*Review*

# STEM CELL THERAPIES, GENE-BASED THERAPIES, OPTOGENETICS, AND RETINAL PROSTHETICS: CURRENT STATE AND IMPLICATIONS FOR THE FUTURE

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**Purpose:** To review and discuss current innovations and future implications of promising biotechnology and biomedical offerings in the field of retina. We focus on therapies that have already emerged as clinical offerings or are poised to do so.

**Methods:** Literature review and commentary focusing on stem cell therapies, gene-based therapies, optogenetic therapies, and retinal prosthetic devices.

**Results:** The technologies discussed herein are some of the more recent promising biotechnology and biomedical developments within the field of retina. Retinal prosthetic devices and gene-based therapies both have an FDA-approved product for ophthalmology, and many other offerings (including optogenetics) are in the pipeline. Stem cell therapies offer personalized medicine through novel regenerative mechanisms but entail complex ethical and reimbursement challenges.

**Conclusion:** Stem cell therapies, gene-based therapies, optogenetics, and retinal prosthetic devices represent a new era of biotechnological and biomedical progress. These bring new ethical, regulatory, care delivery, and reimbursement challenges. By addressing these issues proactively, we may accelerate delivery of care to patients in a safe, efficient, and value-based manner.

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We explore four scientific innovations that are either currently impacting the clinical care of patients with vitreoretinal disease or are being positioned to make a significant near-term impact. The technologies described herein include 1) stem cell therapies, 2) gene-based therapies, 3) optogenetic therapies, and 4) retinal prosthetic devices. We begin with an introduction of each technology, followed by brief review of products currently available or under investigation for clinical use. As opposed to representing a comprehensive review, companies and studies described were selected to highlight the salient features of each technology. We conclude by discussing how these innovations may impact practical patient care and the health care system at large regarding reimbursement, coverage, and the role of regulatory and societal agencies.

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## Stem Cell Therapy

Cellular regenerative therapies are generating interest for the treatment of age-related macular degeneration (AMD) and other degenerative retinal disorders.



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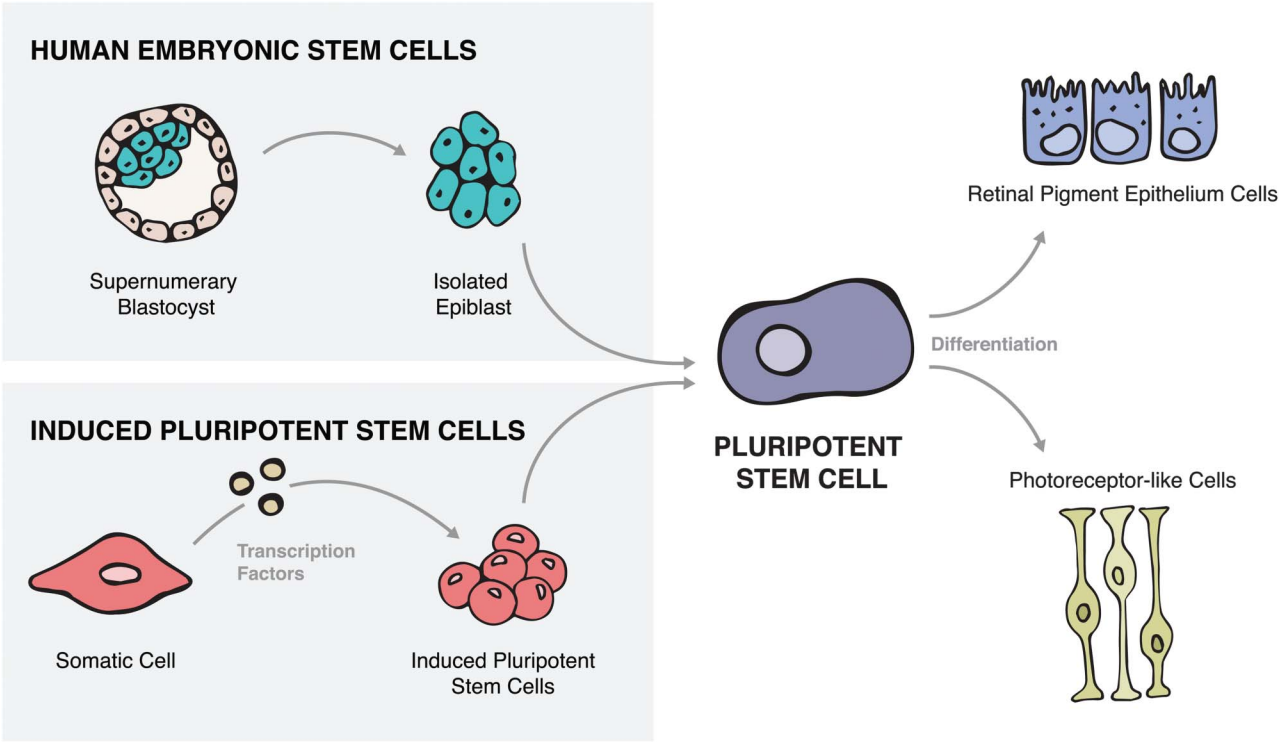


**Fig. 1.** Stem cell therapy. Pluripotent retinal stem cells are most commonly derived from embryonic stem cells and/or iPSCs and may give rise to any assortment of retinal cell types including retinal pigment epithelial cells, photoreceptors, retinal organoids, and others.

Human pluripotent stem cells can produce all the cell types needed for retinal regeneration, and two types that are most commonly used are embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) (Figure 1). Both are defined by an unlimited ability for self-renewal and by the capacity to give rise to any adult cell type.[1,2] Human ESCs are derived from the inner cell mass of a preimplantation embryo produced by in vitro fertilization. Since their discovery in 1998, several human ESC lines have been generated and characterized.[1] The differentiated progeny of ESCs were found to express human leukocyte antigens that can lead to graft rejection after transplantation.[3] Thus, transplantation of human ESC-derived retinal cells currently requires systemic immunosuppression. The creation of a human leukocyte antigen–type bank of ESC lines would potentially allow for selection of a best match for each transplant recipient. However, for ethical and practical reasons, it may be difficult to generate a large and sufficiently diverse collection of human ESC lines to allow for human leukocyte antigens matching.

In 2006, Yamanaka and Takahashi isolated four transcription factors that, when expressed in somatic cells, can induce the formation of pluripotent cells in a process termed reprogramming.[2,4] Induced pluripotent stem cells can provide a source of autologous or allogeneic stem cell lines without ethical concerns poised by human ESCs. Challenges remain in finding the optimum strategy to generate and use iPSCs, as mutations can occur during the extensive culture process required for reprogramming and differentiation.

A third type of pluripotent stem cell can be produced by nuclear transfer. In this technique, the nucleus of an adult somatic cell is transplanted into an enucleated oocyte to produce an ESC genetically identical to the original adult nuclear donor.[4,5] These cells are more difficult to generate than iPSCs; however, this technique has the ability to replace aged or diseased mitochondria with potential clinical applications in mitochondrial-based diseases.

In 2011, a leap forward in retinal differentiation in vitro was made with the discovery that a 3-dimensional (3D) optic cup structure can form autonomously from a 3D culture of ESC aggregates.[6] This discovery has also been replicated with iPSCs,[7,8] and the "organoids" that were produced had the same organized retinal lamination and cell types that are seen in vivo. The photoreceptors within the iPSC-derived 3D retinal tissue achieve advanced maturation, showing outer-segment formation and light reactivity.[8] This technology has advanced the maturation of photoreceptors in vitro and offers the possibility of transplanting sheets of retina.

