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Regenerative Nanomedicine for Vision Restoration

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Abstract

Herein, we discuss recent applications of nanotechnology to ophthalmology, including nanoparticles for drug, gene, and trophic factor delivery; regenerative medicine (in the areas of optogenetics and optic nerve regeneration); and diagnostics (eg, minimally invasive biometric monitoring). Specific applications for the management of choroidal neovascularization, retinal neovascularization, oxidative damage, optic nerve damage, and retinal degenerative disease are considered. Nanotechnology will play an important role in early- and late-stage interventions in the management of blinding diseases.

Nanotechnology involves the creation and use of materials and devices at the size scale of intracellular structures and molecules. The systems and constructs deployed typically are on the order of less than 100 nm. Novel nanosized materials and chemical assemblies, chip-based technologies, and miniaturized devices already provide novel tools that impinge directly on ophthalmology.[1-3] We have reviewed applications of nanotechnology to vision restoration previously,[4-8] and herein we provide an update on recent innovations concerning specific and potential applications of nanotechnology to ophthalmology, including nanoparticles for drug, gene, and trophic factor delivery; regenerative medicine (in the areas of optogenetics and optic nerve regeneration); and diagnostics, (eg, minimally invasive intraocular pressure [IOP] monitoring).

NANOPARTICLES FOR DRUG, GENE, AND TROPHIC FACTOR DELIVERY

Loss of oxygen or its electrons alters the oxidation state of cerium oxide nanoparticles ("nanoceria") and creates defects in their lattice structure. As their size decreases, nanoceria

(3-5 nm in diameter) exhibit more oxygen vacancies in their crystal structure, which can allow them to function as antioxidants. Chen et al[9] found that intravitreal injection of nanoceria prevents light-induced photoreceptor damage in rodents, even if injected after the initiation of light damage. Vacancy-engineered nanoceria also inhibit the development of and promote the regression of pathologic retinal neovascularization in the *Vldlr* knockout mouse, which carries a loss-of-function sequence variation in the very low-density lipoprotein receptor gene and whose phenotype resembles a clinical entity known as *retinal angiomatous proliferation*.[10] Regression occurs even if intravitreal nanoceria treatment is administered after the mutant retinal phenotypes are established. A single injection has a prolonged effect (weeks) because nanoceria are a catalytic and a regenerative antioxidant. Cai et al[11] found that a single intravitreal injection of nanoceria can maintain protective effects on the retina for at least 6 weeks in tubby mice. Tubby mice exhibit rapid photoreceptor degeneration starting on day 14, with undetectable retinal function at 2 months of age secondary to a splice site sequence variation in the *Tub* gene. Tubby mice also exhibit cochlear degeneration, obesity, insulin resistance, and decreased fertility, a constellation of symptoms resembling Usher syndrome. After day 7, tubby mice were intravitreally injected with 172 ng of nanoceria, and retinal structure and function were preserved and only began to decline after the mice reached day 49. Nanoceria inhibit the development of increased vascular endothelial growth factor (VEGF) levels in this model and also up-regulate the expression of genes associated with antioxidant defense and modulated survival vs apoptosis pathways.[12]

Nonviral gene vectors (eg, polymers and lipids) offer low risk of immunogenicity, relatively low cost, and, possibly, greater ease of production compared with viral vectors. In addition, nonviral DNA nanoparticles can accommodate large genes, unlike traditional viral vectors. For example, although gene replacement is a logical strategy to treat autosomal recessive Stargardt disease, which is associated with sequence variations in the photoreceptor-specific flippase ABCA4, the large size of the ABCA4 complementary DNA (6.8 kbp) has hampered progress in the development of genetic treatment. To circumvent the size limitation, Han et al[13] used nanoparticles to subretinally deliver ABCA4 to AbCA4-deficient mice. Afterward, they identified persistent ABCA4 transgene expression for up to 8 months after injection and found marked correction of structure and function, such as reduced lipofuscin accumulation and improved dark adaptation. Polyplexes, DNA nanoparticles that are complexes of cationic polymers (eg, pegylated polylysine) and DNA, have also been used to restore retinal function in the rds$^{+/-}$ mouse,[14] which has sequence variation in peripherin, a photoreceptor-specific glycoprotein that is critical for outer segment disc assembly, outer segment orientation, and photoreceptor structural stability. More than 80 different sequence variations in the *RDS* gene have been identified in humans, and they are associated with multiple retinal diseases, including autosomal dominant retinitis pigmentosa and progressive macular degeneration. The retina in the rds$^{+/-}$ mouse exhibits a classic, well-defined autosomal dominant retinitis pigmentosa phenotype characterized by early-onset rod degeneration and late-onset cone degeneration.

By choosing promoters that are cell specific, one can establish additional specificity in the locus of gene expression. Thus, one can target gene expression to retinal pigment epithelium (RPE) cells, rods, cones, or rods and cones, depending on which promoter is used. Koirala et al[15] took advantage of this principle by documenting that a plasmid, with enhanced green fluorescent protein driven by the RPE-specific promoter VMD2, could be incorporated into nanoparticles and subretinally delivered into mice. Afterward, expression was detected only in the RPE, without change in retinal architecture or function. Notably, mice that had the plasmid delivered via nanoparticles exhibited expression throughout the RPE, whereas mice injected with free DNA exhibited expression only at the site of injection. Furthermore, expression was greater in nanoparticle vs free DNA delivery at all time points assessed, consistent with sustained cytosolic plasmid delivery via nanoparticles.

Liposomes are nanoparticles that can carry hydrophobic or hydrophilic cargo. Liposomes can be coated with ligands that direct them to specific cell surface receptors for cell targeting as well as with polymers that prolong their half-life in the circulatory system. Polyethylene glycol (PEG) can be conjugated with different molecules to enhance their solubility and stability in plasma and to reduce immunogenicity.

Subretinal PEG-substituted lysine peptide nanoparticles containing DNA do not cause tissue damage or inflammation greater than what is observed in normal saline injections.[16] Infiltration of neutrophils and lymphocytes has not been detected in injected retinae, and no elevations in tumor necrosis factor messenger RNA or protein have been detected after treatment.[17]

Liposomes have been used to improve cellular uptake of small interfering RNA (siRNA) by integrin receptor—mediated endocytosis owing to the intrinsic difficulty that free siRNA has in entering cells.[18] Chen et al[19] created a novel functional liposome consisting of arginine-glycine-aspartate motif peptides conjugated to 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide (polyethyleneglycol)-2000] to enhance the uptake of encapsulated siRNA in RPE cells. These investigators found that arginine-glycine-aspartate—PEGylated liposomes had a 4-fold increase in siRNA delivery to RPE cells compared with PEGylated liposomes.

