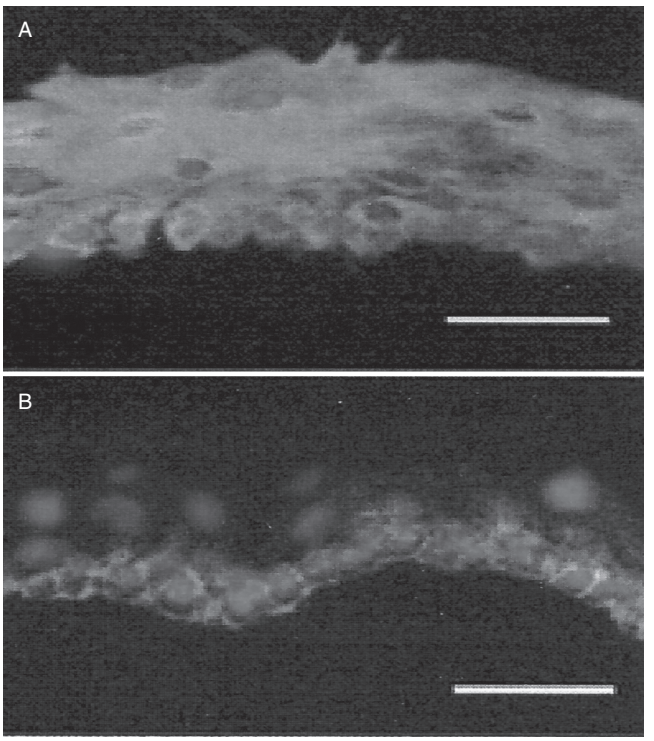
23

OCULAR REGENERATIVE ENGINEERING



Preparation of autologous tissue-engineered epithelial cell sheets fabricated from oral mucosal epithelium. Oral mucosal tissue (3 × 3 mm) was removed from a patient's cheek. Isolated epithelial cells were seeded onto temperature-responsive cell culture inserts. After two weeks of culture at 37°C, the cells grow to form multilayered sheets of epithelial cells. These sheets were used to cover and repair injured corneal epithelium. Specimens were collected for testing the expression of keratin 3 (A) and anti-β1 integrin (B) by immunohistochemistry. Red: cell nuclei. Scale bars: 50 μm. (Reprinted with permission from Nishida K et al: Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium, *New Engl J Med* 351:1187–96, copyright 2004 Massachusetts Medical Society. All rights reserved.) See color insert.

Bioregenerative Engineering: Principles and Applications, by Shu Q. Liu

ANATOMY AND PHYSIOLOGY OF THE OCULAR SYSTEM [23.1]

The *ocular system* is composed of the eyes, optic nerve, and a number of ocular accessory structures, including eyelids, lacrimal apparatus, and ocular muscles. The eye consists of several layered structures: the fibrous tunica, vascular tunica, and the nervous tunica (Fig. 23.1). The *fibrous tunica* is the outmost layer of the eye, composed of sclera and cornea. The *sclera* is a layer of collagen-rich connective tissue, which is distributed around the lateral and posterior sides of the eye and encloses the intraocular structures. It provides structural and mechanical stability to the eye, protects ocular structures from injury, and serves as an anchoring base for the ocular muscles. The *cornea* is an avascular and transparent membrane structure, which is continuous with the sclera and located in the front of the eye. It is composed of three layers: the external stratified epithelium, the middle stroma with a collagen and proteoglycan-rich matrix, and internal endothelium. The cornea is a key optical structure that refracts light to the retina. Since the cornea also serves as a protective barrier for the intraocular structures, it is heavily innervated with sensory nerve endings, which sense mechanical stimuli and temperature changes.

The *vascular tunica* is the middle layer of the eye and contains a rich network of arteries and veins, which supply oxygenated blood to and drain deoxygenated blood from the eye. The arteries of the eye are originated from the internal carotid artery and the veins drain blood to the internal jugular vein. The vascular tunica is composed of choroid, ciliary body, and iris. The *choroid* is the portion associated with the sclera and is distributed around the lateral and posterior sides of the eye. In the front of the eye, the choroid is connected to the *ciliary body*, a structure composed of the *ciliary ring* of smooth muscle cells and *ciliary processes*. The ciliary smooth muscle cells are aligned in the radial direction of the ciliary ring in the outer region, whereas aligned in the circumferential direction in the inner region. The ciliary processes are structures of epithelial cells and are connected to the suspensory ligaments. The suspensory ligaments are fibrous structures linking the ciliary body to the lens. The contraction of the ciliary smooth muscle cells stretches the lens, reducing the thickness of the lens. The lens recoils back to the original shape when the ciliary muscles relax. The contractile activity of the ciliary

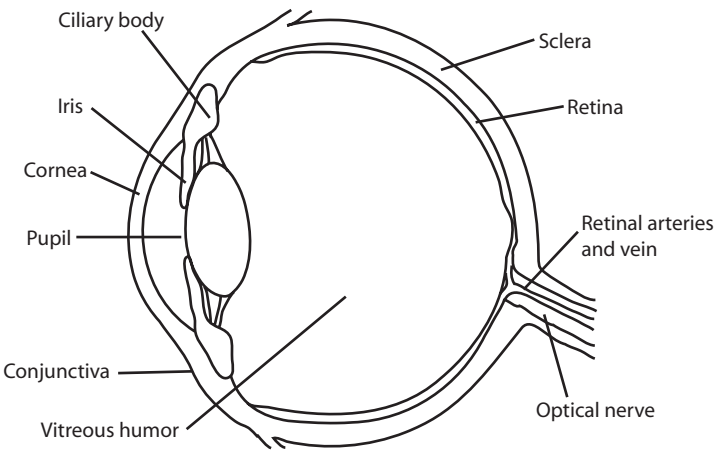


Figure 23.1. Schematic representation of the eye structures.

muscles and the shape change of the lens control the focal distance of the eye. The ciliary body is connected to the *iris*, which contains smooth muscle cells and surrounds the pupil, the central opening of the eye. The iris is composed of circumferentially and radially aligned smooth muscle cells. The circumferential smooth muscle cells are controlled by parasympathetic nerves. The contraction of these muscles reduces the size of the pupil, resulting in a decrease in the amount of light entering the eye. The radial smooth muscle cells are controlled by sympathetic nerves. Their contraction induces the dilation of the pupil, increasing the amount of light entering the eye.

The *nervous tunica* is also called the *retina*, which is the internal layer of the eye. The *retina* is composed of a surface epithelial layer, known as the pigmented retina, and a neuronal layer, known as the sensory retina. The *sensory retina* consists of a large number of rod and cone photoreceptor neurons and relay neurons. The retina covers the internal surface of the eye except for the front area with the lens and the ciliary body. In the posterior center, there are two spots that contain highly concentrated photoreceptor neurons: the macula lutea and fovea centralis. These structures are capable of identifying fine objects and images. Near these structures, there is another spot, known as the optic disc, a location where blood vessels enter and the nerve fiber bundles from photoreceptor neurons leave the eye. The optic disc is not able to sense light because of the lack of photoreceptors.

The *sensory retina* is composed of three layers: photoreceptor neurons, bipolar neurons, and ganglionic neurons. Between these layers, there exist various types of association neurons. The photoreceptor layer contains rod and cone neurons. The *rod neurons* can sense light of low intensity and are insensitive to colors. These cells contain a protein complex, known as rhodopsin, which is responsible for the sensation of dim light. In addition, rhodopsin participates in the regulation of light adaptation. When a person is suddenly exposed to bright light, rhodopsin is rapidly degraded, reducing light-initiated stimulatory signaling activities. In contrast, when a person is suddenly exposed to dim light, the production of rhodopsin increases, enhancing the sensitivity of the retina to dim light.

The *cone neurons* are responsible for the sensation of colors and ordinary light. Color identification by these cells requires the presence of a critical level of bright light. Below such a critical level, these cells lose the capability of color identification. Cone cells contain protein complexes called iodopsins. These complexes are composed of three types of opsin proteins for the sensation of red, blue, and green colors. Each type is only sensitive to a narrow spectrum of light corresponding to a specific color. The distribution of the cone neurons differs from that of the rod neurons. The fovea centralis is primarily composed of cone neurons with almost no rod neurons. Thus this structure is for the sensation of color and bright light and, especially, for accurate identification of images. In contrast, the rod neurons are spread over the remaining retina, a distribution essential for the sensation of dim light.

The *bipolar* and *ganglionic neurons* in other retinal layers play critical roles in the transmission of optic signals from the rod and cone neurons to the central visual centers. The bipolar neurons synapse with the rod and cone neurons at one side and synapse with the ganglionic neurons at the other side. In these layers, there are several types of association neurons, including horizontal neurons, amacrine neurons, and interplexiform neurons. These neurons synapse with the photoreceptor, bipolar, and ganglionic neurons, and relay, integrate, and modify signals from the photoreceptor neurons. Nerve fibers from the ganglionic neurons converge to the optic disc, where they form the *optic nerve*, exit the eye,

and enter the central visual centers of the brain, including the superior colliculi, lateral geniculate nuclei of thalamus, and visual cortex.

Enclosed within the eyeball are two compartments: the anterior and posterior compartments. The *anterior compartment* is the chamber between the cornea and the lens, and is filled with aqueous humor, a fluid that is produced by the ciliary processes, released into the anterior compartment, and returned to the vein through the trabecular meshwork and the canal of Schlemm. The *aqueous humor* circulates constantly with a stable hydrostatic pressure and supplies oxygen and nutrients to the cornea and lens. The obstruction of the trabecular meshwork and the canal of Schlemm results in an increase in the intraocular pressure, a disorder known as *glaucoma* (see page 979). The *posterior compartment* is the chamber surrounded by the retina and filled with *vitreous humor*, a transparent gel-like structure. The vitreous humor plays a critical role in the maintenance of the ocular shape and transmission of light.

