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Dosage forms in therapy of posterior segments of the eye

Diploma Thesis

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Hradec Kralove, 2016

Statement of originality

I declare that this diploma thesis is my own, original, personal work. All literature and other resources I used while processing are listed in the reference list and are properly cited.

Date: 1.5.2016

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1 Abstrakt

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Název diplomové práce: Lékové formy k terapii zadních očních segmentů.

Terapie nemocí postihujících zadní partie oka je obtížná. Protože většina těchto chorob hrozí oslepnutím pacienta, patří v současnosti lékové formy pro terapii zadních očních segmentů k intenzivně se rozvíjející oblasti. Tato řešerská práce si klade za cíl podat přehled anatomických a fyziologických specifík předních a zadních očních segmentů. Jsou referovány nejběžnější patologické stavy zadních partií oka, jako je např. makulární degenerace či cytomegalovirová retinitida. Jsou prezentovány aplikační cesty a lékové formy, které se k terapii využívají, včetně lékových systémů a cílení léčiva.

2 Abstract

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Title of Thesis: Dosage forms in therapy of posterior segments of
the eye.

Therapy of the posterior eye segments is very difficult. Because most of diseases of the posterior eye segments could lead to complete blindness, the research of dosage forms for the drug delivery into this eye part is intensively developing at this moment.

The aim of this thesis is to provide a review of anatomical and physiological specifics of both- anterior and posterior segments of the eye. The most common diseases of the posterior eye are referred to, for example the age related macular degeneration and/or the cytomegalovirus retinitis.

There are various routes of application and drug dosage forms that are used for the therapy and which are mentioned in this work, including the drug delivery and targeting systems.

3 The aim of the study

The aim of this thesis is to compile a literature review focused on the possibilities of the therapy of the posterior segments of the eye. The introduction is dedicated to the anatomical description of anterior and posterior part of the eye. Consequently, the attention is focused on pathologies in the posterior segment. It is compiled a review of the contemporaneous treatment possibilities of the posterior segments including modern approaches on drug targeting.

4 List of Abbreviations

Abbreviation	Meaning (explanation)
AMD	Age related macular degeneration
ATC	Anatomical therapeutic chemical classification
BRB	Blood-retinal barrier
CMV	Cytomegalovirus infection
CNV	Choroidal neovascularization
Da	Dalton
DME	Diabetic macular oedema
ECT	Encapsulated cell technology
EVA	Ethylvinylacetate
FA	Fluocinolone acetonide
GDNF	Glial-cell-line-derived neurotrophic factor
IOP	Intraocular pressure
LDL	Low density lipoprotein
NLCs	Nanostructured lipid carriers
PDT	Photodynamic therapy
PGA	Polyglycolic acid
PLA	Poly(lactic acid)
PLGA	Poly(lactic-co-glycolic acid)
PVA	Polyvinyl alcohol
ROS	Reactive oxygen species
RPE	Retinal pigment epithelium
SEAP	Secretion of alkaline phosphatase
SLNs	Solid lipid nanoparticles
VEGF	Vascular endothelial growth factor
TGFB2	Transforming growth factor B2
TNF	Tumor necrosis factor

5 Introduction

Human sight is very important sense and eye is an organ that provides us to see. A person without the possibility of seeing is losing his or her freedom and self-sufficiency. Although there are many blind people in the world, that are independent, it must be very difficult to live like a normal healthy individual. Serious diseases of posterior eye segments can be also one of the main causes of blindness. Their therapy is very difficult because of many barriers physiologically protecting the eye. This fact is the cause of difficult ophthalmic drugs bioavailability to target tissues.

Generally, eye preparations are made using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of microorganisms.¹ The absence of local toxicity and irritation are the main requirements on ophthalmics, for the aqueous-based formulations represented by the isotonicity value, pH adjustment, protection from the microbial contamination for multidose formulations and thus to avoid any discomfort.^{2,3}

The eye's anatomy is very complicated and specific. Every part of eye collaborates with each other. From a physiological point of view, the eye is divided into two parts- the anterior and posterior sections.⁴ In details, it will be described in this thesis, with the focus, of course, on the posterior segments.

