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Modified cells as potential ocular drug delivery systems

Tatiana Tennikova[1] and Arto Urtti[1,2,3]

[1] Institute of Chemistry, Saint-Petersburg State University, Sankt-Peterburg, Russian Federation
[2] Faculty of Pharmacy, University of Helsinki, Helsinki, Finland
[3] School of Pharmacy, University of Eastern Finland, Kuopio, Finland

Drug delivery to ocular targets is problematic, especially in retinal disease treatment. Therefore, targeted drug delivery, prolonged drug action, and minimally invasive treatments are needed. In this review, we describe cell technologies for drug delivery. These technologies are based on genetic engineering and nongenetic-based approaches for cell modification. In principle, cell technologies enable targeted delivery, long drug action, and minimally invasive administration, but they have only been sparsely studied for ocular drug delivery. Herein, these technologies are discussed in the ocular context.

Introduction

Research at the interface of chemistry and biology has resulted in major advances in drug delivery. Biohybrid materials are based on the combination of unique properties of living systems and rationally designed synthetic compounds. These approaches allow construction of biomaterials for biomedical applications, such as the delivery of therapeutics (drugs, genes, and cells), tissue engineering, diagnostics, and imaging.

Modified cells have been used as transporters of pharmaceuticals. Some cell types have natural homing mechanisms that can be exploited in drug delivery and further optimized chemically or genetically [1]. Drug-loaded cells (pharmacytes) can be based on T lymphocytes, erythrocytes, macrophages, bacteria, lymphocytes, and stem cells [2–8]. Biohybrids utilize the properties of living cells including the ability to home to diseased tissues, long half-life, abundant surface ligands, and flexible morphology. Even though substantial efforts have been made to understand the key features of cell-mediated drug carriers [4,9,10], these approaches have only rarely been used for ocular treatments. In this review, we discuss the potential role of modified cell technologies in ocular drug delivery.

Corresponding author: Tennikova, T. (tennikova@mail.ru)

Cell engineering technologies for drug delivery

Genetically engineered cells

Genetic engineering of the cells is a widely used technique in molecular and cell biology. Cells can be engineered using gene transfer and gene-editing techniques. Gene transfer (or transfection) is based on the introduction of DNA encoding the protein of interest into cells, whereas gene editing is based on the precise repair and modification of genetic sequences in the target cells. For drug delivery purposes, transfection is a more relevant technique and is described here.

Two components are needed for gene transfection: DNA and a carrier. DNA sequences can be designed and modified so that the regulatory DNA elements can be used to control the level of gene expression from the coding sequence for the protein. The regulatory DNA elements enable cell-specific gene expression [11] or use of chemicals (e.g., tetracycline and other drugs) to control the onset of gene expression in the transfected cells [12,13]. Given that DNA does not enter the cell nuclei spontaneously, gene transfer is performed using viral vectors, nonviral carriers (e.g., cationic polymers or cationic lipids), or electroporation. Depending on the gene transfer method, the transfection can be transient or stable and the choice of vector can be used to define the duration of the protein expression. Viral vectors can produce transient (e.g., adenovirus), long-lasting (e.g., adeno-associated virus), or stable transfection (e.g., lentivirus). Nonviral vectors produce usually

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transient *trans*-gene expression, but cell selection procedures allow the generation of stably transfected cell clones for expansion [14].

Even though gene transfer is routinely performed to produce cells that express pharmaceutical proteins, the use of genetically engineered cells *in vivo* is limited by the defense mechanisms of the body. For example, the immune system can lead to cell rejection; the cells might not survive at the site of implantation; or they might proliferate in uncontrolled way. Therefore, polymeric encapsulation techniques have been used to allow diffusion of the secreted therapeutic protein, while protecting the cells from the defense mechanisms of the body [15]. Cell encapsulation has been tested for more than 50 years, but there are still no clinical treatments that are based on the secretion of pharmaceutical proteins from encapsulated cells. In the eye, intravitreal delivery of ciliary neurotrophic factor (CNTF) has been performed with engineered and encapsulated cells in clinical trials [16], but has not yet reached the market.

