Application prospects for synthetic nanoparticles in optogenetic retinal prosthetics

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Abstract: Optogenetic prosthetics is an approach to restore visual function in retinal degenerative diseases. It implies the delivery of genes encoding light-sensitive proteins to retinal cells that survived degeneration, primarily bipolar and ganglion cells. As a result, these cells turn into "pseudophotoreceptors" which are able to take on the function of rods and cones lost in disease. The key element of the optogenetic prosthetic procedure is a vector that delivers exogenous DNA to the nucleus of a retinal cell. There are two main categories of vectors: viral and synthetic. The latter include nanoparticles derived from various polymers, lipids and inert metals. Previously, it was believed that viruses transfect living cells more efficiently than their synthetic counterparts due to the presence of specialized gene delivery mechanisms. However, to date, there have been developed nanoparticles that can effectively penetrate through tissue barriers, get into the cell, and successfully deliver nucleic acid molecules to the nucleus. This review addresses the current approaches to the development of nanocarriers and defines the major requirements for their physicochemical properties to ensure most efficient transfer of the genetic material across the intraocular barriers and its delivery to bipolar and ganglion cells. Based on the literature data, several types of nanoparticles have been selected that appear most promising in terms of optogenetic retinal prosthetics.

Keywords: retina, intraocular injections, optogenetic prosthetics, synthetic nanoparticles, DNA delivery

Optogenetics is an approach that allows regulation of physiological activity of genetically modified cells by light stimulation. For its implementation, it is necessary to deliver exogenous DNA into cells that encodes a light-sensitive protein representing either an ion channel (bacterial opsins) or a metabotropic receptor (animal opsins). Under light exposure with certain spectral characteristics, the membrane of neurons expressing such proteins undergoes hyperpolarization or depolarization [1]. Originally, optogenetics was applied as a tool for fundamental studies of neural activity, as it allows precise and selective control of individual cells and their populations. This approach has acquired its main applicational significance within therapy of retinal degenerative diseases, such as age-related macular degeneration or retinitis pigmentosa. During the development of these pathologies, retinal photoreceptor cells (rods and cones) degenerate either in the area of the macula lutea or starting from the periphery [2]. Quite often, other types of retinal neurons (horizontal, bipolar, amacrine and ganglion) do persist for a long time, but in the absence of photoreceptors that convert light energy into an electrical signal, the primary and critically important link in the light perception chain fails. Optogenetics enables prosthetic vision, i.e. imparts light sensitivity to retinal cells initially unable to transduce light, turning them into "pseudophotoreceptors". The most promising targets for optogenetic prosthetics are bipolar and ganglion cells, as well as survived segments of photoreceptors, since it is through them that the nerve impulse is directly transmitted to the brain [3].

Delivery of DNA encoding light-sensitive proteins into retinal cells occurs via special vehicles, vectors. Non-vector delivery methods, such as direct DNA injection and electroporation, either do not allow transfection of retinal cells or are extremely ineffective. There are two main categories of gene delivery vehicles: viral and synthetic vectors. Viral vectors are constructed on the basis of lentiviruses, rabies virus, adenoviruses and, most often, adeno-associated viruses. Non-viral vectors include a variety of submicron-sized particles that bind to DNA molecules. They can be based on polymers, lipids, and metal nanocrystals [4]. It is traditionally believed that nanoparticles transfect living cells far less effectively than viruses, as they lack special mechanisms for

penetrating into the cell and delivering the gene mate- rial to the nucleus. However, in the recent years, a deeper insight into the mechanisms of interaction between nanoparticles and ocular tissues, as well as its individual cells, has been gained, and researchers have learned to manage with the problem of gene delivery through optimization of the physicochemical properties of individual components and a carrier as a whole.

Synthetic vectors have a number of advantages over viruses, their synthesis is much easier and relatively cheap, they have a higher capacity, especially in comparison with an adeno-associated virus, which is able to transfer DNA fragments no longer than 5000 base pairs (bp), including not only the gene of interest, but also the regulatory sequences required for expression [5]. Nanoparticles can transfer full-length plasmids up to 20000 bp long, which makes it possible to include additional elements into the sequence that enhance expression and accelerate plasmid transport into the nucleus [4]. Despite the fact that the eye is an immunoprivileged organ, the injection of viral vectors into it inevitably induces an immune response and the synthesis of antibodies that block the activity of viruses upon repeated injections [6]. As a result, synthetic nanoparticles represent now a widespread and effective tool to transfect retinal cells. For a more detailed consideration of the issues in this area, see the recent review articles [7, 8].

It is noteworthy that the area of application of synthetic nanoparticles in the delivery of genetic material to retinal cells is currently limited almost entirely to gene therapy of photoreceptors and pigment epithelial cells. In animal models, many approaches have been developed to introduce a healthy copy of a gene whose expression is disrupted, or small RNAs blocking the expression of a pathological product, but there are only a few studies in which nanoparticles are used to modify other cells of the retina through making them light sensitive. The goal of this review is analyze current developments in the field of synthetic DNA vehicles and to assess the possibility of their application for optogenetic prosthetics of bipolar and ganglion cells of the retina. We seek to answer the key question of whether nanoparticles can compete with viruses for the role of a delivery vehicle within the frames of this specific therapy.