6 Anatomy of the eye

The anterior part of the eye is composed of the cornea, conjunctiva, sclera, anterior uvea, ciliary body, iris, aqueous humour, lens and lachrymal system.⁵ The posterior part consists of retina, choroid and vitreous.⁵ For illustration, see Figure 1.

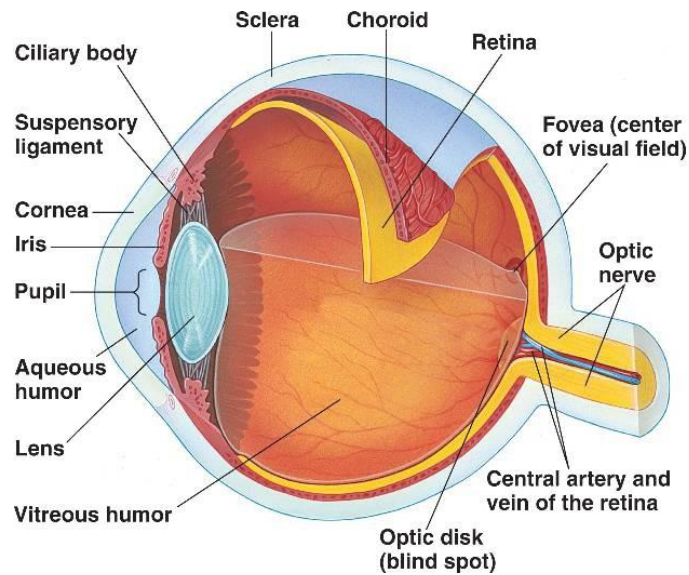


FIG. 1: Anatomy of the eye.⁶

6.1 Anterior eye

The main function of the anterior segment is to focus the light coming into the eye.⁷

Cornea is a clear transparent avascular structure, which is in state of relative dehydration. It is the extension of the outer layer, sclera.⁸

The role of cornea is formation of an impervious barrier between the eye and the external environment. It has a function of mechanical and chemical protection.⁹ The other purpose is focusing the light on the retina.¹⁰

On the surface of cornea, there is a tear film spread for the protection of the corneal epithelium. Tear film has mechanical and immunological function and is composed of three layers that are connected to each other: oleic, aqueous and mucinous.⁹

Three main parts that constitute cornea are epithelium, stroma and endothelium.

Epithelium forms the surface of cornea and is composed of 4-6 different layers. The regeneration of this structure is very fast, it takes only 7 days to recover.⁹ It contains limbal cells that provide the regeneration and has villi that are capable of adherence to the mucinous layer of tear film. It is important to maintain epithelium in an intact state, because only in such state it can perform its role-to protect eye from infection.⁹ The corneal epithelium is composed of lipophilic cells. Therefore, molecules with lipophilic structure will pass this layer easier. On the other hand, hydrophilic substances will not pass this structure. The cells of epithelium are connected with each other by desmosomes and are surrounded by tight junctional complexes that assure reduction of the paracellular permeability to the intracellular space.¹¹

Bowman membrane (*Lamina limitans anterior*) separates epithelium from stroma and is important for the correct setting of basal cells in epithelium. Damage of this structure can cause a scar on the cornea.⁹

Corneal **stroma** is the widest cornea layer. It is composed of extracellular matrix and lamellae that are formed from collagen fibrils. Keratocytes, that are considered the final layer of corneal stroma, are located between collagen lamellae.⁹ Corneal stroma is well hydrated and has a polar character. That is why hydrophilic particles can pass through it. On the other hand, stroma represents a strong barrier for lipophilic molecules.¹¹ Compared to the corneal epithelium, stroma has low regeneration ability.⁹

Descemet membrane (*Lamina limitans posterior*) is a layer between corneal stroma and endothelium. The membrane is composed of collagen fibrils as well, but they are not in a form of lamellae, but in a grid, which is why Descemet membrane is a very rigid structure.⁹

Endothelium is the inner layer of cornea and is composed of cells with hexagonal shape. Endothelium separates stroma from aqueous humour. In contrast with epithelium, there are large spaces and no tight junctions. Therefore, molecules can penetrate more easily through it.¹⁰ The main function of the endothelium is hydration of cornea.⁹