Nevertheless, the concept of engineered cells for continuous protein drug secretion is appealing. In principle, such a system might produce a treatment that is injected only once in lifetime, but there are also hurdles. First, long-term delivery requires cells that survive in the capsule in a nondividing state and secrete the protein of interest for a long time. For example, cell encapsulation therapy for Parkinson's disease was not effective in clinical trials, possibly because of the decreased viability of the implanted cells in the brain [17]. Second, it might be difficult to obtain a high enough expression rate of the therapeutic protein [18]. Third, cells might sometimes escape from the polymeric material, resulting in immunological responses and cell death. Finally, gene transfer techniques do not result in precisely controlled insertion of the transgene into the genome. Therefore, the cells might undergo unwanted changes and so must be strictly controlled before their use. CRISPR/Cas9, a relatively new precise gene-editing technique, might be safer, avoiding risky gene modifications. However, the use of gene editing for improved protein expression is not easy, because precise gene modifications to increase protein secretion must be found. In any case, recent developments in stem cell biology, gene editing, and materials science could foster the development of cell-mediated drug delivery.

Nongenetic cell engineering

The use of nongenetically engineered cells for drug delivery is a complementary approach to genetic engineering methods. Nongenetic engineering applies physical and chemical methods to yield cells with new functions. This approach is suitable for cells that are difficult to transfect (e.g., erythrocytes or T cells). Unlike genetic modification, this technique is also suitable for the delivery of small molecular drugs. Nongenetic cell engineering strategies are described in the following paragraphs.

Drug encapsulation into the cells

Cells have different a circulation times, homing to specific tissues, and immune responses defining the usefulness of each cell type for drug delivery. Erythrocytes have a lifespan of 100–120 days in the blood stream, partly because of the immunosuppressive proteins on their surfaces [19]. Upon swelling in hypotonic solutions, erythrocyte membranes develop holes that allow the loading of drug molecules. Post loading, the erythrocytes adopt their normal shape and close the membrane pores when exposed to isotonic buffer [20,21]. Erythrocytes have a high drug loading capacity because of the absence of a nucleus and other subcellular organelles and also have a longer life-span in the circulatory system than polymeric particles [19]. Senescent erythrocytes are recognized and removed from the circulation by phagocytic cells in the reticuloendothelial system, making erythrocytes suitable carriers for therapeutic delivery to monocytes and macrophages [22].

As immune cells, leukocytes are ideal candidates to deliver therapeutics, because they target to specific tissues and respond to inflammation, which is associated with many diseases [23–25]. For example, macrophages efficiently phagocytose soluble compounds and particulate materials. Thus, nanoparticles with encapsulated drug can be readily loaded into macrophages by simple coincubation [26,27]. After cell administration to the patient, macrophage distribution to the sites of inflammation or infection will also guide the internalized drug to the diseased area [28]. Given that macrophages are able to overcome the blood–retina barrier [29], this approach could be useful in the treatment of ocular diseases.

Stem cells, primarily adult stem cells, are also potential drug carriers [30,31]. Neural stem cells and mesenchymal stem cells (MSCs) are tumoritropic and, therefore, they have been studied for drug delivery and imaging [32–35]. Roger *et al*. loaded poly(lactic acid) nanoparticles, and lipid nanocapsules into human MSCs without affecting their viability, proliferation, differentiation, or tumoritropic migration [36]. Nanoparticles were loaded to the MSCs by simple incubation and trafficked within the cells to brain tumors in a mouse model. Stem cell-mediated drug delivery is subject to the same issues as in stem cell-based transplantations, including the need for complete cell characterization, issues of donor-dependent differences in the cells (e.g., epigenetic background), immunological aspects, and risk of tumor formation.