The *lens* is an avascular, transparent, biconvex structure that is composed of two types of epithelial cells, including the cuboidal and fiber-like epithelial cells. The cuboidal epithelial cells are found on the anterior surface, whereas the fiber-like epithelial cells are found in the remaining body of the lens. The fiber-like cells are specially differentiated cells that do not contain nuclei and cellular organelles. Instead, these cells contain a special type of protein known as crystalline. The presence of crystalline renders the lens highly transparent. The crystalline-containing cells are enclosed by an elastic layer of tissue, which is connected to the ciliary body via the suspensory ligaments.

The *ocular accessory* structures include the eyelid, conjunctiva, lacrimal apparatus, and eye muscles. The *eyelid* is composed of several layers, including the skin, areolar connective tissue, skeletal muscles, tarsal plate, and palpebral conjunctiva. The function of the eyelid is to protect the eye from injury. The *conjunctiva* is a fibrous membrane that covers the internal surface of the eyelid (the palpebral conjunctiva) and the anterior surface of the eyes (the bulbar conjunctiva). The palpebral conjunctiva directly interacts with the cornea. Because of the presence of fluids, the friction between the conjunctiva and cornea is small. The *lacrimal apparatus* is composed of the lacrimal gland, lacrimal canaliculi, lacrimal sac, and nasolacrimal duct. The lacrimal gland produces tears, which are released to the external surface of the eye. The production and release of tears are controlled by the parasympathetic nerves. Tears serve as a lubricant for the interaction of the eyeball with the eyelid. Excessive tears enter the lacrimal canaliculi through two openings called punctas, flow into the nasal cavity via the lacrimal sac and nasolacrimal duct. Each eyeball is associated with six skeletal muscle bundles. These muscles are anchored to the external surface of the sclera and control the movement of the eyeball.

OCULAR DISORDERS

Corneal Injury

Pathogenesis, Pathology, and Clinical Features [23.2]. The cornea is a structure exposed to the exterior environment and is subject to various hazards, such as mechanical injury, chemical corrosion, radiation, and infection by bacteria and viruses. Corneal injury due to mechanical trauma and chemical corrosion is commonly seen. Corneal injury often induces inflammatory reactions, followed by fibrosis and scar formation in the cornea, reducing light transmission. Since the cornea is a collagen-rich structure, disorders with collagen degradation may affect the function of the cornea. Metabolic disorders can also

induce dysfunction of the cornea. For instance, hypercalcemia is associated with calcium precipitation underneath the cornea epithelial cells. Cystinosis can cause the formation of cystine crystals in the cornea. Hypercholesterolemia induces cholesterol deposition in the cornea. All these disorders influence light transmission through the cornea and induce visual impairment.

Conventional Treatment of Cornea Injury [23.2]. There are two strategies for the treatment of corneal disorders: removing the factors that cause corneal abnormalities and conducting corneal transplantation. When causative factors can be identified, these factors should be removed, if possible, to reduce or stop the progression of corneal abnormalities. For example, ocular bacterial infection should be controlled by local administration of antibiotics. When hypercalcemia is identified as a causative disorder, the blood calcium concentration should be reduced to the normal level. When severe corneal scars and opacity develop, the cornea can be replaced with an allogenic corneal specimen, a procedure known as corneal transplantation.

Molecular Regenerative Engineering. Molecular engineering approaches can be applied to corneal disorders. Corneal disorders often involve molecular activities, such as activation of pro-inflammatory factors, upregulation of proliferative genes, and production of extracellular matrix. Thus, molecular strategies for the treatment of corneal disorders are to suppress inflammation and selectively inhibit the proliferation of certain cell types such as fibroblasts. Selected genes can be prepared and used for the treatment of ocular disorders. For example, corneal haze and cloudiness after mechanical injury are due to excessive inflammatory reactions, including leukocyte infiltration, cell proliferation, and extracellular matrix deposition. Genes encoding anti-inflammatory and antiproliferative proteins can be used to suppress inflammatory reactions and fibrous changes. In addition, dominant negative genes for proinflammatory and mitogenic factors can also be used for treating corneal inflammation. Another example is the molecular treatment of primary glaucoma. This disorder is induced by the obstruction of the trabecular meshwork by excessive production of extracellular matrix. Genes encoding matrix metalloproteinases, which degrade extracellular matrix components, can be used for the treatment of glaucoma.

Given the anatomical features of the ocular system, several approaches can be used for gene delivery. For the molecular treatment of the corneal epithelial disorders, a topical gene delivery is effective. For disorders of the iris, ciliary body, and trabecular meshwork, gene injection into the anterior compartment is required. For retinal disorders, it is necessary to conduct intravitreal gene injection. As for other organs and tissues, various methods can be used to mediate gene delivery to the ocular system, depending on the anatomical features of and cell types in the target tissue. For instance, electroporation is an effective method for gene delivery to the corneal epithelial cells, but may not be a suitable method for gene delivery to the intraocular structures. Genetically modified adenoviruses and retroviruses are often used for mediating gene delivery into ocular tissues, including the cornea, trabecular meshwork, and retina. These mediating methods have been shown to be more effective than other mediating methods, such as salt- and liposome-mediated delivery, for the ocular system.

Molecular engineering approaches have been developed and used for treating several corneal disorders, including immune rejection of corneal transplants, corneal inflammation and haze, and corneal complications due to metabolic disorders such as mucopolysaccharidosis. These approaches are discussed in the following sections.

Molecular Therapies for Corneal Immune Rejection [23.3]. Allogenic corneal transplantation is an effective approach for the treatment of corneal dysfunction. However, the presence of functional epithelial cells, which are essential for successful corneal transplantation, often causes immune reactions, resulting in acute rejection. It is often necessary to administrate immune suppressor agents to patients with corneal transplantation. However, these immune suppressor agents induce side effects by inhibiting the activity of the entire immune system. Furthermore, it is required to conduct daily agent deliveries. Molecular engineering approaches can be used to overcome these problems. Genes that encode immune suppressor cytokines and antisense oligonucleotides for immune activator genes can serve as immune suppressor agents. Given the anatomical features of the cornea, it is relatively easier to deliver genes to the cornea than to the internal structures. Three approaches may be used for corneal gene delivery: application of genes to the exterior surface of the cornea, gene injection to the anterior compartment, and augmentation of gene delivery by electroporation (see page 444 for these methods). Typical genes for corneal disorders include the CD152 and interleukin (IL)10 genes, which have been used for the treatment of corneal transplant immune rejection.

The CD152 gene (CTLA-4) encodes a membrane protein in the T lymphocytes. The CD152 protein exerts an inhibitory effect on T lymphocyte-related immune reactions. When allogenic tissues are transplanted to the host, the allogenic antigens activate antigen-presenting cells (APCs), which in turn interact with the T lymphocytes, leading to activation of the T lymphocytes and initiating immune reactions. In particular, a cell membrane protein known as CD80 can interact with another membrane protein CD86 to form complexes. The CD80 and CD86 complexes on the APC surface interact with CD28 (see Table 23.1) on the T lymphocyte surface, eliciting co-stimulating signals for the activation of the T lymphocytes. The CD152 complexes on the T lymphocyte surface, when present, can bind to CD80 and CD86 in antigen-presenting cells, suppressing the activity of these cells as well as the T lymphocytes. The overexpression of the CD152 gene by gene transfer has been shown to induce the arrest of T lymphocyte division, reduce immune responses, prevent corneal immune rejection, and prolong the survival of transplanted allogenic cornea. The CD152 gene can be conjugated with an Ig gene, forming a recombinant gene complex, which can facilitate gene delivery and expression.

Another gene used for the treatment of corneal transplant immune rejection is the interleukin-10 gene (see page 634 for characteristics of IL10). This gene encodes a cytokine that suppresses the activity of T lymphocytes. Experimental investigations have demonstrated that the interleukin-10 gene can be effectively transferred into more than 70% of the epithelial cells of the cornea with a virus-mediated gene transfer approach. The transferred gene can be expressed for about 3 weeks. Such an approach has been shown to reduce immune responses in transplanted allogenic cornea and prolong corneal survival.

Molecular Therapies for Corneal Inflammation and Fibrosis [23.4]. Corneal inflammation is induced by trauma and therapeutic keratectomy. Inflammation often results in epithelial cell proliferation, extracellular matrix production, and fibrosis. Thus, the principle of molecular engineering therapy for corneal inflammation is to introduce genes that encode antiproliferative proteins. A gene encoding the dominant negative cyclin G1 (Table 23.2) protein has been constructed and used to treat corneal inflammation. Cyclin G1 plays a critical role in stimulating the progression of the cell division cycle. The

TABLE 23.1. Characteristics of Selected Molecules that Regulate Immune Responses*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
CD28	Antigen CD28, T-cell antigen CD28	220	25	T cell, B cell	Regulating CD4-positive T-cell survival and proliferation, inducing interleukin-2 production from T cells, and promoting the development of T-helper type-2 (Th2) cells
CD80	CD28 antigen ligand 1 (CD28LG1), B lymphocyte activation antigen B7-1, activation B7-1 antigen, B7-1 antigen	288	33	B cell, dendritic cell, monocyte, mast cell, nervous system, blood vessels	Interacting with CD28 on T cells and regulating T-cell proliferation and activation
CD86	CD86 antigen, CD28 antigen ligand 2 (CD28LG2), B lymphocyte activation antigen B7-2, Activation B7-2 antigen, CTLA4 counterreceptor B7.2, LAB7-2, B70	329	38	T cell, B cell, monocytes, macrophages, dendritic cells, vascular endothelial cells, smooth muscle cells, bone marrow, intestinal epithelial cells	A member of the immunoglobulin superfamily expressed by antigen-presenting cells, binding to CD28 on T cells to regulate T-cell proliferation, survival, and activation, and binding to cytotoxic T-lymphocyte-associated protein 4 on T cells to negatively regulate T-cell activation and diminish T-cell-mediated immune responses
CD152	Cytotoxic T lymphocyte associated 4, cytotoxic T lymphocyte antigen 4 (CTLA4)	223	25	T cell	A member of the immunoglobulin superfamily that inhibits the activity of T cells

*Based on bibliography 23.3.