DELIVERY OF PARTICLES TO THE RETINA

When delivering nanoparticles, it should be considered that the retina in all vertebrates is inverted. This means that the first cells that stand on the path of light propagation, being closest to the vitreous body that fills up most of the eye's volume, are ganglion cells. The light then passes through a layer of retinal neurons (amacrine, bipolar, and horizontal cells) and eventually impinges on the outer layer of photoreceptors that face the pigment epithelium and choroid. The delivery of material (nanoparticles) to retinal cells can be achieved in different ways. Following systemic injection, nanoparticles encounter with the blood-retinal barrier formed by tight junctions between endothelial cells of retinal vessels and cells of the pigment epithelium [9]. Similar difficulties arise when delivering nanoparticles via eye drops; in this case, the eye's cornea acts as a barrier. Although some types of particles manage to overcome these barriers and reach the retina, it is quite obvious that the most preferable way to accomplish this goal is to inject the particle vehicle directly into the eye. Currently, intravitreal and transretinal injections are mainly used to deliver both viral and non-viral vectors. Transretinal injection is a complex ophthalmic operation performed by an eye surgeon, during which a part of the vitreous body is removed (vitrectomy), and then a thin needle is inserted into the subretinal space (jammed between the retina and pigment epithelium) of the target retinal area to infuse a small volume of isotonic saline. Due to this procedure, a bubble forms under the retina, into which the vector is then injected through the same microhole in the retina [10, 11]. This delivery method is fraught with considerable difficulties and risks, including general post-vitrectomy com- plications, subsequent thinning of the retinal outer nuclear layer, as well as side effects of general anesthesia. The degenerating retina, into which it is needed to deliver drugs during optogenetic prosthetics, becomes very fragile, and puncturing the retina with a needle may entail larger ruptures. Moreover, the subretinal injection procedure is very difficult technically, requiring the involvement of highly skilled specialists and the use of sophisticated equipment. Intravitreal injections are an easier and more accessible way to deliver targeted substances to the retina. The procedure is performed under local anesthesia, often on an outpatient basis, and the material is injected directly into the vitreous cavity. In this case, the retina does not detach from the pigment epithelium, and the material to be delivered enters the retina from the side of the inner layers, being faced on its way first with ganglion and then bipolar cells, which are the main targets for optogenetic prosthetics. The

intravitreal route of drug administration is widely used to deliver therapeutic agents suppressing endothelial growth factors, antibiotics and glucocorticoids, as well as viral vectors [12, 13]. When assessing the ability of viral or non-viral vectors to reach retinal cells, it is important to consider the properties of the barriers which they have to pass through *en route* from the vitreous body to ganglion and bipolar cells. The first stage of the passage of particles after intravitreal injection is their movement in the vitreous itself. For instance, in a study by Xu et al. [14], the ability of polystyrene particles to pass through this structure was investigated depending on their size and the charge of the sur- face functional groups. It was shown that particles with a diameter of more than 500 nm have very limited mobility, while among the smaller particles, neutral and negatively charged ones have an advantage in movement, as they do not bind to negatively charged glycosaminoglycan residues that make up the vitreous matrix.

The main barrier in the way of nanoparticles is the inner limiting membrane (ILM), which lines the retina on the inside and borders upon the vitreous cortex. The ILM represents a lamellar basement membrane composed of the extracellular matrix containing such high molecular weight proteins as laminin, type IV collagen, nidogen- / entactin-1 and -2, as well as proteoglycans perlecan, agrin, and type VIII collagen [15]. Proteoglycans exhibit a high degree of glycosylation, while their side chains of glycosaminoglycans determine a high negative charge of the ILM [16]. Apparently, this feature should necessarily be considered when developing non-viral vectors for substance delivery to the retina from the vitreous side, since a neutral or negative charge will prevent particles from binding to the ILM for further penetration, while a too high positive charge will completely immobilize them in the depth of the ILM.

To date, there are no comprehensive data on particle size limits that would allow them to penetrate through the ILM, although there is evidence that a number of monoclonal antibodies, proteins, and peptides weighing up to 150 kDa are able to do this [17]. It has also been shown that for viral vectors, the ILM is both a physical and bio- logical barrier. Heparan sulfate, which is a com- ponent of ILM constituent proteoglycans, binds to adeno-associated virus serotypes 2 and 3, allowing them to accumulate on the surface between the vitreous body and the retina, and exactly this property enables retinal cell transduction with these viruses, in contrast to others serotypes [18, 19]. Although such a specific mechanism of the virus–ILM interaction should be of no significance for the penetration of non- viral vectors, this example, however, shows that the properties of individual components of a given membrane can exert a considerable effect on the passage of nanoparticles through the ILM.

