Nanomedicine and Optic Nerve Regeneration—Implications for Ophthalmology

Rajesh C Rao, MD[1] and Dong Feng Chen, MD, PhD[2]

1. Ophthalmology Resident, Massachusetts Eye and Ear Infirmary, and Research Fellow, Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School; 2. Associate Professor, Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School

Abstract

The optic nerve transmits visual information from the retina to the brain. When injured in adult mammals, the optic nerve does not regenerate. Optic neuropathies such as glaucoma are a leading cause of blindness worldwide. Optic neuropathies can also occur after ischemia, inflammation, infection, neoplasia, trauma, and/or as a result of hereditary conditions. One of the most exciting therapeutic strategies to promote optic nerve regeneration is nanomedicine. Nanomedicine utilizes the assembly and manipulation of structures less than 100 nanometers in size to treat disease. Structural elements such as protein-coated nanofibers and self-assembling peptide scaffolds are designed to enhance axon regeneration. Nanoscale spheres can deliver intraocular pressure-lowering medications and therapeutic proteins. By 'tagging' cells with nanoparticles, stem cell transplants can be tracked and axons redirected via a magnetic field. Finally, nanoparticles with an ability to scavenge the toxic reactive oxygen species generated in hereditary and glaucomatous optic neuropathies may provide a new avenue to treat specific types of optic nerve disorders.

Keywords

Nanomedicine, nanotechnology, nanofibers, optic neuropathy, glaucoma, axon, regeneration

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Correspondence: Dong Feng Chen, MD, PhD, Associate Professor, Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, 20 Staniford Street, Boston, MA 02114. E: dongfeng.chen@schepens.harvard.edu

The optic nerve transmits visual information from the retina to the brain. In humans, the optic nerve is composed of approximately 1.2 million axons of retinal ganglion cells (RGCs). RGC axons course through the nerve fiber layer (NFL) to the optic disc, where they merge to form the axonal bundle of the optic nerve. As RGC axons pass through the disc, they are ensheathed by myelin produced by oligodendrocytes. From the optic nerve, RGC axons form several central projections, including the suprachiasmatic, pretectal and lateral geniculate nuclei, and superior colliculus. Neurons in the lateral geniculate nucleus relay visual information to the visual cortex,[1] while the extra-nuclear pathways are responsible for other visual-associated functions, such as circadian rhythm and orienting movement.[2]

Disorders involving the optic nerve remain an important cause of morbidity. Among them, glaucoma, a group of diseases associated with elevated intraocular pressure (IOP), optic nerve atrophy, RGC and oligodendrocyte death, and a painless, insidious loss of peripheral vision, affects over four million Americans and is the second leading cause of blindness worldwide.[3,4] Vascular insults, due to inflammation of large blood vessels or poor circulation of smaller vessels supplying the optic nerve, can result in ischemic optic neuropathy.[5] Less commonly, optic neuropathies also occur secondary to trauma, during which the optic nerve can be avulsed or transected.[6] In optic neuritis, a condition most commonly associated with multiple sclerosis, immunologic attack results in denuded, demyelinated RGC axons and eventual death of oligodendrocytes and RGCs whose axons comprise the optic nerve.[7] Bacterial, fungal and viral infection of the optic nerve, whether from the eye, brain, or sinus, or as a result of a systemic infection or in an immunocompromised host, can result in extensive inflammation and necrosis. In contrast to glaucoma, these latter conditions typically present with a more acute loss of vision.

Glaucomatous, hereditary, ischemic, infectious, inflammatory, and traumatic injuries in the optic nerve, as in the spinal cord and other parts of the central nervous system (CNS), can be associated with axonal drop-out and (RGC) neuron and oligodendrocyte cell death. Macrophages infiltrate the lesion site and phagocytose cell debris, such as degenerated myelin. In response to these events, reactive gliosis occurs, in which neighboring astrocytes undergo hypertrophy and proliferate. On a larger scale, axonal injury and cell death result in a generalized loss of tissue architecture, which can manifest as widespread demyelination, widened subdural spaces, and formation of cystic structures. In non-infectious and infectious inflammatory disorders, liquefactive necrosis of the optic nerve can occur. Over time, glial scars resulting from astrocyte proliferation often fill in the tissue defect.