To pass through cornea, substances must have amphiphilic character. Molecules with this arrangement have both hydrophilic and lipophilic part. Therefore, they can cross all layers of the cornea.¹¹

Positively charged drug formulations are able to enhance penetration thanks to interaction with the negative charge of corneal membrane.⁸

Metabolism of cornea is slow, so it is evident, that its regeneration would take more time. Amino acids and glucose are very important for the regeneration and nutrition of cornea and, of course, oxygen is essential for this eye part as well as for many other structures.⁹

Conjunctiva is a transparent membrane, which has the function to produce mucus, an important substance for lubrication of the ocular surface. Conjunctiva is very sensitive, because it is well innervated. If irritated by inflammation, mechanical or chemical stimulus, it induces painful feelings including cutting, burning-like phenomena, tearing, etc.¹²

It is well-vascularized structure, which allows the elimination of active ingredients into systemic circulation.⁴ Blood and lymphatic capillaries that surround conjunctiva are a problem for the bioavailability of drugs, because they can cause higher drug absorption to systemic circulation leading to reduction of drug's concentration.¹¹

The intracellular conjunctival space is wide and permeable for molecules, especially the larger ones. Therefore, several drugs can be administered this way.¹⁰

Aqueous humour is a clear watery fluid, which is situated both in the anterior and posterior chambers between the posterior surface of the cornea and anterior lens. Aqueous humour is being secreted thanks to the ciliary body at the posterior part, and it goes to the anterior chamber through the pupil. Aqueous humour is important for delivering nutrients, removing the waste from avascular tissues and regulating the intraocular pressure, which is important for maintaining the convex shape of the cornea.⁴

Uvea is a layer composed of iris and ciliary body at the anterior part and choroid at the posterior segment.¹⁰ Because of its very good vascularization, it is important for supplementation of the eye by blood.⁹

The main function of iris is to line the anterior and posterior part of the eye. In the centre of the iris, there is an aperture, pupil maintained by muscles, which allow it to dilate and extend due to the intensity of the light. Less light leads to mydriasis. On the other hand, miosis is caused by inducing the light. Due to the innervation of the sympathetic, reactions to the stress occur and cause the change of pupil's shape.⁹

Ciliary body is a structure in the middle layer of the eyeball placed just behind iris. Ciliary muscles, whose contractions regulate accommodation of lens, form a part of ciliary body.^{4,10}

Lens is a tissue situated between aqueous and vitreous humour. It has a fibric structure thanks to epithelial cells; therefore, it is very plastic and can change the curvature radius and refractive index. The lens allows active control of the light penetration.⁴

Lachrymal system is useful for production of tears and drainage of the ocular surface. Lachrymal system comprises lachrymal gland, which secretes tears. Tears are composed of water, electrolytes, lipids, proteins, glucose and mucins.⁴ During inflammation, inflammatory mediators, antigens and cytokines can be found in tears. Tears' physiological function is to protect, hydrate and cover the ocular surface.

The tear film is approximately 4-9µm wide and consists of three layers.¹³ The upper lipophilic layer, which regulates the tears' evaporation, contains primarily neutral oils (4%), phospholipids (16%), sterol esters (32%), waxes (35%) and other lipids (13%). It contains also surfactants that simplify spreading tears over ocular tissues. The middle layer contains water, electrolytes and proteins. The osmotic pressure is about 311-350 mOsmol and it is maintained thanks to the presence of Na⁺, K⁺, Cl⁻, HCO₃⁻ and proteins. Tears have antibacterial effect thanks to high levels of enzyme lysozyme. The lower layer is in contact with cells of conjunctival epithelium and

contains glycoprotein mucin, which is composed of approximately 25% proteins and 75% of saccharides.¹⁴

Usual daily volume of created tears is about 7–10 μl and they are produced by secretory cells.⁴ Lachrymal fluid flows through cornea and its residues are collected in lachrymal sac, which has the capacity of 20–30 μl . Thanks to winking, the excess of tears is drained to the nasolachrymal duct and consequently to the nose. This tear drainage is a reason of systemic adverse effects of drugs.