TABLE 23.2. Characteristics of Cyclin G1*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Cyclin G1	Cyclin G, CCNG	295	34	Lymphocytes, lung, kidney, intestine, spleen, thymus, testis, ovary, prostate gland, skeletal muscle	Regulating cell division

*Based on bibliography 23.4.

negative dominant cyclin G1 protein competes with the wild-type or natural form of cyclin G1 for substrate binding, but does not activate the substrate. Thus, the introduction of this dominant negative gene to the corneal epithelial cells suppresses the activity of the natural cyclin G1, induces the arrest of the cell division cycle, and reduces cell proliferation and matrix production. These processes are associated with a decrease in inflammatory reactions and suppression of extracellular matrix production and haze development. Experimental investigations have demonstrated promising results for the transfer of the dominant negative cyclin G1 gene into the cornea with laser keratectomy-induced injury. Dominant negative genes constructed for other types of mitogenic signaling factors, such as growth factor receptors, protein tyrosine kinases, and cell cycle regulators, can also be used to inhibit corneal inflammatory reactions.

Pharmacological inhibitors can be used to suppress the activities of inflammatory factors. One example is the use of the nuclear factor κB inhibitor SN50. Nuclear factor κB is a transcription factor that stimulate the expression of inflammatory genes. The suppression of the activity of nuclear factor κB may reduce inflammatory reactions in corneal injury. Experimental investigations have demonstrated that the topical application of SN50 can facilitate the healing process of alkali-induced corneal injury (Fig. 23.2).

Molecular Therapies for Corneal Complications Due to Mucopolysaccharidosis Type VII (MPSVII) [23.5]. Mucopolysaccharidosis type Vll is an hereditary disorder due to the deficiency of the enzyme β-glucuronidase (GUSB) (Table 23.3), which breaks down proteoglycans. This disorder is associated with the lysosomal accumulation of undegraded glycosominogycans (GAGs), resulting in corneal abnormalities and opacity. The transfer of the β-glucuronidase gene into the corneal epithelial cells induces the over-expression of β-glucuronidase, prevents the accumulation of undegraded glycosominogycans, and reduces corneal opacity.

Cellular and Tissue Engineering. Cellular and tissue engineering approaches have been established and used to treat ocular disorders in experimental models and preliminary clinical trials. As for other organ and tissue systems, a successful replacement of a malfunctioned ocular tissue requires the construction of a functional cellular and tissue structure and the integration of the constructed structure into the ocular system. To date, cellular and tissue engineering approaches have been used to repair and reconstruct disordered cornea in experimental models and clinical trials. However, the application of cellular and tissue approaches to other ocular tissues has been limited because of difficulties in the construction and assembly of functional ocular structures, such as the retina and lens.

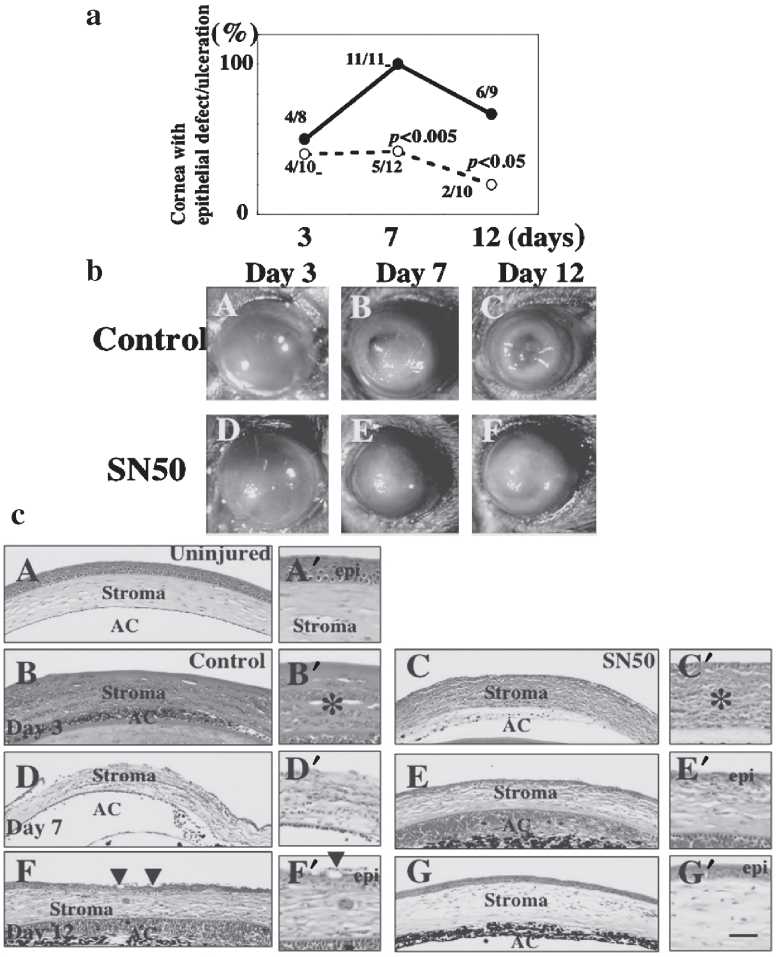


Figure 23.2. Healing of alkali-burned mouse cornea treated with topical SN50, an inhibitor of NFκB. (a) Percentage of corneas with epithelial defect (including ulceration) at each healing interval. The incidence of epithelial defect/ulceration is significantly higher in the control group than in the SN50-treated group at days 7 and 12 as judged by the χ^2 test. (b) Macroscopic observation shows similar initial resurfacing in both control (A) and SN50-treated groups (D) at day 3 after alkali burning. Recurrence of the epithelial defect with stromal opacification is observed more frequently in the control group at days 7 (B) and 12 (C) as compared with SN50-treated group (E, F). (c) Histology of burned corneas stained with H&E. (A) An uninjured cornea. Stratified epithelium and stroma are seen. There is no histological difference between central corneas in the control (B) and SN50-treated group (C) at day 3. The epithelium shows a large defect and many inflammatory cells are observed. At day 7 the burned cornea in the control (D) shows more stromal inflammation, and a large epithelial defect as compared with the SN50-treated corneas that has been resurfaced with a thin epithelium (E). At day 12 the control cornea still shows marked inflammation and hypercellularity in the stroma (F), whereas the treated cornea exhibits a well-regenerated epithelium with a less stromal inflammation (G). Regenerated epithelium in control exhibits conjunctiva-like appearance with goblet cells (arrowheads). A′–G′ are high-magnification pictures of the central area of the healing corneas in A–G, respectively. Scale bar: 100 μm (A–G), 25 μm (A′–G′). (Reprinted with permission from Saika S et al: *Am J Pathol* 166:1393–1403, copyrights 2005.)

TABLE 23.3. Characteristics of β-Glucuronidase*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
β-Glucuronidase	βG1	651	75	Retinal pigmented epithelial cells, leukocyte, liver, kidney, spleen, placenta, intestine, pancreas	An enzyme that degrades proteoglycans

*Based on bibliography 23.5.

Corneal dysfunction requires corneal replacement by transplantation. Allogenic cornea is often used for such a purpose. However, there two major problems for allogenic cornea transplantation: immune rejection and shortage of cornea donors. In particular, immune responses induce injury and death of corneal epithelial and endothelial cells, leading to opacification of the transplanted cornea. Several cellular and tissue engineering strategies have been developed and used to overcome these problems, including: (1) corneal surface reconstruction with epithelial stem cells or autogenous epithelial cells, which can be applied to native or transplanted corneas with injured epithelial cells and (2) corneal reconstruction with extracellular matrix and polymeric materials.

Corneal Surface Reconstruction [23.6]. The cornea is covered at the external surface with an epithelial layer. The injury or denudation of this layer induces inflammatory reactions and fibrosis, resulting in alterations in the optical properties of the cornea and visual acuity. In such a case, it is necessary to reconstruct the corneal surface. Corneal surface reconstruction can be accomplished by using an epithelial cell layer constructed in vitro. Several types of cells can be used to construct an epithelial cell layer: limbal epithelial stem cells, adult epithelial cells, and stem cells. Epithelial stem cells are present in the limbal region or the junction of the cornea and conjunctiva. These cells can differentiate into corneal epithelial cells in corneal injury. The deficiency of limbal stem cells can cause ocular surface disorders, leading to blindness. In corneal injury, limbal stem cells can be collected and used for enhancing corneal cell regeneration by cell transplantation.