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A major concern in all cell-based regenerative therapies is the potential for neoplastic changes.[9] Transplanting undifferentiated human ESCs or iPSCs, which may coexist with progeny in a differentiating colony, can result in the formation of a teratoma.[10] Sorting for markers that identify remaining pluripotent stem cells before transplantation can reduce this risk. Highly proliferative cells, such as iPSCs, also require assessment of genomic integrity before differentiation. Differentiated progeny, including iPSC-derived retinal pigment epithelium (RPE) cells and photoreceptors, requires in vivo tumorigenicity testing in animal models before transplantation.

Cell-based regeneration strategies require not only the ability to produce and isolate the desired cell type, but also the ability to deliver the cells successfully to the subretinal space. Two approaches are currently being used for the replacement of the RPE: 1) injection of RPE cell suspension into the subretinal space and 2) transplantation of sheets of RPE. In 2015, Advanced Cell Technology (ACT), later renamed Ocata Therapeutics (Marlborough, MA), and subsequently purchased by Astellas Pharma (Tokyo, Japan), performed several Phase I/II trials to evaluate human ESC-derived RPE cell suspensions delivered subretinally in 18 eyes of 18 patients with advanced nonexudative AMD, Stargardt macular dystrophy, or myopic macular degeneration.[11,12] Systemic immunosuppression with tacrolimus and mycophenolate was used to prevent graft rejection. The RPE cells were injected into the subretinal space at preselected sites in the transition zone between areas of atrophic and healthy RPE. A majority of patients developed subretinal pigmented clusters within the injection areas and corresponding hyperreflective areas lining Bruch membrane on optical coherence tomography, although only one patient had an increase in autofluorescence suggestive of the development of viable RPE. No changes were observed between pretransplantation and posttransplantation visits in static perimetry, electroretinography, visual field, and reading speed. Although this trial was primarily a safety analysis, visual outcome at 12 months in patients with AMD was significantly different for treated eyes (14 letters gained vs. 1 letter, $P > 0.0117$) and trended positive in eyes with Stargardt macular dystrophy. Furthermore, adverse events associated with this surgical procedure included one case of culture-positive postoperative endophthalmitis and another of vitreous inflammation at posttransplant week 3 that resolved with medical management. Systemic immunosuppression was discontinued prematurely for two patients because of adverse events.

The first Phase I clinical trial to use autologous human iPSC-derived RPE cells was conducted by the RIKEN Institute in Japan and presented in 2017.[13] The iPSCs were generated and differentiated into RPE cells from skin fibroblasts of two patients with advanced neovascular AMD. The surgical approach in the first patient included removal of the neovascular membrane and transplantation of the iPSC-derived RPE sheet subretinally. As the transplant was autologous, immunosuppression was not needed. One year after surgery, the transplanted sheet remained intact, and best-corrected visual acuity had not changed. Transplantation was not performed in the second patient because copy number mutations were identified in the iPSCs that were not detected in the patient's original skin fibroblasts. The RIKEN trial's enrollment was halted primarily because of Japan's enactment of their regenerative medicine law in 2014, which limited regenerative medicine clinical studies to medical institutions. The trial investigator subsequently applied for regulatory permission in Japan to use partially matched allogeneic rather than autologous iPSC-derived cells.[14]

A second Phase I RPE sheet transplantation trial in patients with AMD was conducted in the United Kingdom and reported in 2018. The London Project to Cure Blindness trial used a human ESC-derived RPE monolayer on a coated, synthetic membrane.[15] The RPE patch was delivered into the subretinal space, under the fovea, in the affected eye of two patients with neovascular AMD that subsequently developed subretinal hemorrhage. Silicone oil was used for tamponade and removed several months after transplantation. Immunosuppression was achieved with a combination of oral prednisone and the insertion of a fluocinolone acetonide intravitreal implant. Primary endpoints were incidence and severity of adverse events and proportion of subjects with improved best-corrected visual acuity of 15 letters or more. The authors reported retention of the RPE sheet and best-corrected visual acuity gains of 29 and 21 letters in the two patients, respectively. Adverse events included a retinal detachment with proliferative vitreoretinopathy in one of the two eyes in the trial and exposure of the fluocinolone implant suture in the other eye. These required membrane peel with inferior retinectomy and conjunctival revision surgeries, respectively. Systemic immunosuppression was associated with worsening diabetes in one of the two patients, who was treated medically.

This approach has been shown to be successful by Kashani et al,[16] who surgically implanted a monolayer of human embryonic stem cell–derived RPE on an ultrathin, synthetic basement membrane–like substrate into five nonneovascular AMD eyes. All eyes showed changes consistent with integration of the composite implant based with host photoreceptors on optical coherence tomography imaging. The implants

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remained in stable location at 180 days. One eye showed visual acuity improvement (17 letters), two eyes demonstrated improved fixation, and all eyes showed improved anatomical RPE-host photoreceptor integration. Furthermore, in contrast to stable or improved vision in eyes that were implanted, visual acuity in fellow eyes of implanted subjects decreased in three of the five patients. Although the study authors concede that these serve as imperfect controls, this may show stabilization of disease process in this limited sample size. Patients were immunosuppressed with tacrolimus, and no serious adverse events were reported.

Clinical and histological studies suggest that RPE replacement strategies may delay disease progression or restore vision. A prospective, interventional, US Food and Drug Administration (FDA)-cleared, Phase 1/2a study is being conducted to assess the safety and efficacy of a composite subretinal implant in subjects with advanced nonexudative AMD. The composite implant, termed the California Project to Cure Blindness–Retinal Pigment Epithelium 1 (CPCB-RPE1), consists of a polarized monolayer of human embryonic stem cell–derived RPE on an ultrathin, synthetic parylene substrate designed to mimic Bruch membrane. An interim analysis of the Phase 1 cohort consisting of five subjects enrolled in the study showed that four successfully received the composite implant. In all implanted subjects, optical coherence tomography imaging showed changes consistent with human embryonic stem cell–derived RPE and host photoreceptor integration. None of the implanted eyes showed progression of vision loss, 1 eye improved by 17 letters, and 2 eyes demonstrated improved fixation. The concurrent structural and functional findings suggest that CPCB-RPE1 may improve visual function, at least in the short term, in some patients with severe vision loss from advanced nonexudative AMD.

To date, no clinical trials of transplanting pluripotent stem cell–derived photoreceptor cells have been completed, although this will likely change based on the volume and results of preclinical studies. Transplanted photoreceptors, if harvested at the appropriate developmental stage, integrate and form synaptic connections and improved retinal function in rodent models.[17] In degenerative retinal disorders, the stage of the disease, the causative mutation, and the host retinal and subretinal microenvironment all have a profound impact on the survival and function of transplanted photoreceptors.[18] Transplant survival can be improved by modifying the host environment and by using apoptosis inhibitors such as X-linked inhibitor of apoptosis protein (XIAP)[19] and immunosuppression.[19,20]

Transplantation of retinal sheets derived from 3D cultures of ESC aggregates is a promising strategy that may facilitate the survival of transplanted photoreceptors. Takahashi et al transplanted human ESC-derived retinal sheets into the subretinal space of monkey injury–induced retinal degeneration models.[21] Immunosuppression was achieved with cyclosporine. Retinal sheet transplants were monitored in vivo for up to 5 months in 4 eyes of 3 monkeys. Focal ERGs performed over the time course of the study were negative; however, histological analysis performed subsequently demonstrated that the transplanted sheets were beginning to form synaptic connections with the host retinas.

An additional concern when transplanting tissue is rejection. All patients discussed above who received stem cell–derived RPE cells were immunosuppressed to some degree to prevent classical immunologic rejection mediated initially by microglia/macrophages and T cells.[22,23] Although histopathologic rejection may be concrete, there is less consensus on what defines clinical rejection, which may include subretinal fibrosis, vitritis, subretinal fluid, RPE pigmentary changes, transplant encapsulation, etc. The trials discussed herein did not experience a significant incidence of rejection, but this will be considered in further trials with larger enrollment.

