Nanotechnology in Retinal Diseases

Several promising areas under study, including drug and gene delivery.

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Nanotechnology involves the creation and use of devices and materials on a very small scale, the scale of intracellular structures and molecules. The dimensions of these systems and constructs are generally 100 nm or smaller.

More specific to the field of medicine, nanomedicine is defined as the monitoring, repair, construction and control of human biological systems at the molecular level, using engineered nanodevices and nanostructures.[1]

Nanomedicine involves the application of nanoscale technologies to the practice of medicine, whether in diagnosis, treatment, or prevention.[2] Nanotechnology may have an impact on the treatment of retinal disease through gene delivery, drug delivery, cell delivery, retinal neural prosthetics, and nanosurgery. This article gives an overview of what we may have to look forward to in the field of vitreoretinal therapy when the promise of nanomedicine is fulfilled.

GENE DELIVERY

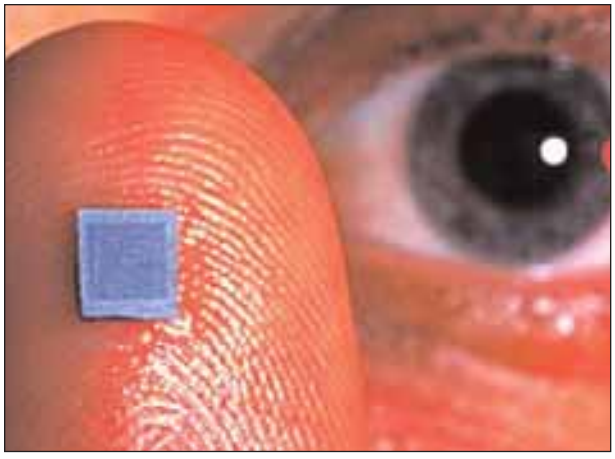
Gene delivery has been attempted with viral vectors, but these approaches are associated with risks such as immunogenicity and mutagenesis. Nonviral vectors such as polymers and lipids also have the capability to carry genes, but with lower risk of immunogenicity, lower cost, and greater ease of production than viral vectors.

The electrostatic interaction of cationic polymers with RNA or DNA molecules carrying a negative charge results in condensation and formation of the material into particles in the nanoscale range (ie, several hundred nanometers in diameter). These polymer nanoparticles can protect genes from enzymes and mediate their entry into cells.

Incani and colleagues[3] found that polypexes—complexes of cationic polymers with plasmid DNA—can have transfection efficiencies comparable to adenoviral vectors but with reduced safety risks.

Courtesy of Dr. Chih-Ming Ho (http://www.n-base.org/news/index.html).

Figure 1. Biocompatible film for drug delivery. Twenty-micron-thick biocompatible film with multiple drug-loaded nanoparticles for sustained drug delivery.



Courtesy of Dr. Chih-Ming Ho (http://gtresearchnews.gatech.edu/newsrelease/NEEDLES.html).

Figure 2. Microneedle array. The size of the experimental microneedle array is shown by its placement on the researcher's finger. There are 400 needles in the array.

Incani was working with gene delivery to bone marrow stromal cells, but nanoparticles have also been investigated as a means to deliver genes for the treatment of ocular disease.[4-7] Thus far, nanoparticles have been stable, noninflammatory, nonimmunogenic, and nontoxic in the eye.

Farjo and colleagues, led by Muna I. Naash, PhD, at the University of Oklahoma, used a particular polypex, polyethylene glycol, to deliver a gene in the retinal degeneration slow (rds) mouse. RDS (also known as peripherin 2) is a glycoprotein involved in photoreceptor structural development, stability, and outer segment disc renewal. Mutations in the RDS protein in humans can be associated with retinitis pigmentosa (RP) and with adult-onset hereditary macular degeneration. Mice heterozygous for *rds* mutation develop a typical autosomal dominant RP phenotype with early-onset rod and late-onset cone degeneration.

Naash's group made nanoparticles about 150 nm long and 8 nm wide using polyethylene glycol and complementary DNA of the normal mouse protein that is mutated in the *rds* mouse.[8] The particles were injected into the subretinal space of the heterozygous *rds* mouse, and the gene was taken on and specifically expressed by the photoreceptors. Electroretinogram (ERG) results under scotopic conditions show moderate improvement in the polypex-treated mice, but under photopic conditions the ERG amplitude in the treated mice is almost equal to that in the normal mouse.

Ultrastructurally, 120 days after injection, mice treated with the normal gene have a thicker outer photoreceptor layer compared with untreated animals. The inner segments are longer, and the outer segments are more healthy, as can be seen on transmission electron microscopy.

DRUG DELIVERY

Nanoparticles are also being investigated for use in drug delivery. Colloidal nanoparticle carrier systems can improve the efficacy of drug delivery by overcoming diffusion barriers, reducing the size of drug doses through more efficient tissue targeting, and allowing sustained delivery. These features are all attractive for the treatment of chronic diseases such as glaucoma or macular edema due to choroidal neovascularization or venous occlusion. Nanoparticles are also promising for targeted delivery of drugs to intraocular tumors.

Sustained submacular delivery might be enhanced with the use of a biocompatible film that serves as a carrier for drug-loaded nanoparticles (Figure 1). For an extraocular approach, arrays of hundreds of microneedles that can penetrate the sclera to deliver drugs have been designed using microfabrication technology (Figure 2).