Gene therapy by targeted nanoparticles via intravenous injection

- Integrin-binding linear RGD peptide
- PLGA nanoparticle
- Flt23K intracepter plasmid

FIGURE 1. A single intravenous injection of targeted, biodegradable nanoparticles delivering a recombinant Flt23k intraceptor plasmid homes to neovascular lesions in the retina and regresses choroidal new vessels in primate and murine age-related macular degeneration models. Moreover, this treatment suppressed subretinal fibrosis, which is not addressed by currently approved clinical therapies. Murine vision, as tested by OptoMotry (CerebralMechanics Inc), significantly improved, with nearly 40% restoration of visual loss induced by choroidal new vessels. These findings offer a nanoparticle-based platform for targeted, vitreous-sparing, extended-release, nonviral gene therapy. PLGA = poly(lactic-co-glycolic acid); RGD = arginine-glycine-aspartate. From *ACS Nano*,[22] with permission.

Similarly, Suen and Chau[20] identified increased receptor-mediated endocytosis uptake of novel folate-decorated PEG-b-polycaprolactone nanoparticles compared with standard nanoparticles. In vitro, the folate-decorated nanoparticles were found to deliver triamcinolone steadily to ARPE-19 cells in tissue culture, which resulted in decreased levels of VEGF and increased levels of pigment epithelium—derived factor (PEDF) over 3 weeks.[20] Of note, the nanoparticles containing triamcinolone resulted in less cytotoxicity compared with direct administration of triamcinolone alone. Pharmacologic blockade of VEGF plays a critical role in treating choroidal neovascularization, which is the major blinding complication of age-related macular degeneration.[21]

Luo et al[22] developed an intracellular antiangiogenic therapy relying on a 3-component system: (1) plasmids expressing Flt23k intraceptors that consist of the VEGF-binding domains 2 and 3 of Flt (the highest-affinity VEGF receptor), (2) poly(lactic-co-glyocolic acid) biodegradable nanoparticles as a delivery system, and (3) the tripeptide adhesion motif arginine-glycine-aspartate to coat nanoparticles and facilitate selective homing to choroidal new vessels (CNVs) after systemic intravenous injection (Figure 1). They previously found that these antiangiogenic nanoparticles inhibit laser-induced CNVs in rats.[23] The nanoparticles targeted CNVs, induced CNV regression, decreased fibrotic scarring, improved visual acuity, and documented safety in 2 murine CNV models and a primate CNV model (Figure 2).[22] This delivery system provides extended-release, nonviral gene therapy that inhibits CNV formation and subretinal scarring.

Cationic liposomes can be used to improve therapy. Gross et al[24] used an argon laser to induce CNVs bilaterally in mice on day 0. On day 10, either verteporfin (Visudyne, Valeant Pharmaceuticals International, Inc.) or verteporfin loaded in cationic liposomes was injected intravenously. For each mouse, one eye was treated with photodynamic therapy and the other served as a control. The specimens were examined histologically 1 week later for response to treatment. Liposome-loaded verteporfin was as efficacious as verteporfin alone in treating neovascularization but resulted in less tissue damage, possibly secondary to cationic liposomes' affinity for active angiogenesis.
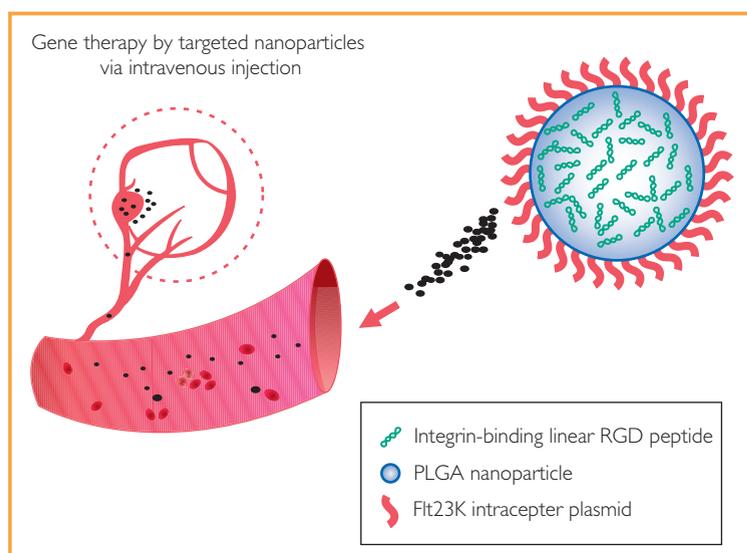
Intravitreal injection of nanoparticles containing PEDF into Royal College of Surgeons rats resulted in greater preservation of opsin and photoreceptors with reduced numbers of terminal deoxynucleotidyl transferase dUTP nick end labeling—positive cells over 8 weeks compared with injection of PEDF alone.[25] These rats have a sequence variation that is found in some patients with retinitis pigmentosa,[26] and there is some evidence that sustained neurotrophic factor therapy might benefit selected patients with retinitis pigmentosa.[27] Nanoparticles also provide sustained delivery of basic fibroblast growth factor and rescue the retina in Royal College of Surgeons rats.[28]

Jiang et al[29] found that intravitreal glial cell line—derived neurotrophic factor (GDNF)—loaded biodegradable poly(lactic-co-glycolic acid) microspheres provide sustained retinal ganglion cell (RGC) protection in a rodent model of glaucoma. Microspheres (approximately 8 μm in diameter) containing GDNF were fabricated using a modification of a spontaneous emulsion technique. Moreover, the use of a novel solid-in-oil-in-water emulsion solvent evaporation technique with incorporation of vitamin E into the microspheres has a protective effect on GDNF. Checa-Casalengua et al[30] found that this revised formulation allowed for release of active GDNF for up to 3 months and improved survival of photoreceptors and RGCs in vitro. Intravitreal injection of these microspheres in a mouse model of glaucoma resulted in improved RGC survival compared with GDNF, vitamin E, or blank microspheres, an effect that was present for up to 11 weeks after injection.