The loading efficiency of nanoparticles into cells depends on particle surface properties (i.e., charge and hydrophobicity) that determine the particle–cell interactions [8]. Nonspecific and receptor-mediated uptake determine the extent of cellular nanoparticle internalization. Positively charged nanoparticles are more effectively taken up by cells than are anionic or neutral nanoparticles. Polyethylene glycol coating partly masks positive and negative charges on the nanoparticle surface, leading to nearly neutral zeta-potential and slower cellular uptake. The size and shape of nanocarriers affect their uptake in a cell type-dependent manner. Macrophages and retinal pigment epithelial cells are phagocytic, internalizing even micron-sized particles, whereas most other cell types ingest only particles <100–200 nm in diameter.

Drug stability in cells depends on the intracellular trafficking of the nanocarriers. Lysosomes host enzymes that degrade RNA, DNA, and many proteins [37]. Drug release from cell carriers controls the dosing rate and duration of drug action. The release rate is controlled mostly by drug release from the nanoparticles within the cells and is further modulated by passive diffusion or efflux transport across the plasma membrane. Drug release from the nanoparticles (and particle-bearing cells) can be optimized by polymer modifications. The eye has small compartments that allow local delivery at dosing rates that are orders of magnitude smaller than in the systemic drug delivery.

Drug encapsulation into the cells or ghost membranes (i.e., plasma membrane vesicles without intracellular organelles) has

some advantages. First, there is no need for genetic engineering. Second, nanoparticles enable an extra level of control of drug release. Third, clinically accepted drugs can be used without chemical derivatization, which would change their regulatory status to a new compound.

Modification of plasma membrane

Nongenetic modification of plasma membrane is a powerful strategy for cell modification. Membrane engineering enables anchoring of different molecules or particles to the cell surfaces. The membrane-anchored compounds are designed to be retained on the cell membranes, because their interactions with the extracellular environment are essential for their function.

The plasma membrane comprises lipids, proteins, and polysaccharides, and is crucial for the communication of cells with the environment [38]. For drug delivery, cell membranes can be engineered using three strategies (Fig. 1).

Biochemical methods are based on the natural protein anchors that form selective affinity pairs with their counterparts in the solution (e.g., peptides) [39–42]. Each reaction requires a unique acceptor peptide, which is usually not available in mammalian cells; thus, genetic modification is needed to generate the fusion proteins for anchoring. A range of chemical functionalities can be integrated into proteins with unnatural amino acids. The post-translational modification of proteins (e.g., glycosylation) depends on the enzymes and these reactions can be affected by unnatural components. Biochemical approaches are thoroughly reviewed by Sletten and Bertozzi [43].

Physical modification causes milder interference to cell functions compared with biochemical approaches. Similar to natural proteins, GPI anchor-containing proteins can be integrated into plasma membrane. In addition, drug molecules can be conjugated with lipids and similarly attached to the cell membranes [44,45] by simple incubation with a drug–lipid conjugate in serum-free buffer. The most stable integration is achieved with long and saturated lipid chains. The molecules with lipid chains have also been fabricated into vesicles that fuse the conjugates into plasma membrane [46]. Molecules and nanostructures can also be anchored to the cell membrane proteins. For example, polymeric nanoparticles coated with hyaluronic acid attached to cell membranes via the CD44 receptor [47]. The efficacy and stability of molecular loading to the cell membrane proteins and lipids depend on the quantity of target protein and binding affinity and length of the lipid anchor.

Chemical methods for cell membrane modification are mostly based on reactive primary amines on the cell membrane, which form covalent bonds with activated carboxylic acids. Cyanuric chloride can be used to convert carboxyl groups to acyl chlorides, which react with the amines. To avoid producing HCl as a by-product, mild alkaline buffers are used [48]. A reagent, 1-ethyl-3-(3′-dimethyl aminopropyl)-carbodiimide (EDC), is utilized to convert the carboxyl group to a reactive *O*-acylisourea intermediate that forms amide bonds with the amines. EDC has usually been applied to conjugate proteins on the cell surfaces [49]. *N*-hydroxysuccinimide (NHS) and *N*-hydroxysulfosuccinimide (sulfo-NHS) can transform unstable intermediates to relatively stable amine-reactive esters of carboxylates. When drug or drug-loaded nanoparticles contain primary amines (e.g., acetazolamide, brinzolamide, or ranibizumab), two step reactions are sometimes required. In the first step, biotin, maleimide, or other reactive groups are introduced onto the cell membranes using the aforementioned reactions. Biotins bind to avidin or streptavidin, which are tetramers serving as the linkers for biotinylated therapeutic molecules or nanoparticles [50,51].

