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Review article

Nanocarriers of nanotechnology in retinal diseases

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Abstract

We are approaching a new era of retinal pharmacotherapy where new drugs are rapidly being worked out for the treatment of posterior-segment disease. Recent development in ocular drug delivery systems research has provided new insights into drug development, and the use of nanoparticles for drug delivery is thus a promising excellent approach for advanced therapy of ocular diseases. The primary goal is to develop a variety of drug delivery systems to complement and further enhance the efficacy of the available new medications. The ideal sustained release technology will provide a high level of safety with continuous release over an extended period of time while maintaining almost total drug bioactivity.
The use of nanocarriers, such as cyclodextrin nanoparticle suspension, liposomes, nanospheres and, nanoemulsions for gene therapy of retinal diseases has been highlighted in this review.

Keywords: Nanotechnology, Intravitreal injection, Drug delivery system, Nanoparticles, Posterior segment eye disease

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Introduction

Nanotechnology involves creation and utilization of materials, devices or systems on the nanometer scale. This field is currently undergoing explosive development on many fronts. The technology is expected to create innovations and play a critical role in various biomedical applications, not only in drug delivery, but also in molecular imaging, biomarkers and biosensors.

The eye is a relatively isolated organ divided into anterior and posterior segments with numerous avascular structures.[1] In this regard, the efficacy of topical drug delivery via eye-drops is only limited to the treatment of anterior segment eye diseases. Drugs can enter the posterior segment of the eye via three distinctive noninvasive routes: (1) through conjunctiva/sclera after topical application; (2) from the cornea and aqueous humor after topical application; and (3) from the systemic circulation after topical, parenteral, oral, or other administration routes that deliver drug to the blood circulation.

The eye, particularly the posterior segment, is composed of tissues that are difficult for drugs to penetrate because of structural peculiarities such as the barrier function. Thus, many research studies on nano-sized drug carriers have been conducted in the field of ophthalmology.[2,3]

In this review, the focus will be on the use of nanocarriers, such as cyclodextrin nanoparticle suspension, liposomes, nanospheres and nanoemulsions, and highlights the use of nanoparticles for gene therapy of retinal diseases.

What is micro and nano scale?

Nanotechnology refers to a field of science whose unifying materials are close to molecular scale on dimensions between 1 and 1000 nm (Fig. 1). The macroscopic classification of ophthalmic dosage forms is not related to their microscopic structure and they cannot per se be defined as micro- or nano-technology such as gel-forming solutions, powders for solutions, ophthalmic suspensions, ophthalmic ointments,

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ophthalmic emulsions (e.g., creams), ophthalmic gels, and ocular inserts.[4]

Micro- and nano-technological drug formulations are classified according to the diameter of their particulates. Microtechnology usually refers to technological devices with dimensions close to 0.1–100 μm (102–105 nm) and particles between 0.1 and 100 μm are called microparticles. Drop size in pharmaceutical emulsions is between 0.1 and 1 μm (102–103 nm). Microemulsions form droplets that are between 5 and 140 nm and, thus, should be more correctly referred to as nanoemulsions. Microparticles can consist of micronized drug particles for intravitreal injection but more often such particles consist of micronized polymer/drug matrix-like structures, i.e., microspheres, or polymer-coated microparticles.

Nanoparticles are particles with at least one dimension less than 100 nm for example, the size of liposomes is frequently between 10 and 1000 nm. Nanoparticles can refer to nanospheres (e.g., drug/polymer matrix) or nanocapsules (e.g., polymer-coated drug particles). Examples of nanoparticles for intravitreal drug delivery include albumin nanoparticles for delivery of ganciclovir and a formivirsen analog,[5] and tamoxifen-loaded nanoparticles.[6] Nanoclusters are nanoparticles formed by molecular aggregation and nanospheres are nanoparticles where the active ingredient is encapsulated.