The ability of nanoparticles to deliver siRNA over prolonged intervals and selectively to tissues may improve treatment efficacy and specificity. Du et al[31] found, for example, that in vivo knockdown of *EphB4* (erythropoietin-producing hepatocellular carcinoma receptor B4) levels by short hairpin RNA (shRNA) delivered by a lentiviral system decreased CNVs. Laser-induced CNVs in C57BL/6 mice were treated with phosphate-buffered saline, *EphB4* shRNA, or lentiviral empty vector. Fluorescein angiography and histologic analysis revealed that down-regulation of *EphB4* resulted in decreased areas of leakage and CNV mean thickness compared with phosphate-buffered saline and empty vector alone.
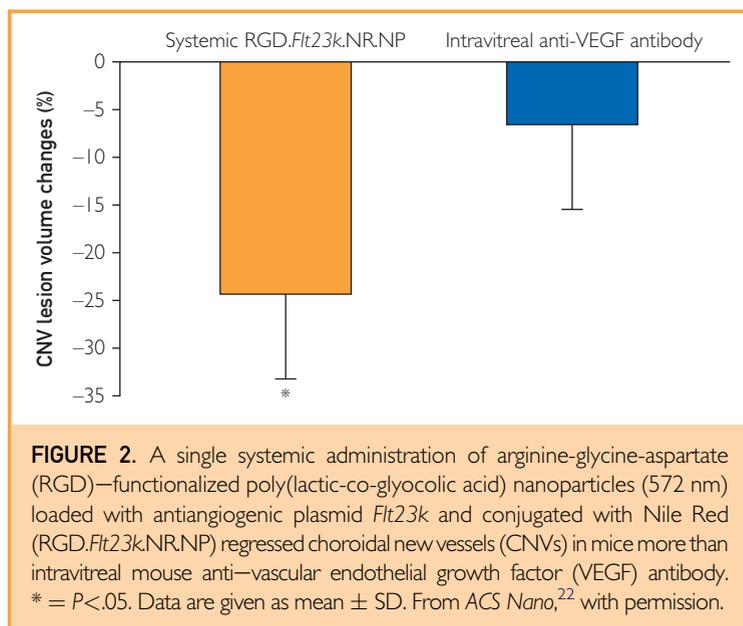
FIGURE 2. A single systemic administration of arginine-glycine-aspartate (RGD)—functionalized poly(lactic-co-glyocolic acid) nanoparticles (572 nm) loaded with antiangiogenic plasmid *Flt23k* and conjugated with Nile Red (RGD.*Flt23k*.NR.NP) regressed choroidal new vessels (CNVs) in mice more than intravitreal mouse anti—vascular endothelial growth factor (VEGF) antibody. * = *P*<.05. Data are given as mean ± SD. From *ACS Nano*,[22] with permission.

Albumin-derived nanoparticles that deliver plasmids containing genes for the Flt receptor (VEGFR1), which binds free VEGF, penetrate keratocyte cytoplasm and provide sustained inhibition of injury-induced corneal neovascularization.[32] Flt1 can be alternatively spliced to produce soluble FLT1, the soluble VEGF receptor 1 that lacks the transmembrane and intracellular kinase domains and, hence, acts as a soluble sink for free VEGF. Owen et al[33] took advantage of this phenomenon and designed morpholinos directed against splice site targets in FLT1, the hypothesis being that this approach could result in increased levels of soluble FLT1 as opposed to membrane-bound FLT1, thereby decreasing VEGF signaling. As predicted, intravitreal injections of the FLT1 morpholinos increased the soluble to membrane-bound FLT1 ratio 5-fold and decreased laser-induced CNVs by 50%. Similarly, Lai et al[34] delivered solulable FLT1 via recombinant adeno-associated virus subretinally in a mouse model of retinal neovascularization and found inhibition of abnormal blood vessel growth. Flt23k is an anti-VEGF intraceptor similar to the soluble VEGF binding domain of soluble FLT1. Cho et al[35] found that nanoparticle delivery of FLT23K-expressing plasmids improved the corneal transplant survival rate and decreased corneal neovascularization in mice. This rescue was enhanced by the addition of topical triamcinolone. Instead of

posttranslationally inhibiting VEGF, Qazi et al[36] created nanoparticles loaded with plasmids expressing shRNA against VEGF-A to decrease the signal pretranslationally. They found that nanoparticles loaded with VEGF-A shRNA reduced corneal neovascularization (as per the CD31 marker) twice as well as solitary plasmid treatment in mice after mechanical alkali injury.

Nanoparticle delivery systems that deliver relevant shRNAs might serve as useful treatment for retinal degenerative disease, diabetic retinopathy, or glaucoma. Regarding retinal degenerative disease, production of the photoreceptor determinant *Nrl* transcription factor results in rod development, whereas the absence of *Nrl* causes cones to develop from photoreceptor precursors. Retinitis pigmentosa results in early rod photoreceptor death and loss of vision. Montana et al[37] found that knocking down *Nrl* expression partially reprograms rods to adopt the molecular, morphologic, and functional characteristics of cones. Reprogramming rods into cones was sufficient to reduce photoreceptor cell death in the rho$^{-/-}$ mouse model of retinitis pigmentosa. This work suggests that future knockdown of *Nrl* with shRNA may serve as a useful therapeutic approach to some types of retinitis pigmentosa. Regarding diabetic retinopathy, severe retinopathy can be associated with retinal neovascularization. Connective tissue growth factor is produced by retinal Muller cells in diabetic rats and results in increased deposition of extracellular matrix (ECM) and angiogenesis. Intravitreal injection of shRNA against connective tissue growth factor decreases ECM production, suggesting that this growth factor may be another potential future target of shRNA clinically.[38] Similarly, siRNA targeted against *Smad*7 decreases production of ECM in the aqueous outflow pathway and may be a potential treatment for glaucoma.[39]

REGENERATIVE MEDICINE: OPTOGENETICS AND OPTIC NERVE REGENERATION

Optogenetics involves the use of light-sensitive ion channels (vs electrodes) to make neurons light sensitive. This approach to visual rehabilitation has been reviewed extensively.[6-8,40] Stimulation of RGCs or bipolar cells provides an alternative approach to retinal cell stimulation in lieu of that provided by the retinal prosthesis.[41] In contrast to the currently available retinal prosthesis, optogenetics has the potential for minimally invasive neuronal stimulation with high spatial resolution. In principle, for example, one can convert a large fraction of the approximately 1 million RGCs into photosensitive neurons vs the number one might stimulate with a 60- or 200-electrode array. Optogenetics may play an important role in ophthalmic regenerative medicine.