When injured from these insults, the nerve fibers that comprise the optic nerve, as in other parts of the mammalian CNS, do not efficiently repair themselves or regenerate. Damaged axons from surviving RGCs may initially attempt to arborize, but are inhibited from doing so by a variety

of cell intrinsic and extrinsic factors, including the presence of myelin-associated inhibitory proteins and traversing a dense glial scar.[8,9] Without the ability to reach their axonal targets, neurons lose their neurotrophic support (protein growth factors) and die.[10] New approaches to enhance CNS repair remain important clinical goals in regenerative medicine.

Nanomedicine

One of the most exciting emerging therapeutic strategies to promote optic nerve (and CNS) regeneration is nanomedicine. Nanomedicine uses the production, assembly, and control of structures, devices, or molecules less than 100 nanometers (nm) in size to treat disease. The dimensions of nanoscale materials are particularly amenable to treatment of optic nerve neuropathies because they can penetrate intracellularly and be fabricated into dimensions mimicking the extracellular matrix (ECM) (e.g. collagen, laminin, fibronectin) disrupted in CNS injury (resulting from axonal dropout, demyelination, apoptosis, necrosis, and glial scarring).

Nanoscaffolds

One experimental approach involves engineering a variety of so-called nanofiber scaffolds for transplantation into the CNS. Prefabricated nanofiber scaffolds can be constructed *ex vivo* by a method known as electrospinning. In this technique, a polymer or composite material is extruded out of a thin syringe at a constant rate in the presence of a strong electrical field. Electrostatic forces 'stretch and whip' the electrified jet of polymer to form aligned or non-aligned fibers with nanoscale diameters.[11] Popular biodegradable materials used to fabricate these scaffolds include poly(lactic-co-glycolic acid) (PLGA), poly(L-lactide-co-epsilon-caprolactone) (PLCL) and poly(L-lactic acid) (PLLA).[12] These fibers have been used as a substrate for neural cells, where their growth *in vitro* more closely models 3D growth seen *in vivo* than standard 2D culture.[13]

The combination of nanofibers coated with proteins has been shown to promote nerve regeneration in recent studies. Polymers can be mixed with neurotrophic proteins to generate coupled growth factor-encapsulated fibers with defined protein release kinetics. Recent *in vivo* transplantation of a glial-derived neurotrophic factor (GDNF)–nanofiber composite scaffold into transected rat sciatic nerve (peripheral nervous system) showed accelerated histologic regeneration of the nerve, increased myelinated axons, and improved electrophysiologic function.[14] In a study involving optic nerve injury, a polymer composed of polyglycolic acid (PGA, synthetic) and chitosan (a natural material derived from the exoskeleton of crustaceans), was coated with a recombinant neuronal adhesion protein called L1. This molecule is expressed on elongating axons of CNS neurons during development and regeneration.[15] An earlier study demonstrated that rats treated with soluble L1 following spinal cord injury achieve some degree of locomotor recovery.[16] L1-coated PGA–chitosan conduits were placed to bridge transected optic nerve stumps *in vivo* and compared with non-L1-coated PGA–chitosan, and showed less macrophage invasion and improved axonal re-growth and myelination.[17] Furthermore, the protein-coated nanofibers promoted more RGC axon regeneration, as shown by retrograde labeling from the superior colliculus, than nanofibers without L1 coating.[17] While this particular study did not address functional visual recovery, it showed that the regenerated axons align along the protein-coated nanofibers and that the nanofiber conduit was absorbed and degraded in two months without significant local toxicity.[17]