Finally, as with any new therapy, we should remain cautious and hold a high degree of responsibility over how potential outcomes are marketed to the public. Although this technology is still in its infancy, several clinics have independently promoted stem cell therapy to patients by injecting adipose-derived "stem cells" into the vitreous. These interventions have resulted in serious complications including ocular hypertension, retinal atrophy, and proliferative vitreoretinopathy–induced retinal detachments leading to severe loss of vision.[24,25] Based on this, the FDA issued a formal letter[26] condemning the lack of good manufacturing practice requirements and highlighting the importance of patient safety in these and all clinics.

Cellular therapy research aims to deliver on the promise of replacing or rescuing dead or dying retina. iPSC technology and 3D retinal organoid production are important developments but require further refinement. The best sources and ages of cells that are used must be identified, and the cells must be sorted, so that the desired types are isolated before transplantation. Delivery techniques can also be improved. Sheet transplantation currently requires retinotomy formation with associated risks of retinal detachment, proliferative vitreoretinopathy, and subretinal fibrosis. Cell suspensions involve risks of cell death during delivery, reflux during injection, and poor cellular

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distribution within the subretinal space. Cell survival and integration after delivery should also be improved, and better local prosurvival and antirejection agents need to be developed.

## Gene-Based Therapies

A genetic mutation often leads to the production of a protein that either exhibits a decrease or absence of function, or takes on a new or detrimental role. This serves as the pathophysiology for a large number of congenital retinal dystrophies that decrease vision and may lead to permanent blindness. Recent advances have produced two therapeutic approaches to target these diseases at a molecular level: gene therapy and gene editing.

Gene therapy refers to the strategy of restoring or augmenting a specific genetic mutation that underlies the pathophysiology of disease. This involves encoding the normal (wild-type) DNA sequence of a target gene into a small and circular molecule called a plasmid that is then packaged into a delivery vector. Often, the vector is a recombinant virus, where the viral DNA is replaced by wild-type DNA. Once delivered into the cell, the plasmid containing the wild-type DNA is expressed, generating normal protein to restore cellular function and address the underlying pathophysiology (Figures 2 and 3). Common indications include inherited retinal dystrophies, but heterogeneous diseases (such as AMD) may also be targeted with approaches such as gene therapy–based anti–vascular endothelial growth factor pharmacotherapy.[27] Gene therapy is best thought of as gene product supplementation and does not impact dominant-negative genetic mutations (which require repression or elimination).[28] Therefore, a major limitation of this method is that it is typically limited to treating autosomal recessively inherited genetic mutations.

This powerful therapeutic method can potentially cure a wide range of devastating genetic diseases ranging from Type 1 diabetes mellitus to cystic fibrosis. The private-sector interest in gene therapy as a pharmaceutical product has grown alongside advancements in basic science research, with the first approved experiments conducted in 1990, the first human trials in 2003, and the first gene therapy product approved by the European Union in 2013 (alipogene tiparvovec; uniQure N.V., Amsterdam, Netherlands).[29]

Ophthalmology is the vanguard of commercial gene therapy development, leading to the way with dramatic innovations in treating Leber congenital amaurosis (LCA). Although LCA displays significant genetic heterogeneity (many different mutations resulting in a similar phenotype), one causative mutation involves the *Rpe65* gene that normally encodes a pivotal protein for using vitamin A derivatives for phototransduction in the visual cycle.[30] Children born with LCA have significant early vision impairment that often progresses to complete vision loss by the third or fourth decade of life.[31] Clinical trials were undertaken by three separate groups with such promising preliminary[32–34] and long-term results[35–39] that the FDA approved voretigene neparvovec-rzyl (Luxturna) on December 19, 2017. This is an adenoviral vector-based treatment for LCA representing the first ocular gene therapy product to market. The developer, Spark Therapeutics (Philadelphia, PA), estimates that approximately 1,000 to 2,000 individuals within the United States would be eligible for this treatment, with potentially 6,000 eligible individuals globally. Although its scope is limited, this is an important development and paves the path forward for other therapies to be commercialized.

A second strategy, gene editing, has been recently shown to have great promise using clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated systems (Cas). Gene editing methods in addition to CRISPR include zinc-finger nucleases and transcription activator–like effector nucleases, which link nonspecific DNA molecular scissors to specific DNA sequence recognizing peptides such as zinc fingers and transcription activator–like effectors. The fundamental principles underlying all gene-editing technologies are 1) the creation of double-stranded DNA (dsDNA) breaks (at specific, predetermined sites) and 2) dsDNA breaks correction with gene correction and/or introduction (Figure 4). Once a specific dsDNA break has been created, the nucleic acid sequence is either repaired randomly by "non-homologous end-joining" (NHEJ), or specifically by "homology-directed repair" (HDR).[27,40]

CRISPRs are a family of DNA sequences originally found in bacteria that protect against viruses by storing a DNA copy of the invading viral sequence and then using it to cleave further invading viruses.[41] CRISPRs interface with CRISPR-associated systems (Cas) to form an RNA-guided protein complex that is designed to cleave dsDNA with very high specificity (Figure 5). A component in the target DNA (protospacer adjacent motif [PAM]) is required for Cas9 to recognize and cleave at the target location. Seminal studies in the last 5 years have expanded our understanding of CRISPR-Cas, allowing us to modify it for use as an important genomic editing tool.[41–44] CRISPR-Cas can be used to knock down expression of specific genes, activate the expression of others, aid in the introduction of

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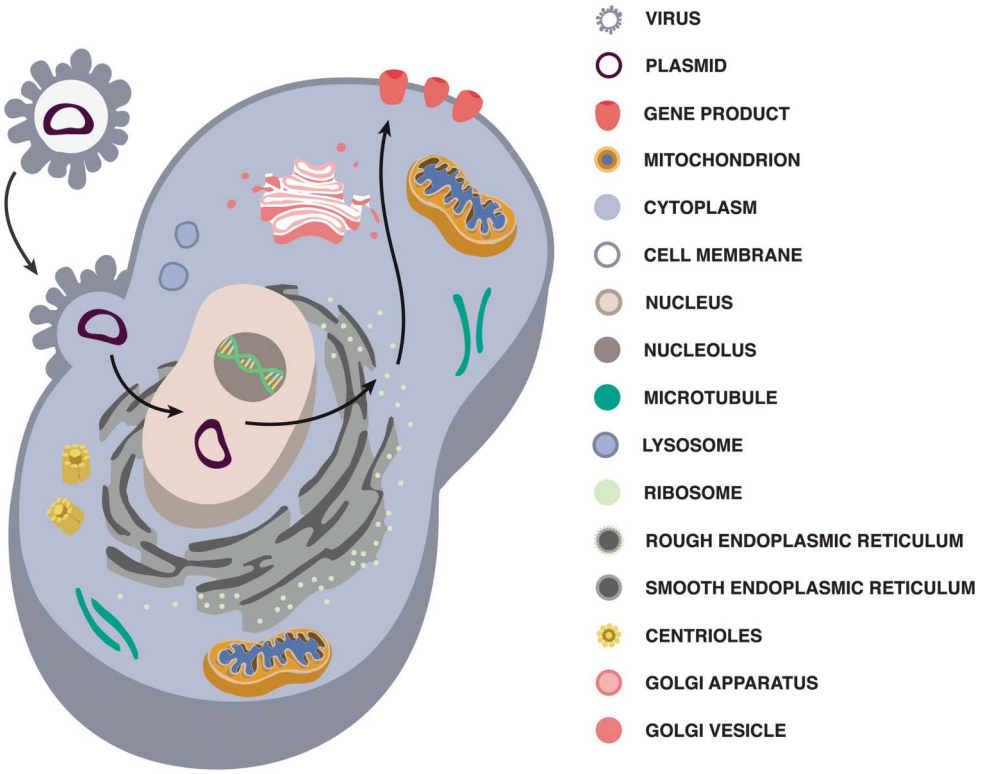


