and their relative ease of use on a practical level.

One obvious goal would be the potential engineering of an artificial trabecular meshwork. Structures based on the human elastin polypeptides could be used for the formation of a spongiform scaffold, the 3-dimensional configuration of which would offer the required resistance to aqueous humor outflow. The scaffold would need to be pressure sensitive and conditions such the potential retention of protein carried by the aqueous humor need to be considered. Addition of trabecular meshwork stem cells to an elastin polymer could help repopulate the meshwork and maintain its physiological properties; once the scaffold is set, replenishment of trabecular meshwork cells could be considered. Other important considerations include the location of surgical insertion and immune responses that could induce inflammation and scarring. Because of the scarcity and difficulty of obtaining human trabecular stem cells, the use of allogenic embryonic stem cells also needs to be considered. As usual, the development of appropriate animal models occupied an important part of the discussion and also invoked the idea of using biopolymers for the generation of elevated pressure models.

To apply these approaches to replace lost RGC, a different and exceedingly difficult set of goals must be attained. If one were to graft stem cell/polymer constructs as a means of replacing lost RGC, several conditions for success must be met. First, the stem cells must take up residence in the appropriate retinal lamina, the RGC layer. Experiments suggest that in animal models of optic nerve disease, grafted stem cells do indeed migrate into the damaged cell layer.\(^51-53\) These cells must then differentiate along a neuronal lineage, make contacts with cells in the inner plexiform layer, and send neuronal processes into the optic nerve. While many of these conditions have been met by cells derived from the rodent hippocampus, it remains to be proven that the contacts in the inner plexiform layer represent functional synaptic contacts with appropriate inner nuclear layer cells, and the differentiation into mature neurons is a relatively rare event. Moreover, it is not yet clear how one would evaluate whether a grafted stem cell differentiates into a RGC. Although some have used Thy 1.1 as a specific marker for RGC in the retina, this molecule is expressed by other neurons in the CNS.
The Glaucoma Foundation sponsored an international conference entitled “Tissue Engineering and Glaucoma,” Boston, Mass, September 19–20, 2003. The meeting organizers were Terete Borris, PhD, (University of North Carolina School of Medicine, Chapel Hill), Julia E. Richards, PhD (University of Michigan, Ann Arbor, Robert Ritch, MD (The New York Eye and Ear Infirmary, New York), Michael Walter, PhD (University of Alberta, Edmonton), and Michael J. Young, PhD (Scheeps Eye Research Institute Boston, Mass). Participants included: Michael Anderson, PhD (Postdoctoral Fellow, Howard Hughes Medical Institute, Bar Harbor, Maine), Henry F. Edelhauser, PhD, (Director of Ophthalmic Research, Emory Eye Center, Atlanta, Ga); Rutledge Ellis-Behnke, PhD, (Research Scientist, Department of Brain & Cognitive Science, Massachusetts Institute of Technology, Cambridge); M. Rosario Hernandez, DDS, (Associate Professor, Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, Mo), Raymond Iezzi, MD, (Assistant Professor, Kresge Eye Institute, Detroit, Mich); Daniel G. Jay, PhD (Professor of Physiology and Neuroscience, Department of Physiology, Tufts University School of Medicine, Boston), Simon John, PhD (Associate Investigator, Jackson Laboratories, Howard Hughes Medical Institute, Bar Harbor; Jonathan Kipnis, PhD, (Weizmann Institute of Science, Rehovot, Israel); Theodore Krupin, MD (Clinical Professor of Ophthalmology, Northwestern University School of Medicine, Chicago); Erin B. Lavik, ScD (Assistant Professor, Department of Biomedical Engineering, Yale University, New Haven, Conn); Shulamit Levenberg, PhD, (Research Associate, Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge); Jeffrey M. Liebmann, MD (Professor of Clinical Ophthalmology, The New York Eye and Ear Infirmary, New York); Cynthia Mattox, MD, (Assistant Professor, Department of Ophthalmology, Tufts-New England Medical Center, Boston); Erin Rubin Ochoa, MD, (Attending Pathologist, Department of Pathology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY); Louis R. Pasquale, MD, (Assistant Professor, Department of Ophthalmology, Harvard Medical School, Boston), Michael P. Ratibbone, PhD, (Professor, Department of Medicine, McMaster University Health Sciences Centre, Hamilton, Ontario); Buddy D. Ratner, PhD, (Distinguished Professor of Bioengineering, University of Washington, Seattle); Gerald E. Schneider, PhD, (Professor, Department of Brain and Cognitive Science, Massachusetts Institute of Technology, Cambridge); Molly S. Schochet, PhD (Professor, Institute for Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario); J. Wayne Streilein, MD (President, Scheeps Eye Research Institute, Harvard Medical School, Boston); Ernst Tamm, MD (Associate Professor of Molecular Anatomy and Embryology, Department of Anatomy, University of Erlangen-Nürnberg, Erlangen, Germany); Sheffer C. Tseng, MD, PhD (President, Ocular Surface Center, Miami, Fla); Liliana Werner, MD, PhD (Research Assistant Professor, John A. Moran Eye Center, Salt Lake City, Utah); Scott R. Whittemore, PhD (Professor of Neurological Surgery, University of Louisville School of Medicine, Louisville, Ky); Kimberly A. Woodhouse, PhD, (Associate Professor, Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto); Ting Xie, PhD (Associate Scientist, The Stowers Institute for Medical Research, Kansas City, Mo), Shaguang Zhang, PhD (Principal Investigator & Associate Director, Center for Biomedical Engineering, Massachusetts Institute of Technology, Cambridge); Steven Bartels, PhD (Vice President, Pharmaceutical New Product Development, Bausch & Lomb, Rochester, NY); Michael Niesman, MD, Ophthalmic Disease Drug Discovery, Pfizer Global Research & Development/La Jolla Labs, San Diego, Calif); Michael Stern, PhD (Research Investigator, Allergan, Irvine, Calif); Karyn Siemasko, PhD (Scientist, Allergan, Irvine).

