EDUCATIONAL ARTICLE

Nanotechnology in regenerative ophthalmology and imaging

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ABSTRACT

Nanomedicine, the reincarnation of Feynman's vision into a highly evolving medical branch, is revolutionizing medical research and offers solutions in many previously unresolved medical problems. The contribution of this branch in the field of regenerative ophthalmology and imaging is vast and is expected to deliver major breakthroughs. The present review not only summarizes, but offers a comprehensive insight into the use of nanomaterials for both therapeutics and imaging of major ophthalmological degenerative diseases. The result of various studies utilizing a plethora of nanomaterials for diseases that concern retinal regeneration, corneal tissue, ocular surface and diseases like glaucoma and cataract have been summarized accordingly. Also, the miscellaneous use of nanomaterials in imaging, both as contrast agents alone and in conjunction with a functional imaging platform, have been presented. The purpose of this review is to provide accurate and comprehensive insight on the rapidly evolving field of "nano-ophthalmology", so that existing and future obstacles can be surpassed.

Keywords: nanotechnology, nanoparticles, nanomedicine, nanodisks, nanorods, dendrimers, nanoscaffolds, nanoceria, ophthalmology, regenerative medicine, ocular regeneration, cell regeneration, retinal regeneration, nanotherapeutics, imaging, corneal tissue, glaucoma, cataract, imaging platform.

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INTRODUCTION

Nanotechnology is a highly evolving field of modern science with roots in chemistry, physics, biology and engineering and applications that can be seen in everyday life scenarios to highly specialized and demanding fields of science. The term nanotechnology has been used since the mid-1980s to describe a concept that Richard Feynman has first described in his lecture "There's plenty room at the bottom". Since Feynman's vision, the development of nanomachines that are able to develop other nanomachines and products by using atom-by-atom control (molecular manufacturing), the term nanotechnology has broadened. According to US National Science and Technology Council (NSTS), 2000- "the essence of nanotechnology is the

ability to work at the molecular level, atom by atom, to create large structures with fundamentally new molecular organization."[1] Nanomedicine is term describing the applications of nanotechnology to medicine. Since most workings on a cellular level occur on the nanoscale level and many significant molecules have nanoscale dimensions, it is obvious that technological products in that same scale could have many applications on medical treatments.[2,3] The first nanomedical technique for tissue regeneration took place in 1902 by Alexis Carrel, by replacing or repairing inflamed tissues by transferring cells and tissue constructs to the body.[4,5] The goals of nanomedicine are to develop nanoparticles (NPs) for prophylactic, diagnostic and therapeutic applications. Some of the already FDA approved nanomedicine applications in the field of ophthalmology are: Pegaptanib, a PEG-anti-VEGF aptamer for wet age-related macular degeneration and a Liposomal verteporfin for wet age-related macular degeneration, pathological myopia and ocular histoplasmosis syndrome.[3] The field of regenerative ophthalmology is rapidly evolving and in conjunction with the field of nanomedicine it is only a matter of time before a massive breakthrough. Not only the nanotherapeutics though, but also the contribution of nanomedicine in imaging techniques (in this article specifically in the field of ophthalmology) may have a huge impact on diagnostics as well as on the follow up of therapeutic acts. This article highlights the current nanomedicine leaps on tackling major ophthalmological degenerative diseases, not only in the therapeutic aspect, but also in the field of imaging.

NANOTHERAPEUTICS

Retinal regeneration

Many retinal degenerative diseases (age-related macular degeneration, retinitis pigmentosa, Stargardt's disease and diabetic retinopathy) manifest clinically as retinal degeneration, with retinal progenitor cell (RPC) and photoreceptor degeneration as most common outcomes that leads to visual disability or vision loss.[5] Various nanostructures have been used for targeting specific tissues and cells for retinal regeneration. The most common are: nanoparticles, nanowires, hybrid nanostructures and nanoscaffolds.