Non-prefabricated peptides that are self-assembled *in vivo* to form nanofiber scaffolds (called self-assembling peptide nanofiber scaffolds [SAPNS]) have seen successfully used in a model of optic nerve regeneration. SAPNS are L-amino acids with alternating ionic charges. In physiologic solutions, such as cerebrospinal fluid (CSF) and other human body fluids, saline and tissue culture media, SAPNS form ~10nm-diameter interwoven fibers. When a solution of SAPNS was injected into a tissue gap created after transection of the optic tract in hamsters, resulting in the interruption of signals from the retina to the superior colliculus, the peptides rapidly formed a scaffold to bridge the tissue gap, whereas in saline-injected controls the tissue gap persisted in all animals. Injection of anterograde axon tracers into the eye showed that RGC axonal terminals extended into the superior colliculus, demonstrating axonal regeneration, and re-targeting to their distal projections in the brain, in nearly all the young (P2) hamsters treated with SAPNS versus none in the controls. In fact, the innervation density through the lesion was nearly 80% that of normal (non-lesioned) animals. A similar pattern, though somewhat less impressive an effect, was seen in lesioned SAPNS-treated adult hamsters. Most importantly, 75% of the SAPNS-treated adult animals showed return of functional orienting movement, as assayed by the ability to turn to a small object, whereas all the sham-treated controls remained blind.[18]

Nanosphere Delivery of Therapeutic Agents

Glaucoma is associated with increased IOP, and the reduction of IOP can slow the progression of glaucomatous optic neuropathy.[19,20] To this end, topical pharmacologic agents (beta-blockers, carbonic anhydrase inhibitors, muscarinic receptor agonists, etc.) targeting aqueous fluid production and its drainage through the trabecular meshwork have been used as therapy to decrease IOP. Since many of these topical agents need to be instilled into the eye multiple times per day, one practical challenge in medical therapy of glaucoma remains patient compliance. Recently, PLGA–PLLA nano- and microspheres have been developed to deliver encapsulated timolol (a beta-blocker) and neurotrophic proteins (known to enhance neural cell survival, differentiation, and regeneration) with sustained release over three months.[21,22] These polymeric nano- and microspheres can be injected subconjunctivally and may offer a novel approach to improving patient compliance and progression of glaucomatous disease. Nanoparticle-mediated introduction of foreign DNA into cells (transfection), in contrast to other gene delivery methods such as electroporation and viral-mediated transduction, may offer another route to 'tag' particular cell types for imaging or to supply missing genes to cells in specific hereditary disorders. A pilot study comparing DNA constructs complexed with chitosan, PCEP (poly(((cholesteryl oxocarbonylamido ethyl) methyl bis(ethylene) ammonium iodide) ethyl phosphate)), and magnetic nanoparticles to intravitreally and subretinally deliver DNA encoding fluorescent proteins into the retina was conducted. The study showed that while all three constructs transfected cells, only PCEP and magnetic nanoparticles did not induce an inflammatory response and, of the two, magnetic nanoparticles had a superior transfection rate.[23] One can imagine delivering genes encoding for neurotrophic proteins to RGCs and other retinal neurons and glia to enhance ganglion cell survival.

Nanomedicine Adjuncts to Surgery

Nanomedicine may also have application in surgical therapy of glaucomatous optic neuropathy. Currently, in patients with poor

compliance or those who are otherwise refractory to glaucoma medication, surgical approaches are used to drain aqueous humor, generally by placing one end of a polymeric tube into the anterior chamber and the other to the conjunctiva (external part of the eye); such approaches have been limited by protein plugging and bacterial ingression (as one end of the tube is exposed to the external environment). A recent study has detailed the fabrication of a so-called 'nano-drainage' implant.[24] Here, pores with diameters of 100nm are etched onto extremely thin silicon wafers using electron-beam lithography. The size of the pores essentially blocks bacterial penetration, and the silicon surface is modified with low molecular weight coatings to decrease protein clogging. While these nano-drains remain to be tested, they potentially represent a safer, less invasive, and cheaper alternative to current surgical treatment for refractory glaucoma and prevention of optic neuropathy.