Structural barriers of the eye

Eye structures that function as a barrier to decrease the permeability of the eye to pharmacological agents include the corneal epithelium and endothelium, the sclerocorneal parenchyma, the inner and outer blood–retinal barriers, and the retinal inner limiting membrane.[7]

Drugs penetrate the epithelium either transcellularly or paracellularly. The transcellular route predominates for lipophilic drug molecules whereas the paracellular route predominates for hydrophilic molecules and small ions. The pore size has been estimated to be about 1 nm (permeable for drugs with molecular weight (MW) less than about 700 Dalton) although studies have indicated that some pores could be up to 5 nm in diameter.[8,9] It is accepted that most drugs permeate the epithelium via passive diffusion and, although drug transporters have been located in the epithelium, their significance is still unclear.[10] As a result, less than 5% of the drug can cross the corneal barrier and gain access to the inner eye.[11–13]

The endothelium is a membrane one cell layer with large intracellular junctions. It can be considered as a leaky lipophilic barrier that offers no permeation resistance toward hydrophilic drugs but may offer some resistance toward lipophilic drugs.[14] Conjunctiva is approximately 15–25 times more permeable than the sclera and the sclera is

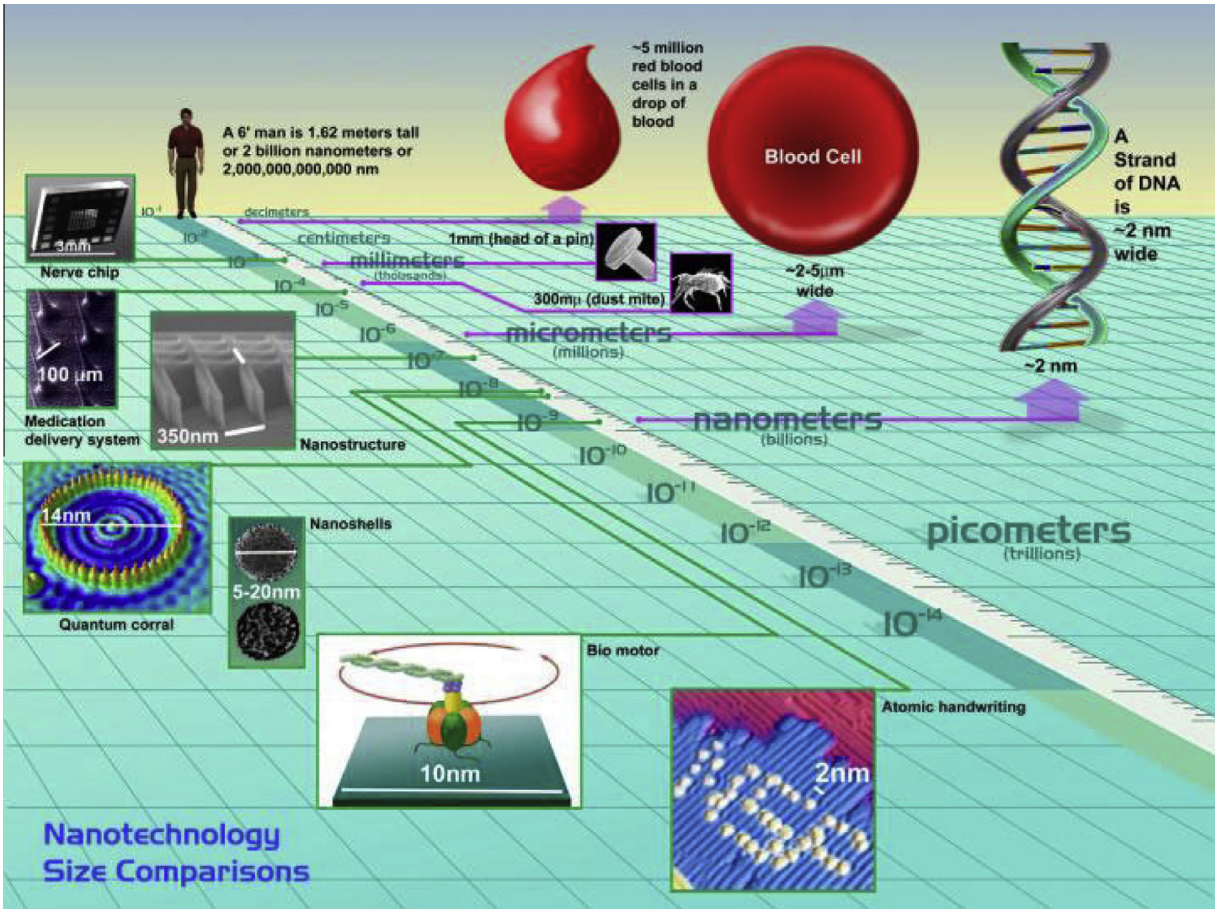

Figure 1. Nano scale (This picture is from http://www.tzhealth.com/health/Nanometer/2007-10-17/15240.html).