**Fig. 2.** Gene therapy. Nonfunctioning cell containing the mutated gene of interest is transduced with a normal-functioning copy of the gene in the form of a plasmid packaged within a viral vector. After viral transduction, the plasmid is read, and the gene product is shuttled to the site of interest (in this case plasma membrane).

foreign DNA into the genome, and epigenetically modify DNA in vivo.[45]

A major advantage of using CRISPR-Cas instead of a plasmid is the ability to treat autosomal dominantly inherited mutations (with gene elimination/replacement/repression). Similar to gene therapy, recombinant viral vectors are typically used to deliver CRISPR-Cas components to the cells of interest, but there are other methods of delivery including physical techniques (electroporation, hydrodynamic therapy, and microinjection), liposomes and lipoplexes, lipid and gold nanoparticles, cell-penetrating peptides, and

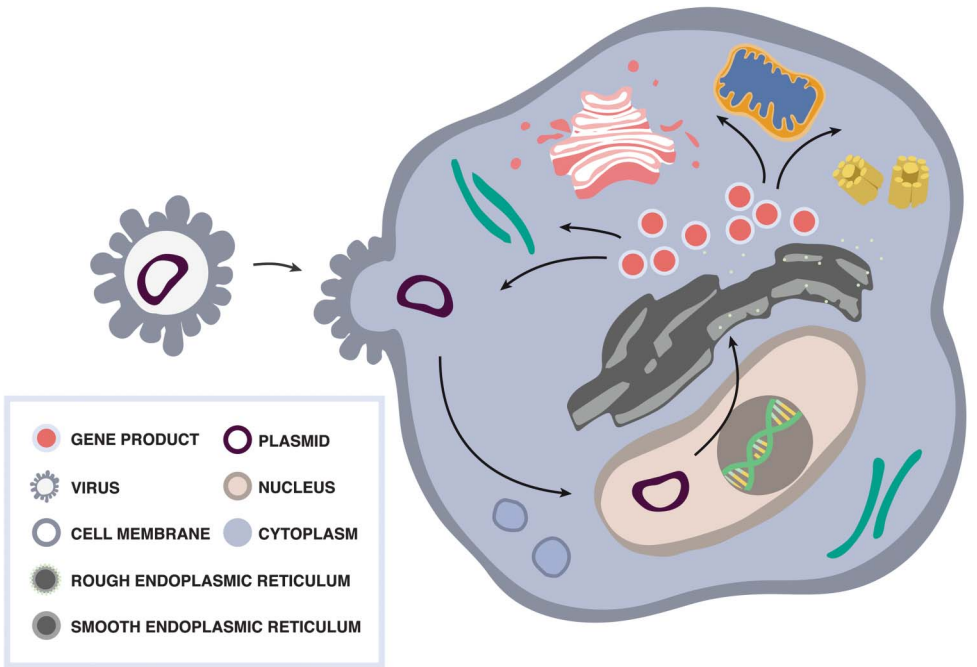


**Fig. 3.** Gene therapy with subcellular targeting. Gene products may also be shuttled to subcellular compartments for specific organelle modulation.

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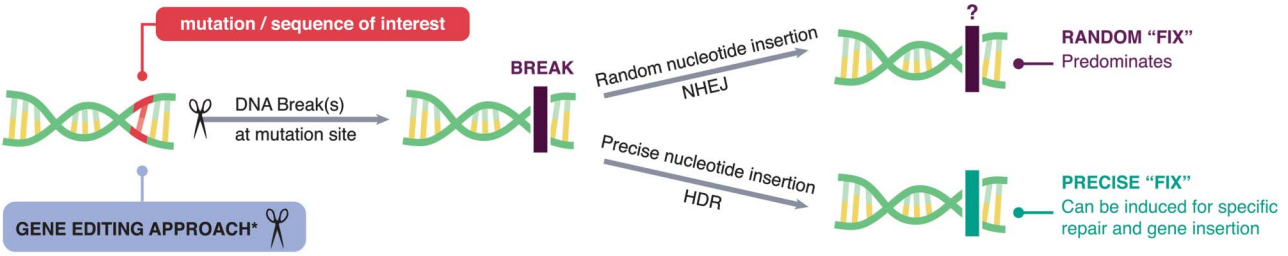


**Fig. 4.** Gene editing approach. It is possible to use a variety of molecular scissors to induce specific dsDNA breaks at sites of DNA mutations. After the break occurs, the nucleic acid sequence is either repaired randomly by "non-homologous end-joining" (NHEJ), or specifically by "homology-directed repair" (HDR).

others.[46] CRISPR-Cas has already been successfully implemented in ophthalmology through ex vivo treatment of iPSCs derived from diseased patients with RP, LCA, and others.[47,48] Furthermore, this technology has been used in vivo to correct a variety of mutations in mouse models for a variety of retinal dystrophies.[47–49] An active area of investigation is using CRISPR-Cas to generate animal models for studying human disease.[50,51] A major concern with CRISPR-Cas is off-targeting effects, which are unintended mutations that arise in remote dsDNA sites[47] typically by aberrant RNA guidance and faulty PAM recognition. Active areas of research include increasing the specificity of CRISPR-Cas and detecting off-target mutations through in silico algorithm predictions, in vitro selection, and genome-wide assays.[47]

Although private-sector interests in gene editing have not progressed as far as gene therapy, it is poised to make an impact. Two companies are at the forefront of CRISPR-Cas–based clinical trials. Editas Medicine (Cambridge, MA) recently announced its intention to file an investigational new drug application later this year for a therapeutic candidate to treat LCA. Furthermore, the company plans to have three early stage clinical trials and two late-stage clinical trials underway by 2022. CRISPR Therapeutics (Zug, Switzerland) may soon receive permission from European regulators to begin clinical trials to treat beta thalassemia, a type of inherited blood disorder characterized by the reduced or absent synthesis of beta chains of hemoglobin that result in significant clinical disease such as anemia. Furthermore, the company intends to file an investigational new drug application with the FDA for the same therapeutic agent to treat sickle cell disease. Although developments in CRISPR-Cas are still in its infancy, there is great potential for this technology to make an impact within ophthalmology.

## Optogenetics

Optogenetics is broadly defined as the creation or inhibition of well-defined events in specific cells using the combination of optical and genetic methods.[52] In the field of ophthalmology, this classically involves inserting light-sensitive proteins into subpopulations of retinal neurons that have no intrinsic light sensitivity. This allows one to jump start the process of phototransduction in the retina when native photoreceptors and/or other crucial retinal neural elements are damaged. Optogenetics thereby represents an additional strategy for the treatment of retinal dystrophic[53] and degenerative diseases.

To achieve this, optogenetics makes use of several scientific tools including 1) light-sensitive proteins, 2) light of sufficient intensity and wavelength for cellular stimulation, and 3) methods (typically viral-based gene therapy) for opsin introduction into cells.

