THE CONVERGENCE OF NANOTECHNOLOGY AND RETINAL DISEASE

A look at the next generation of diagnostics and therapeutics.

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Nanobiotechnology can prove useful in retinal diagnostics, pharmacologic and surgical interventions, and drug delivery (Table). Regenerative medicine also stands to benefit from nanoscience. The retina—for which therapeutics and surgery are in the range of micrograms and microns, respectively, combined with its relative immune privilege and accessibility—is ideally suited for nanobiotechnological innovations. Here, we review the state of nanotechnology in the field of retina.

DIAGNOSTICS

Today's retinal imaging, although a far cry from the tools used decades ago, remains imperfect in several ways. For example, the specificity of neovascular structures on OCT can be limited by optical artifacts from adjacent vascular structures. Furthermore, OCT angiography is unable to directly demonstrate leakage. Nanotechnology may be a useful innovation to combat these limitations.

Researchers are exploring the utility of gold nanoparticles (AuNPs) as contrast agents for OCT because they are small enough (almost 3 µm in diameter) to pass through retinal and choroidal vessels, with wavelength absorption efficiencies higher than conventional angiographic dyes.[1] For example, researchers used AuNPs to enhance boundary contrast and increase OCT signaling when examining rabbit skin.[1] Iron-titanium dioxide NPs have also been investigated as a viable contrast agent in swept-source OCT.[2]

Atomic force microscopy can create 3D surface profiles of retinal cells, organelle membranes, and nanostructures such as rhodopsin molecules.[3] Using this technology, researchers discovered that rhodopsin assembles in rows of dimers and paracrystals; they also realized that the rhodopsin dimer is the building block of higher-order structures.[3]

Another tool under investigation, photoacoustic microscopy, uses high-resolution identification of endogenous chromophores (such as hemoglobin and melanin) which, when integrated with OCT, can enhance chorioretinal localization of oxygen saturation measurements.[4,5]

Transistor-like circuitry incorporating functionalized carbon nanotubules and gold nanoarrays (with dimensions less than 10 nm) can detect single molecules of DNA and other biologic molecules by changing surface conductivity.[6] Similarly, nanopore DNA sequencing takes advantage of changes in conductivity when DNA nucleotides are sequentially passed through 2 nm nanopores.[7] By increasing diagnostic sensitivity at the molecular level,

Check out a listing of retinal nanodiagnostics in the pipeline at *retinatoday.com:*

these next-generation sequencing technologies may one day replace time-consuming, off-site analyses with rapid point-of-service testing.

THERAPEUTICS: DRUGS, DELIVERY SYSTEMS, AND TARGETING

Nanoscale therapeutics allow for successful penetration of the blood-brain barrier via endocytosis without the inflammation noted with viral vectors. Self-assembled nanoparticles are coated to form poly(lactic-co-glycolic acid) nanoparticles with slow-release properties.[8] One study found that the biodegradable biopolymer poly(E-caprolactone) embedded with resveratrol demonstrates antioxidant and antiinflammatory properties. The surface was coated with metformin (known to inhibit choroidal neovascularization) and cell-penetrating peptides that increased retinal permeability 15-fold after intravitreal injection.[9]

Topical nanoparticles deliver high potency because of their high surface area-to-volume ratio.[10] In one AMD model, a nanoparticle formulation of aminocaproic acid-Diosgenin delivered antiangiogenic activity with low cellular toxicity.[11] Similarly, research has shown that topical celecoxib-loaded poly(ortho ester) nanoparticles possess antiinflammatory and antiproliferative properties.[12] Another study found that nanoparticles of apatinib—an anti-VEGFR2 tyrosine kinase inhibitor—can penetrate the cornea and target retinal cells.[13]

Dendrimers are highly branched molecules as small as 1.5 nm in diameter that can allow for delivery of multiple drugs, as well as effective sustained delivery. Systemic dendrimer-based molecules have demonstrated selective, passive retinal pigment epithelium (RPE) uptake of a steroid in damaged cells, which can suppress both inflammation and choroidal neovascularization.[14]

SURGICAL ADJUNCTS

Researchers have investigated the utility of coating surgical instruments with silver nanoparticles, which confer antiinfective and antioxidative properties.[15] Other investigators have designed nanotube tweezers by fusing together two carbon nanotubes with a spacing of 10 nm.[16] Others have proposed direct nerve fiber axon repairs using a knife edge with a 20 nm radius of curvature, with attachment of a transplanted axon segment performed by electrofusion.[17]

Hyaluronic acid-coated AuNPs have been found to cluster on vitreous opacities in vivo. Sauvage et al found that low-energy nanosecond laser pulses can create vitreous nanobubbles by heating the AuNPs and ablating vitreous opacities with lower energy levels, reducing the risk of complications compared with Nd:YAG vitreolysis.[18]

NANOPROSTHESES

The smallest nanoscale transistors are 1 nm long, and diodes are as small as one molecule in size—technology that could increase the resolution of current subretinal implants. For example, researchers are investigating poly(3-hexylthiophene) nanoparticles injected into the subretinal space, mimicking the spatial distribution of photoreceptors, to form a light-sensitive interface.[19] Others are exploring carbon nanotubule prosthetics integrated with neural tissue to guide synaptic development during neuronal repair,[20] which could help patients with trauma or retinal degeneration.