A thiol–maleimide reaction has been suggested for conjugation on cell surfaces [52]. In this approach, membrane-impermeable linkers formed of succinimidyl-[(*N*-maleimidopropionamido)-diethylene glycol] esters are used to introduce maleimide groups to the cell membranes. Compounds with a thiol group [e.g., thiol-

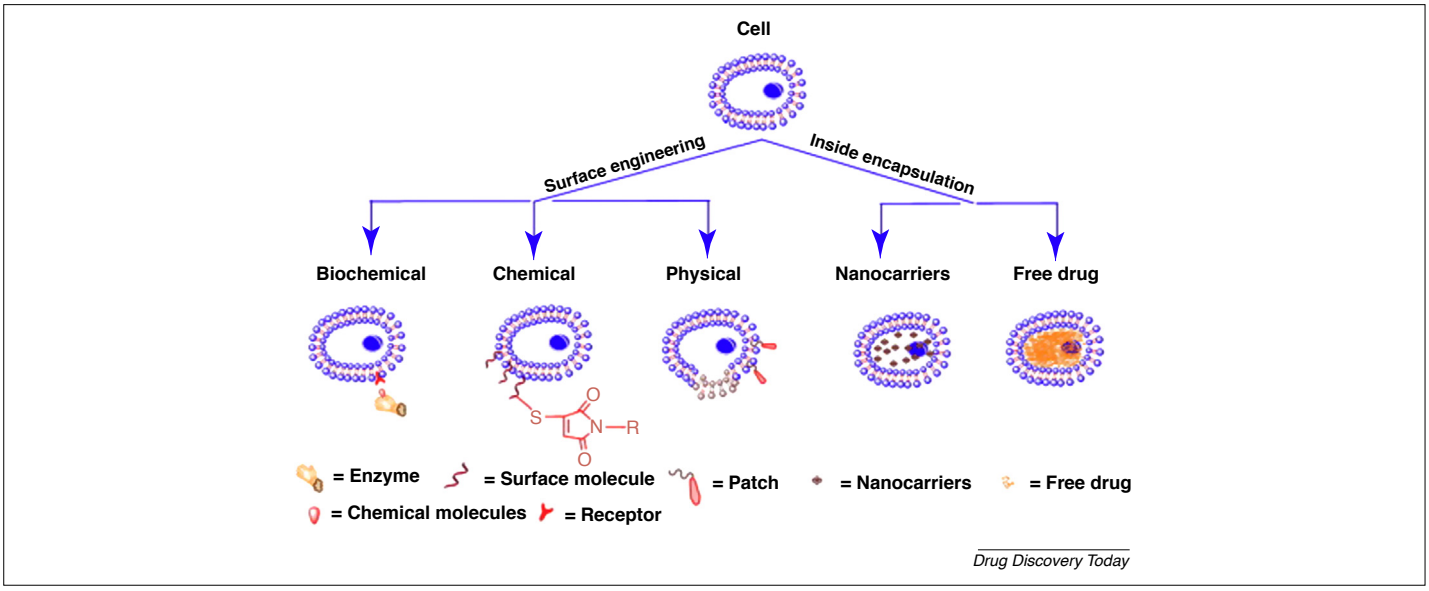

FIGURE 1

Schematic illustration of different approaches for the nongenetic engineering of cells for drug delivery and cell-based therapy [8].

containing oligonucleotides or antibodies for vascular endothelial growth factor (VEGF) inhibition] can then be covalently linked to the cell surface with the maleimide groups. For instance, peptides have been conjugated onto MSCs in this way without changes to other cell properties [52]. Unfortunately, the direct reaction approaches are not specific, because any cell membrane protein with amine or thiol groups can react.