Light-sensitive proteins encompass a broad range of molecules designed to interface with light and produce a principal cellular event. By far, the most studied and applied light-sensitive proteins are the rhodopsins, a large family of proteins consisting of seven transmembrane domains that covalently bind the chromophore retinaldehyde (RAL).[54] Upon the absorption of light, RAL undergoes an isomerization event that enacts a conformational change in the opsin. In microbial or Type 1 opsins, this results in the passage of an ion by either opening a channel or powering a pump. In Type 2 opsins, which includes human rhodopsin and cone opsins in the visual system, this results in the initiation of intracellular G-protein–coupled signaling cascades (Figure 6). Modern optogenetic approaches in the field of neuroscience classically use Type 1 opsins such as channelrhodopsin-2[55,56] to control the depolarization state of a neuron. By directing the passage of ions into and/or out of a neuron with light, this allows scientists to analyze and even direct the functionality of neural networks. Within ophthalmology specifically, the most commonly applied opsins are the channelrhodopsins,[57–59] halorhodopsin,[60,61] human rhodopsin,[62] and melanopsin.[63] Using these opsins, one may choose to target various neuronal subpopulations within the retina (Figure 7).

For example, it is possible to target damaged cones without outer segments but with remaining cone tips

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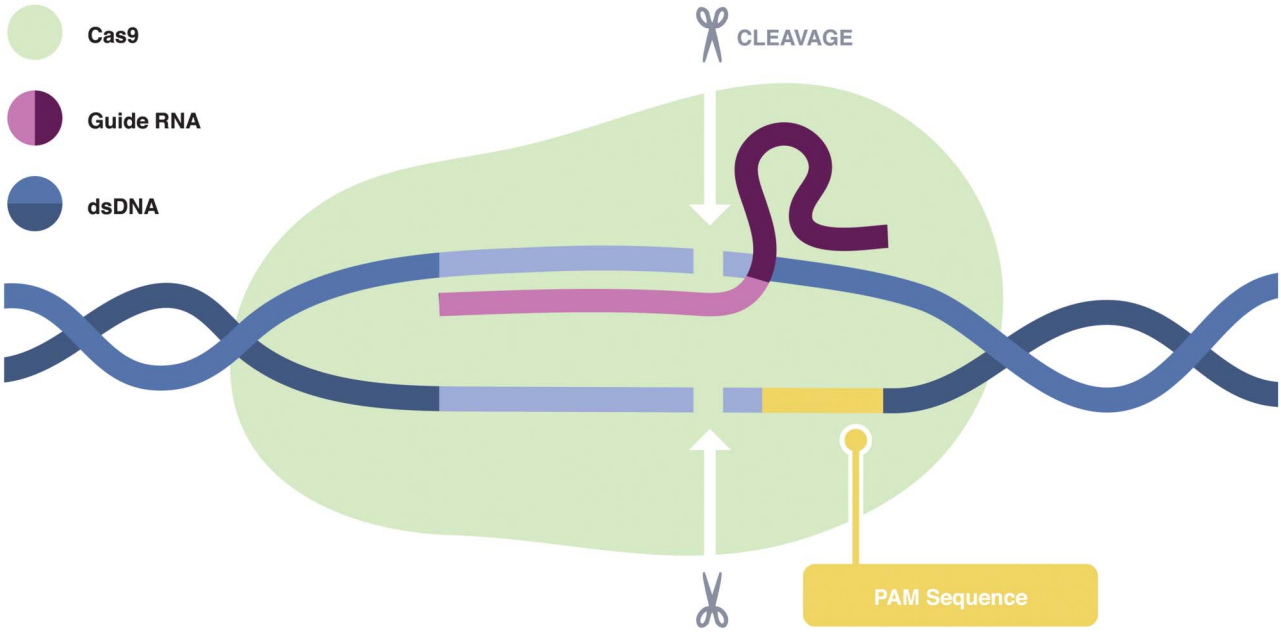


**Fig. 5.** Gene editing with clustered regularly interspaced short palindromic repeats (CRISPR). CRISPRs interface with CRISPR-associated systems (Cas) that act as molecular scissors to cleave-targeted DNA segments with very high specificity. The most commonly used CRISPR-Cas9 system uses a guide RNA. A component in the target DNA (protospacer adjacent motif [PAM]) is required for Cas9 to recognize and cleave at the target location.

by initiating photoreceptor hyperpolarization using halorhodopsin.[60,61,64] This will result in phototransduction by using the remaining cellular architecture. Alternatively, when photoreceptors are damaged, it is possible to apply optogenetics to retinal bipolar cells using ChR[59,65] and human rhodopsin.[62] When more significant retinal damage has occurred, one may target retinal ganglion cells (RGCs) with ChR[66–70] and/or melanopsin.[71] Yet another alternative is to use synthetic molecules that change confirmation under bright light, collectively known as chemical photoswitches.[72] Generally, these synthetic molecules contain light-activated azobenzene moieties. When introduced to a cell, they bind to plasma membrane channels and confer the ability for light activation. The aforementioned approaches are only a subset of the vast work being performed in vivo and in vitro in this area.

Limitations and considerations when applying optogenetics to ophthalmology involve the high level of light typically required to activate microbial opsins.[73] This intense high level of light required may necessitate an external light source, which often has its own drawbacks in terms of phototoxicity and clinical applicability. Further limitations include the partial or complete loss of retinal visual processing when light transduction is initiated by bipolar cells or RGCs as opposed to photoreceptors. This may result in a distorted and unusual visual experience, which may be addressed by predistorting the visual experience as described below. Additional limitations are those implicit to any viral-based gene therapy as discussed above. In the future, opsins may also be applied to subcellular regions to change the physiology of organelles and secondary messenger systems.

Although significant progress is being made with in this field, there are currently five companies with optogenetic product candidates under investigation for human visual restoration: 1) Retrosense therapeutics (Ann Arbor, MI), which was recently purchased by Allergan, 2) GenSight Biologics (Paris, France) in collaboration with Pixium Vision (Paris, France), 3) Applied Genetic Technologies Corporation (AGTC) (Alachua, FL) in collaboration with Bionic Sight, 4) Acucela Inc (Seattle, WA), and 5) LambdaVision (Hartford, CT).

Retrosense Therapeutics has created an AAV-2–based vector encoding the Type 1 opsin channelrhodopsin-2 (ChR2) for RGC transduction (RST-001) through intravitreal injection. Retrosense acquired the patent rights for this technology, developed at both Wayne State and Salus Universities.[74] In 2014, RST-001 received an Orphan Drug Designation by the FDA for the treatment of retinitis pigmentosa (RP) and is currently undergoing a Phase I/IIa, open-label, Dose-Escalation Study of Safety and Tolerability of Uniocular Intravitreal RST-001 in patients with RP (https://clinicaltrials.gov/ct2/show/NCT02556736). In 2016, Retrosense Therapeutics was acquired by Allergan and plans to expand the therapeutic scope of RST-001 to include dry AMD as a follow-on indication.