REGENERATIVE NANOBIOTECHNOLOGY

Future therapies will not only protect retinal tissues, but also repair and regenerate structure and function. AuNPs, for instance, are relatively inert and possess intrinsic antiinflammatory and antiangiogenic properties.[21] Nanoceria is another powerful example of a reactive oxygen species scavenger that can help to promote and regulate healthy angiogenesis while demonstrating anti-VEGF properties.[22] Dexamethasone-conjugated dendrimers demonstrate selective affinity for damaged retinal Müller glial cells, promoting regenerative stem cell-like properties in mammals while minimizing the potential for systemic toxicity.[23]

Carbon nanotubules are not only useful in diagnostics, but also can function as radical scavengers,[24] potentially reducing oxidative stress thought to play a vital role in progression to wet AMD. Similarly, fullerenes are inherently antioxidative and antiinflammatory and are showing promise as a therapeutic approach to arthritis.[25]

Intravitreal oxygen nanobubbles have been used to deliver oxygen to the inner retina for rescue from ischemic damage.[26] Furthermore, platinum nanozymes have been used to counteract light-induced photoreceptor degeneration and inflammation in a rodent AMD model.[27]

Magnetic nanoparticles are being used for targeted stem cell delivery within the eye. Yanai et al describe a technique of magnetizing rat mesenchymal stem cells and, after intravenous injection, inducing migration and localization to the inner and outer retina with a magnet placed in the orbit.[28]

Researchers are also exploring natural nanofiber scaffolds from gelatin, chitosan, collagen, and hyaluronic acid. These

AT A GLANCE

- Nanotechnology, such as gold nanoparticles, atomic force microscopy, and photoacoustic microscopy, may help to address the limitations of current imaging modalities.
- Nanoscale therapeutics allow for successful penetration of the blood-brain barrier via endocytosis without the inflammation common with viral vectors.
- Inorganic nanoparticles hold much promise for improving the success of various gene therapies.

TABLE. POTENTIAL RETINAL NANOTHERAPEUTICS AND THERANOSTICS

Retinal Application	Nanoparticle	Model	Conclusion
Precision cell ablation (ie, tumors)	Gold nanoparticles[14]	Various cellular targets	Aggregate gold nanoparticles enhance detection and treatment over single nanoparticles
Gene therapy	Gold nanoparticles[15]	Various plasmid, minivector DNA and siRNA vectors	Gold nanoparticles enhance nucleic acid delivery
AMD	Antiangiogenic peptides[19]	Murine	Angiogenesis decreases for at least 14 weeks after a single dose
AMD	Cell-penetrating peptides[20]	Rat	Single dose improves retinal permeability, increases antioxidant retention, and suppresses neovascularization for 56 days
Ocular tumors	Functionalized Q-dots[16]	Human osteosarcoma cells	For single-cell microscopy, exhibit strong fluorescence and hypersensitivity and are non-toxic and biologically inactive
Ocular tumors	Magnetic nanoparticle (MNP) hyperthermia[17,18]	Zebrafish embryos,[17] humans[18]	Functionalized MNPs preferentially localize to the choroid and RPE,[17] thermotherapy using MNPs was proven safe and effective[18]
AMD	Topical nanoemulsions[22,23,25]	Primate,[22] human embryonic kidney cells, rat Müller cells,[23] rats[25]	Topical drug could penetrate the cornea and blood-retina barrier,[22] improve long-acting intraocular bioavailability of hydrophobic celecoxib,[23] and enhance retinal accumulation of anti-VEGF apatinib[25]
AMD	Dendrimers[31]	Human donor eyes, rats	Pathology-dependent biodistribution, suppression of choroidal neovascularization, and cytokine suppression
AMD	Self-assembling polymeric micelles[28]	Various in vitro and in vivo	Improved bioavailability, bioactivity, intracellular penetration, controlled delivery, and retention time
AMD	Nanoceria[22]	Human ARPE-19 and umbilical endothelium cell lines	Antiinflammatory, antiangiogenic, and antiapoptotic properties

have been shown to promote RPE growth and release of regenerative factors, with superior cellular adhesion to that of synthetic scaffolds.[29] Electrospinning, a technique whereby nanofibers are created by extrusion of a polymer solution, has recently been used to create a nanofiber scaffold upon which RPE cells can be cultured with the possibility of subsequent subretinal transplantation.[30]