Channelrhodopsin-2 (ChR2) and halorhodopsin (HaloR) are naturally occurring light-activated ion channels[42]; ChR2 is a cation channel, and HaloR is an anion channel. Using viral delivery systems, these molecules can be expressed in vivo in RGCs, amacrine cells, biopolar cells, and photoreceptors.[43-48] In preclinical models, the kinetics of ChR2- and HaloR-mediated light responses is compatible with the retina's temporal information-processing requirements. However, ChR2 and HaloR exhibit low light sensitivity, with threshold activation light intensities approximately 5 to 6 log units higher than those of cones. Furthermore, the light intensity operating range of ChR2 and HaloR is 2 to 3 log units, in contrast to the normal retinal dynamic range of 10 log units. Low light sensitivity may hinder the use of ChR2 and HaloR clinically. Recently, a new ChR2 variant, the calcium translocation ChR, has been identified that has an accelerated response time and a voltage response that is 70-fold more light sensitive than that of ChR2.[49] Numerous other "next-generation" ChRs have been engineered via site-directed mutagenesis and provide improved parameters for experimental and clinical approaches, and these advances have been well summarized by Lin.[50]

A genetically and chemically engineered light-gated mammalian ion channel, the light-activated glutamate receptor, has been expressed selectively in RGCs of the *rd1* mouse.[51] In these mice, the light-activated glutamate receptor restores light sensitivity to the RGCs, reinstates light responsiveness to the primary visual cortex, and restores the pupillary light reflex and natural light-avoidance behavior. Optogenetic recovery of vision also can be achieved with nonviral approaches, such as intravitreal injection of acrylamide-azobenzene-quaternary ammonium (AAQ). The AAQ acts as a synthetic K$^+$ channel

photoswitch. Polosukhina et al[52] reported that intravitreal injection of AAQ restored the pupillary light reflex and light-avoidance behavior in mice lacking retinal photoreceptors by interacting with multiple types of cells in the retina, primarily RGCs (Figure 3). This therapeutic modality does not require exogenous gene delivery or manipulation, suggesting limited hurdles in delivery to patients. This approach is reversible (vs viral therapy), repeatable, and apparently nontoxic.

BIOMATERIALS AND REGENERATIVE MEDICINE

Retinal progenitor cells can be delivered to the eye, migrate to correct regions in the retina, and differentiate into photoreceptors with appropriate markers and morphologic features. It is essential, however, that these photoreceptors can transmit a signal centrally for vision perception. Pearson et al[53] used Gnat1$^{-/-}$ mice, which lack rod function, to

address this issue. They found that transplanted rod precursors could form classic triad synaptic connections with second-order bipolar and horizontal cells in the recipient retina, which were then able to transmit visual signals to higher-order visual processing centers in the brain and induce meaningful optokinetic head tracking and visually guided behavior. Similarly, de Lima et al[54] found that a combination of methods that synergistically activate RGCs' intrinsic growth state enables these cells to regenerate axons over the entire length of the optic nerve, across the optic chiasm, and into the brain in mature mice that have undergone optic nerve crush injuries. Axons innervated the dorsal lateral geniculate nucleus, the superior colliculus, and other visual target areas, leading to partial recovery of depth perception, the optomotor response, and circadian photoentrainment. In addition, olfactory stem cells can rescue the optic nerve in an animal model of glaucoma.[55,56] The use of stem cells to
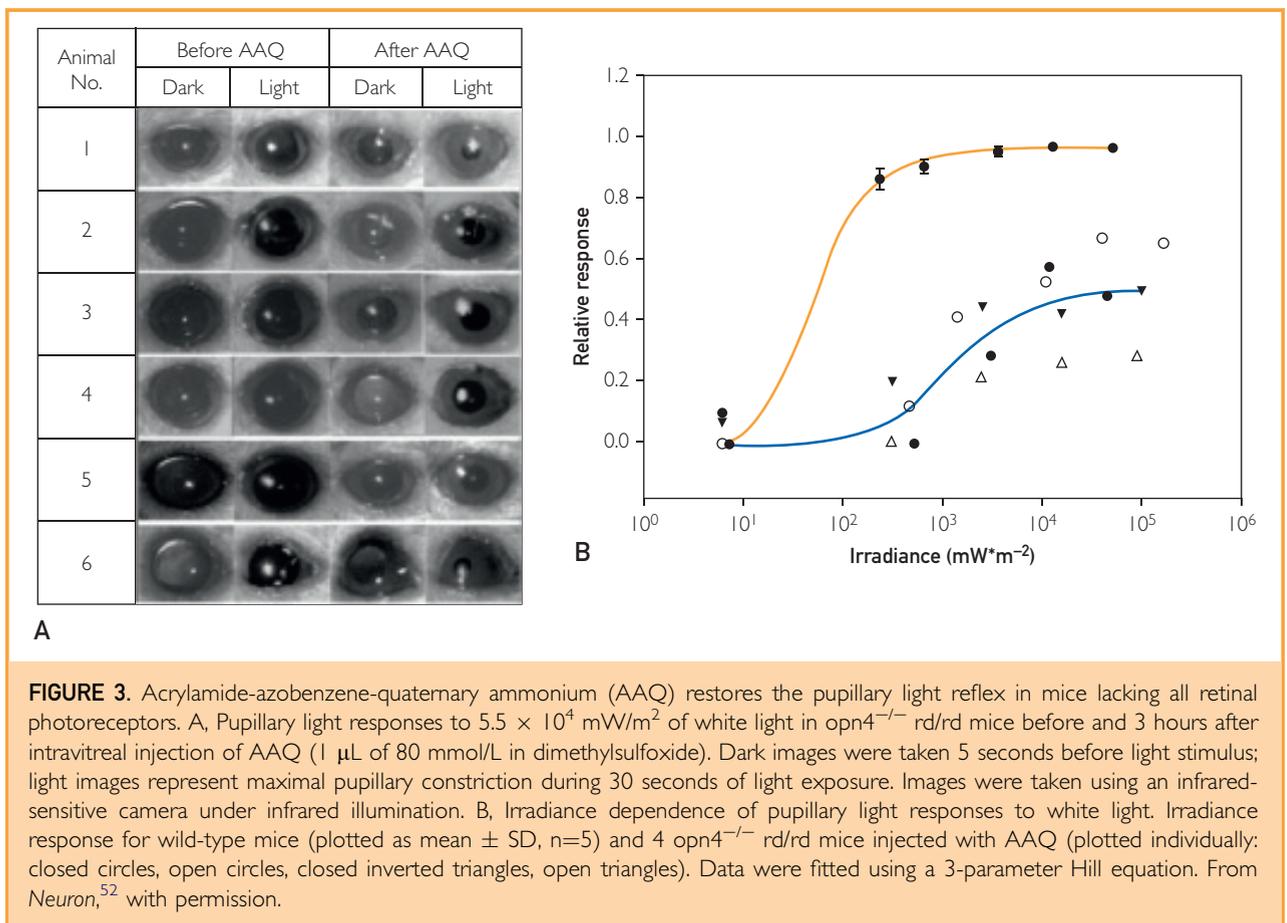
FIGURE 3. Acrylamide-azobenzene-quaternary ammonium (AAQ) restores the pupillary light reflex in mice lacking all retinal photoreceptors. A, Pupillary light responses to 5.5 × 10^4 mW/m^2 of white light in opn4$^{-/-}$ rd/rd mice before and 3 hours after intravitreal injection of AAQ (1 μL of 80 mmol/L in dimethylsulfoxide). Dark images were taken 5 seconds before light stimulus; light images represent maximal pupillary constriction during 30 seconds of light exposure. Images were taken using an infrared-sensitive camera under infrared illumination. B, Irradiance dependence of pupillary light responses to white light. Irradiance response for wild-type mice (plotted as mean ± SD, n=5) and 4 opn4$^{-/-}$ rd/rd mice injected with AAQ (plotted individually: closed circles, open circles, closed inverted triangles, open triangles). Data were fitted using a 3-parameter Hill equation. From Neuron,[52] with permission.