INTERACTION OF NANOPARTICLES WITH CELLS

The uptake of nanoparticles from the extracellular space by retinal cells occurs via phagocytosis or endocytosis [20]. The implementation of one or another mechanism is associated with both the physicochemical properties of particles (size, charge, shape, the presence of specific surface groups) and the type of cells that take them up [21, 22]. For example, among the retinal cells, it is only pigment epithelial and Müller glial cells that are capable of phagocytosis, functioning actually as macrophages that remove fragments of rod and cone outer segments or apoptotic cells [23, 24]. This feature allows them to take up pretty large objects, such as bacteria, yeast, and even algae (1–8 μm in diameter) [25]. However, in excitable retinal cells, which are of primary interest as targets for optogenetic prosthetics (photoreceptors, bipolar and ganglion cells), the main route for particles to enter the cell is endocytosis, and they can capture particles up to 250 nm in diameter [26–29]. A number of studies demonstrate that the optimal particle size for their fastest and most efficient internalization into the cell is about 50 nm [30, 31].

Depending on the proteins and lipids involved, as well as the morphology of resulting membrane structures, several types of endocytosis are distinguished (see review [20]). Clathrin- or caveolin- mediated endocytosis is a major type, but other clathrin/caveolin-independent mechanisms also contribute to extracellular particle uptake. Caveolin-dependent endocytosis is regulated by proteins of the caveolin family, and in most cell types, the caveolin-1 isoform is primarily responsible for the formation of vesicles. In the retina, this isoform is most actively expressed in Müller and vascular cells, as well as in the pigment epithelium, while in photoreceptor cells and other retinal neurons, it is only found in small amounts [32]. Thus, it is unlikely that nanoparticles, destined for the transfection of excitable cells, will be taken up in a caveolin-mediated manner. The clathrin-dependent pathway is triggered either by the interaction of particles with specific receptors on the cell sur- face (receptor-

mediated endocytosis), or by the occurrence of ionic or dipole–dipole interactions of particles with the plasma membrane (receptor- independent endocytosis) [33]. In both cases, the formation of vesicles is mediated by clathrin complexes, which form on the intracellular side of the plasma membrane and, along with a number of adapter proteins, make up a basket-like structure around the nanoparticle. As a result, the particle becomes enclosed in a membrane vesicle (endo- some), which is then internalized into the intra- cellular space.

To increase the likelihood for a nanoparticle to be taken up via receptor-mediated endocytosis, its surface can be conjugated with membrane receptor ligands, such as lactoferrin or folate [34, 35]. For the same purpose, nanoparticles can be coated with specific antibodies that bind to receptors on the target cell [36]. As a rule, unmodified nanoparticles penetrate into the cell via receptor- independent endocytosis due to ionic interactions between the cell plasma membrane and its own surface charge. Since the sites of phospholipids that form the membrane surface have a negative charge, positively charged (cationic) particles undergo endocytosis more efficiently than anionic or neutral ones [37, 38]. However, the uptake of a large number of positively charged particles can lead to a disruption of cell mem- brane integrity and cause a toxic effect or even cell death [39, 40]. It should also be pointed out that negatively charged nanoparticles penetrate into the cell more efficiently than neutral ones [41]. The nanoparticles taken up in the course of clathrin-dependent endocytosis are usually destroyed during endosome maturation and fusion with lysosomes under the action of hydro- lases operating in an acidic milieu (pH 4.5–6). Therefore, when constructing nanocarriers, it is necessary to provide for the ways by which the particle will be able to leave the endosome (see review [42]). For example, some types of lipid and polymeric nanoparticles are able to fuse with the endosome membrane and thus release their con- tents into the cytosol [43, 44]. The most common mechanism of endosomal escape is associated with the destruction of its membrane due to an increase in internal pressure, and it was previously assumed that this phenomenon is associated with the so-called “proton sponge” effect. According to this hypothesis, polymers with a high buffer capacity, which are part of some particles, are able to suppress the decreasing in the endosome pH, which forces the cell to continue pumping protons together with chloride anions and water molecules into it, increasing thereby its internal pressure [45]. However, a number of experimental studies have shown that this model not always predicts accurately the intracellular behavior of particles of various compositions, suggesting that, if this effect does exist, its role in endosome destruction is not leading [46, 47]. The possible alternative ways considered in different studies are as follows: (1) decomposition of polymeric particles into a large number of oligomers, leading to an intraendosomal osmotic pressure jump [48]; (2) increasing the particle size under low pH conditions [49]; (3) direct destruction of the endo- some membrane by some polymeric components of the particle [50]. The mechanisms underlying the endosomal escape of nanoparticles may turn out to be toxic to the cell if they affect its plasma membrane. In order to avoid this, nanoparticles should be constructed from the polymers that initiate membrane destruction only in an acidic environment typical for maturing endosomes and endolysosomes. If the nanoparticle manages to be released from the endolysosome, then the plasmid DNA ends up in the cytosol. However, before the expression of the gene it transfers can occur, DNA must get into the cell nucleus. Since retinal neurons are mature postmitotic cells, the penetration of DNA into the nucleus is a critical step that significantly influences the efficacy of light-sensitive protein expression (see review [51]). Studies have shown that only short DNA fragments (200– 300 bp) are able to penetrate through the nuclear pores independently [52]; however, the use of transport proteins, cytosolic histones and chaperones enables the transport of longer fragments as well [53, 54]. It is possible to improve the ability of exogenous DNA, delivered by nanoparticles, to penetrate into the nucleus by incorporating nuclear localization signals into its nucleotide sequence, which are recognized by cellular transcription factors. For example, the simian virus 40 (SV40) enhancer, commonly used within plasmids to enhance gene expression, contains a nuclear localization signal recognized by several transcription factors (AP-1, 2, 3, Tef-1, 2, Oct1, NFkB) [55]. The plasmid–transcription factor complex is then recognized by importin proteins, which bind to this complex and deliver it to the nuclear pores. It has been shown that plasmids lacking the sites recognizable by transcription fac- tors do not move into the nucleus but remain in the cytosol [56]. Thus, the construction of plasmid DNA is no less important for efficient cell transfection than the composition and structure of the vehicles, nanoparticles.