Limbal stem cells can be collected from several sources, including the cadaver eyes, the conjunctival limbal tissue from the donors, and autogenous limbal tissue. Harvested limbal epithelial stem cells by biopsy can be cultured and expanded in vitro on a suitable carrier membrane, such as a polymeric or natural matrix membrane, forming a transplantable epithelial membrane structure. The cultured epithelial cells can produce extracellular matrix, which serve as a basal meshwork for the formation of a stable epithelial membrane structure. The epithelial membrane is readily adhesive and can be used for the construction of a corneal epithelium-like structure (Fig. 23.3). Clinical investigations have shown that this approach can be used to effectively prevent corneal inflammatory reactions and improve visual acuity (Fig. 23.4).

Natural biological membranes can also be used to serve as epithelial cell carriers. A typical example is the human amniotic membrane, which can be used as a basal membrane

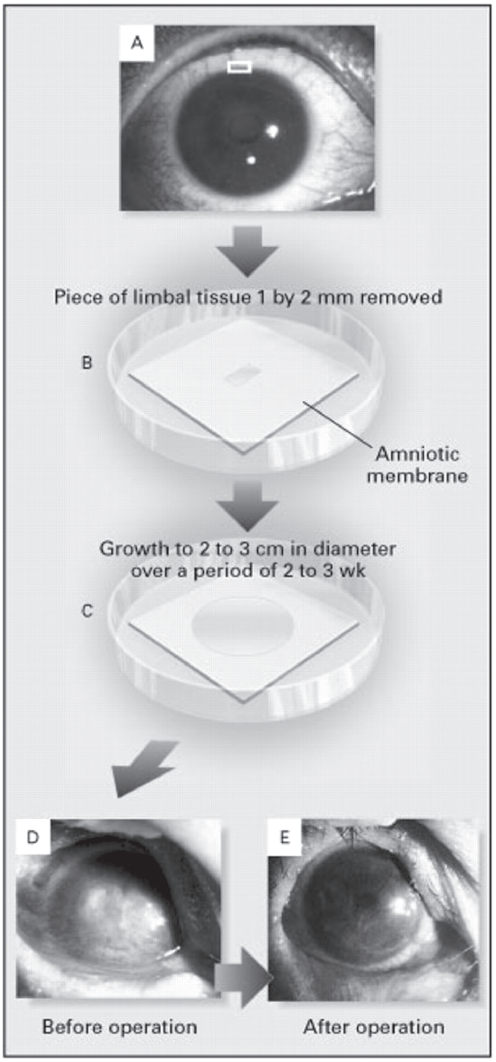


Figure 23.3. Transplantation of autologous limbal epithelial cells cultured on amniotic membrane. Limbal tissue (1 × 2 mm) was removed by lamellar keratectomy from the superior limbus of the healthy contralateral eye (panel A). The explanted tissue was placed on amniotic membrane in a 35-mm dish containing 1.5 mL of culture medium (panel B). After 2–3 weeks, the epithelial cells had grown and spread to form a circular sheet of cells with a diameter of 2–3 cm (panel C). The cultured limbal epithelial cells with amniotic membrane were then transplanted to the diseased eye (panels D and E). (Reprinted with permission from Tsai RJ, Li LM, Chen JK: *New Engl J Med* 343:86–93, copyright 2000 Massachusetts Medical Society. All rights reserved.)

for culturing epithelial cells and constructing corneal epithelial layers. The constructed epithelial layer can be directly applied to the exterior surface of the cornea. Since the epithelial cell layer is usually thin and self-adhesive, it is not necessary to fast the cell layer with suture stitches or adhesives. Compared to synthetic biomaterials, a biological membrane is compatible with cells and provides a suitable substrate for the formation of an epithelial cell layer.

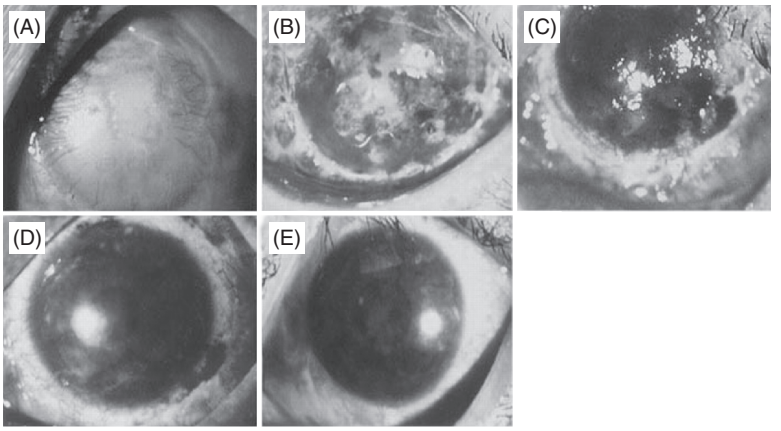


Figure 23.4. Serial photographs of the eye of patient 6 before and after transplantation initial examination. Panel A reveals corneal opacity with central corneal erosion and neovascular growth extending into the entire cornea for 4–6 mm before operation. Lamellar keratectomy was performed to remove the entire opacified limbal and corneal area to a thickness of ~$^1/_3$rd of the corneal layer (panel B). Limbal epithelial cells with the amniotic membrane substrate were transplanted onto the denuded limbal and corneal area. Photographs were taken 1 day (panel B), 7 days (panel C), 30 days (panel D), and 450 days (panel E) after the operation. (Reprinted with permission from Tsai RJ, Li LM, Chen JK: *New Engl J Med* 343:86–93, copyright 2000 Massachusetts Medical Society. All rights reserved.)

Adult epithelial cells can proliferate and can be used for constructing the corneal external surface. Ideally, autogenous corneal epithelial cells should be used for corneal reconstruction. However, in patients with corneal disorders, corneal epithelial cells are usually not available. Given the fact that most epithelial cell types on the exterior surface of the body exhibit certain common phenotypes, it is conceivable that epithelial cells from other exterior tissues may be used for constructing a corneal epithelial structure. A candidate epithelial cell type is the oral mucosal epithelial cells. These cells can be easily identified, harvested, manipulated, and expanded in culture. A major advantage of using the oral mucosal epithelial cells is that cells can be harvested from the host patients, thus avoiding immune rejection responses.

Prepared oral mucosal epithelial cells can be seeded on a membrane for the construction of an epithelial layer. Researchers have developed a temperature-sensitive synthetic polymer material, which can be used to construct membranous cell carriers (Fig. 23.5). Epithelial cells or stem cells can be seeded on the carrier for expansion and formation of an epithelial membrane. A reduction in temperature causes shrinkage of the carrier polymeric material, inducing the separation of the cells from the carrier membrane. Since this approach does not require the use of proteinases for cell separation from the membrane carrier, epithelial cells remain intact and functional.

Epithelial membranes constructed with autogenous oral epithelial cells have been shown to express corneal epithelial markers, such as keratin-3 (Chapter 23 opening figure), and have been applied to patients with complete denudation of the corneal epithelium with severe impairment of visual acuity. These investigations have demonstrated that reepithelialization of the corneal surface occurs within one week, resulting in the restoration of the corneal transparency and significant improvement of visual acuity (Fig. 23.6). In

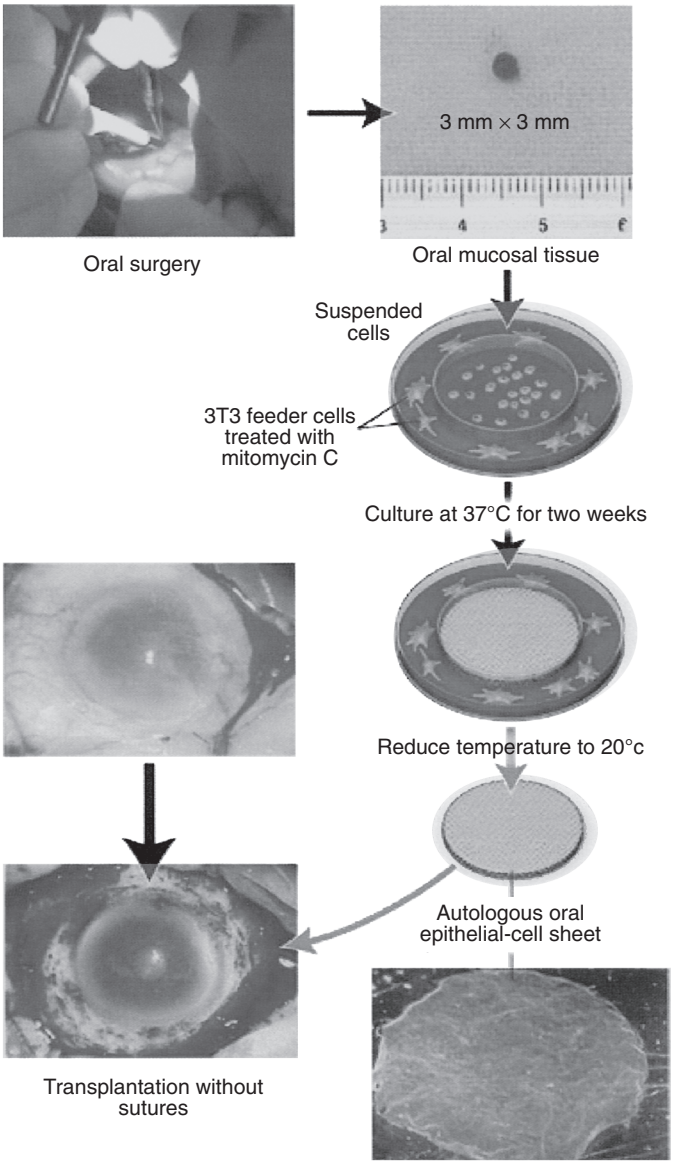


Figure 23.5. Transplantation of autologous tissue-engineered epithelial cell sheets fabricated from oral mucosal epithelium to injured cornea. Oral mucosal tissue (3 × 3 mm) was removed from a patient's cheek. Isolated epithelial cells are seeded onto temperature-responsive cell culture inserts. After 2 weeks at 37ºC, these cells grow to form multilayered sheets of epithelial cells. The viable cell sheet was harvested with intact cell-to-cell junctions and extracellular matrix in a transplantable form simply by reducing the temperature of the culture to 20ºC for 30 min. The cell sheet is then transplanted directly to the diseased eye without sutures. (Reprinted with permission from Nishida K et al: Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium, *New Engl J Med* 351:1187–96, copyright 2004 Massachusetts Medical Society. All rights reserved.)