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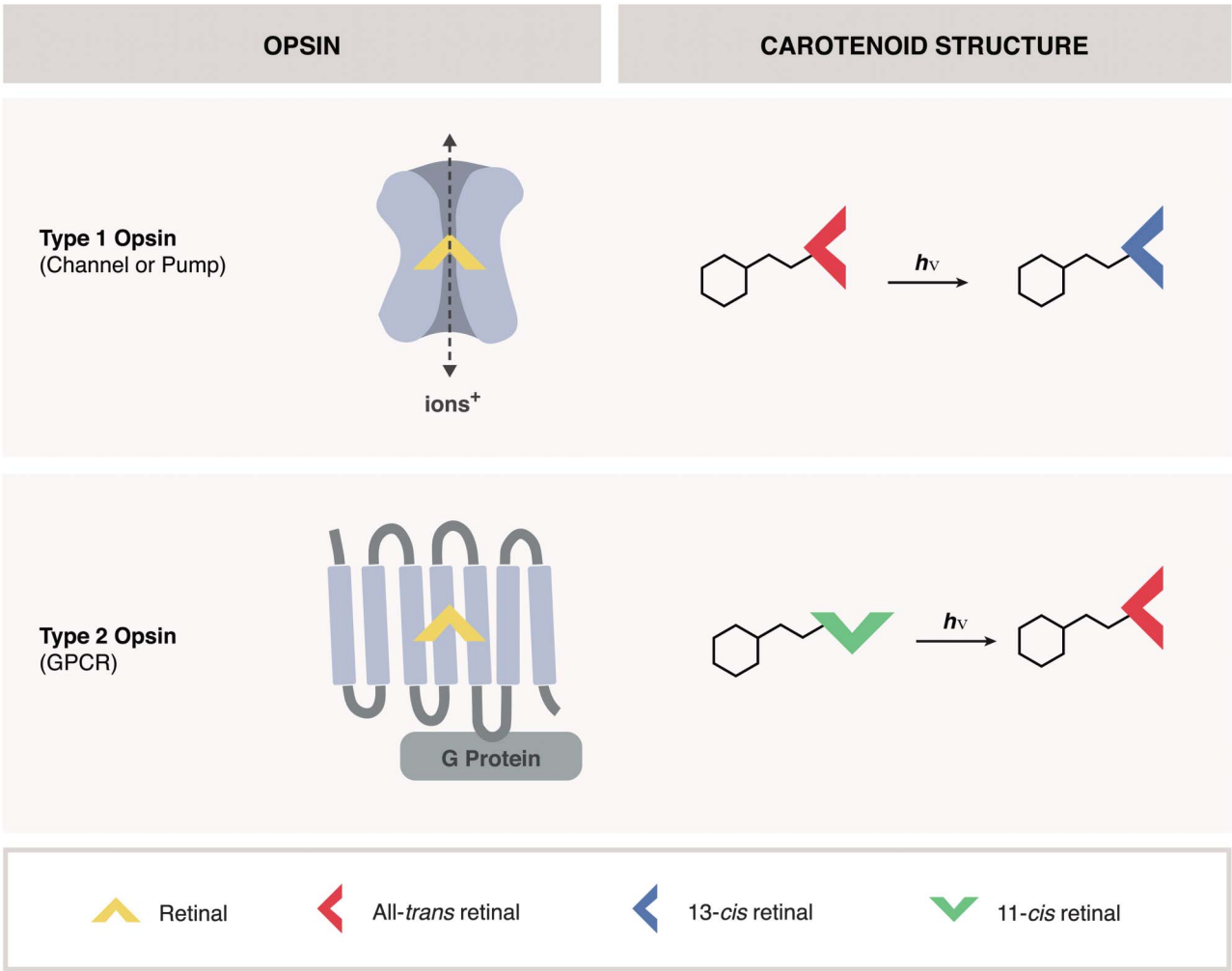


**Fig. 6.** Opsin classification. Type 1 opsins are microbial transmembrane proteins that serve as either channels or pumps to facilitate ion flow across membranes in response to light, whereas Type 2 opsins are transmembrane G-protein–coupled receptors that facilitate intracellular signaling in response to light (such as human rhodopsin).

GenSight biologics has created a therapeutic agent (GS030) comprising a modified AAV-2 viral vector with a peptide on its heparin-binding site[59] (AAV2 7m8). The vector transduces RGCs with a modified ChR called ChrimsonR-tdTomato (ChrR-tdT), derived from the algal light-gated cation channel ChrimsonR (Ed Boyden, MIT).[59] Although there are many ChR variants, ChrR-tdT is a new red-light drivable channelrhodopsin with an absorption spectrum 45 nm more red-shifted than any previous ChR. Red-shifting the opsin absorption spectrum allows for theoretical greater safety (long as opposed to short wavelength light) and decreased remnant photoreceptor cross-talk. Gensight has been able to transduce RGCs efficiently and safely with GS030 in vivo macaque retinas after intravitreal administration.[57] Along with a light stimulation device provided by Pixium Vision, light responses through opsin-transduced RGCs were induced in normal monkey retinas under pharmacological block of endogenous phototransduction, suggesting that the combination of light-stimulating goggles and optogenetics is a viable method for treating retinal dystrophic diseases.

Applied Genetic Technologies Corporation (AGTC) (Alachua, FL) is currently developing an additional combinational optogenetic approach in collaboration with Bionic Sight. By using an AAV delivery system in concert with a neuroprosthetic "retinal code decipherer" developed by Bionic Sight,[75] this group aims to optogenetically endow the retina with opsins and stimulate the transduced cells with patterns of light that are visually meaningful. AGTC is currently seeking investigational new drug approval for this combinational approach, and further details are pending at this time.

Acucela Inc, a Kubota Pharmaceutical company, has also developed an optogenetic approach for the

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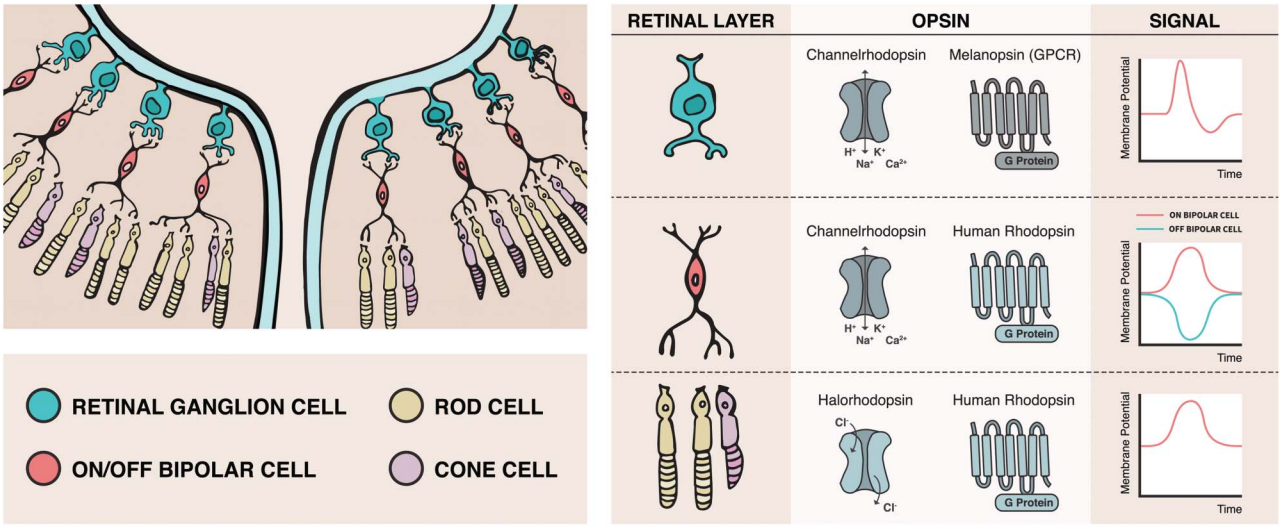


**Fig. 7.** Opsin and neuronal subpopulation of choice. This figure depicts three main retinal cell types targeted for optogenetics (RGCs, bipolar cells, and photoreceptors) along with representative opsins most commonly targeted to these distinct cellular layers.

treatment of RP. Similar to the approaches of Retrosense and Gensight, Acucela will use an AAV-2 viral vector. Unlike the above approaches that use modified ChRs, Acucela's vector will transduce retinal ON bipolar cells with human rhodopsin (hRho).[57] Acucela believes that targeting cells upstream to RGCs will provide superior signal quality and amplification, perhaps with lower light levels, and possibly without the need for external light-emitting devices. Given that rhodopsin is an endogenous protein native to the human retina, there is a theoretically lower risk of immunologic reaction. Acucela is currently in preclinical testing, with estimated proof of concept in 2019.