treat blinding disease has been reviewed elsewhere.[57] Nanofabrication techniques and judicious use of various biomaterials will probably play an important role in operationalizing cell-based therapy for regenerative ophthalmic medicine.

Poly(ε-caprolactone) (PCL) is biodegradable, is biocompatible, can be spin-cast to a thin film (5 μm) with controlled microtopography (that favors cell adherence), and promotes the differentiation of retinal progenitor cells. Additionally, these scaffolds can support the adhesion, proliferation, and differentiation of mouse retinal progenitor cells in vitro and migration into mouse retinal explants.[58] Christiansen et al[59] found that PCL scaffolds could be implanted subretinally in porcine eyes and were well tolerated, without signs of tissue disruption or inflammation. Furthermore, this study revealed that PCL short nanowires preserved the overlying outer retina better than PCL electrospun and PCL smooth scaffolds and exhibited the most suitable degree of stiffness for surgical delivery. By sealing protein between two layers of PCL film, Bernards et al[60] found that PCL scaffolds can act as a reservoir that steadily releases active protein for up to 70 days. This function may be used in the future as an effective means to deliver biological therapies to cells in the scaffold.

Three-dimensional hyaluronic acid—based hydrogels with encapsulated retinal progenitor cells have been delivered to the subretinal space without perturbing retinal architecture and have resorbed, allowing retinal progenitor cells to differentiate and to express the mature photoreceptor marker recoverin within 3 weeks of implantation.[61] In addition to providing structural and nutrient support, scaffolds and nanoparticles have been shown to secrete a variety of agents that promote survival and integration of retinal precursors, notably chondroitinase ABC,[62] matrix metalloprotease 2,[63] and AG1478, an investigational epidermal growth factor receptor tyrosine kinase inhibitor.[64]

Although generation of RGCs from embryonic and induced pluripotent stem cells is in an early stage of development, one can envision combining cell-based therapy with composite nanofabricated cell conduits or hydrogel matrices (which not only provide signals to cells but also provide structural support to encapsulated cells and which eventually can be metabolized into nutrients) to provide RGC regenerative therapy for various optic neuropathies, including glaucoma.

Chitosan is a nontoxic polysaccharide with mucoadhesive properties that can increase drug delivery to human corneal epithelium[65] and may serve as an excellent means to treat retinal disease[66] and glaucoma and other ocular pathologic disorders.[67] Wang et al[68] devised a biodegradable scaffold composed of a 75:25 ratio of chitosan to polycaprolactone that had the mechanical and biocompatibility properties that allowed for growth, differentiation, and production of tight junctions of corneal epithelial cells. With further optimization, such a system may be able to create corneal grafts for transplantation.

DIAGNOSTICS

Some ways in which nanotechnology has improved diagnostic imaging have been reviewed previously.[4] Progress in this area continues. For example, monitoring active angiogenesis in neovascular eye diseases is essential for gauging a patient's disease progression and response to treatment.[69] Thus far, no in vivo imaging methods are available to label active angiogenesis specifically. Hua et al[70] demonstrated that cationic (but not neutral) liposomes labeled with indocyanine green could, with high affinity, stain active CNV lesions in C57BL/6 mice when viewed through a scanning laser ophthalmoscope.

Microelectromechanical and nanoelectromechanical systems engineering have been used to manufacture IOP monitors. The IOP exhibits diurnal and instantaneous fluctuations. Nocturnal IOP measurement may be more critical to glaucoma management than the typical daytime measurement in an outpatient setting.[71] The results of frequent IOP measurement over a 24-hour period often lead to a change in glaucoma management.[72,73] The wireless contact lens is a microelectromechanical systems—engineered, noninvasive IOP fluctuation monitor.[74] Mansouri et al[75] developed a disposable highly oxygen-permeable silicone soft contact lens with an embedded sensor (Triggerfish; Sensimed AG). Microfabricated strain gauges (170-nm platinum, 25-nm titanium) are embedded in the contact lens and measure circumferential changes in the area of the corneoscleral junction that can reflect changes in IOP (Figure 4). Wireless powering and

FIGURE 4. Contact lens used to measure changes in intraocular pressure (Triggerfish; Sensimed AG). A, Diagram of the contact lens sensor showing the location of the sensor-active strain gauges (which are placed circumferentially to measure changes in the corneal curvature caused by intraocular pressure variations) and the sensor-passive strain gauges for thermal compensation (which are placed radially, where no strain is measured). For wireless powering and communication, a microprocessor and an antenna have been integrated into the soft contact lens. B, The contact lens sensor resting on the cornea. C, Wireless power and data transfer are achieved using a patched periorbital antenna, from which a cable is connected to a portable recorder (black box shown in the center of the photograph). Courtesy of Sonja Simon-Zoula, PhD, Sensimed AG. From *Curr Opin Pharmacol*,[7] with permission.

communication between the contact lens and the recording unit are achieved with a microprocessor and a 9-mm mean diameter, 8-μm-thick gold loop antenna integrated into the lens. Wireless power and data transfer occur via a patched periorbital antenna from which a cable is connected to a portable recorder, which is worn around the patient's waist. The silicone contact lens has a diameter of 14.1 mm and central and peripheral thicknesses of 585 and 260 μm, respectively. Three hundred data points are acquired during a 30-second measurement period, which is repeated every 5 minutes.[75] Variations in central corneal thickness and rigidity and, possibly, motion artifacts may limit the utility of this approach. The contact lens sensor can essentially provide continuous IOP monitoring so that the patient does not need to be awakened from sleep to monitor IOP.