Nanoparticles:

Gold and Silver nanoparticles have antiangiogenic and anti-inflammatory properties, low or no cytotoxicity, plasmon band, inert nature and good biocompatibility, properties that makes them useful for therapeutics.[5] In vitro studies using 2D and 3D confocal imaging have shown that gold nanoparticles with retinal pigment epithelial (ARPE-19) cells have internalization and biocompatibility dependent to the shape and size of the particles[6] and that gold nanoparticles have no observable inhibitory effect ARPE-19 cells proliferation.[7] Detailed assessment of silver and gold nanoparticle uptake and toxicity (in a mouse's retina in vitro culture, analyzed with transmission electron microscopy) revealed the presence of silver and gold nanoparticles in all neuronal layers of the retina.[5] Gold nanodisk's antiangiogenic effect was evaluated in vitro and indicated an attenuation of VEGF-induced human retinal microvascular endothelial cells, more effective at concentrations of 3 pM and less effective at 1 pM, but with no toxic effects at the endothelial cells from the gold nanodisks even at concentrations of 104 per cell after 48h of incubation.[8] In vivo studies in a murine model of oxygen-induced retinopathy show that intravitreal injection of gold nanodisks attenuate retinal neovascularization in a dose-dependent manner (as indicated by immunostaining of the retina). No signs of inflammation or disruption of the retinal integrity were shown by histological observation as well as no sign of apoptosis.[5] In vivo intravenous administration of gold nanoparticles showed that they pass the blood-retinal barrier with no signs of cytotoxicity in mice.[5] Gold nanoparticles that were intravitreally administrated in Dutch belted rabbits indicated no signs of optic nerve and retina toxicity even 1 month after administration.[9] Therapeutic agents that target directly angiogenesis signaling molecules, are of a great scientific interest lately. Gold nanodisks bind

with VEGF and inhibit VEGF-induced angiogenesis in human retinal microvascular endothelial cells[5] and inhibit aberrant retinal angiogenesis in mice with oxygen-induced retinopathy.[10] Gold nanoparticles also inhibit VEGF-induced migration of choroid retina endothelial cells (RF/6A) through the inhibition of Akt/eNOS signaling pathways.[11] Silver nanoparticles act on the same pathway and inhibit VEGM-induced migration, proliferation and tube formation of bovine retinal endothelial cells.[12] Silver nanoparticles inhibit VEGF-induced matrigel plug angiogenesis in mice. [12,13]

Nanoscale zirconium-porphyrin metal-organic framework (NPMOF) loaded with methylprednisolone (common drug for retinal degenerative diseases), after intraocular administration in adult zebrafish showed faster photoreceptor regeneration with excellent in vivo biocompatibility and low biotoxicity.[14,15]

Recent study on conjugated polymer [poly(3-hexylthiophene), P3HT] nanoparticles showed that the subretinal injection of them mediated light-stimulus-induced stimulation in photoreceptors and rescued vision in a rat model of retinitis pigmentosa.[16]

Vacancy engineered, mixed-valence state cerium oxide (CeO2) nanoparticles, also called nanoceria particles, scavenge reactive oxygen intermediates and utilizing their high surface area:volume ratio they act catalytically, regenerating their activity.[17] Treatment of conditions associated with oxidative damage (AMD, diabetic retinopathy, retinitis pigmentosa) may find nanoceria particles to be of high value.[18]

The development of a diagnostic device by Prow et al[19], has started a new era in the field of theragnostics (individualized for every patient diagnosis of a disease state). A biosensor is based on an enhanced green fluorescent protein (EGFP) reporter gene, driven by an antioxidant response element (ARE) which is then activated by oxidative stress and enhances the expression of genes downstream in its sequence.[18,19] With this nanoparticle, clinicians could diagnose patients who might need therapy later on, at a time where no symptoms were to be observed. This nanoparticle when coupled with a therapeutic gene could create a combined diagnostic-therapeutic device.[18]

Polyplexes (compacted DNA nanoparticles) containing the Rds gene (wild-type retinal degeneration slow), showed an induced photoreceptor rescue in animal model with retinitis pigmentosa.[20,21]

Collagen based nanoparticles were prepared by Sakai et al for the treatment of degenerative disease in rats. With the use of these nanoparticles, basic fibroblast growth factor was delivered to the rats, resulting in sustained retinal rescue.[22,23]

Nanowires:

Nanowires are engineered nanostructural materials with structure and morphology similar to the photoreceptors and comparable photoabsorption and charge separation properties to those of photodetectors or solar cells. In vitro seeding of RPCs on small and large poly (ε-caprolactone) nanowires, showed that they maintained their spheroid shape, whereas individual RPCs create cell-to-cell contact with lamellipodia like structures. Long term in vitro cultures of postnatal retinal cells (including ganglion cells, photoreceptors and bipolar cells), were enabled by nanowire arrays of gallium phosphide that also provided a better attachment for neurons.[14] An in vivo experiment in pigs, where various modifications of PCL (poly-ε-caprolactone) scaffolds where subretinally implanted, indicated that the PCL short nanowire was the most suitable for subretinal implantation.[14] An artificial photoreceptor (Au-TiO2 nanowire array) after subretinal implantation, evoked light activities in the primary cortex of blind mice without affecting the retina and retinal ganglion cells even 5 months after the implantation.[5]

Nanoscaffolds:

Nanoscaffolds consist of synthetic or natural polymers and are self-assembled or electrospun nanofibers that provide an influential microenvironment for proliferation, migration and differentiation of various cells.[24] Natural nanoscaffolds made of ultrathin collagen I membranes were found to be viable substrate for subretinal implantation and retinal

pigment epithelium cell attachment.[25] Stable and suitable for retinal sheet implantation proved to be 1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide cross-linked gelatin scaffolds.[26] Used for human RPCs cell attachment and growth were also decellularized retinal scaffolds.[27] Increased proliferation and survival of human embryonic stem cells was observed with the use of collagen IV-coated porous honeycomb PLA films.[5] A biohybrid scaffold consisting of poly (L-lactic acid-co-ε-caprolactone) and silk fibroin at a ratio of 1:1 was found to promote the proliferation and differentiation of RPCs into photoreceptors[28]. SrAl2O4:Eu2+, Dy3+/CS-PCL (30%) electrospun scaffolds for the repair and reformation of damaged retinal tissues showed better cytocompatibility, proliferation rates and adequate differentiation towards mice retinal progenitor cells, proving their suitability for curing retinal diseases.[29] A recent study in patients with age-related macular degeneration (AMD) used Poly (lactic-co-glycolic acid) nanoscaffold to deliver clinical-grade AMD-patient-derived induced pluripotent stem cell (iPSC) RPE in rodent and porcine laser-induced RPE injury. The iPSC-RPE patches proved to be safe and when the biodegradable PLGA scaffold degraded, the patch was integrated on Burch's membrane while being fully functional.[30]

Dendrimers are highly branched polymers with nanoscale scaffolding and nanocontainer properties that are fully controllable.[31] An in vivo experiment in rats carried out by Marano et al showed that the injection of a lipophilic amino acid dendrimer loaded with anti-VEGF oligonucleotide, inhibited the development of choroidal new vessels for 4-6 months by up to 95% (the anti-VEGF alone showed no treatment benefit), in rats with laser-induced choroidal new vessels while maintaining a good in vivo toleration.[32] The efficacy of photodynamic therapy in the treatment of laser-induced choroidal new vessels was found significantly enhanced with the use of dendrimer porphyrin encapsulated by a polymeric micelle. (Ideta et al.)[33]

In vivo experiment in hamsters conducted by Ellis-Behnke et al[34] showed that a self-assembling peptide nanofiber scaffold, promoted axonal regeneration in the severed optic tract of hamsters, axons that reconnected with target tissues and promoted visual recovery.[34]