In the synthesis of biohybrid materials, specificity and biocompatibility are essential issues. Click chemistry is a valuable tool for specific cell surface modifications [53]. In most cases, copper-catalyzed azide-alkyne cycloaddition has been used. In azide-alkyne chemistry, both functionalities are biocompatible and show complete mutual reactivity in mild conditions in the presence of catalytic transition metals [mainly Cu(I)]. Copper can cause problems *in vivo* in the retina, because elevated free copper can inhibit enzyme functions, accelerate the formation of oxidative species, damage mitochondria, and cause inflammation and death of retinal neurons [54]. Alternatively, metal-free methods for azide functionalization (Staudinger ligation) can be used in mild conditions [55,56].

Before membrane modification by click chemistry, new functional groups must be introduced to the cell surface. Tetra-acetyl-*N*-azidoacetylmannosamine and tetra-acetyl-*N*-azidoacetylglucosamine are the most suitable and well-known labeling agents for cells. Azido monosaccharides can be fed to cells, resulting in their integration into glycoconjugates. Incubation time, concentrations of azido monosaccharides, and labeling reagent should be adequate for surface labeling. For example, folic acid moieties have been used for the delivery of cells to tumors (e.g., retinoblastoma cells), which are known to overexpress the folate receptor [57,58].

Chemical modification of the cells or ghost membranes can result in the modified distribution and elimination of cells from the site of administration. In principle, drugs could also be attached to the cell surface, but the drug should be released intact at a controlled rate. Applicability of this approach depends on the chemical conjugation possibilities; conjugation efficacy and intact drug release are particularly important features.

Ocular drug delivery with engineered cells

Ocular cells have been widely engineered with gene transfer. Most ocular gene therapy research is based on the use of viral vectors, most often adeno-associated virus, to transfect retinal cells. The first ocular gene therapy product (Luxturna, Spark Therapeutics) received market authorization in December 2017 [59]. In addition, cell transplantation is widely studied in ophthalmology. For example, stem cell-derived retinal pigment epithelial cells have reached clinical trials for the treatment of age-related macular degeneration (AMD) [60] and the first stem cell product was accepted in 2015 for corneal clinical transplantations in the European Union (Holoclar, Holostem Terapie Avanzante) [61]. However, cell-mediated ocular drug delivery has been only sparsely studied.

The most significant and systematic effort for cell-mediated ocular drug delivery was carried out at Neurotech [62–64], even though a Phase II trial of anti-VEGF cell capsules for wet AMD (NT-503) was discontinued. Neurotech technology is based on the genetic engineering of ARPE-19 cells for stable expression of therapeutic proteins, such as ciliary neurotrophic factor (CNTF). The cells are embedded in a gel matrix surrounded by a cylinder-shaped membrane wall that allows diffusion of CNTF (20 ng/day) from the cell capsule to the vitreous humor. CNTF was released for at least 2 years, representing, in total, 15 mg of protein, after single implantation. It would be challenging to formulate 15 mg of CNTF in a single-injection formulation and it is not certain whether the protein would remain stable for 2 years at +35 °C in the vitreous. Furthermore, it would be difficult with conventional methods to maintain the protein release rate at such constant levels for 2 years. This technology entered Phases II and II/III many years ago, but has not yet reached the market. Retinitis pigmentosa, geographic atrophy, and glaucoma have been the target indications in the clinical trials of CNTF-releasing encapsulated cells [64]. The system increased macular volume and modestly improved vision in patients with geographic atrophy and retinitis pigmentosa [62]. Another clinical trial showed slowing of photoreceptor loss in the patients with macular telangiectasia [63].