There are also several groups approaching various forms of optogenetically endowed sheets, polymers, or prosthetics,[76,77] including LambdaVision, Inc, which has developed a protein-based subretinal implant coated with bacteriorhodopsin (a light-activated proton pump). This allows for the creation of an ion gradient that is used to stimulate the bipolar and ganglion cells. The flexible implant consists of multiple layers of oriented bacteriorhodopsin that are between two ion-permeable membranes. Preclinical trials are currently underway.

## Retinal Prosthetic Devices

An additional approach to restoring sight is based on introduction of visual information by patterned electrical stimulation of the remaining inner retinal neurons. In the setting of retinal degeneration, inner retinal neurons and RGCs survive retinal degeneration to a large extent, providing a pathway for reintroducing information into the visual system. Cells can be polarized in an electric field, causing the opening of the voltage-sensitive ion channels on the depolarized side of the cell. This increases the cell potential as a whole and can result in generation of an action potential in spiking neurons.

Retinal implants are usually classified based on their anatomical placement as epiretinal, subretinal, and suprachoroidal (Figure 8). In an epiretinal approach, a stimulating array is placed on top of the retinal surface and typically stimulates RGCs.[78] Epiretinal arrays can be implanted with relative ease and can also be removed in case of postsurgical complications or device failure. The ultimate goal of direct RGC stimulation is to emulate the natural retinal code.[79] Rapid (1–3 ms) response of the RGCs to electrical stimulation with a single action potential enables precise control of the elicited spike sequence. However, because different types of RGCs respond to different aspects of the image (light intensity, direction of motion, etc.), they require different codes. Identification and selective activation of cell types in the diseased retina pose great challenges. In particular, epiretinal electrodes stimulate not only the nearby cells, but also the axons from distant cells passing through the adjacent nerve fiber layer (Figure 8). Consequently, patients may report distorted arcuate visual percepts instead of round localized spots of light.[80]

In a subretinal implant, electrodes are located underneath the inner nuclear layer and replace the degenerated photoreceptors (Figure 8B).[81] Graded responses induced in the inner retinal neurons by electrical stimulation are transmitted through the retinal network to the RGCs, which convert them into trains of action potentials. In this fashion, the retinal signal

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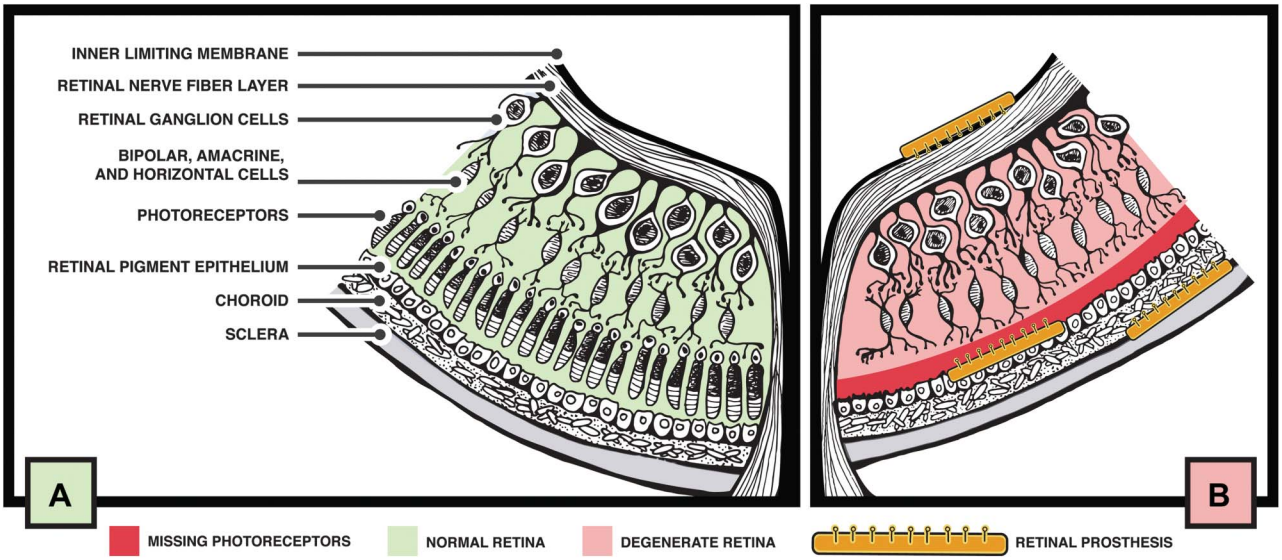


**Fig. 8.** Retinal prosthetic implant placement. **A.** Cross-sectional depiction of a healthy retina. **B.** Cross-sectional depiction of a degenerate retina lacking photoreceptors. Epiretinal implants are placed on top of the nerve fiber layer, whereas subretinal implants replace missing photoreceptors in a degenerate retina and hence are in direct contact with the inner nuclear layer. Suprachoroidal implants are placed between the choroid and the sclera.

process is partially preserved, and encoding of visual information by the subretinal implant is simplified. Preserved features include flicker fusion at high frequencies,[82] antagonistic center-surround organization[83] and nonlinear summation of subunits in receptive fields,[82] as well as ON and OFF responses.[84] Another theoretical advantage of the subretinal placement is that it provides close and stable proximity of electrodes to the target neurons. However, implantation in the subretinal space can be more difficult than the epiretinal approach, and removal of the implant is also more challenging.

With the suprachoroidal approach, the implant is placed between the choroid and the sclera (Figure 8B). Such implants are designed to help with low-resolution peripheral vision,[85] which cannot be easily attained with the other approaches. However, the larger distance between stimulating electrodes and retinal neurons restricts attainable spatial resolution.

A further distinction is the way information and power are delivered to the implant (1–3).

1. In the ARGUS II (Second Sight, Inc, Sylmar, CA), information from a head-mounted camera, as well as power, is transmitted through a radiofrequency (RF) antenna to the implant.[78] The signals are decoded and processed inside the extraocular implant before being delivered to the 6 × 10 stimulating electrode array through a *trans*-scleral cable. A flexible foil with an array of 200-$\mu$m diameter electrodes spaced by 575 $\mu$m is attached to the epiretinal surface with a *trans*-retinal tack. The Argus II has now been implanted in more than 200 patients, with a best reported grating visual acuity of 20/1,260.[86] Despite this level of resolution, the device does tend to improve the spatial mobility of the patient. Lack of connection between the visual information that is transmitted to the implant and eye movements may create a perceptual mismatch because the brain expects the images to shift on the retina accordingly. In principle, this effect can be rectified using eye tracking to control the shift of the image transmitted from the camera to the implant. Suprachoroidal RF-powered devices developed and implanted by Bionic Vision Australia (3 patients[85]) and by Osaka University (2 patients[87]) have extremely large electrode spacing (2 mm), so that the equivalent visual acuity was in the range of 20/4,000 to 20/20,000.
2. In Alpha AMS (Retina Implant AG, Reutlingen, Germany),[81] a subretinal camera, consisting of 1,600 pixels of 72 $\mu$m in size,[88] converts naturally projected images on the retina into electrical currents that stimulate neurons within the inner nuclear layer. Power is delivered to the implant through a *trans*-scleral cable connected to an RF power receiver positioned behind the ear similar to cochlear implants. Optical transmission of the images retains the natural coupling of the visual information to eye movements. Typically, visual acuity with this device is about 20/1,200; however, a few patients have demonstrated visual acuity up to 20/550.[89]
3. In the PRIMA system (Pixium Vision), images captured by the camera are projected onto the retina

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from augmented-reality goggles using pulsed near-infrared (~880 nm) light. Wireless photovoltaic subretinal prosthesis directly converts light into pulsed electric current in each pixel, stimulating the nearby neurons.[82,89] Preclinical studies with 70-$\mu$m and 55-$\mu$m pixels demonstrated grating visual acuity matching the pixel pitch.[82,90] Clinical study has begun in 2018 with subretinal implantation of a 2-mm wide 30-$\mu$m thin chip in patients with geographic atrophy. Measurements of the prosthetic visual function with 100-$\mu$m pixels are now in progress. In future versions of the implant, the pixel pitch is expected to decrease to below 50 $\mu$m to potentially provide visual acuity above the threshold of legal blindness (20/200).