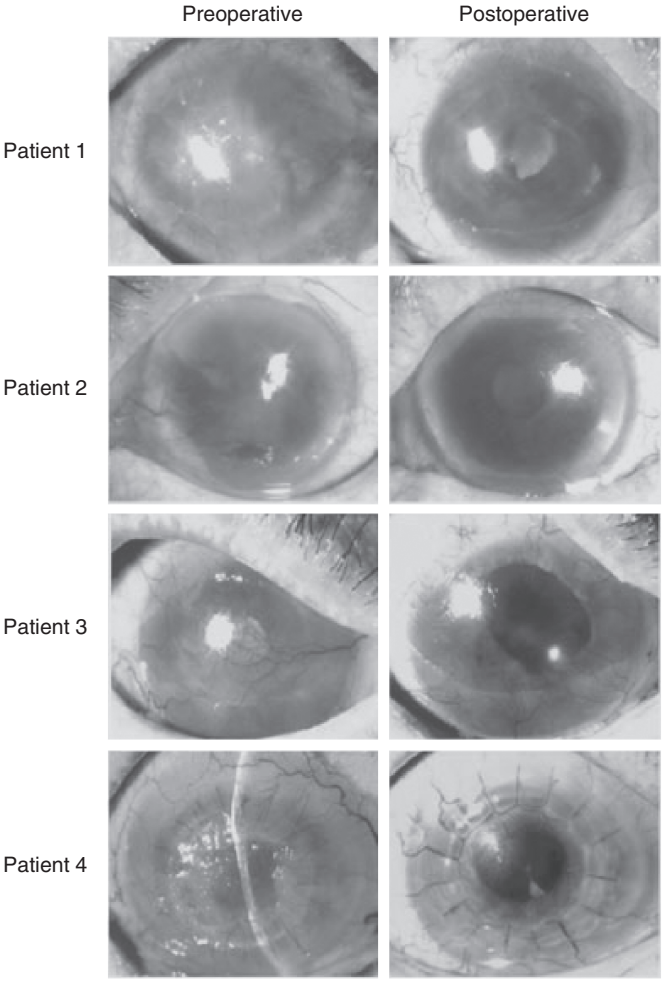
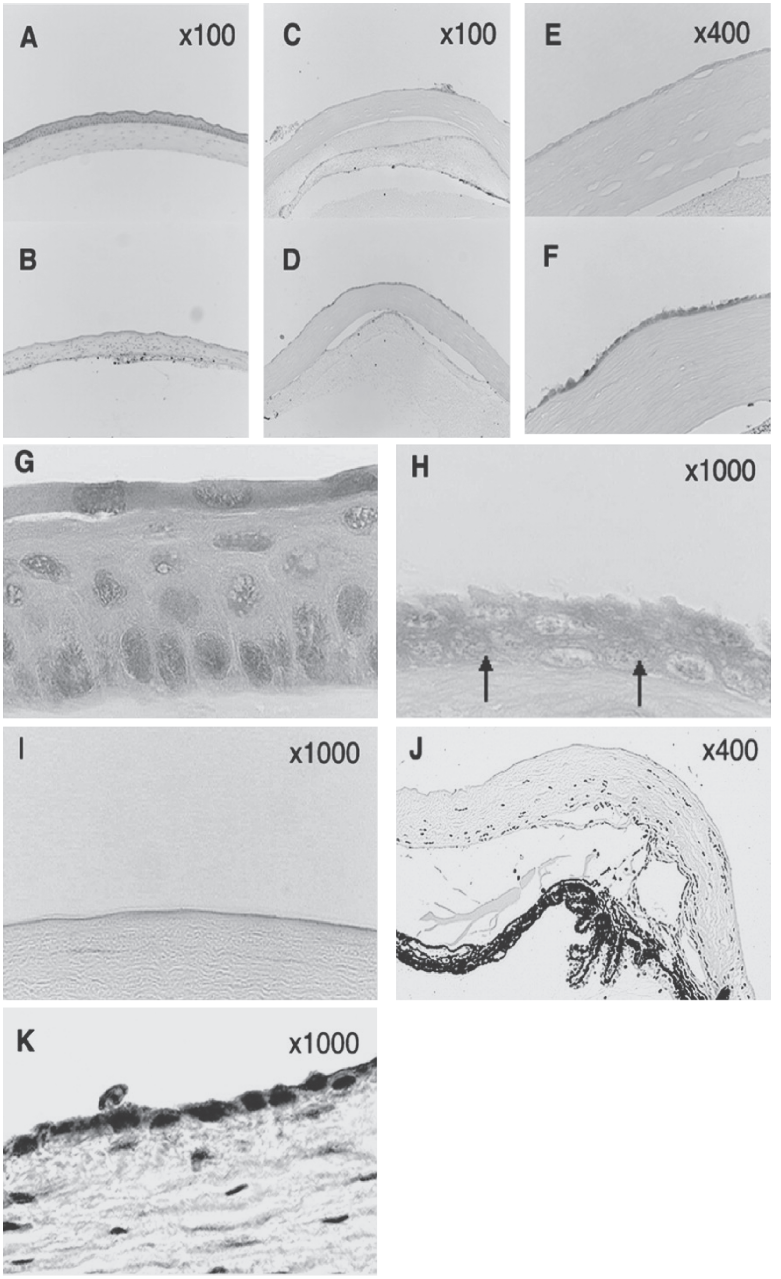


Figure 23.6. Eyes of patients before and after transplantation of sheets of tissue-engineered autologous epithelial cells. These photographs were taken just before transplantation of the cell sheets and postoperatively at 13, 14, or 15 months. (Reprinted with permission from Nishida K et al: Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium, *New Engl J Med* 351:1187–96, copyright 2004 Massachusetts Medical Society. All rights reserved.)

addition to epithelial progenitor cells and mature epithelial cells, embryonic stem cells can also be used for corneal reconstruction. An example is shown in Fig. 23.7.

Corneal Reconstruction [23.7]. When severe opacity exists in the cornea, it is necessary to conduct complete corneal reconstruction. Although allogenic corneal transplantation is an option, there is a shortage of the supply of allogenic corneas. Thus, it is necessary to construct artificial corneal substitutes. Extracellular matrix components, including collagen and proteoglycans, are potential constituents for the construction of artificial corneas. Collagen type I and a type of glycosaminoglycan called chondroitin sulfate can be blended together and molded into a cornea-like structure in vitro. The presence of chondroitin



sulfate enhances the transparency of the corneal construct. However, these matrix components cannot be naturally crosslinked to form a structure with sufficient mechanical strength. It is often required to enhance the mechanical strength by adding crosslinking agents, such as glutaraldehyde and formaldehyde. The constructed matrix scaffold can be used for seeding and culturing corneal epithelial cells, keratocytes, and endothelial cells, forming a functional corneal substitute. Preliminary studies have demonstrated that this

Figure 23.7. Histologic analysis of injured cornea, with or without transplantation of the embryonic stem (ES)-cell-derived epithelial progenitor cells. The ES-cell-derived epithelial progenitor cells (day 8 culture) were transplanted to *n*-heptanol-injured cornea of mice. (A) Normal mouse cornea. (B). *n*-Heptanol-injured cornea without transplantation. (C–F). Mouse eyes were injured with *n*-heptanol. At 1 h (C, E) and 12 h (D, F) after transplantation, the eyes were enucleated. Cryostat sections were fixed with 20% formaldehyde in methanol, stained with H&E, and compared with those of normal cornea. (G) Higher magnification of the normal corneal epithelium shown in (A). (H) Higher magnification of another preparation of the ES-cell-derived epithelial progenitor cells at 12 h after transplantation. *Arrows*: the basal or wing-cell–like transplanted cells. (I) Higher magnification of *n*-heptanol-injured cornea without transplantation 12 hours after the injury. No corneal epithelial cells were observed. (J) Limbus of *n*-heptanol-injured cornea without transplantation 24 h after the injury. Migration of the host-originated progenitor cells onto the corneal surface was not observed. (K) Immunostaining for E-cadherin of the corneal epithelial cells 12 h after transplantation of ES-cell-derived graft cells. E-cadherin-positive epithelial cells are stained red. (Reprinted with permission from Homma R et al: Induction of epithelial progenitors in vitro from mouse embryonic stem cells and application for reconstruction of damaged cornea in mice, *Invest Ophthalmol Vis Sci* 45:4320–6, copyright 2004.)

type of corneal scaffold is suitable for the growth and expansion of corneal cells and can be potentially used for corneal reconstruction. However, matrix structures generated by aldehyde-induced cross-linking are not natural and may exert side effects on the ocular system.