Nanoparticle Tagging

The transplantation of neural stem and other precursor cells represents a promising strategy for cell therapy of CNS diseases, including retinal degenerations, spinal cord injury, and Parkinson's disease.[25–27] Ideally, the transplanted cells mature into photoreceptors, oligodendrocytes, and dopaminergic neurons that replace the endogenous cell types lost in these diseases; however, their therapeutic effect may also be exerted through trophic signals they secrete (such as neurotrophic factors). The recent use of superparamagnetic nanoparticles to magnetically tag cells has allowed non-invasive *in vivo* imaging of grafted cells by magnetic resonance imaging (MRI). In this process, prior to transplantation, cells are incubated in a solution containing nanosize particles such as iron oxide. Cells take up the nanoparticles by endocytosis and are 'tagged'.[28] Following transplantation, the grafted cells, such as oligodendocyte precursors, can be imaged *in vivo* as they divide, migrate, differentiate, and remyelinate areas of the injured spinal cord.[29] One can imagine a similar process to correlate cellular behavior of grafted nanoparticle-tagged stem cell derived-RGCs or oligodendrocytes with functional visual recovery in transplanted patients with glaucoma, optic neuritis, or other optic neuropathies, using MRI. Moreover, there may be ways to deliver superparamagnetic nanoparticles into endogenous CNS cells such as RGCs or oligodendrocyte precursors. An external magnetic field may then exert tensile forces on regenerating axons or processes of oligodendrocytes, to retarget axons and enhance remyelination after optic nerve injury (www.nsti.org/procs/Nanotech2007v2/10/X68.03).

Nanoscale Scavenging of Reactive Oxygen Species

Oxidative stress (the generation of reactive oxygen species (ROS) from aerobic metabolism and/or intracellular signaling cascades) may contribute to the pathogenesis of a variety of optic neuropathies[30] and may be a target for the application of nanomedicine. Mutations in enzymes that regulate oxidative phosphorylation (source of ROS) are the cause of a hereditary disease known as Leber's optic neuropathy. Acute, chronic, and moderate IOP elevation is associated with oxidative stress in the retina. Sera from glaucoma patients show upregulation of enzymes associated with increased oxidative stress, including changes in antioxidant metabolism and oxidative modification of retinal proteins.[31–34] Modulating the activity of antioxidant enzymes in animal models has been shown to protect RGCs in a specific type of glaucoma and Leber's optic neuropathy and reduce demyelination in optic neuritis.[35–37] Rare-earth cerium nanoparticles (called 'nanoceria'), some 5nm in diameter, exhibit a high affinity to scavenge ROS. Importantly, intravitreal injection of nanoceria protects and rescues photoreceptors from light-induced degeneration, as assayed by calculating rates of photoreceptor apoptosis and by electroretinography.[38] It will be interesting to determine whether nanoceria exert a protective effect on RGCs and oligodendrocytes in glaucoma, optic neuritis, and mitochondrial or other optic neuropathies.

Conclusion

The translation of nanotechnology to therapy has been eagerly pursued. The principal advantages of nanomedicine lie in the tiny size of nanoparticles to penetrate into cells, thereby delivering therapeutic genes, proteins, and drugs. These particles can mimic extracellular structures to direct or enhance cell growth, migration, survival, and axonal regeneration.

By contrast, the facile integration of nanoparticles into the body also raises questions of toxicity. For example, chitosan remains a promising nanoscale, biodegradable material, with success *in vitro* and *in vivo* in peripheral nerve and optic nerve (see discussion above). However, *in vivo* introduction of DNA–chitosan complexes intravitreally causes monocyte infiltration into the normally acellular vitreous and perivascular inflammation with phagocystosis of the nanoparticles within the retinal substance, with subsequent retinal neuron degeneration.[23] Other types of nanomaterials, such as carbon nanotubes, have been shown to incite human T-cell death and stimulate increased intracellular signaling associated with an immune response.[39] Future studies will require rigorous testing of these and other, novel nanoscale materials in a variety of animal models (not solely rodents) to ensure that the therapeutic benefits of nanomedicine will be optimized and predictable and not be outweighed by toxic side effects.