TYPES OF NANOPARTICLES AND THEIR APPLICATION FOR DNA DELIVERY TO RETINAL CELLS

Below, there will be addressed the nanoparticles based on three types of substances: polymer molecules (most

heterogeneous group), lipids, and inert metals (see Fig. 1). For each type, the characteristic features will be described that deter- mine the mode of their application. Also, experimental studies will be described, in which the given type of nanoparticles was used to transfect cells of the retinal or other eye tissues.

Polymeric nanoparticles

Polypeptides

Gelatin is a partially hydrolyzed collagen, which is characterized by high biocompatibility, biodegradability, and the ability to attach a variety of active groups for targeted delivery. Due to these properties, it began to be used in the synthesis of nanoparticles for the delivery of drugs and genes to human and animal tissues. Gelatin represents a special polypeptide whose cationic, anionic and hydrophobic groups are presented in a ratio of 1:1:1 [57]. As pure gelatin nanoparticles are unstable and prone to aggregation, they are usually modified by crosslinking with other compounds, such as aldehydes, microbial transglutaminase, etc. For binding to anionic plasmid DNA, a favorable factor is the positive charge, which characterizes gelatin type A nanoparticles, although nanoparticles with a high positive charge are often toxic to cells. A compromise solution was proposed by Zorzi et al. [58], who developed hybrid nanoparticles on the basis of cationized gelatin and polyanions of dextran sulfate and chondroitin sulfate. These particles bind plasmid DNA, protecting it from the effect of DNases, exhibit reduced toxicity in in vitro experiments, and are able to deliver DNA to cells of the human (ex vivo) and mouse (in vivo) cornea [59].

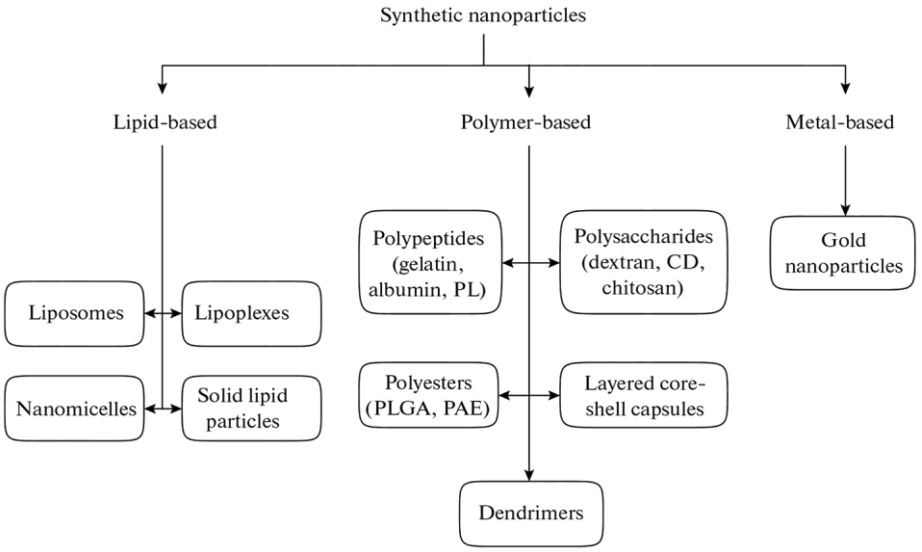

Fig. 1. Types of synthetic vectors for nucleic acid delivery to the retina. PL - polylysine, CD - cyclodextrins, PLGA - poly (lactic-co-glycolic acid), PAE - polyaminoesters.