Another approach for the construction of corneal substitutes is to integrate extracellular matrix components into a synthetic polymer material, forming a natural and synthetic copolymer material. Scientists have used hydrated collagen and a polymeric material called poly(*N*-isopropylacrylamide–coacrylic acid–coacryloxysuccinimide) to fabricate a copolymer stromal scaffold. A cornea-like structure can be generated by casting a corneal mold with mixed collagen gel and synthetic polymer. Corneal cells can be seeded and grown on the matrix scaffold to establish a functional corneal substitute. Such a matrix scaffold can also be used for nerve innervation. In addition to collagen and glycosaminoglycans, other biological matrix molecules, such as fibrin and fibronectin, have been used for the construction of corneal substitutes. These investigations have demonstrated that corneal substitutes generated with these materials can be potentially used for the reconstruction of malfunctioned cornea.

Glaucoma

Pathogenesis, Pathology, and Clinical Features [23.8]. *Glaucoma* is a disorder characterized by an increase in the pressure of the intraocular aqueous humor. Under physiological conditions, the aqueous humor maintains a narrow range of pressure about 15 mm Hg. When the outflow of the aqueous humor is obstructed, the intraocular pressure increases, resulting in *glaucoma*. Such a disorder is often induced by the abnormality and occlusion of the trabecular meshwork and the canal of Schlemm. Glaucoma is diagnosed when the aqueous humor pressure is increased over 22 mm Hg. Increased aqueous humor pressure in the anterior compartment is transmitted to the posterior compartment via the vitreous humor, resulting in the compression and impairment of the retina and optic nerves. These alterations exert harmful effects on the retinal neurons, eventually leading to blindness. In the United States, glaucoma is the second leading cause of blindness.

Based of on pathogenic mechanisms, glaucoma can be classified into two types: primary glaucoma due to the obstruction of the trabecular meshwork and secondary glaucoma as a complication of other disorders such as leukemia, rheumatoid arthritis (collagen disorder), infectious diseases (rubella and onchocerciasis), amyloidosis, cancer metastases, asthma, emphysema, renal disorders, administration of corticosteroids, chemical toxicity, Marfan's syndrome, and ocular trauma. The pathogenic mechanisms of the primary obstruction of the trabecular meshwork are not fully understood. Glaucoma may be associated with ocular pain and corneal edema. The diagnosis of glaucoma relies on the measurement of intraocular pressure and visual field tests. In severe cases, partial or complete visual loss may occur.

Conventional Treatment of Glaucoma [23.8]. Strategies for glaucoma treatment are to reduce the resistance of the trabecular meshwork to the outflow of the aqueous humor and reduce the intraocular pressure. There are two conventional approaches that can be used to achieve these goals: administration of pharmacological agents and conduction of laser trabeculoplasty. Several types of agents, including cholinergic agonists and β-adrenergic antagonists, have been used to reduce the resistance of the trabecular meshwork. Cholinergic agonists, such as pilocarpine and carbachol, stimulate the contraction of the circumferential smooth muscle cells of the iris. This action shrinks the pupil, decreases the thickness of the iris, and increases the diameter of the canal of Schlemm, thus reducing the resistance to the outflow of the aqueous humor. A treatment with β-adrenergic antagonists, such as timolol maleate, reduces the formation of aqueous humor, thus lowering the intraocular pressure. When these drugs are ineffective, it is necessary to carry out trabeculoplasty, a laser-based surgical procedure that widens the trabecular meshwork and reduces resistance to the outflow of the aqueous humor. Another surgical intervention is to create a fistula from the anterior chamber to the subconjunctival gap, a procedure known as *filtration surgery.* This approach facilitates the outflow of the aqueous humor.

Molecular Regenerative Engineering. There are several strategies for the molecular treatment of glaucoma. These include facilitation of the aqueous humor outflow through the trabecular meshwork, prevention of scar formation and occlusion of surgically created aqueous humor fistula, and protection of retinal neurons from glaucoma-induced injury and death. A number of genes can be used for these purposes. These genes are discussed as follows.

Facilitation of Aqueous Humor Outflow through the Trabecular Meshwork [23.9]. Primary glaucoma is induced by increased resistance of the trabecular meshwork to the outflow of the aqueous humor. Extracellular matrix components, including proteoglycans, in the trabecular meshwork contribute to the resistance. It has been thought that an increase in the production and a decrease in the degradation of proteoglycans may play a role in the induction of primary glaucoma. Extracellular matrix components are degraded by a class of proteinases, known as matrix metalloproteinases (MMPs). Stromelysins (Table 23.4) are a group of matrix metalloproteinases that degrade proteoglycans, several types of collagen, and other matrix components. This group of proteinases includes three known members: stromelysin 1, 2, and 3. Stromelysin 1 and 2, also known as matrix metalloproteinase 3 and 10, respectively, degrade proteoglycans, collagen types III, IV, V, and IX, fibronectin, and laminin. Stromelysin 3, known as *matrix metalloproteinase* 11, degrades primarily fibronectin and laminin. The transfer of stromelysin genes into the cells of the

TABLE 23.4. Characteristics of Stromelysin Isoforms*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Locus	Expression	Functions
Stromelysin 1	Matrix metalloproteinase-3 (MMP 3), transin, progelatinase	477	54	11q22.3	Skin, connective tissue, heart	Degrading fibronectin, laminin, collagens III, IV, IX, and X, and proteoglycans
Stromelysin 2	Matrix metalloproteinase-10, MMP10	476	54	11q22.3q23	Heart, lung, liver, kidney	Similar to those of stromelysin 1
Stromelysin 3	Matrix metalloproteinase-11 (MMP11)	488	55	22q11.2	Skin, connective tissue	Degrading fibronectin and laminin

*Based on bibliography 23.9.

trabecular meshwork may upregulate the expression of these proteinases, enhance the degradation of extracellular matrix, and reduce the resistance of the trabecular meshwork. Experimental investigations have provided promising results for the use of these proteinases. Thus, the genes encoding stromelysins are candidate genes for the molecular therapy of human primary glaucoma. Another potential gene is the interleukin-1 gene. Interleukin-1 is known to stimulate the expression of trabecular matrix metalloproteinases. The overexpression of the interleukin-1 gene in the cells of the trabecular meshwork has been shown to enhance the outflow of the aqueous humor in experimental models. For gene transfer into the eye, electroporation has been proven an effective method (Fig. 23.8).

Prevention of the Occlusion of Surgically Created Aqueous Humor Fistula [23.10]. Surgical construction of a fistula through the trabecular meshwork is an effective approach for the treatment of glaucoma. However, surgical trauma induces inflammatory reactions, cell proliferation, extracellular matrix production, and scar formation, ultimately leading to the occlusion and failure of the fistula. To resolve such a problem, it is necessary to apply anti-inflammatory and anti-proliferative agents to the fistula. While pharmacological agents have been used for such a purpose, these agents can be degraded rapidly and it is necessary to conduct daily multiple deliveries. The delivery of therapeutic genes to the surgical site can provide long-term effects.

A large number of proteins have been known to participate in the regulation of inflammatory and proliferative activities. While some proteins, such as growth factors, cell cycle regulatory proteins, and mitogenic protein kinases, enhance inflammatory and proliferative activities, others exert an opposite effect. A typical example of negative regulators is the p21 WAF-1/Cip-1 protein (Table 23.5). This protein induces cell cycle arrest, thus suppressing cell proliferation and production of extracellular matrix. The gene encoding p21 WAF-1/Cip-1 has been tested extensively in experimental glaucoma. The transfer of the p21 WAF-1/Cip-1 gene results in a reduction in inflammatory reactions, cell proliferation, and scar formation in the surgical site of ocular fistula. Genes that encode matrix metalloproteinases can also be used to prevent fistula occlusion. As discussed above,

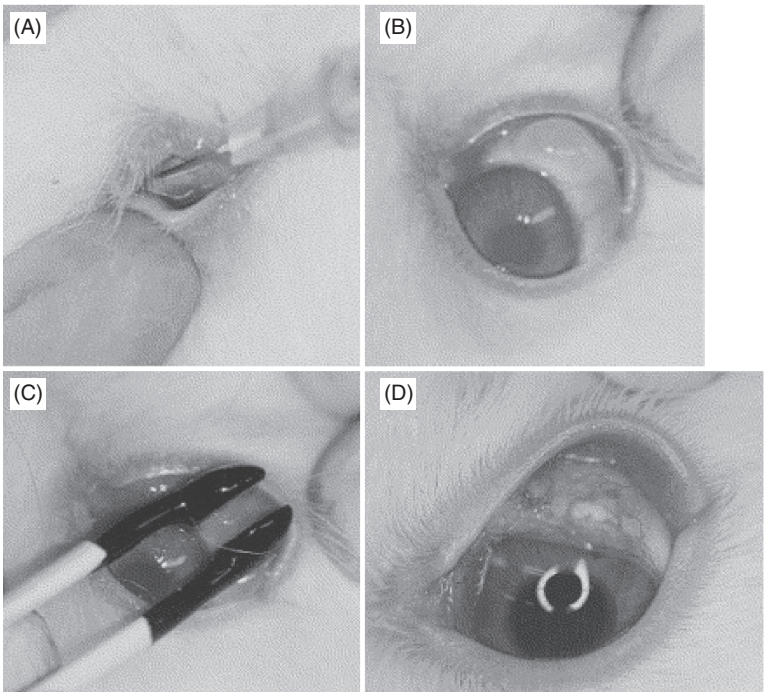


Figure 23.8. Photographic demonstration of surgical procedures of electroporation of MMP-3 cDNA into rabbit conjunctiva followed by trabeculectomy: (A) Injection of 0.1 mL of PBS containing CMV/MMP-3 vector (0.5 mg DNA/mL) into superior conjunctiva with 26G needle; (B) bleb formation; (C) electroporation-mediated transfection using cup-shaped electrodes; (D) 7 days after trabeculectomy, which was performed 3 days after electroporation. (Reprinted from Mamiya K et al: Effects of matrix metalloproteinase-3 gene transfer by electroporation in glaucoma filter surgery, *Exp Eye Res* 79:405–10, copyright 2004, with permission from Elsevier.)

matrix metalloproteinases degrade extracellular matrix, thus reducing the rate of occlusion of surgically established ocular fistula. The stromelysin genes have been used for such a purpose in experimental models.