Despite these concerns, nanomedicine represents a promising and diverse modality for a variety of human disorders, and may have a special place in the treatment of eye diseases. From the transplantation of nanofibers and ROS-scavenging nanomaterials to nanosphere delivery of drugs and trophic proteins, nanomedicine offers multipronged strategies for the treatment of optic neuropathies. ■

1. Vrabec JP, Levin LA, The neurobiology of cell death in glaucoma, *Eye*, 2007;21(Suppl. 1):S11–4.
2. Hankins MW, Peirson SN, Foster RG, Melanopsin: an exciting photopigment, *Trends Neurosci*, 2008;31(1):27–36.
3. Resnikoff S, Pascolini D, Etya'ale D, et al., Global data on visual impairment in the year 2002, *Bull World Health Organ*, 2004;82(11):844–51.
4. Nakazawa T, Nakazawa C, Matsubara A, et al., Tumor necrosis factor-alpha mediates oligodendrocyte death and delayed retinal ganglion cell loss in a mouse model of glaucoma, *J Neurosci*, 2006;26(49):12633–41.
5. Fontal MR, Kerrison JB, Garcia R, Oria V, Ischemic optic neuropathy, *Semin Neurol*, 2007;27(3):221–32.
6. Sarkies N, Traumatic optic neuropathy, *Eye*, 2004;18(11):1122–5.
7. Compston A, Mechanisms of axon–glial injury of the optic nerve, *Eye*, 2004;18(11):1182–7.
8. Cho KS, Yang L, Lu B, et al., Re-establishing the regenerative potential of central nervous system axons in postnatal mice, *J Cell Sci*, 2005;118(Pt 5):863–72.
9. Nakazawa T, Takeda M, Lewis GP, et al., Attenuated glial reactions and photoreceptor degeneration after retinal detachment in mice deficient in glial fibrillary acidic protein and vimentin, *Invest Ophthalmol Vis Sci*, 2007;48(6):2760–8.
10. Caleo M, Menna E, Chierzi S, et al., Brain-derived neurotrophic factor is an anterograde survival factor in the rat visual system, *Curr Biol*, 2000;10(19):1155–61.
11. Ashammakhi N, Ndreu A, Nikkola L, et al., Advancing tissue engineering by using electrospun nanofibers, *Regen Med*, 2008;3(4):547–74.
12. Bhang SH, Lim JS, Choi CY, et al., The behavior of neural stem