Albumin is also a protein vehicle that is used to obtain non-toxic, non-immunogenic, biocompatible and biodegradable nanoparticles. Such nanoparticles have a high binding capacity, as well as a good safety and tolerance profile. The primary structure of albumin is well characterized, and a high content of charged amino acids allows drugs with a positive or negative charge to bind without the additional involvement of other agents [60]. Nanoparticles of a relatively small size (50–300 nm) can be synthesized from albumin. Since albumin contains a wide range of functional groups, this allows various surface modifications of albumin-based nanoparticles, for example, by covalent binding with surfactants, polylysine, polyethylene glycol (PEG), transferrin, monoclonal antibodies, etc. However, over the recent years, those works have only been published, in which albumin nanoparticles were used to deliver proteins and drugs into the retina [61, 62]. The first and still the only documented attempt to deliver genetic material into eye tissues using such particles was made in 2007 [63], demonstrating that 120-nm nanoparticles based on human serum albumin successfully deliver plasmids with the Cu/Zn-superoxide dismutase gene into mouse retinal cells after intravitreal injection, as additionally confirmed by Western blotting.

Among the peptide polymers used for the delivery of various substances into eye tissues, CK30-PEG

stands out. It consists of 30 lysine residues, giving a positive charge for DNA binding, to which PEG is attached via a cysteine residue, ensuring thereby the structural stability and improving the cellular uptake of particles [64]. Since such a nanoparticle thus acquires a large number of cationic groups, it is able to compensate almost completely (more than by 90%) the negative charge of a large plasmid DNA molecule, as a result of which the latter spontaneously condenses into a compact DNase-resistant nano- structure. It has been shown that particles based on CK30-PEG, once captured by a cell, are immediately transported to the nucleus, bypassing endosomal uptake [65]. This occurs due to the formation of complexes with a nuclear protein nucleolin, which is also present on the plasma membrane in some amount. Subsequently, the nucleolin-nanoparticle complex moves along the microtubules directly into the nucleolus, and plasmid DNA launches the expression of its constituent genes. It has been shown that nucleolin is expressed in all cell layers of the mouse retina, suggesting that both ganglion and bipolar cells can be efficiently transfected with particles based on CK30-PEG [64]. An experiment on intravitreal injection of nanoparticles carrying a plasmid that encodes a fluorescent marker under a nonspecific promoter confirmed this assumption, because the expression of green fluorescent protein (GFP) was observed primarily in ganglion cells [66]. Particles based on CK30-PEG have been repeatedly used for gene therapy in mice with abnormal expression of the genes, such as *Rds*, *Rpr65*, and *Abca4*, and led to a significant improvement in the state of the retina compared to the control [67– 69]. A quantitative assessment of the effectiveness of such particles also showed that they are able to lead to a target protein expression level comparable with that of viral vectors [70].

Polysaccharides

Polysaccharides are biodegradable, biocompatible and non-immunogenic polymers; their monomers are linked to each other through glycosidic bonds. Dextran, chitosan, and hyaluronic acid are most often used to create vehicles [71]; however, the latter, due to its negative charge within the physiological pH range, poorly binds nucleic acids and is used for their transportation only in combination with other polymers [72]. Dextran is a polymer composed of glucose residues and is not charged under normal conditions. As a result, the efficiency of DNA binding is significantly reduced, and additional cationic com- ponents must be included to transfer plasmids into dextran-based particles. For example, the ability to successfully penetrate into cells and deliver the genetic material has been demonstrated for dextran-based particles modified with diethylaminoethyl, spermine, and polyethyleneimine (PEI) [73–75]. Despite their widespread application as a transfection tool, dextran-based particles have not yet been used for eye tissues. A related group of polymers based on glucose residues, cyclodextrins, has a number of advantages over dextran: they readily acquire a positive charge due to chemical modifications, accelerate particle passage through the cell membrane, and are able to form the inclusion complexes, encapsulating the transfer molecule into their cavity [76]. Cyclodextrins are typically used as vehicles for drug delivery to various eye tissues in the form of drops applied onto the cornea [77, 78]. How-ever, recent studies have shown that some of the modified forms of these polymers have a toxic effect on retinal cells [79], which limits their application for genetic material delivery.

Chitosan consists of N-acetyl-D-glucosamine monomers and contains a large number of amino groups, which determines its positive charge within the physiological pH range. This property makes chitosan-based nanoparticles extremely attractive for plasmid DNA transfer; however, their use is limited by a low buffering capacity, which does not allow the particle to leave the endosome via the "proton sponge" mechanism [80]. This problem can, in part, be solved by including additional amino groups into the polymer; as a result, the expression of the protein encoded by the delivered DNA is enhanced com- pared to nanoparticles based on unmodified chitosan [81, 82]. Mitra et al. [83] uncovered another problem that reduces the effectiveness of chitosan-based particles as a vector: once in the vitreous body, chitosan-based polymers form a viscous gel that impedes the distribution of particles and, accordingly, DNA throughout the eye. As a result, protein expression in the retina is limited to a small area near the injection site, which does not allow creating a sufficiently wide visual field during optogenetic prosthetics. The situation can be improved by including additional cationic polymers to nanoparticles that prevents gel formation. It should be noted that Mitra et al. [83] also demonstrated that chitosan-based nanoparticles modified by the addition of ethylene glycol successfully transfect pigment epithelial cells after subretinal injection without disturbing retinal morphology and function. Other studies have shown the possibility of intravitreal delivery of genes (plasminogen and GFP) into retinal ganglion cells, as well as the cells of the inner nuclear layer, using particles based on short chitosan oligomers and hybrid particles additionally containing poly(lactic-*co*-glycolic acid) [84, 85]. Thus, modified chitosan appears the most attractive

polysaccharide to serve as a basis for genetic vectors. For more details about the factors influencing the transfection of polyplexes based on chitosan and its derivatives see the review [86].