Protection of Retinal Neurons from Glaucoma-Induced Injury and Death [23.11]. A glaucoma-associated increase in the intraocular pressure, when reaching a certain level, often induces injury and apoptosis of the retinal neurons, a common cause for blindness. A strategy to prevent retinal neuron injury and death is to deliver genes encoding survival factors or antiapoptotic factors to the retina. Most growth factors are known to promote cell survival and their genes can be used for such a purpose. An example of cell survival factors is the brain-derived neurotrophic factor (BDNF). The transfer of the gene encoding this factor into the retina can effectively protect the retinal neurons from apoptosis and prolong the survival of these cells. In addition, genes encode ciliary neurotrophic factor (CNTF) and glial cell line-derived neurotrophic factor (GDNF) can also be used for such a purpose. These genes have been tested in experimental models, demonstrating promising results. Characteristics of several growth factors are listed in Table 23.6.

TABLE 23.5. Characteristics of p21*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
P21 WAF1/ Cip-1	p21, CDK-interaction protein 1 (CIP1), wildtype p53-activated fragment 1 (WAF1), melanoma-differentiation-associated protein 6 (MDA6), DNA synthesis inhibitor, cyclin-dependent kinase inhibitor 1	164	18	Heart, eye, bone marrow, mammary gland, kidney	Binding to and inhibiting the activity of cyclin-CDK2 or cyclin-CDK4 and inducing cell cycle arrest at G1 and G2

*Based on bibliography 23.10.

Cataract

Pathogenesis, Pathology, and Clinical Features [23.12]. A major lens disorder is *cataract*, which is characterized by progressive opacification of the lens. Lens opacity may be found in the center or the peripheral region of the lens. Pathogenic factors that cause cataract include infectious diseases (such as rubella, herpes simplex, and syphilis), ocular trauma, exposure to radiation, chemical toxicity, diabetes-related metabolic disorders, and aging (senile cataract). Senile cataract occurs much earlier in diabetics than in the general population. Long-term administration of corticosteroids enhances the progression of cataract. Cataract is often associated with visual impairment, such as image blurriness and duplication, alterations in color perception, and reduction in visual acuity.

Conventional Treatment of Cataract [23.12]. When cataract is a secondary disorder due to other diseases, such as infectious diseases, metabolic disorders, and diabetes, it is necessary to treat these causative diseases. The alleviation of these diseases prevents or slows down the progression of cataract. When the transparency of the lens is significantly reduced and the visual acuity is severely impaired, surgical removal of the lens is an effective approach for the treatment of cataract.

Molecular Regenerative Engineering [23.13]. The development of cataract is related to cell proliferation in the lens, often induced by diabetes-induced pathogenic alterations or inflammatory reactions in infectious diseases. An important strategy in molecular therapy for cataract is to deliver genes that encode anti-inflammatory and antiproliferative or proapoptotic proteins. There are two approaches that have been used for the treatment of cataract: transferring genes encoding proteins that activate cell mitosis-inhibiting or pro-apoptotic mechanisms and delivering antisense oligonucleotides or siRNA that inhibit the translation of mitogenic mRNAs.

TABLE 23.6. Characteristics of Selected Growth Factors*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Gene Locus	Expression	Functions
Brain-derived neurotrophic factor	BDNF	247	28	11p13	Central nervous system (cortex, retina, and spinal cord), fetal testis	Regulating the survival of neurons and stimulating embryonic development
Ciliary neurotrophic factor	CNTF	200	23	11q12.2	Brain	Promoting neurotransmitter synthesis and neurite outgrowth and regulating the survival of neurons and oligodendrocytes
Glial cell line-derived neurotrophic factor	Astrocyte-derived trophic factor 1, and glial-cell-derived neurotrophic factor	211	24	5p13.1-p12	Nervous system, kidney	Promoting the survival and differentiation of dopaminergic neurons, and preventing the apoptosis of motor neurons

*Based on bibliography 23.11.

For the first approach, the herpes simplex virus-thymidine kinase (HSV-tk) gene is a typical example. This gene encodes a protein kinase that catalyzes the phosphorylation of deoxynucleosides. When a deoxynucleoside analogue such as ganciclovir (an analog of 2′-deoxyguanosine) is present, the analogue can be phosphorylated into a deoxynucleotide, which can be incorporated into the genome of newly formed cells during mitosis. Unlike the natural types of deoxynucleotides, the incorporation of the deoxynucleotide analogues results in the termination of DNA synthesis, an effective process suppressing cell proliferation. Thus the herpes simplex virus-thymidine kinase gene and ganciclovir can be codelivered to the lens cells for the inhibition of cell proliferation. Such an approach prevents or reduces the progression of cataract.

For the second approach, an example is the use of the antisense oligonucleotides specific to the mRNAs of cell cycle regulatory proteins, such as cyclin G1. The selected genes can be injected into the anterior compartment of the eye. Experimental investigations have demonstrated that the transfer of antisense cyclin G1 oligonucleotides into the human lens epithelial cells in vitro induces downregulation of the cyclin G1 gene, an increase in cell apoptosis, and a decrease in cell proliferation. These observations suggest that antisense oligonucleotides to cell cycle regulators or mitogenic factors may be potentially used for the treatment of cataract.

Retinopathy

Pathogenesis, Pathology, and Clinical Features [23.14]. *Retinopathy* is a retinal disorder characterized by retinal arterial stenosis and occlusion, hemorrhage, edema, neuronal injury and apoptosis, reduction in visual acuity, and blindness. Retinopathy occurs due to several diseases, including atherosclerosis in the retinal arteries, systemic hypertension, and diabetes. Atherosclerosis induces stenosis and occlusion of the retinal arteries, leading to injury and death of retinal neurons. Systemic hypertension can induce pathological alterations in the retinal structures, including arteriolar reduction, hemorrhage, retinal edema, and focal ischemia. These alterations influence the function of the retinal neurons, reducing visual acuity. Long-term hypertension may cause severe retinal damage and blindness. Diabetes often induces wall thickening of the retinal arterioles, microaneurysms, hemorrhage, and neovascularization or angiogenesis. These changes result in the injury and death of retinal neurons and thus reduce visual acuity. The ultimate consequence of retinopathy is blindness. Retinopathy is the most common cause of visual loss in the elders.

Another major type of retinal disorder is inherited retinal degeneration. This disorder is characterized by progressive apoptosis of retinal neurons, ultimately leading to blindness. Retinitis pigmentosa is a common form of retinal degeneration. The pathogenic mechanisms of this disorder remain poorly understood. Molecular research has demonstrated that the mutation of several genes may contribute to the development of retinal degeneration. Potential genes include the retinal cyclic GMP phosphodiesterase (PDE) gene, the peripherin 2 gene, and the retinal pigmented epithelium (RPE) 65 gene. In transgenic animal models, the mutation of these genes is associated with retinal degeneration and visual impairment. The retinal cyclic GMP phosphodiesterase gene encodes a protein that regulates phototransduction in the retinal neurons. This protein is composed of catalytic α and β subunits and two inhibitory γ subunits. The mutation of the γ gene results in the loss of the catalytic activity of the α and β units, in association with retinal degeneration similar to that found in human retinitis pigmentosa. The mutation of other subunits also induces retinal degenerative changes. Peripherin 2 is a membrane

glycoprotein that is necessary for the formation of the external segment discs of the retinal photoreceptors. The mutation of the gene encoding this protein results in changes leading to retinal degeneration. In addition, the mutation of the RPE65 gene, encoding a protein participating in the regulation of retinoid metabolism, is associated with retinal degeneration. In general, the mutation of the genes that are involved in regulating the function of the visual system often results in visual impairment.

Conventional Treatment of Retinopathy [23.14]. The principle of treating diabetic retinopathy is to control the primary causative disease: diabetes. The alleviation of diabetes can prevent or reduce significantly the progression of retinopathy. When neovascularization is a factor causing retinopathy, photocoagulation can be carried out to reduce the progression of neovascularization. However, only a small fraction of patients are eligible for this treatment, and about half of treated patients experience recurrence of retinopathy. When the retina is severely impaired, there are few conventional approaches available for the treatment of the disorder.

Molecular Regenerative Engineering [23.15]

Molecular Therapy for Diabetic Retinopathy. Diabetic retinopathy is associated with choroid neovascularization, which reduces visual acuity and causes blindness. Such a process is induced and enhanced by the activation of angiogenic factors. Thus, a strategy in molecular therapy for retinopathy is to prevent or suppress the activity of angiogenic factors. Several genes have been used for such a purpose: the angiostatin, endostatin, and pigment epithelium-derived factor (PEDF) genes. Angiostatin is a 38-kDa fragment of plasminogen (number of amino acids 98–440), which exerts an inhibitory effect on the proliferation of vascular endothelial cells and angiogenesis. An in vivo injection of angiostatin into tumor models induces the suppression of tumor growth and angiogenesis. The angiostatin gene has been prepared and transferred into the subretinal space by using an adeno-associated virus vector in animal models with laser injury-induced neovascularization. Such a procedure induces sustained expression of the gene in the chorioretinal tissue for up to several months, and results in a reduction in the degree of neovascularization. Other factors, including endostatin (a fragment of collagen type XVIII) and pigment epithelium-derived factor, also exert an inhibitory effect on endothelial cell proliferation and angiogenesis in tumor tissues. The genes encoding these factors can be delivered into the subretinal space and used to suppress retinal neovascularization. Several angiogenesis-inhibiting proteins are listed in Table 23.7.