cells on biodegradable synthetic polymers, *J Biomater Sci Polym Ed*, 2007;18(2):223–39.
13. Yang F, Murugan R, Wang S, Ramakrishna S, Electrospinning of nano/micro scale poly(L-lactic acid) aligned fibers and their potential in neural tissue engineering, *Biomaterials*, 2005;26(15):2603–10.
14. Chew SY, Mi R, Hoke A, Leong KW, Aligned protein–polymer composite fibers enhance nerve regeneration: a potential tissue-engineering platform, *Adv Funct Mater*, 2007;17(8):1288–96.
15. Doherty P, Williams E, Walsh FS, A soluble chimeric form of the L1 glycoprotein stimulates neurite outgrowth, *Neuron*, 1995;14(1):57–66.
16. Roonprapunt C, Huang W, Grill R, et al., Soluble cell adhesion molecule L1-Fc promotes locomotor recovery in rats after spinal cord injury, *J Neurotrauma*, 2003;20(9):871–82.
17. Xu G, Nie DY, Wang WZ, et al., Optic nerve regeneration in polyglycolic acid–chitosan conduits coated with recombinant L1-Fc, *Neuroreport*, 2004;15(14):2167–72.
18. Ellis-Behnke RG, Liang YX, You SW, et al., Nano neuro knitting: peptide nanofiber scaffold for brain repair and axon regeneration with functional return of vision, *Proc Natl Acad Sci U S A*, 2006;103(13):5054–9.
19. Comparison of glaucomatous progression between untreated patients with normal-tension glaucoma and patients with therapeutically reduced intraocular pressures. Collaborative Normal-Tension Glaucoma Study Group, *Am J Ophthalmol*, 1998;126(4):487–97.
20. Nickells RW, Schlamp CL, Li Y, et al., Surgical lowering of elevated intraocular pressure in monkeys prevents progression of glaucomatous disease, *Exp Eye Res*, 2007;84(4):729–36.
21. Bertram JP, Saluja SS, McKain J, Lavik EB, Sustained delivery of timolol maleate from poly(lactic-co-glycolic acid)/poly(lactic acid) microspheres for over 3 months, *J Microencapsul*, 2009;26(1):26.
22. Nkansah MK, Tzeng SY, Holdt AM, Lavik EB, Poly(lactic-co-glycolic acid) nanospheres and microspheres for short- and long-term delivery of bioactive ciliary neurotrophic factor, *Biotechnol Bioeng*, 2008;100(5):1010–9.
23. Prow TW, Bhutto I, Kim SY, et al., Ocular nanoparticle toxicity and transfection of the retina and retinal pigment epithelium, *Nanomedicine*, 2008;4(4):340–9.
24. Pan T, Brown JD, Ziaie B, An artificial nano-drainage implant (ANDI) for glaucoma treatment, *Conf Proc IEEE Eng Med Biol Soc*, 2006;1:3174–7.
25. Kim JH, Auerbach JM, Rodriguez-Gomez JA, et al., Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease, *Nature*, 2002;418(6893):50–6.
26. Keirstead HS, Nistor G, Bernal G, et al., Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury, *J Neurosci*, 2005;25(19):4694–705.
27. MacLaren RE, Pearson RA, MacNeil A, et al., Retinal repair by transplantation of photoreceptor precursors, *Nature*, 2006;444(7116):203–7.
28. Bulte JW, Douglas T, Witwer B, et al., Magnetodendrimers allow endosomal magnetic labeling and *in vivo* tracking of stem cells, *Nat Biotechnol*, 2001;19(12):1141–7.
29. Bulte JW, Zhang S, van Gelderen P, et al., Neurotransplantation of magnetically labeled oligodendrocyte progenitors: magnetic resonance tracking of cell migration and myelination, *Proc Natl Acad Sci U S A*, 1999;96(26):15256–61.
30. Tezel G, Oxidative stress in glaucomatous neurodegeneration: mechanisms and consequences, *Prog Retin Eye Res*, 2006;25(5):490–513.
31. Yang J, Tezel G, Patil RV, Romano C, Wax MB, Serum autoantibody against glutathione S-transferase in patients with glaucoma, *Invest Ophthalmol Vis Sci*, 2001;42(6):1273–6.
32. Wax MB, Tezel G, Kawase K, Kitazawa Y, Serum autoantibodies to heat shock proteins in glaucoma patients from Japan and the United States, *Ophthalmology*, 2001;108(2):296–302.
33. Farkas RH, Chowers I, Hackam AS, et al., Increased expression of iron-regulating genes in monkey and human glaucoma, *Invest Ophthalmol Vis Sci*, 2004;45(5):1410–7.
34. Tezel G, Yang X, Cai J, Proteomic identification of oxidatively modified retinal proteins in a chronic pressure-induced rat model of glaucoma, *Invest Ophthalmol Vis Sci*, 2005;46(9):3177–87.
35. Chalasani ML, Radha V, Gupta V, et al., A glaucoma-associated mutant of optineurin selectively induces death of retinal ganglion cells which is inhibited by antioxidants, *Invest Ophthalmol Vis Sci*, 2007;48(4):1607–14.
36. Qi X, Lewin AS, Sun L, et al., Suppression of mitochondrial oxidative stress provides long-term neuroprotection in experimental optic neuritis, *Invest Ophthalmol Vis Sci*, 2007;48(2):681–91.
37. Qi X, Sun L, Hauswirth WW, et al., Use of mitochondrial antioxidant defenses for rescue of cells with a Leber hereditary optic neuropathy-causing mutation, *Arch Ophthalmol*, 2007;125(2):268–72.
38. Chen J, Patil S, Seal S, McGinnis JF, Rare earth nanoparticles prevent retinal degeneration induced by intracellular peroxides, *Nat Nanotechnol*, 2006;1(2):142–50.
39. Bottini M, Bruckner S, Nika K, et al., Multi-walled carbon nanotubes induce T lymphocyte apoptosis, *Toxicol Lett*, 2006;160(2):121–6.