Other plyomers

Poly(lactic-*co*-glycolic acid) (PLGA) is a copolymer composed of lactic and glycolic acid residues, both of which are typical intracellular metabolites, which imparts high biocompatibility and biodegradability to PLGA-based particles. The ratio of lactide to glycolide can vary up to the nanoparticles based a pure lactic acid polymer, which is most resistant to endolysosomal degradation [87]. The safety of using PLGA-based particles was tested in various in vivo experiments, and their toxicity toward body tissues was found to be very low [88]. However, Thackaberry et al. [89] showed that PLGA toxicity toward eye tissues can strongly vary depending on the shape of nanoparticles, being the lowest for the rod-like shape. One of the drawbacks of PLGA as a potential DNA delivery vehicle is its neutral charge at physiological pH values, which deteriorates its binding of plasmid DNA. For this reason, particles are usually constructed with the addition of another, positively charged, polymer, for example, chitosan or PEG [90, 91]. On the other hand, in an acidic environment inside the endolysosome, intermediate products of PLGA hydrolysis acquire a positive charge, which rises as the nanoparticle degrades. This feature allows particles to efficiently escape from endosomes, since the accumulated charge leads to their destabilization [92]. Despite their popularity as drug delivery vehicles, PLGA-based particles have been used for genetic material delivery into retinal cells only in a limited number of experimental studies. In addition to the above investigation of particles containing chitosan and PLGA, it is worth mentioning a study by Zhang et al. [93], who used nanoparticles based on unmodified PLGA to deliver a plasmid encoding short hairpin RNA against the transcriptional regulator HIF-1 (an important participant in angiogenesis during retinal injuries) and GFP. After intravitreal injection of nanoparticles to rats with induced neovascularization of the choroid, GFP was expressed in photoreceptors, as well as in pigment epithelial cells. The PLGA-related compounds, polyaminoesters, are also used as a basis for nanovehicles, having an advantage in DNA binding due to the presence of cationic amino groups [94].

Dendrimers are a unique class of nanoparticles that represent branching monomeric chains diverging from the central molecule [95]. They have the appearance of a ramified spherical structure with internal cavities which can retain various macromolecules, including plasmid DNA. Although the most popular for DNA transportation are polyamidoamine (PAA) dendrimers having a large number of positively charged amino groups, PEI and polylysine are also used for this purpose [96]. Dendrimers typically range in size from 1 to 100 nm (depending on the length of branching chains), are taken up by the cell via clathrin-mediated endocytosis, and finally leave the endosome due to the "proton sponge" effect [97]. In in vivo studies, PAA and PEI dendrimers showed high efficiency; however, with an increase in the size, they became toxic to cells due to a large positive charge [98, 99]. Also, PEI dendrimers were used for intravitreal delivery of the plasmid encoding short hairpin RNA into retinal ganglion cells [100].

Yet another promising type of polymeric vehicles is represented by multilayer nanocapsules. For their synthesis, a rigid core is required (its role is usually played by vaterite nanocrystals, one of the polymorphic modifications of $CaCO_3$), onto which cationic (polyarginine/polylysine) and anionic (dextran sulfate) polymers are deposited alternately, layer by layer [101]. The transported nucleic acids form a complex with the very first positively charged layer and are released into the intracellular space after the destruction of the polymeric shell. To prevent the toxic effect associated with an increase in the intracellular Ca^{2+} concentration after the destruction of the vaterite core, it can be pre-removed from the capsules using a chelating agent (sodium ethylenediaminetetraacetate). The important assets of such vehicles are their increased stability and capacity. A number of studies have shown the efficacy of nanocapsules for the delivery of miRNA, mRNA, and plasmid DNA into cell cultures of various types [102, 103]. We have also obtained pilot results showing that capsules with a diameter of 50 nm successfully deliver mRNA into mouse retinal cells after intravitreal injection [104] and are predominantly localized in photoreceptors. Additional modifications aimed at increasing the selectivity of such particles toward bipolar and ganglion cells may make this type of vehicles suit- able for being used in optogenetic prosthetics.