BARRIERS TO CLINICAL APPLICATION

Several obstacles to the incorporation of nanotechnology into medicine are recognized.[4] The biodistribution of nanoparticles and their persistence in tissues despite immune surveillance is a concern.[76] Safe bionanomanufacturing techniques also must be identified. This issue is particularly relevant when scaling up production for commercial distribution of products. Clean room processes similar to those used for semiconductor device manufacture may be needed in some cases. Although nanotechnology permits precise targeting of therapeutic modalities and

minimizes the doses of drugs delivered in vivo, nanomaterial toxicity may still occur. Test protocols to assess nanomaterial safety have been developed.[77] However, this field is nascent. In general, nanoparticle toxicity reflects the underlying chemistry.[78] Carbon nanotubes have toxicity that reflects their shape, size, surface coating, and concentration.[79,80]

CONCLUSION

Nanomedicine has already had an impact in the areas of biopharmaceuticals (eg, glaucoma drugs and neurotrophic factors), implantable materials (eg, tissue regeneration scaffolds), and diagnostic tools (eg, IOP monitors) in ophthalmology. The examples provided herein demonstrate that nanotechnology will play an important role in early- and late-stage interventions in the management of blinding diseases. Nanotechnology already has been applied to the measurement and treatment of different disease states in ophthalmology, including choroidal neovascularization, retinal neovascularization, oxidative damage, optic nerve damage, and retinal degenerative disease. During the next few years, as these discoveries transition from laboratory experiments to clinical practice, they are likely to have a major effect on the development of sight-preserving and sight-restoring treatments for conditions that currently are among the major causes of blindness.

The Symposium on Regenerative Medicine will continue in an upcoming issue.

REFERENCES

1. Prow T, Salazar JH, Rose WA, et al. Nanomedicine-nanoparticles, molecular biosensors and targeted gene/drug delivery for combined single-cell diagnostics and therapeutics. *Proc SPIE.* 2004;5318:1-11.
2. Prow T, Grebe R, Merges C, et al. Nanoparticle tethered antioxidant response element as a biosensor for oxygen induced toxicity in retinal endothelial cells. *Mol Vis.* 2006;12:616-625.
3. Prow TW, Bhutto I, Grebe R, et al. Nanoparticle-delivered biosensor for reactive oxygen species in diabetes. *Vision Res.* 2008;48(3):478-485.
4. Zarbin MA, Montemagno C, Leary JF, Ritch R. Nanomedicine in ophthalmology: the new frontier. *Am J Ophthalmol.* 2010;150(2):144-162.e2.
5. Zarbin MA, Montemagno C, Leary JF, Ritch R. Nanotechnology in ophthalmology. *Can J Ophthalmol.* 2010;45(5):457-476.
6. Zarbin MA, Montemagno C, Leary JF, Ritch R. Regenerative nanomedicine and the treatment of degenerative retinal diseases. *Wiley Interdiscip Rev Nanomed Nanobiotechnol.* 2012;4(1):113-137.
7. Zarbin MA, Montemagno C, Leary JF, Ritch R. Nanomedicine for the treatment of retinal and optic nerve diseases. *Curr Opin Pharmacol.* 2013;13(1):134-148.
8. Zarbin M, Montemagno C, Leary J, Ritch R. Artificial vision. *Panminerva Med.* 2011;53(3):167-177.
9. Chen J, Patil S, Seal S, McGinnis JF. Rare earth nanoparticles prevent retinal degeneration induced by intracellular peroxides. *Nat Nanotechnol.* 2006;1(2):142-150.
10. Zhou X, Wong LL, Karakoti AS, Seal S, McGinnis JF. Nanoceria inhibit the development and promote the regression of pathologic retinal neovascularization in the vldlr knockout mouse. *PLoS One.* 2011;6(2):e16733.
11. Cai X, Sezate SA, Seal S, McGinnis JF. Sustained protection against photoreceptor degeneration in tubby mice by intravitreal injection of nanoceria. *Biomaterials.* 2012;33(34):8771-8781.
12. Kong L, Cai X, Zhou X, et al. Nanoceria extend photoreceptor cell lifespan in tubby mice by modulation of apoptosis/survival signaling pathways. *Neurobiol Dis.* 2011;42(3):514-523.
13. Han Z, Conley SM, Makkia RS, Cooper MJ, Naash MI. DNA nanoparticle-mediated ABCA4 delivery rescues Stargardt dystrophy in mice. *J Clin Invest.* 2012;122(9):3221-3226.
14. Cai X, Nash Z, Conley SM, Fliesler SJ, Cooper MJ, Naash MI. A partial structural and functional rescue of a retinitis pigmentosa model with compacted DNA nanoparticles. *PLoS One.* 2009;4(4):e5290.
15. Koirala A, Makkia RS, Cooper MJ, Naash MI. Nanoparticle-mediated gene transfer specific to retinal pigment epithelial cells. *Biomaterials.* 2011;32(35):9483-9493.
16. Han Z, Koirala A, Makkia R, Cooper MJ, Naash MI. Direct gene transfer with compacted DNA nanoparticles in retinal pigment epithelial cells: expression, repeat delivery and lack of toxicity. *Nanomedicine (Lond).* 2012;7(4):521-539.
17. Ding XQ, Quiambao AB, Fitzgerald JB, Cooper MJ, Conley SM, Naash MI. Ocular delivery of compacted DNA-nanoparticles does not elicit toxicity in the mouse retina. *PLoS One.* 2009;4(10):e7410.
18. Pirollo KF, Chang EH. Targeted delivery of small interfering RNA: approaching effective cancer therapies. *Cancer Res.* 2008;68(5):1247-1250.
19. Chen CW, Lu DW, Yeh MK, Shiau CY, Chiang CH. Novel RGD-lipid conjugate-modified liposomes for enhancing siRNA delivery in human retinal pigment epithelial cells. *Int J Nanomedicine.* 2011;6:2567-2580.

20. Suen W-LL, Chau Y. Specific uptake of folate-decorated triamcinolone-encapsulating nanoparticles by retinal pigment epithelium cells enhances and prolongs antiangiogenic activity. *J Control Release*. 2013;167(1):21-28.

21. Zarbin MA, Rosenfeld PJ. Pathway-based therapies for age-related macular degeneration: an integrated survey of emerging treatment alternatives. *Retina*. 2010;30(9):1350-1367.

22. Luo L, Zhang X, Hirano Y, et al. Targeted intraceptor nanoparticle therapy reduces angiogenesis and fibrosis in primate and murine macular degeneration. *ACS Nano*. 2013;7(4):3264-3275.

23. Singh SR, Grossniklaus HE, Kang SJ, Edelhauser HF, Ambati BK, Kompella UB. Intravenous transferrin, RGD peptide and dual-targeted nanoparticles enhance anti-VEGF intraceptor gene delivery to laser-induced CNV. *Gene Ther*. 2009;16(5):645-659.