CELL DELIVERY

Polymer scaffolds, engineered on the nano- and microscale, can increase the survival and differentiation of cells for retinal transplantation. These structures allow the spatial targeting of transplanted cells and can alter cellular attachment and mobility and attenuate the recipient's foreign body response. The scaffolds can also be impregnated with growth factors or designed with specific topographic patterns to enhance cell survival and differentiation.

Tao and colleagues[9] have developed a process to produce microscale thin films, 6 µm to 10 µm thick, made of polycaprolactone (PCL). This is a biocompatible, slowly degrading polymer that may be ideal for transplanting retinal progenitor cells. The polymer is hydrophobic, yet highly permeable by water, and it is well-tolerated in the subretinal space.[10]

The same group also created poly(methyl methacrylate) (PMMA) scaffolds 6 µm thick and used them as a substrate for the growth of mouse retinal progenitor cells, creating composite grafts for transplantation into the subretinal space in the mouse.[11]

Tao and colleagues created pores in the PMMA material about 11 µm in diameter with 63 µm pore separation. They found that the holes enhanced the adherence of cells during transplantation and also allowed greater outgrowth of transplanted cells into the surrounding host tissue. They concluded that the porous PMMA scaffolds could serve as a biocompatible substrate for cell delivery in vivo.[11]

In addition, the surface chemistry of the scaffold can be altered to improve cell transplantation. Working with the Tao group, we pretreated thin PCL sheets with a conditioning medium. In culture, retinal pigment epithelial (RPE) cells did not grow well on scaffolds made with untreated PCL sheets, but on pretreated PCL sheets the RPE proliferated and differentiated (Sugino, Tao, and Zarbin, unpublished observations).

RETINAL PROSTHETICS

Retinal prosthetic devices have been investigated by several centers with the aim of rejuvenating, bypassing, or somehow taking advantage of residual retinal function in patients with retinitis pigmentosa and other inherited degenerative retinal diseases. Challenges to these approaches have included the amount of current needed to create nerve impulses in the retina, which can actually damage the retina, and the density of the electrodes needed to produce meaningful and helpful images in the brain. Nonetheless, much progress has been made using retinal prostheses.[12]

Researchers at the University of California, Berkeley, developed a mechanism to remotely control neuronal activity with a light-gated glutamate receptor.[13,14] A light-activated channel allows manipulation of cell activity with synthetic optical switches. The key part of the molecule is azobenzene. When azobenzene is irradiated with high-energy light, at 380 nm, it undergoes a trans-cis conformational change,

and when irradiated with lower-energy light, at 500 nm, it reverts to the trans configuration. An agonist is tethered to the ligand binding domain via the azobenzene moiety. In the cis conformation, the agonist is moved into the ligand binding pocket, which activates the ion channel. In the trans conformation, the agonist is moved out of the binding pocket. Photoswitching can be performed on a millisecond timescale. Light pulses of 1 to 5 ms length at 380 nm trigger action potentials or sustained depolarizations that persist for minutes in the dark until extinguished by a short pulse of approximately 500 nm light.

The researchers introduced these allosteric photoswitches into zebrafish larvae and found that the switch turns off and on the touch response.[14] Under normal circumstances, if the larvae are touched, they will move away. With the 380-nm light shining on them, the switch is in the cis configuration, which activates the glutamate channel and turns off the touch response.

The promise of these light-activated ion channels for treatment of retinal pathologies is that they may provide a means of controlling the activity of specific neurons downstream from the site of neural damage or degeneration. These channels might restore light-regulated activity in relatively healthy retinal neurons (eg, bipolar cells or ganglion cells) even after the rods and cones have degenerated.

NANOSURGERY

The inability of neuronal cells to regenerate after axonal damage is a challenge not only in ophthalmology but in other areas of neuroscience, neurosurgery, and neurobiology.

Sretavan and colleagues[15] have proposed direct axon repair. This is surgery on the microscale, performed on an axon surgery platform that is a 1-mm cube. The cutting device used is a silicon nitride knife with an ultrasharp edge, mounted on a silicon-based flexion suspension. The radius of curvature of the knife edge is on the order of 20 nm, about the diameter of a single microtubule.

In the microsurgical procedure, the injured axon is incised, and either a healthy donor axon segment is moved into its place or the two ends of the severed axon are mobilized and brought together. Dielectrophoresis is used to mobilize the neural tissue, and cell fusion is stimulated with electrofusion, a technology used to transfer fetal nuclei into oocytes.

Nanotweezers, another device with potential for use in nanosurgery, were described in 1999.[16] They are made by fusing two carbon nanotubes, 4 µm long and 50 nanometers wide, onto a glass micropipette with conducting electrodes connected to it, spaced about 10 nm apart. Because carbon nanotubes conduct electricity, when a voltage gradient is applied and increased across the electrodes, the two ends of the nanotubes come together. The nanotweezers are capable of grabbing and manipulating submicron polystyrene nanoclusters and nanowires.

CONCLUSION

This paper offered only a brief overview of the areas where nanotechnology is beginning to have an effect on ophthalmology. The earliest area of practical application of nanotechnology promises to be in drug and gene delivery, eventually in cell delivery and retinal prosthetics, and ultimately in nanosurgery. ■

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