Lipid-based nanoparticles

Liposomes represent spherical particles consisting of at least one lipid bilayer and enclosing a hydrophilic (aqueous) phase. The bilayers are formed predominantly by phospholipids and sterols, similar to cell

membranes. Liposomes can act as a vehicle for hydrophilic, hydrophobic, and amphiphilic compounds [105]; their advantages include the lack of toxicity, low antigenicity, the possibility of having their size, lipid composition and electric charge accurately controlled during synthesis, as well as to modify their surface by polymers and antibodies [106]. Particles based on cationic lipids are able not only to be effectively captured by the cell via endocytosis, but also to fuse with the negatively charged membrane of the endosome, releasing DNA into the cytosol. Liposomes have been using for drug delivery into the eye, including the retina, for several decades. They served as a delivery vehicle for such intravitreally injected compounds as antibiotics [107], antiviral drugs [108, 109], angiogenesis-suppressing drugs [110]. The applicability of liposomes to deliver genes into pigment epithelial cells was demonstrated in vivo by Lajunen et al. [111], where plasmid DNA was effectively delivered within transferrin-labeled liposomes; this drug was applied to rats in the form of eye drops. Furthermore, it was shown that intraocular injections of liposomes that carried plasmid DNA encoding the galactosidase gene resulted in its successful delivery into retinal ganglion cells and pigment epithelium [112].

Nanomycelles are self-assembling lipid particles; unlike liposomes, they are formed by a lipid monolayer and have a hydrophobic core and a hydrophilic shell. Nanomycelles have low eye tis- sue toxicity, which, for example, has been shown for the vehicles consisting of polyoxyethylene 40 hydrogenated castor oil and octoxynol 40 [113], as well as for complex carriers of octoxynol 40 and tocopherol PEG succinate used for rapamycin delivery into eyes tissues [114]. Although these facts indicate the attractiveness of lipid nanomycelles as vehicles for gene delivery into retinal cells, no studies have been published so far that would address the ability of nanomycelles to transport DNA into eye tissues.

Another promising type of lipid-based transport nanoparticles are lipoplexes. This term defines compact structures which result from the interaction of positively charged (due to the presence of additional chemical groups) hydrophilic phospholipid heads with negatively charged molecules of nucleic acids. In such a complex, nucleic acids are protected from degradation [115]. In the literature, there have been described the attempts to use lipoplexes for delivering interfering microRNAs (miRNAs) into eye tissue. One of these studies evaluated the effect of the surface charge of PEGylated miRNA lipoplexes on their penetration and distribution in the retina after intravitreal injections to mice, and it was shown that positively charged lipoplexes are optimal for this purpose [116]. In a study by Amadio et al. [117], miRNA against human antigen R, a protein from the ELAV (embryonic lethal abnormal vision) family, was delivered by lipoplexes injected intravitreally to rats with a model of diabetic retinopathy. Lipoplexes with the above miRNA demonstrated a successful transfection, and their injection led to a decrease in the human antigen R level. In another study [118] on an ex vivo model (isolated bovine eyes, human pigment epithelium cell culture), it was shown that the coating of plasmid DNA lipoplexes with hyaluronic acid results in a noticeable increase in the internalization and transfection efficiency, and also increases the mobility of such lipoplexes in the vitreous matrix. Quite promising results have also been obtained in one of the recent studies [119], where hyaluronic acid-coated lipoplexes after intravitreal injection to rats delivered miRNA against caspase-3 (activated in retinal degeneration) into various retinal cells, exerting thereby a neuroprotective effect. Besides, in this work, potential retinotoxicity of such particles was separately evaluated retinographically, and it was shown that they have no toxic effects. In nanoparticles based on solid lipids, there is a core of such lipids as octadecylamine, dioleolyl-3- trimethylammonium propane, and dioleolylphosphatidylethanolamine, which is stabilized by surfactants in an aqueous suspension. The mobility of the drug being delivered in such particles is appreciably reduced, and its release can be more con- trolled [120]. Solid lipid-based nanoparticles share their low toxicity with liquid lipid-based particles, but at the same time, have a number of advantages, specifically, they appear promising vehicles for the delivery of macromolecules. To date, several works have already been published, devoted to using solid lipid-based nanoparticles for genetic material delivery into eye tissues. For example, Torrecilla et al. [121] demonstrated the ability of solid lipid- based nanoparticles to deliver short hairpin RNAs, aimed to combat neovascularization, into human corneal epithelial cell culture. In another study [122], solid lipid-based nanoparticles coated with hyaluronic acid were used to deliver the retinoschisin gene into mice with a model of juvenile retinoschisis. The transfection level resulted from intravitreal injection of these particles was the highest in photoreceptor cells, although transfection was also noted in ganglion cells.

Summarizing the results described in this section, it should be noted that the approach combining a lipid base with a polymer coating seems to be a particularly promising means of delivering nucleic acids into retinal cells. The data published by now on such particle types also appear very convincing, and it would be advisable to use these vehicles for the delivery of constructs for optogenetic retinal prosthetics.