Clinically tested retinal implants represent an important proof of concept that sight can be restored even after decades of profound blindness because of retinal degeneration, albeit currently with rather low resolution. Significant research efforts are under way to increase the number of pixels in implants to thousands, to improve the localization of electric stimulation for high-resolution interfaces, and to better encode neural activity. Continuous progress in 3-dimensional electroneural interfaces, novel materials, and image processing will help advance the field of prosthetic vision toward functional restoration of sight in patients suffering from retinal degeneration.

## Discussion

Although the aforementioned technologies have been frequently labeled as buzzwords in medicine and ophthalmology as a whole, the field of retina is making tangible advancements in using them in clinical practice. For example, gene-based therapies and retinal prosthetic devices have both found use in properly selected, albeit thus far limited, patient populations. However, when compared with the development of small molecules and macromolecules by large-scale biopharmaceutical companies, the technologies discussed herein present unique challenges to the health care ecosystem in regard to regulation, reimbursement, and realization of value.[91] Despite this, the future is promising. The reimbursement road is complex, and accommodating such seminal technologies may require new payment models. Hopefully, economies of scale will eventually reduce the cost burden.

In addition to the scientific and clinical challenges presented by stem cell therapy, effective solutions in the emergent field of stem cell therapy for the retina must address unique regulatory challenges. First of all, the structure, composition, potency, and purity of cell-based therapies are complex and difficult to measure. In addition, many cell-based therapies are developed by smaller pharmaceutical companies that often do not have the resources or scope to perform large, controlled, and appropriately powered clinical trials.[91] As opposed to drugs, stem cell–based therapies may also continue to reside in the patient in perpetuity. Finally, close regulatory oversight is necessary to prevent patient harm and ensure only proven therapies are offered.

To address these regulatory challenges, the FDA Commissioner Scott Gottlieb, MD, released a comprehensive new policy to facilitate the development of innovative regenerative medicine products on November 17, 2018. This policy by the FDA's Center for Biologics Evaluation and Research (CBER) (https://www.fda.gov/BiologicsBloodVaccines/CellularGeneTherapyProducts/ucm537670.htm) attempts to strike a balance between enhancing the approval of promising technologies while simultaneously limiting the involvement of unscrupulous individuals or companies preying on desperate patients. Essentially, the regenerative medicine advanced therapy (RMAT) designation provides all the benefits of fast-track and breakthrough therapy designations, with the additional bonus of early interactions to guide the creation and satisfaction of intermediate and surrogate endpoints. So far, no offering has passed through the FDA with the RMAT designation,[92] but the CBER (which also manages gene-based therapies) has approved 16 therapies to date.[93] Although several offerings are in the pipeline as discussed above, there is currently no FDA-approved stem cell–based therapy for ophthalmology.

Reimbursement for stem cells will also be complicated because such treatments may potentially recover and prevent significant cost over a patient's lifetime despite possibly being a one-time treatment. Consider the analogy to sustained-release drugs. As part of complicated lifecycle management plans, pharmaceutical companies frequently seek 505(b)(2) approval for drugs to extend market exclusivity by making relatively modest changes to already approved drugs. One way of doing so is making the drug into an extended release formulation. By seeking to recoup the value of sustained-release medications that require less-frequent dosing, those medications frequently end up becoming more expensive. By extrapolating this model to stem cell-based therapies, it is easy to see how companies may seek to recoup the lifetime value of these offerings by pricing them accordingly highly. However, the technology for creating effective cell-based therapies is becoming less expensive as

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competition rapidly increases, and thus, we expect the price for cell-based therapies to decrease in the future as well.

Many consider Japan to be leading the international biotechnology market in not only regulatory efforts for regenerative medicine (with the Pharmaceuticals and Medical Devices Act [PMD Act], the Act on the Safety of Regenerative Medicine, and the "Sakigake Package" of policy changes, which accelerates the introduction of innovative medical products), but also in reimbursement for biotech companies creating cell-based therapies. For example, regenerative medicine offerings in Japan may undergo a rapid (2–3 years) route to conditional approval with immediate reimbursement.[94] This requires an initial study to demonstrate safety with indication for efficacy, with long-term approval based on further follow-up studies. Foreign companies who perform regenerative medicine research in Japan are also entitled to grants from the Japanese Ministry of Trade, Economy, and Industry (METI), and support from local governmental grants. Japan also contains the world's most rapidly aging population. Although the FDA's RMAT designation does make the United States more competitive on a global scale than it would have been otherwise, many reimbursement questions for companies seeking to develop regenerative medicine products remain unanswered.

Regarding gene-based therapies, the voretigene neparvovec-rzyl (Luxturna) approval was expedited by several designations such as with priority review, orphan drug, and breakthrough therapy.[95] Now approved, Luxturna is currently the most expensive "drug" ever introduced in the United States, at a total cost of $850,000 to treat both eyes. Although its cost is high, it is interesting to note that the physician reimbursement for this time-consuming procedure is not modified or unique. Insurance companies (depending on the plan) may cover a significant amount of the expense for the patient, but there is very likely a large sum that reduces one's deductible or out-of-pocket maximum. The Centers for Medicare and Medicaid Services (CMS) will provide a large share of reimbursement and will set the example for how current and future gene-based therapies will be reimbursed. Spark Therapeutics is working on several solutions to this problem[96] for Luxturna including 1) rebate programs based on effectiveness at set time intervals, 2) working with CMS on eliminating the base Medicaid drug rebate, and 3) allowing for installment payment plan reimbursement. They are also working directly with commercial payers and specialty pharmacies to negotiate payment while leaving those parties to separately negotiate with treatment centers for reimbursement. Large pharmaceutical companies are surely monitoring the negotiations of new payment models by this relatively small company with a first-of-its-kind commercial product and will likely base their strategies on its success or failure. In addition, the regulatory and reimbursement issues for optogenetic therapies will most likely parallel those of gene-based therapies at large.

The only currently FDA-approved retinal prosthetic device is the Argus II Retinal Prostheses (which also carries a CE mark). Despite its relatively limited patient application when compared with drugs (implantation in more than 200 patients[86] to date, including 75 implantations in 2017[97]), the Argus II is a technological tour de force. The device is reliable and stable, with no reported device failures within 3 years after implantation[86] (a total of 88.2 subject-years). The device and company paved the way forward not only scientifically and regarding the clinical application of retinal prosthetic devices, but also from a regulatory and reimbursement perspective. The insertion of the device is associated with its own current procedural terminology code, with further codes available for device tuning and maintenance. Second sight is currently enrolling patients in a clinical trial for the implantation of a cortical visual prosthetic device called Orion I (NCT03344848), which may serve as another groundbreaking advancement in our field.

## Conclusion

Stem cell therapies, gene-based therapies, optogenetics, and retinal prosthetic devices represent a new era of biotechnological and biomedical progress spearheaded by the field of vitreoretinal surgery. This significant progress is accompanied by new ethical, care delivery, and reimbursement challenges that are necessary to address to ensure delivery of care to patients in a safe, efficient, and value-based manner. The future of care for vitreoretinal patients is promising, and vitreoretinal specialists have the unique privilege of developing and delivering this future.

**Key words:** innovation, stem cells, optogenetics, gene therapy, CRISPR, gene editing, retinal prosthetics, future, biotechnology.

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