Molecular Therapy for Retinal Degeneration [23.16]. Retinal degeneration is induced by mutation of several genes, including the retinal cyclic GMP (see Table 23.8) phosphodiesterase (PDE), peripherin, and Bcl2 genes. Thus the correction of the mutated genes is a potential approach for the treatment of retinal degeneration. The transfer of the retinal cyclic GMP phosphodiesterase β gene into the subretinal space of animal models of retinal degeneration induces sustained expression of the gene for several months, reduces the rate of retinal apoptosis, promotes the survival of retinal neurons, and enhances the function of the retinal neurons. The transfer of the peripherin and Bcl2 genes into the subretinal space results in similar changes.

Another approach for the treatment of retinal degeneration is to transfect the retinal neurons with cell survival-stimulating genes, such as the nerve growth factor (NGF),

TABLE 23.7. Characteristics of Selected Proteins that Inhibit Angiogenesis*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Plasminogen	Angiostatin, microplasmin	810	91	Liver, kidney, brain	Forming plasmin, a hydrolase capable of converting coagulant fibrin into soluble forms
Collagen type XVIII	COL18A1, endostatin, human type XVIII collagen	1516	154	Liver, kidney, placenta, ovary, heart, skeletal muscle, intestine	Generating a *C*-terminal fragment known as endostatin, which is a potent antiangiogenic factor
Pigment-epithelium-derived factor	PEDF, serine (or cysteine) proteinase inhibitor, serpin peptidase inhibitor	418	46	Retina, cornea, brain, heart, lung, liver, kidney, intestine, ovary, pancreas, prostate gland, bone marrow	Inhibiting angiogenesis and promoting neurite growth

*Based on bibliography 23.15.

TABLE 23.8. Characteristics of Selected Proteins that Regulate the Growth of Retinal Neurons*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Retinal rod photoreceptor cGMP phosphodiesterase β subunit	Rod cGMP-specific 3′,5′-cyclic phosphodiesterase β subunit	854	98	Retina, brain	Promoting the survival of retinal neurons and preventing retinal neuronal apoptosis
Peripherin		470	54	Nervous system, retina, fetus	A type III intermediate filament protein that regulates nerve cell development, activation, growth, and motility

*Based on bibliography 23.16.

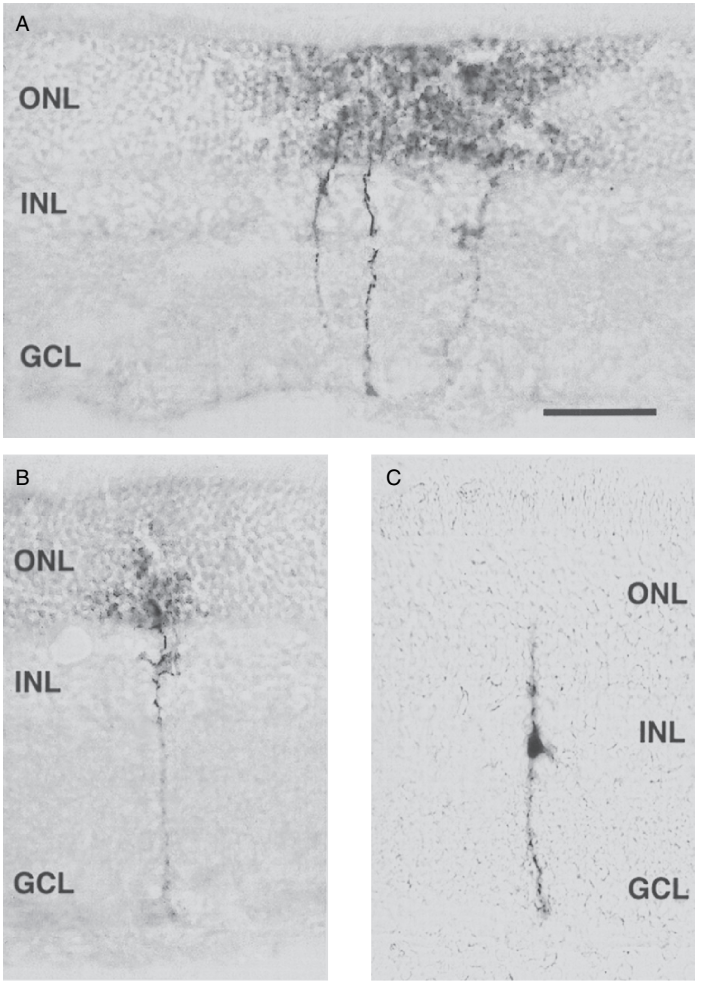


Figure 23.9. Retinal radial cryosections showing typical Müller cell transgene expression at 7 days after intravitreal administration of Ad vectors (5 μL = 10^7 plaque-forming units/mL) in the adult rat eye. (A) A group of Müller cells and (B) a single Müller cell transduced in vivo with Ad.BDNF were visualized with an anti-c-myc antibody. (C) A single Müller cell infected with the control virus Ad.LacZ was visualized by 5-bromo-4-chloro-3-indolyl b-D-galactoside staining. Note the diffuse reaction product at the level of the ONL only in cells exposed to Ad.BDNF. INL: inner nuclear layer, GCL: ganglion cell layer. (Scale bar = 50 μm). (Reprinted with permission from Di Polo A et al: Prolonged delivery of brain-derived neurotrophic factor by adenovirus-infected Muller cells temporarily rescues injured retinal ganglion cells, *Proc Natl Acad Sci USA* 95:3978–83, copyright 1998.)

brain-derived neurotrophic factor (BDNF), and fibroblast growth factor (FGF) genes. The proteins encoded by these genes not only promote cell survival, but also prevent cell apoptosis. Figures 23.9 and 23.10 show the effectiveness of BDNF gene transfer in improving the survival of the retinal ganglion cells.

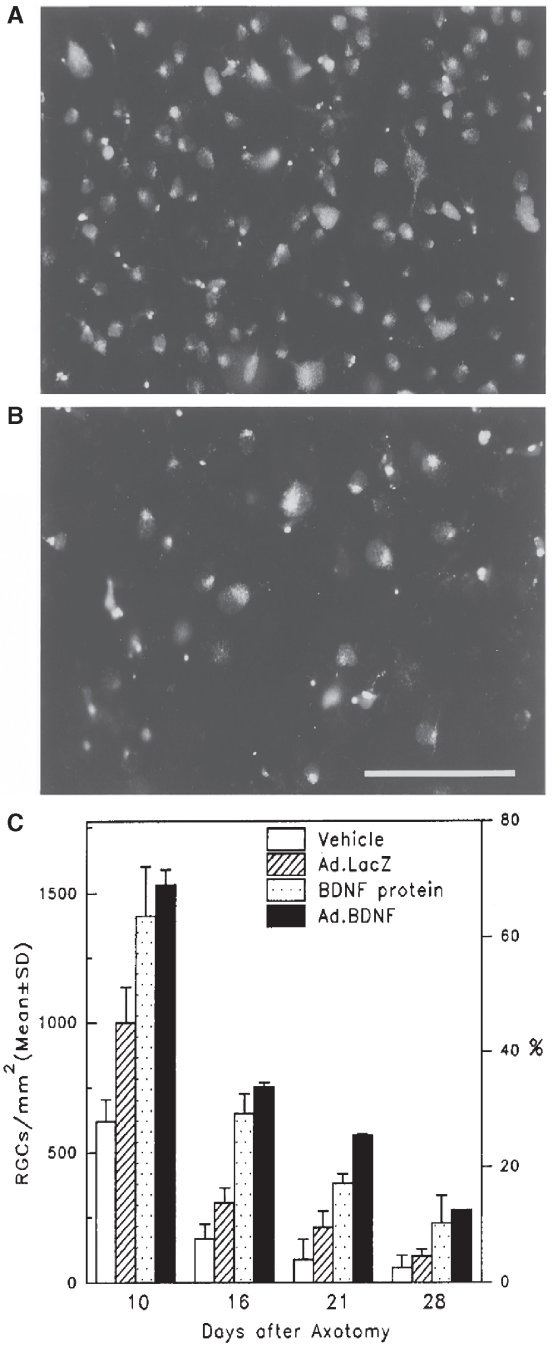


Figure 23.10. Flat-mounted retinas showing Fluorogold-labeled retinal ganglion cells (RGCs) at 10 days after optic nerve transection and intravitreal injection of Ad.BDNF (A) or vehicle (B). Note that BDNF is for brain-derived neurotrophic factor. Scale bar:100 µm. (C) Quantitative analysis of RGC survival in vivo after axotomy and intravitreal administration of 5 µL of Ad.BDNF, recombinant BDNF, Ad.LacZ, or vehicle (n = 3–8 rats per group). At all times examined, significantly greater numbers of RGCs survived in the retinas treated with Ad.BDNF (solid bars) than in the retinas exposed to Ad.LacZ (hatched bars), or vehicle (open bars) (Student's t-test, $P < 0.001$). RGC densities were similar for the groups of retinas treated with Ad.BDNF (solid bars) or recombinant BDNF (stippled bars) but decreased in all groups at longer times after axotomy. (Reprinted with permission from Di Polo A et al: Prolonged delivery of brain-derived neurotrophic factor by adenovirus-infected Muller cells temporarily rescues injured retinal ganglion cells, *Proc Natl Acad Sci USA* 95:3978–83, copyright 1998.)

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