Nanocarriers investigated for gene delivery are lipoplexes, polyplexes, mesoporous nanoparticles, organic-inorganic hybrid nanocrystals, NanoScripts, self-assembling DNA and magnetic nanoparticles.[35] LPD complexes (lipoplexes) are lipid bilayers that contain a highly condensed DNA core.[36] Rajala et al designed a lipoplex for the delivery of RPE65 gene to the retina.[37] After subretinal administration in 5-day old RPE65 knockout mice, the test group expressed the RPE65 gene whereas the control group didn't.[38,39] Unfortunately, most of the lipoplexes are toxic and interact non-specifically with serum proteins and cells among other limitations.[40] Polyplexes are complexes that form by electrostatic interaction of cationic polymers with negatively charged DNA in aqueous solution, resulting in reversible linear to globule transition of the DNA.[35] Mesoporous nanoparticles are nanocarriers, containing pores with diameters between 2 and 50 nm, that enable high gene loading/encapsulation and enhanced transfection.[35] Cao et al showed that plasmid DNA encoding VEGF-loaded mesoporous iron oxide nanoparticles had better transfection than free plasmid DNA.[41] NanoScripts are nanoparticles with attached specific small molecules, so that they mimic natural transcription factor proteins.[35] Kutsuzawa et al showed that a hybrid organic-inorganic nanocrystal had enhanced gene transfection and transgene expression, 20 times when compared with the inorganic non-modified nanocrystal and 3 times when compared with Lipofectamine (commercially available).[42] Ma et al showed that self-assembled DNA tetrahedronnanostructures had enhanced proliferation of neuroectodermal stem cell in vitro.[43] Lee et al used paramagnetic nanoparticles to transfect mouse embryonic fibroblast cells, showing that induced pluripotent stem cells and the transfected embryonic stem cells had similar properties.[44]

PVA hydrogel's ability to serve as a long term vitreous substitute was studied after the injection of PVA hydrogel in the foldable capsular vitreous body and the study indicated that the hydrogel supported the retina and remained

transparent 180 days after injection, while showing good viscoelasticity and biocompatibility.[45]

Corneal tissue

Commonly investigated natural polymers are chitosan, gelatin, collagen, keratin and silk fibrin,[35] whereas commonly studied synthetic polymers are poly(ethylene glycol), PLGA, poly(vinyl acetate), PGS and PCL.[35]

In the case of injury induced corneal neovascularization, nanoparticles (Albumin-derived) that deliver plasmids containing genes for the Flt receptor (binds free VEGF) provide sustained inhibition of the neovascularization.[46]

Dry eye disease (DE) is a condition in which tears cannot properly lubricate the eye. If left untreated, it can lead to lasting damage in the corneal surface and declining vision. Yu et al[47,48] developed a water soluble CNP (cerium oxide nanoparticle-nanoceria) nanocarrier loaded with Glycol Chitosan (GCCNP). After histological evaluations, it was clarified that treatment with 10 μM of GCCNP and Xiidra (an FDA approved DE drug) was effective for the recovery of damaged corneal epithelial layers and returning the corneal status to an almost normal morphology.[47,48]

When replacing corneal endothelial cells, these cells are first cultured in a nano-structured surface (cell sheet engineering approach). Just before the cells are removed from the culture, to get implanted, a transfer medium that reduces the temperature is used. This use of nanotechnology enables the growth and harvest of the corneal endothelial cells, but never touch the patient. (Yang et al)[49]

Aslan et al on comparing the cell proliferation on aligned collagen type I nanoscaffolds and poly (L-lactic acid) scaffolds indicated that light transmittance was better on the collagen nanoscaffolds (close to native cornea), but with a faster degradation rate than in the PLA nanoscaffolds.[50] Stafiej et al on investigating the growth of human corneal endothelial cells (HCECs) and human corneal keratocytes (HCKs) on aligned PCL-PGS and PCL-chitosan nanoscaffolds indicated that both types support the growth and elongation of both cells, but HCECs on PCL-PGS scaffolds had a metabolic activity approximately 40% higher than the PCL-chitosan, while HCKs metabolic activity was pretty much the same on both scaffolds.[51] Arabpour et al observed a continued cell growth in PCL-keratin based nanoscaffolds after day 7, while in PCL based nanoscaffolds it has stopped.[52] It is observed that nanofiber scaffolds cause less inflammatory responses than microfiber scaffolds, due to nanoscaffolds mechanical properties, that mimic those of the native cornea.[53] Muhammad et al on comparing the growth of human corneal endothelial cells found that, cell density was higher on 1000nm pillars but the hexagonal morphology was obtained on the 250nm pillars[54], while other study reported that best HCECs proliferation was found on 1000nm pillars with fibronectin-collagen coated surface.[55]