24. Gross N, Ranjbar M, Evers C, et al. Choroidal neovascularization reduced by targeted drug delivery with cationic liposome-encapsulated paclitaxel or targeted photodynamic therapy with verteporfin encapsulated in cationic liposomes. *Mol Vis*. 2013;19:54-61.

25. Akiyama G, Sakai T, Kuno N, et al. Photoreceptor rescue of pigment epithelium-derived factor-impregnated nanoparticles in Royal College of Surgeons rats. *Mol Vis*. 2012;18:3079-3086.

26. Gal A, Li Y, Thompson DA, et al. Mutations in MERTK, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa. *Nat Genet*. 2000;26(3):270-271.

27. Sieving PA, Caruso RC, Tao W, et al. Ciliary neurotrophic factor (CNTF) for human retinal degeneration: phase I trial of CNTF delivered by encapsulated cell intraocular implants. *Proc Natl Acad Sci U S A*. 2006;103(10):3896-3901.

28. Sakai T, Kuno N, Takamatsu F, et al. Prolonged protective effect of basic fibroblast growth factor-impregnated nanoparticles in Royal College of Surgeons rats. *Invest Ophthalmol Vis Sci*. 2007;48(7):3381-3387.

29. Jiang C, Moore MJ, Zhang X, Klassen H, Langer R, Young M. Intravitreal injections of GDNF-loaded biodegradable microspheres are neuroprotective in a rat model of glaucoma. *Mol Vis*. 2007;13:1783-1792.

30. Checa-Casalengua P, Jiang C, Bravo-Osuna I, et al. Retinal ganglion cells survival in a glaucoma model by GDNF/Vit E PLGA microspheres prepared according to a novel microencapsulation procedure. *J Control Release*. 2011;156(1):92-100.

31. Du J, Zhao W, Wang Y, Cai Y. Lentivirus vector-mediated knockdown of erythropoietin-producing hepatocellular carcinoma receptors B4 inhibits laser-induced choroidal neovascularization. *J Ocular Pharmacol Ther*. 2013;29(1):14-22.

32. Jani PD, Singh N, Jenkins C, et al. Nanoparticles sustain expression of Flt intraceptors in the cornea and inhibit injury-induced corneal angiogenesis. *Invest Ophthalmol Vis Sci*. 2007;48(5):2030-2036.

33. Owen LA, Uehara H, Cahoon J, Huang W, Simonis J, Ambati BK. Morpholino-mediated increase in soluble Flt-1 expression results in decreased ocular and tumor neovascularization. *PLoS One*. 2012;7(3):e33576.

34. Lai CM, Estcourt MJ, Wikstrom M, et al. rAAV.sFlt-1 gene therapy achieves lasting reversal of retinal neovascularization in the absence of a strong immune response to the viral vector. *Invest Ophthalmol Vis Sci*. 2009;50(9):4279-4287.

35. Cho YK, Uehara H, Young JR, et al. Flt23k nanoparticles offer additive benefit in graft survival and anti-angiogenic effects when combined with triamcinolone. *Invest Ophthalmol Vis Sci*. 2012;53(4):2328-2336.

36. Qazi Y, Stagg B, Singh N, et al. Nanoparticle-mediated delivery of shRNA: VEGF-a plasmids regresses corneal neovascularization. *Invest Ophthalmol Vis Sci*. 2012;53(6):2837-2844.

37. Montana CL, Kolesnikov AV, Shen SQ, Myers CA, Kefalov VJ, Corbo JC. Reprogramming of adult rod photoreceptors prevents retinal degeneration. *Proc Natl Acad Sci U S A*. 2013;110(5):1732-1737.

38. Winkler JL, Kedees MH, Guz Y, Teitelman G. Inhibition of connective tissue growth factor by small interfering ribonucleic acid

39. prevents increase in extracellular matrix molecules in a rodent model of diabetic retinopathy. *Mol Vis*. 2012;18:874-886.

39. Su Y, Yang CY, Li Z, et al. Smad7 siRNA inhibit expression of extracellular matrix in trabecular meshwork cells treated with TGF-β2. *Mol Vis*. 2012;18:1881-1884.

40. Busskamp V, Picaud S, Sahel JA, Roska B. Optogenetic therapy for retinitis pigmentosa. *Gene Ther*. 2012;19(2):169-175.

41. Ahuja AK, Dorn JD, Caspi A, et al. Blind subjects implanted with the Argus II retinal prosthesis are able to improve performance in a spatial-motor task. *Br J Ophthalmol*. 2011;95(4):539-543.

42. Zhang H, Morrison MA, Dewan A, et al. The NEI/NCBI dbGAP database: genotypes and haplotypes that may specifically predispose to risk of neovascular age-related macular degeneration. *BMC Med Genet*. 2008;9:51.

43. Tomita H, Sugano E, Yawo H, et al. Restoration of visual response in aged dystrophic RCS rats using AAV-mediated channelopsin-2 gene transfer. *Invest Ophthalmol Vis Sci*. 2007;48(8):3821-3826.

44. Bi A, Cui J, Ma YP, et al. Ectopic expression of a microbial-type rhodopsin restores visual responses in mice with photoreceptor degeneration. *Neuron*. 2006;50(1):23-33.

45. Doroudchi MM, Greenberg KP, Liu J, et al. Virally delivered channelrhodopsin-2 safely and effectively restores visual function in multiple mouse models of blindness. *Mol Ther*. 2011;19(7):1220-1229.

46. Lagali PS, Balya D, Awatramani GB, et al. Light-activated channels targeted to ON bipolar cells restore visual function in retinal degeneration. *Nat Neurosci*. 2008;11(6):667-675.

47. Zhang Y, Ivanova E, Bi A, Pan ZH. Ectopic expression of multiple microbial rhodopsins restores ON and OFF light responses in retinas with photoreceptor degeneration. *J Neurosci*. 2009;29(29):9186-9196.

48. Busskamp V, Duebel J, Balya D, et al. Genetic reactivation of cone photoreceptors restores visual responses in retinitis pigmentosa. *Science*. 2010;329(5990):413-417.

49. Kleinlogel S, Feldbauer K, Dempski RE, et al. Ultra light-sensitive and fast neuronal activation with the Ca^2+-permeable channelrhodopsin CatCh. *Nat Neurosci*. 2011;14(4):513-518.

50. Lin JY. Optogenetic excitation of neurons with channelrhodopsins: light instrumentation, expression systems, and channelrhodopsin variants. *Prog Brain Res*. 2012;196:29-47.

51. Caporale N, Kolstad KD, Lee T, et al. LiGluR restores visual responses in rodent models of inherited blindness. *Mol Ther*. 2011;19(7):1212-1219.