A functional definition, in which synaptic connection occurs between the retina and the brain, remains the standard by which one must judge complete RGC differentiation. Herein lies the challenge. In an experiment in which stem cells differentiated into neurons and sent axon like projections toward the brain, only modest ingrowth into the optic nerve was noted.\(^{52,53}\) Growing axons have never made it to the chiasm, much less the optic tectum. Innervation of the brain is the obstacle to be overcome in these experiments, but simply innervating the brain is not enough. Contacts must be made with the appropriate retinorecipient structures in the lateral geniculate. A topographic map must be formed by these connections for useful visual information to be conveyed. This will be quite difficult to achieve in the mature brain, as the wiring and organization of the retinal-brain circuitry takes place during the critical period of early development. While these challenges are indeed daunting, they remain worthy goals to aim for. When neurons are lost due to disease or injury, functional replacement is difficult and fraught with obstacles. The lack of regeneration in the mature mammalian CNS makes cell replacement one of the only avenues of treatment. Tissue engineering strategies offer the hope of fundamentally new approaches to the treatment of cell death throughout the neuraxis, and we remain hopeful that repair of the optic nerve will someday be possible through this technique.

While cell replacement is the most obvious tissue engineering strategy in optic nerve disease, other options must also be considered. In almost all circumstances, it is easier and preferable to rescue diseased neurons rather than replace them with new ones. Glaucoma is a perfect example of this truism. Tissue engineering strategies aimed at delaying RGC death, by delivering growth factors, apoptotic agents, or any number of potentially therapeutic drugs, should be applied to glaucoma. Preliminary studies suggest that both stem cells and biodegradable polymers can decrease the tempo of degeneration in a mouse model of pigmentary glaucoma. Biomechanical constructs could also be used to deliver therapeutic genes to the injured retina. The accessibility of the eye has allowed the retina become something of a proving ground in gene therapy, with clinical trials set to begin in patients with the RPE65 type of Leber congenital amaurosis following the startling success in the dog model of this disease.\(^{54,55}\) While such a straightforward loss-of-function mutation is unlikely to exist in optic nerve disease, we must move ahead with gene therapy in glaucoma.
Our discussions conclude with cautious optimism. While the challenges of treating optic nerve disease are daunting, the progress in tissue engineering and the related field of regenerative medicine is providing the tools to contemplate what seemed only a few years ago to be impossible. Although the replacement of the degenerating or diseased structures of the glaucomatous eye has yet to be realized, we are now entering, to paraphrase Winston Churchill, the end of the beginning, if not the beginning of the end.

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