The median corneal allograft survival time was significantly prolonged in a Sprague-Dawley rat xenotransplantation, after administration of 0.1% tacrolimus loaded niosomes (derived from prionosomes, containing as surfactants poloxamer 188 and lecithin and as stabilizer cholesterol).[56]

A PEG-based doxycycline laden transparent hydrogel was evaluated for the corneal tissue wound healing.[57]Immunofluorescence and histology studies indicated that the hydrogel indicated superior healing properties in comparison to conventional topically administrated drugs.[51]

A PEG hydrogel containing Tyr-Arg-Gly-Asp-Ser (YRGDS) peptides was created as a culturing base for keratocytes, that later would be transplanted for a treatment of keratoconus. The hydrogel wasn't successful on restoring the keratocytes phenotype and further investigation should be conducted.[57]

Ocular surface applications

In a study by Choi et al[58] a water-soluble ROS scavenging nanoceria-embedded contact lens for the prevention of ocular surface diseases[47] showed excellent extracellular ROS scavenging properties and protective effects in a mouse model after the administration of 3% H2O2 eye drops.

A study by Pierscionek et al revealed that nanoceria at

doses of 5 and 10 μg/ml caused no damage in the DNA and no chromosomal changes when cultured in human crystalline lens epithelial cells.[59] Low concentrations of nanoceria present protective effects against oxidative stress, whereas high concentrations (400 μg/mL) increased intracellular levels of ROS and activated the apoptotic process (Hanafy and colleagues).[60] Therapeutic concentrations of nanoceria must be studied on various types of cells.[47]

Adaption of the microfabrication technology has resulted in the creation of arrays of microneedles that penetrate the sclera or cornea to deliver drugs. This biocompatible film could be filled with drug-loaded nanoparticles and accomplish sustained drug delivery in the inner parts of the eye.[61,62]

Glaucoma

Microspheres consisting of biodegradable (poly)lactic-co-glycolic acid (PLGA-approved for human use by the US FDA[18]), loaded with intravitreal glial-derived neurotrophic factor showed a sustained ganglion cell protection in rodent models of glaucoma.[63]

The nanoencapsulation of a BCL-2 plasmid with a targeting sequence for the retinal ganglion cells, aim to block the apoptotic pathway in glaucoma and keep the retinal ganglion cells alive.[64]

Small interfering RNA (siRNA) is a 20-25 nucleotide long double stranded RNA with an observed effectiveness in stopping the proliferation of blood vessels. It is suggested to use a nanoencapsulated and targeted sequence to deliver siRNA in the cytoplasm, with similar work principles of the BCL-2 plasmid above, but nonviral.[64]

De et al[65] prepared nanoparticles loaded with brimonidine (approved medication for glaucoma). Two groups, polyacrylic and polyitaconic acid nanoparticles were used in this in vitro trial that revealed polyacrylic acid nanoparticles as biocompatible, with good adherence to human cornea and with controlled release of the medication through the cornea, while polyitaconic acid nanoparticles were found to be toxic.[22,65] In vivo study (rabbits) with the same medication conducted by Bhagav et al[66], with the use of nanoparticles of brimonidine tartrate combined with inert polymeric resin copolymers of poly (ethacylate, methylmethacrylate and chlorotrimethyl ammonioethyl methacylate) showed no irritation while accomplishing high drug loading and efficient release of the drug.[22,66]

Dos Santos et al[67] designed microspheres to reduce scarring after glaucoma surgery. Poly (lactide-co-glycolide) microspheres containing antisense transforming growth factor-β2 phosphorothioate oligonucleotides with polyethilenimine subconjuctivally administrated, improving bleb survival in rabbits after trabeculectomy.[22,67]

Injectable hydrogels are being studied as the delivery system of stem cells for stem cell treatment of glaucoma.[57] Embryonic stem cells and induced pluripotent stem cells were cultured within an alginate-RGD-hydrogel and this study showed the maintenance of cell survival for 45 days while the expression of retinal pigment epithelium markers and retinal ganglion cell marker were upregulated.[68]