With standard speed of 1–2.2 $\mu\text{l}/\text{min}$, the mechanism of tear drainage is one of the most powerful in human body.¹⁵ The absorption of drug into the eye is slower process than its elimination, which results in a fast decrease of drug concentration.¹⁶

6.2 Posterior eye

Retina (Fig. 2) is the inner layer of the eyeball.¹⁰ It is transparent structure, which is connected with choroid on the outer side and vitreous on the inner one.⁴ The function of this tissue is to transfer light signals into the electrochemical ones and to send them to the brain through optic nerve.¹⁰

Retina is composed of several layers-retinal ganglion cell layer, interneuron's layer and photoreceptor's layer.¹⁰

In the outer layer (photoreceptor's layer) of retina there are important receptors called rod cells and cone cells. They are very specialized and connected with ganglion cells of retina by three-neuronal line. The photoreceptors can change the light stimuli into the electrochemical response and send the signal to the optic nerve and further to the brain.⁹

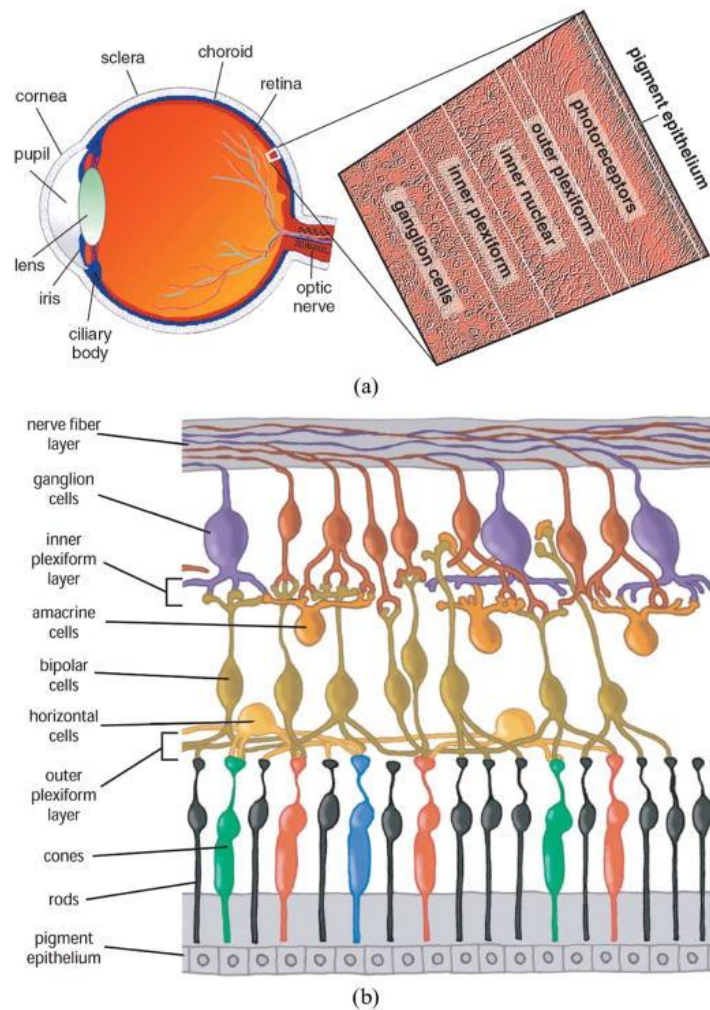


FIG. 2: Structure of the retina.¹⁷

Photoreceptor rod cells (rods) involve pigment rhodopsin, which is responsible for scotopic vision (seeing during poor light conditions).¹⁸ There are approximately 140 million rod cells in retina. With increase of age, the number of rods is reducing up to 30 % of the original amount.