Inert metal-based nanoparticles

Another trend in developing vehicles for the delivery of drugs and genetic materials into target cells is the creation of nanoparticles from inert metals. Such particles are potentially non-toxic, biocompatible, and provide ample opportunities for their modification using other materials (polymers, antibodies, etc.). One more advantage is the relative ease of their synthesis. Among inert metals, gold is the most widely used in manufacturing of nanoparticles for biomedical purposes. The mechanism of internalization of gold nanoparticles depends on their surface characteristics, such as charge and size, and most often it is endocytosis. If gold nanoparticles are coated with antibodies, they enter the cell via the mechanism of receptor-mediated endocytosis [123]. Gold nanoparticles successfully cope with the task of delivering large peptides and nucleic acids, with no severe restrictions imposed on the size of the molecules to be transferred [124]. Protocols have been developed, in which gold particles bind to plasmid DNA through non-covalent electrostatic interactions, thus protecting DNA from enzymatic cleavage [125, 126]. It should be noted that this implies additional functionalization of the surface of gold particles with ligands containing quaternized amino groups, PEI, etc. An alternative approach is modification of nucleic acid strands in such a way that they covalently bind to the surface of nanoparticles. For this purpose, nucleic acids are modified with thiol groups and, in this form, attached to gold nanoparticles coated with a cationic polymer. For example, in the case of miRNA, this led to efficient transfection of cells in culture [127]. Furthermore, gold nanoparticles can be conjugated to various ligands, including transferrin and antibodies, which ensure targeted delivery [128, 129].

There are only few studies published to date on the delivery of genetic material to eye tissues using gold nanoparticles. In one of them, Sharma et al. [130] showed that PEI-coated gold nanoparticles are able to transfer plasmid DNA into corneal cells in an in vitro model (human cornea), and also do not exhibit toxicity toward the rabbit cornea in in vivo experiments. Recently, a method for the delivery of plasmid DNA by gold nanoparticles has been proposed for optogenetic retinal prosthetics [131]. As a vehicle, there were used gold nanorods conjugated to an antibody against either PKCα (protein kinase C-alpha, a protein specifically expressed in bipolar retinal cells) or Thy1 (a protein specifically expressed in ganglion cells), which made it possible to achieve targeted delivery. In addition, the nanoparticles differed in the surface plasmon resonance, which peaked at 780 nm for the nanoparticles intended for gene delivery into ganglion cells, and 850 nm for those directed to bipolar cells. Next, the internalization of nanoparticles after intravireal injection to mice was initiated by laser radiation at a wavelength depending on what type of retinal cells was to be transfected. In this study, gold nanoparticles demonstrated a selective transfection and, no less importantly, an excellent safety profile: potential toxic effects were assessed retinographically, by optical coherence tomography, for the structural integrity of eye tissues after exposure, and immunohistochemically. A study by Batabyal et al. [131] remains the only experiment published thus far on optogenetic retinal prosthetics with the use of synthetic vectors.

CONCLUSION

Based on the properties of the intraocular barriers the synthetic vector must overcome before reaching the retina, as well as the mechanisms of its interaction with target cells, the optimal parameters of such a vector can be inferred. Relatively small nanoparticles with a diameter of no more than 100–150 nm are most successful in passing the vitreous body through and being taken up by retinal cells. The most favorable charge of these particles is difficult to determine due to the following contradiction: with a negative charge, the particle is less effective in being captured by cells and bound to DNA; with a positive charge, it is more impeded by the vitreous body and has a potentially higher toxicity. Therefore, an effective particle must be either electrically neutral or have only a small positive or negative charge. Each of these cases allows various modifications which make it possible to counterbalance the drawbacks of a charge of one sign or another. For example, the low endocytosis efficiency of anionic particles can be compensated by adding surface ligands and increasing the chances of uptake on the receptor- mediated endocytosis pathway. Analysis of the studies of nanoparticles as vehicles for gene delivery into retinal cells that have been published in the recent years shows that currently most of these vehicles are oriented toward the needs of gene therapy. They predominantly transfect photoreceptor and pigment epithelial cells, i.e. those types of cells where mutations occur most often, leading to death of the photosensitive periphery of the visual system and, as a consequence, loss of vision [132]. At the same time, optogenetic prosthetics is applied in the clinical picture corresponding to the late stage of retinal degeneration, when photoreceptor cells have already died completely, and gene therapy to eliminate the pernicious effect of one mutation or another is no longer giving any positive effect. Such a therapy requires the development of vehicles able to deliver the genes of light-sensitive proteins to the inner layers of the retina, bipolar and ganglion cells.

Among the nanoparticles considered in this review, the most promising ones are polymeric CK30-PEG

particles and those based on PLGA- chitosan or hyaluronic acid-coated lipoplexes, as well as antibody-modified gold nanoparticles. All of them have been shown to be able to successfully deliver nucleic acids (DNA or RNA) at least to ganglion cells following intravitreal injection. Despite this, even they, like all the other types of nanoparticles considered above, require additional research and modifications before they can be considered specialized vectors for optogenetic retinal prosthetics.

AUTHORS' CONTRIBUTION

Analysis of relevant publications (A.Yu.R., I.S.R., L.A.A., Y.V.T.); writing a manuscript (A.Yu.R., I.S.R., L.A.A.); editing a manuscript (A.Yu.R., Y.V.T., L.A.A.).

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CONFLICT OF INTEREST

The authors declare that they have neither evident nor potential conflict of interest related to the publication of this material.

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