52. Polosukhina A, Litt J, Tochitsky I, et al. Photochemical restoration of visual responses in blind mice. *Neuron*. 2012;75(2):271-282.

53. Pearson RA, Barber AC, Rizzi M, et al. Restoration of vision after transplantation of photoreceptors. *Nature*. 2012;485(7396):99-103.

54. de Lima S, Koriyama Y, Kurimoto T, et al. Full-length axon regeneration in the adult mouse optic nerve and partial recovery of simple visual behaviors. *Proc Natl Acad Sci U S A*. 2012;109(23):9149-9154.

55. Dai C, Khaw PT, Yin ZQ, Li D, Raisman G, Li Y. Structural basis of glaucoma: the fortified astrocytes of the optic nerve head are the target of raised intraocular pressure. *Glia*. 2012;60(1):13-28.

56. Dai C, Khaw KT, Yin C, Li D, Raisman G, Li Y. Olfactory ensheathing cells rescue optic nerve fibers in a rat glaucoma model. *Trans Vis Sci Tech*. 2012;1(2):3.

57. Zarbin M. The promise of stem cells for age-related macular degeneration and other retinal degenerative diseases. *Drug Discovery Today Ther Strategies*. 2012. http://dx.doi.org/10.1016/j.ddstr.2012.08.001. Accessed July 30, 2013.

58. Cai S, Smith ME, Redenti SM, Wnek GE, Young MJ. Mouse retinal progenitor cell dynamics on electrospun poly (ε-caprolactone). *J Biomater Sci Polym Ed*. 2011;22(4-6):443-456.

59. Christiansen AT, Tao SL, Smith M, et al. Subretinal implantation of electrospun, short nanowire, and smooth poly(ε-

caprolactone) scaffolds to the subretinal space of porcine eyes. *Stem Cells Int.* 2012;2012:454295.

60. Bernards DA, Lance KD, Ciaccio NA, Desai TA. Nanostructured thin film polymer devices for constant-rate protein delivery. *Nano Lett.* 2012;12(10):5355-5361.

61. Liu Y, Wang R, Zarembinski TI, et al. The application of hyaluronic acid hydrogels to retinal progenitor cell transplantation. *Tissue Eng Part A.* 2013;19(1-2):135-142.

62. Ma J, Kabiel M, Tucker BA, Ge J, Young MJ. Combining chondroitinase ABC and growth factors promotes the integration of murine retinal progenitor cells transplanted into Rho(-/-) mice. *Mol Vis.* 2011;17:1759-1770.

63. Yao J, Tucker BA, Zhang X, Checa-Casalengua P, Herrero-Vanrell R, Young MJ. Robust cell integration from co-transplantation of biodegradable MMP2-PLGA microspheres with retinal progenitor cells. *Biomaterials.* 2011;32(4): 1041-1050.

64. Robinson R, Viviano SR, Criscione JM, et al. Nanospheres delivering the EGFR TKI AG1478 promote optic nerve regeneration: the role of size for intraocular drug delivery. *ACS Nano.* 2011;5(6):4392-4400.

65. Zhu X, Su M, Tang S, et al. Synthesis of thiolated chitosan and preparation nanoparticles with sodium alginate for ocular drug delivery. *Mol Vis.* 2012;18:1973-1982.

66. Jayaraman MS, Bharali DJ, Sudha T, Mousa SA. Nano chitosan peptide as a potential therapeutic carrier for retinal delivery to treat age-related macular degeneration. *Mol Vis.* 2012;18: 2300-2308.

67. Fulgêncio Gde O, Viana FA, Ribeiro RR, Yoshida MI, Faraco AG, Cunha-Júnior Ada S. New mucoadhesive chitosan film for ophthalmic drug delivery of timolol maleate: in vivo evaluation. *J Ocul Pharmacol Ther.* 2012;28(4):350-358.

68. Wang TJ, Wang IJ, Lu JN, Young TH. Novel chitosan-polycaprolactone blends as potential scaffold and carrier for corneal endothelial transplantation. *Mol Vis.* 2012;18:255-264.

69. Lalwani GA, Rosenfeld PJ, Fung AE, et al. A variable-dosing regimen with intravitreal ranibizumab for neovascular age-related macular degeneration: year 2 of the PrONTO Study. *Am J Ophthalmol.* 2009;148(1):43-58.e41.

70. Hua J, Gross N, Schulze B, et al. In vivo imaging of choroidal angiogenesis using fluorescence-labeled cationic liposomes. *Mol Vis.* 2012;18:1045-1054.

71. Barkana Y, Anis S, Liebmann J, Tello C, Ritch R. Clinical utility of intraocular pressure monitoring outside of normal office hours in patients with glaucoma. *Arch Ophthalmol.* 2006;124(6): 793-797.

72. Graham SL, Drance SM. Nocturnal hypotension: role in glaucoma progression. *Surv Ophthalmol.* 1999;43(suppl 1):S10-S16.

73. Wax MB, Camras CB, Fiscella RG, Girkin C, Singh K, Weinreb RN. Emerging perspectives in glaucoma: optimizing 24-hour control of intraocular pressure. *Am J Ophthalmol.* 2002;133(suppl):S1-S10.

74. Leonardi M, Pitchon EM, Bertsch A, Renaud P, Mermoud A. Wireless contact lens sensor for intraocular pressure monitoring: assessment on enucleated pig eyes. *Acta Ophthalmol.* 2009;87(4):433-437.

75. Mansouri K, Medeiros FA, Tafreshi A, Weinreb RN. Continuous 24-hour monitoring of intraocular pressure patterns with a contact lens sensor: safety, tolerability, and reproducibility in patients with glaucoma. *Arch Ophthalmol.* 2012; 130(12):1534-1539.

76. Zhang LW, Monteiro-Riviere NA. Mechanisms of quantum dot nanoparticle cellular uptake. *Toxicol Sci.* 2009;110(1):138-155.

77. Hoet P, Legiest B, Geys J, Nemery B. Do nanomedicines require novel safety assessments to ensure their safety for long-term human use? *Drug Saf.* 2009;32(8):625-636.

78. Ferreira L, Karp JM, Nobre L, Langer R. New opportunities: the use of nanotechnologies to manipulate and track stem cells. *Cell Stem Cell.* 2008;3(2):136-146.

79. Magrez A, Kasas S, Salicio V, et al. Cellular toxicity of carbon-based nanomaterials. *Nano Lett.* 2006;6(6):1121-1125.

80. Zhu L, Chang DW, Dai L, Hong Y. DNA damage induced by multiwalled carbon nanotubes in mouse embryonic stem cells. *Nano Lett.* 2007;7(12):3592-3597.