They differ in their function and shape from the cone cells. Unlike every thin rods, the cones have a cylindrical shape.⁹ Thanks to their pigment opsin, the function of cones is to provide sight during the daylight (photopic vision).¹⁸ They differ from rods also in their quantity; their amount is much lower. There are approximately 5 million of cones in retina, but their advantage is constant amount despite the aging of organism. Most cones are in the fovea and they are in the *macula lutea* too. Colour distinction and focused view is provided thanks to the cone cells.⁹

Both of the receptor types contain visual pigment, which is located in the outer segment of photoreceptor's layer that is continually recovering. The rods are eliminated during the day compared to the cones that are removed at night. The inner segment of photoreceptor's layer is very important for metabolic processes. This is relevant for nutrition of retina and keeping it in good condition. This mechanism can function due to mitochondria, where oxidation reactions occur.⁹

The change of light signal into the electrochemical one is being processed in the outer segment of photoreceptor's layer.⁹

The receptors are connected with the pigment epithelium (RPE), which is composed by one layer of pigment cells. Retinal pigment epithelium contains melanin, an important pigment for the protection of cell nuclei, which could be damaged by the strong effect of the light. With an increase in age, the shape and size of RPE cells are changing and the amount of these cells is reducing.⁹

RPE has several functions: (1) based on hemoretinal barrier's principle, (2) a metabolic function, which provides necessary fluids and nutrients for the retina, (3) the synthesis and regeneration of the retinal pigment cells, (4) the synthesis of growth factors, e.g. the best-known growth factor VEGF.⁹

Thanks to the fact, the retina is formed by several layers; it is well protected and is a good barrier against the exogenous substances. However, it also lowers the possibility of the drug to get to the posterior segment, which can be unfortunate for the treatment of the eye diseases.⁹

Choroid is a pigmented medium layer of the eyeball, which is situated between retina and sclera and is extended from optic nerve to the ciliary body.⁹

Choroid is important for carrying oxygen and nutrition to the retina and maintaining intraocular pressure and thermal equilibrium.⁴

Vitreous humour is a transparent structure composed of 99% water. It has a gel constitution and occupies 2/3 of the volume of the eyeball.⁴ The vitreous is composed mainly from solutes, ions and compounds with low molecular weight, including predominantly collagen, hyaluronic acid and glycosaminoglycan hyaluronan, which carries the negative charge.¹¹

In general, molecules with anionic negative charge will pass better through the vitreous.¹¹ In opposite, the positively charged molecules as lipids, polymers and DNA liposomal complexes, can interact with glycosaminoglycan. The attractive forces between molecules and glycosaminoglycan lead to the creation of aggregates, stopping the movement of these molecules.¹¹

Sclera is a part of the eye, formed by many proteoglycans and filaments of collagen, which maintains the right conformation of the eye globe.¹¹ Compared to the cornea, sclera is predominantly on the bulb providing the protective role.⁴

The tissue is permeable for proteins and other macromolecules.⁵ Therefore, permeation depends on a structure of molecule in space. It is more permeable for substances with globular formation. On the other hand, linear molecules cannot pass through the sclera so easily. Positively charged molecules are poorly permeable due to their binding to the negatively charged proteoglycan matrix.¹¹

7 Bioavailability of drugs after ocular application

7.1 Ocular barriers

The eye is well protected from the external influences and pathogens thanks to its barriers as illustrated in Fig.3. Therefore, it is a strong barrier also for the drugs, limiting their bioavailability and pharmacological effects.