Cataract

Lens epithelial progenitor cells and pigment epithelial cells of the dorsal iris have been studied in the past for the regeneration of lens without surgery in animal models and human infants with congenital cataract.[69,70] An ex vivo porcine eye study by Nibourg et al used a supramolecular hydrogelator with low molecular weight to form self-assembled nanofiber based nanogels that would be used as extracellular environment for lens epithelial cell growth. The results indicated that the nanogels helped the cells to maintain their normal epithelial morphology with less capsular opacification.[71] Xi et al showed that aligned nanofibrous membranes had greater cell attachment of human umbilical vein endothelial cells, when compared with planar and randomly aligned membranes, while also maintaining the, more favorable, bipolar morphology.[72]

A PEG-based formulation is already FDA approved as a sealant and has been studied in multiple cases.[57,73] At the same time, hydrogels have been studied for lens replacement[57]

and a siloxane polymer has been designed as an injectable intraocular lens with consistency suitable for injection in the empty lens capsule.[74]

Imaging

Due to nanoparticle's physical and chemical properties, they have been recently introduced as contrast enhancement agents in MRI, fluorescence imaging, photoacoustic imaging, ultrasound imaging and computed tomography.[5] Nanoparticles absorb or scatter light at specific frequencies due to their physical and chemical properties, something that qualifies them as well suited for bioimaging.[5] Gold nanoparticle and nanorods intravitreal injection has been used for retinal imaging, but due to the obscuring of retinal signal and induced ocular inflammation of the nanorods[75], gold nanodisks are preferred and are considered more optically suitable, because they produce signals that are detectable irrespective of the direction of polarization of the light source and minimize the production of reactive oxygen species (ROS).[5] Gold nanoparticle labeling enabled visualization of the photoreceptor precursors in subretinal space using CT imaging.[76] An in vivo experiment in 4-8-week-old pigmented rats showed that fluorescently labeled photoreceptor precursors did not cause any inflammation intraocularly or other toxic effects on the retina and vitreous space, after 1 month of optical coherence tomography monitoring.[5]

Platinum nanowires have been threaded into the vascular system of tissue samples and have successfully detected the activity of neurons that are adjacent to the blood vessels in the CNS.[77] Similarly, platinum nanowires could be used to determine the viability of the retinal ganglion cells in the retina.[78]

Prototypes of polyamidoamine dendrimers could be used as contrast agents for targeted diagnostic magnetic resonance imaging (MRI).[61] Superparamagnetic iron oxide nanoparticles (SPIO) are FDA approved as contrast agents for MRI imaging (SPIO labeled stem cells have been visualized in patients with brain trauma).[79,80]

Alternative labeling material are Qdots (Quantum dots) that are light emitting nanocrystals[81] and outweigh SPIO nanoparticles because they can be visualized with Ocular CT rather than MRI.[82] Qdots could be used for the in vivo monitoring of the survival, distribution and differentiation of stem cells.[83,84] Coupling of the SPIO nanoparticles or the Qdots with antibodies that recognize components that are released specifically during an ocular degenerative disease could provide the means to imaging the abnormalities that arise from the degenerative disease.[61]

Kelsey et al[85] successfully developed an ultrasound and photoacoustic imaging platform for cell tracking, aiming to assess mesenchymal stem cell delivery in the anterior eye. Gold nanoparticles (AuNSs) (whose cytotoxicity was previously determined as minimal) were used as contrast agents and incubated them with adipose derived mesenchymal stem cells for 24 hours. All imaging experiments used a Vevo 2100/LAZR imaging system that incorporates both ultrasound and photoacoustic imaging (US/PA). This study showed that a US/PA tracking platform has the ability to longitudinally monitor stem cell delivery in the anterior eye, while excluding many of the problems that other stem cell detection techniques in the anterior eye have.[85,86]

Conclusion

The use of nanomaterials in regenerative ophthalmology and imaging is not only a matter of research, but has already various FDA approved applications. The fusion of nanomedicine and ophthalmology has a highly active research interest and an immense number of studies are being or have been conducted, underlying the importance and the severe need of breakthroughs in this field, especially in the branch of regenerative ophthalmology. Although many issues are yet to be resolved, nanomaterials seem to be the way for treating major ophthalmological diseases and for bringing hope in the, otherwise dark, scenery of regenerative medicine.

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