approximately 10 times more permeable than the cornea.[15] The choroidal vasculature can contribute to drug clearance from the eye and, thus, constitute a permeation barrier during drug permeation from the eye surface to the retina and vitreous. Although systematically administered drugs can reach the choroid membrane, drug delivery into the retina or the vitreous body is difficult to achieve through conventional methods because of the presence of the blood–aqueous barrier and the inner and outer blood–retinal barriers in those structures.[16] Direct intravitreal injection of drugs into the vitreous cavity is employed to achieve higher drug concentrations in the vitreous and the retina.[17–20] However, repeated injections are required to maintain drug concentrations at an effective therapeutic level over a certain period of time because the half-life of drugs in the vitreous is relatively short.

Nanoparticles and retinal diseases

Particles can interact with cells via different mechanisms which, in turn, results in exposure of nanoparticles to different intra cellular environments.[21]

The development of a drug delivery system (DDS) that can be used for the posterior segment of the eye that involves nanocarriers to overcome the issue of frequent intravitreal administration has received great consideration. In this review, a description of nanocarriers used in the field of retina, focusing on cyclodextrin nanoparticle suspension, liposomes, nanospheres and, nanoemulsions has been provided. The use of nanoparticles for gene therapy has also been highlighted.

Cyclodextrin

Cyclodextrin nanoparticle suspension in eye drops offers a new and promising drug delivery method for the posterior segment of the eye. It is effective with a variety of drugs, including steroids and sulfonamides, such as carbonic anhydrase inhibitors, and may be used with many small lipophilic molecules. Cyclodextrin derivatives which have been applied in ophthalmology include the hydroxypropyl derivatives of β- and γ-cyclodextrin, the randomly methylated β-cyclodextrin, and sulfobutylether β-cyclodextrin.[22]

Liposomes

Liposomes vesicles are 25–10,000 nm in diameter[23] and, composed of a phospholipid bilayer, and water-soluble drugs can be incorporated into their aqueous phase, whereas lipid-soluble drugs can be incorporated into their lipid phase.[24] Their size, lipid composition, and electric charge can be easily controlled.[25] Liposomes have low antigenicity and almost no toxicity.[26] The liposome membrane structure is highly stable and can be deformed during injection through a 27- or 30-gauge needle.[23,27] Studies have been conducted on the intravitreal injection of drug-bearing liposomes and have demonstrated that toxicity of the drug can be reduced, the half-life of the drug inside the vitreous body can be prolonged, and the release of the drug can be controlled.[28–30] A liposome delivery system can be used to encapsulate large antibody fragments and even small unstable siRNA molecules without degradation and loss of bioactivity.[23,27,31] Liposome-encapsulated ganciclovir (GCV) is used for the treatment of cytomegalovirus retinitis in acquired immune deficiency syndrome (AIDS) patients, liposome-encapsulated GCV and free GCV were injected into the vitreous body of rabbits in a previous study.[32] In contrast, no retinopathy was found in the group that received 1 mg of liposome-encapsulated GCV, and the concentration of the drug demonstrated therapeutic levels up to 14 days after injection.[32] In addition, previous reports have shown that fewer intravitreal injections were required for liposome-encapsulated GCV than for free GCV.[33] Other reports have also demonstrated the usefulness of liposomal formulations after intravitreal injection and the drugs delivered in these studies have included amikacin,[34] amphotericin B[35] a model antisense oligonucleotide[36] bevacizumab,[37] cyclosporine,[38] 5-fluorouridine 5′-monophosphate,[39,40] fluconazole,[41] tacrolimus,[42] tobramycin,[43] vasoactive intestinal peptide,[44] an angiogenesis inhibitor,[45] tilisolol,[46] and ofloxacin.[47]

Nanospheres

Nanospheres are of truly uniform sizes ranging from ~50 nm to 1000 nm.[48] Drugs are encapsulated in synthetic and natural polymers to permit sustained local release and tissue targeting of the drugs. The most common substrates are (poly lactic acid) (PLA), polyglycolic acid (PGA), and their copolymer, and poly (lactic-co-glycolic acid) (PLGA). Injected intravitreally PLA and PLGA do not show electrophysiological or histological toxicity in the retina.[49,50] A GCV intraocular implant is the first FDA-approved sustained-release formulation (Vitrasert®; Bausch and Lomb, Rochester, NY, USA) that is nondegradable in vivo and is being used in the treatment of cytomegalovirus retinitis in AIDS patients. When fluorescent 2000 nm, 200 nm, and 50 nm nanospheres were injected into the vitreous body of rabbits, the 2000 nm particles were found in the intravitreal cavity and the trabecula, whereas the 200 nm and 50 nm particles were found even inside the retina.[51]