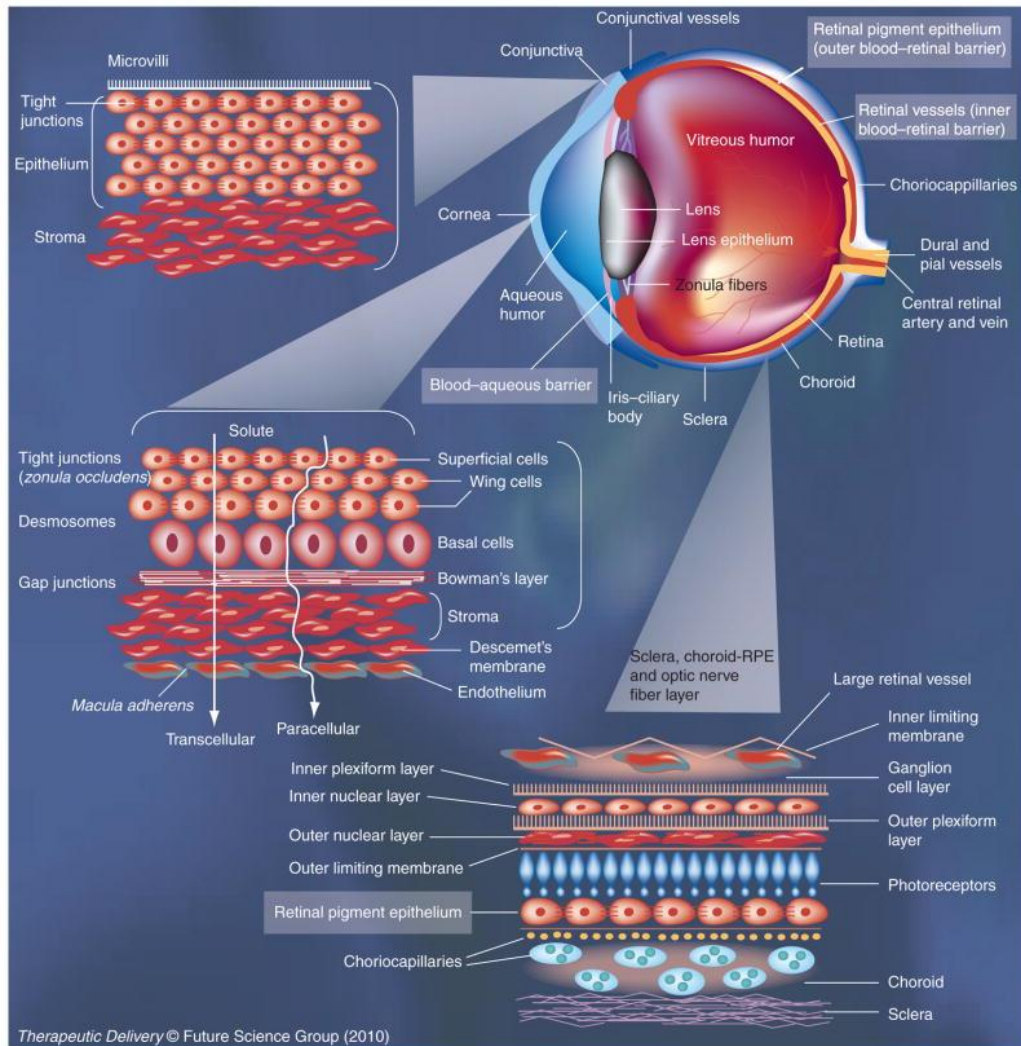


FIG. 3: Barriers for ocular drug delivery.¹⁹

7.1.1 Precorneal barriers

Generally, there are precorneal barriers, which are characterized by **dosage spill-over, nasolachrymal drainage, blinking, tear film, tear mucins and low corneal permeability.**^{9,20}

The most of drugs penetrate into the eye by transcorneal diffusion. Except corneal barrier, they have to pass also the precorneal barrier that consists of tear film.²¹ The precorneal barrier is characterized by high turnover rate, 5–6 minutes are enough for washing the drug away, and thus time for drug's absorption is very short.⁴

Tears contain buffering systems-carbonates and weak organic acids. They can change ionization and thus bioavailability of the drug.⁴ Because of the fact that pH of tears is 7.4, there is an effort to manufacture drug solutions of this pH. However, most of eye drugs are weak acids and they are unstable in neutral and alkaline solution. Therefore, it can be added an acid to keep the chemical stability, but it can cause irritation after application, lachrymation, consequently dilution, and lower absorption of the drug.²²

Tears contain also proteins and mucins. Therefore, drugs with the affinity to proteins have a tendency to bind to these structures, which leads to a lower concentration and bioavailability of drug.²³

In addition, several enzymes and enzymatic antioxidants that protect the eye from the oxidative stress and formation of reactive oxygen species (ROS) may be found in tears.²⁴

Nasolachrymal drainage of tears and systemic absorption through a mucous membrane of nasolachrymal ductus lead to decrease in drug concentration in precorneal area.⁵