Alginate-collagen gels have been used for the encapsulation of glial cell-derived neurotrophic factor (GDNF) [65]. The cell-containing gels were administered intravitreally to rats and GDNF release-associated beneficial effects on photoreceptors were observed for 28 days. It is uncertain whether the pharmacological actions could be further extended. In any case, 1 month would be too short a clinical dosing interval for such a system. A longer duration (up to 90 days) of photoreceptor rescue in diseased rats was seen with genetically engineered embryonic stem cells that were injected into the vitreous without any polymeric carrier [66]. The inner limiting membrane hindered the access of most cells to the retina, but adverse effects were also reported (e.g., tractional detachment and hyperplasia).

So far, there are no ocular examples of drug delivery with nongenetically engineered cells; nevertheless, but this approach remains an interesting option for ocular drug delivery.

Prospects for ocular drug delivery

The eye is an attractive target for drug delivery with modified cells. Importantly, experience from the clinical studies of Neurotech [62–64] demonstrates that genetically engineered cells can be used safely in the eye for long-term drug treatment. The eye includes small compartments where therapeutics can be administered locally, including topical, subconjunctival, intravitreal, suprachoroidal, and subretinal routes of delivery. In general, the required dosing rates are lower than with systemic drug treatments. Thus, drug loading, release rate, and required cell quantities are small, but depend on the potency of the drug. In general, the required dosing levels are lower for intraocular delivery (intravitreal and subretinal) than for topical and periocular (subconjunctival and suprachoroidal) routes of administration. Several membrane barriers and flow factors (e.g., blood flow and tear turnover) limit drug bioavailability to intraocular targets after topical and periocular administration [67]. Required drug payload and release rate can be estimated *in silico* based on the route of ocular drug administration and required drug concentration at the target site [18,67]. Furthermore, the blood–ocular barriers generate immune privilege to the intraocular compartments [68], thereby facilitating the use of modified cells for drug delivery. Genetically modified cells can deliver therapeutic proteins, but nongenetic engineering could be used also for the delivery of small-molecular drugs (e.g., cortico-

steroids for diabetic macular edema and tyrosine kinase inhibitors for AMD treatment).

Modified cells have attractive properties, including long retention, prolonged drug release, and homing to diseased tissue. Surface modifications of cells or plasma membrane vesicles can be tuned to graft the surface of the plasma membrane with affinity compounds for the vitreous (e.g., vitreous-binding peptides) [69]. A combination of drug encapsulation and plasma membrane modification could result in optimized localization and/or retention and prolonged drug release, key aspects for the long duration of drug action.

Properties of the modified cells can be utilized for homing to target site; for example, polymorphonuclear cells and macrophages to the ocular inflammatory regions in uveitis, AMD, and diabetic retinopathy [70]. In addition, magnetic particles within cells have been used for the site-specific localization of cells in the body [71], targeting ligands can be used for tissue-specific homing of the modified cells, and convective flow in the vitreous can guide cells to the macular region [72]. Some parts of the eye are transparent, allowing monitoring of the implanted cells after their delivery, for instance in the conjunctival sac, vitreous, and subretinal space. Optical properties also allow the use of external signals to control drug delivery (e.g., light, magnetic field, or ultrasound) [73,74].

Even though modified cells have many promising features, more research is needed to reveal the true potential of the cells as ocular drug delivery systems. It is important to understand how chemical modifications change the properties of the cells, such as immunogenicity, interactions with other cells and extracellular matrix, cell plasticity, and viability. For example, macrophages have two subclasses (M1 and M2) that have different biological effects, and purity of the cell population then becomes an important issue. Large-scale manufacturing, sterility, storing, and transportation of such products are not easy. Logistics would be easier if the technology could be prepared on site in hospitals.

Modified cells are more complex than physicochemical drug delivery systems, such as implants, microspheres, and nanoparticles. However, they have potential advantages over traditional drug delivery systems. In particular, genetic and nongenetic modifications offer versatile means for prolonged drug delivery (even lifetime) and chemotactic tissue homing features that might not be attainable with traditional methods.

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