Nanoemulsions

The diameter of the micelles is approximately 100 nm or less. Micro/nanoemulsions have good tissue permeability because of the small size of the micelles and the presence of a surfactant among the components; as a result, studies on DDSs have been conducted mainly in the field of ophthalmic drugs.[52] The instillation of dexamethasone-containing microemulsions in the eyes of rabbits has been shown to result in enhanced intraocular permeability.[53] In comparison with nanospheres and liposomes, nanoemulsions are also unsuitable for long-term sustained drug release.

Nanoparticles and gene therapy

Scientists and clinicians have used nanoparticles that contain gene transcription factors and other modulating molecules that permit reprogramming of cells in vivo,[54] as well as nanomaterials to induce selective differentiation of neural progenitor cells[55] and to create neural–mechanical interfaces.[56–58]

A tyrosine kinase mutation is present in some patients with retinitis pigmentosa in the Royal College of Surgeons (RCS) rats.[59–61] The PCQretinal pigment epithelium of RCS rats

has difficulty in phagocytose photoreceptor outer segments properly, resulting in progressive rod and cone photoreceptor degeneration.[62] About 585-nm-diameter basic fibroblast growth factor (bFGF) nanoparticles using bovine gelatin and recombinant human bFGF was prepared by Sakai et al.[63] bFGF nanoparticles were still present in the outer retina after 8 weeks of intravitreal injection into RCS rat eyes.

Oxidative damage has a role in the pathogenesis of many retinal diseases, including diabetic retinopathy, age-related macular degeneration, retinopathy of prematurity, and phototoxicity.[25,26,64–67] Cerium oxide (CeO_2) nanoparticles (''nanoceria'') demonstrate the formation of more oxygen vacancies in their crystal structure, particularly at 3–5 nm diameter.[28,29] As a result, nanoceria can scavenge reactive oxygen intermediates.

Nanoparticles can deliver genes efficiently to stem cells[30] and have been explored as a means for gene delivery in the diagnosis and treatment of ocular disease.[68–71] Used DNA nanoparticles consisting of single molecules of DNA compacted with 10-kDa polyethylene glycol (PEG)-substituted lysine 30-mer peptides containing the wild-type retinal degeneration slow (Rds) gene, peripherin/rds, to induce cone photoreceptor rescue in an animal model (rds+/−) of human retinitis pigmentosa with promising results.[72,73]

Nanoengineering of viral vectors via site-directed mutagenesis has produced modifications of the virus capsid that may play a role in its clinical utility. Adeno-associated viruses (AAVs) are nonpathogenic, small, single-stranded DNA parvo-viruses that can transduce dividing and nondividing cells.[56] Nanoengineering of the AAV capsid may provide an important tool for facilitating gene therapy to photoreceptors. Because it is a relatively immune-privileged site, subretinal delivery may still have some advantages in comparing with intravitreal virus delivery.[74] Subretinal virus delivery requires pars plana vitrectomy and has a higher rate of complications (e.g., retinal tears) than intravitreal delivery, which can be done in a clinical setting under topical anesthesia. Tyrosine-to-phenylalanine capsid AAV2 mutants showed 10- to 20-fold higher transgene expression of the entire retina after intravitreal injection, compared to AAV with wild-type capsids.[75] Recombinant AAVs (rAAVs) have relatively low immunogenicity, can target many nondividing cells, and can provide sustained efficient therapeutic gene expression after a single treatment.[76] Recombinant AAVs have been used to treat humans with Leber congenital amaurosis.[77–79]

Conclusion

Nanotechnology has moved into our present life with smart materials, nanoscale biostructure and drug delivery. Innovations in nanotechnology have already shown large medical applications. DDSs are considered to be essential for overcoming the current limitations of the frequent and long-term intravitreal injection of drugs with regard to drug efficacy, and the currently used method of sustained release of retinal medications. These systems should be flexible for combining several drugs without the need for multiple procedures with minimizing the invasive procedure for the sustained-release drug devices.

Conflict of interest

The authors declared that there is no conflict of interest.

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