FUTURE DIRECTIONS

Nanotechnology holds much promise for improving the success of various gene therapies. For example, carefully designed inorganic nanoparticles (eg, polymers, silicone, and organometallic composites) may overcome the challenges of crossing the blood-retina barrier, rapid degradation, and gene/cellular toxicity.[31] Researchers have also harvested IPSC-derived RPE cells from fibroblasts or mononuclear blood cells by incubation with various protein-coding genes, such as *LEFTY2*.[32] Müller glial cells, furthermore, have been shown to transdifferentiate to rod photoreceptors using the sonic hedgehog gene, *SHH*.[33] Targeted delivery of mRNA to the RPE, Müller glia, and neural retina has been accomplished using lipid nanoparticles.[34,35] Laser-enhanced delivery of genetic material to precise areas of retinal degeneration using optoporation is a novel technique that could be used to deliver gene-laden lipid nanoparticles or other non-viral nanoparticles.[36,37]

Exosomes, bilayered nanovesicles that can be as small as 30 nm in size, are showing promise as disease biomarkers, intercellular communication vehicles, and drug delivery vehicles.[38] Exosomes have been shown to transport microRNA between RPE cells and retinal glial cells, which could prove useful in modulating senescence and apoptosis of the retina in conditions such as AMD and diabetic retinopathy.[39]

Scientists have successfully used external magnetic fields to guide magnetic nanoscale micropropellers to traverse the vitreous cavity with the potential to deliver therapeutics to the retina (Figure); such nanorobots could conceivably be engineered to diameters approaching 2 nm.[40,41]

Others have investigated a polyacrylamide nanoparticle integrated with neurotrophin nerve growth factor with an affinity for retinal rods and cones to prevent retinal cell apoptosis.[42] Similarly, oligochitosan-coated nanoceria demonstrates antiangiogenic, antiinflammatory, and antiapoptotic characteristics in cellular AMD models.[43]

Researchers have found that lipid nanoparticles combined with targeting peptides and messenger RNA can bypass retinal barriers that have otherwise limited access to photoreceptors, a critical target in designing gene therapies for inherited retinal diseases.[44]

Simna et al recently engineered photoreceptors using

a nanoparticle-mediated delivery of a full-length human rhodopsin gene to murine rod photoreceptors, which could be useful in future treatment of retinitis pigmentosa.[45] Kwon et al synthesized melanin-like nanoparticles that could be used as an artificial melanin substitute in murine RPE cells.[46]

ONLY THE BEGINNING

We have only begun to scratch the surface of what is possible with therapeutic and regenerative applications of nanotechnology in the field of retina. We look forward to seeing where this field of investigation takes us. ■

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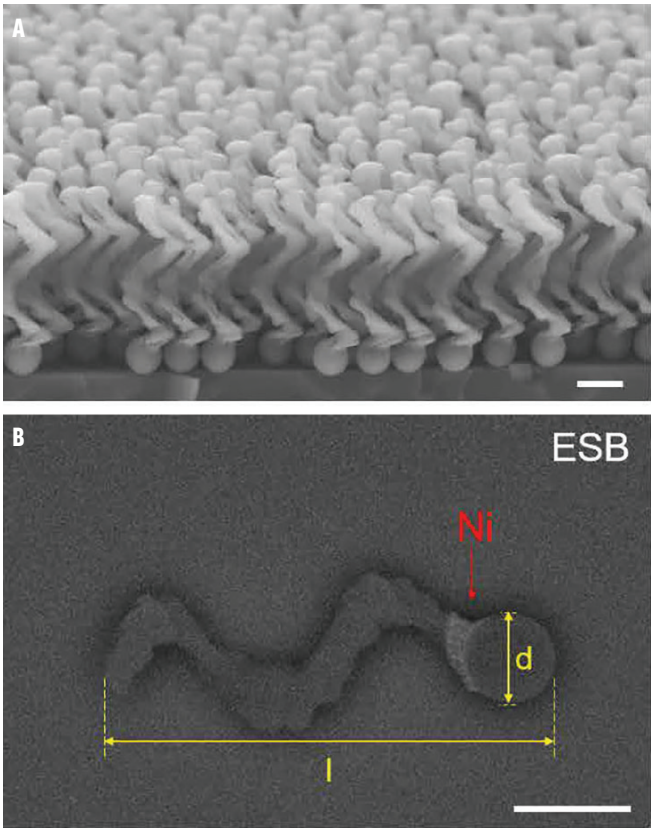

Figure. These scanning electron microscope (A) and energy-selective backscatter scanning electron microscope (B) images depict micropropellers that can cross the vitreous cavity. Scale bar = 500 nm. Reprinted with permission from Wu et al.[40]

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