For the reason mentioned above, traditional ophthalmic preparations such as eye drops have poor bioavailability of the drug, usually referred to less than 5%.²⁵ New formulations, therefore, are being developed to prolong contact time of the preparations and to increase drugs efficiency and bioavailability. The most common ones are gels or gels *in situ*, ointments and ocular inserts.²⁶

7.1.2 Corneal and conjunctival barrier

Cornea is a barrier of the eye, which is considered a mechanical barrier blocking passage of substances to the eye.⁴

Corneal epithelium can be understood as the main barrier limiting drug diffusion into the eye.¹¹ It is composed of lipids; therefore, it shows lipophilic properties. The diffusion of the administered drug depends directly on its structure.¹¹ Due to the cornea lipophilicity, hydrophilic substances cannot pass through this layer.⁴ However, there are also tight junctions, which decrease paracellular transport.

Penetration of drugs is also limited by molecular weight. Only molecules with less than 50 000 Da are able to diffuse through epithelium.⁴

Stroma is a medium layer of cornea, which has a hydrophilic nature, thus only polar molecules can get through it. Either stroma is permeable only for molecules smaller than 50 000Da.

Endothelium has the same lipophilic character as epithelium and contains many phospholipids. Therefore, permeation of ionized molecules is reduced.

In order to pass through all three layers of the cornea, molecule must have amphiphilic character.⁴ The rate of absorption depends on its physicochemical parameters: solubility, lipophilicity and partition coefficient, molecular size and shape, ionization degree and charge.²⁷

Due to high vascularization, also **conjunctiva** is considered a barrier for drug transport.⁴ It is a leaky tissue with a surface twenty times bigger compared to the cornea. Larger bioorganic molecules like peptides or proteins can pass through it and be absorbed to systemic circulation reducing drug's bioavailability.⁵

The principles of passive diffusion and active transport of the cornea and conjunctiva are crucial for the future scientific developments of new ophthalmic formulations.²⁸

7.1.3 Posterior barriers

The eye is protected from exogenous substances by biological **blood-ocular barriers** (see Fig 3.), which have two parts: blood–aqueous and blood–retinal barrier.^{5, 20}

Blood–aqueous barrier is formed by two cell layers located in the anterior segment of the eye, the endothelium of the iris and the non pigmented ciliary epithelium. Both cell layers express tight junctional complexes and prevent the entry of solutes into aqueous humour.^{11,29}

Blood–retinal barrier (BRB) is constituted of **retinal pigment epithelium (RPE)** and tight **retinal capillary walls** and is formed by tight junctions between the endothelial cells of the retinal vessels (the inner BRB) and by similar tight junctions in the retinal pigment epithelium (the outer BRB).³⁰ The inner BRB impedes molecules larger than 20–30 kDa to pass through retinal vessels. Small molecules,

such as glucose and ascorbate, can pass by facilitated diffusion. In the outer BRB, there are tight intercellular junctional complexes-*zonula occludens*. There are two types of molecular movement across the blood-retinal barrier: (1) active transport from the vitreous into the blood (amino acids, organic anions, prostaglandins, and fluorescein), (2) passive transfer across the BRB and into the vitreous due to the concentration gradient. Although, the second transport is very limited, it occurs with sodium, phosphate, glucose and potassium.²⁹

The absorption (and, however, bioavailability) to the retina and vitreous in posterior segments after systemic and intravitreal application could also be influenced by melanin. This is a pigment, namely presented in choroid (uvea) and RPE, which binds to free radicals and xenobiotics thanks to Van der Waals forces or attractive forces.¹¹ Lipophilic and alkaline molecules are powerfully attracted. For the treatment of eye diseases, it is important not to forget this process and it is necessary to consider the medication very thoroughly.¹¹

7.2 Elimination of drugs from eye tissue

Drug elimination from the vitreous after intravitreal injection, is determined by anterior bulk flow of aqueous humour, posterior elimination via retinochoroidal flow and transcellular transportation mediated by specific carrier protein in the retinal pigment epithelial cell membranes.³¹

Although blood-retinal barrier is considered fundamental barrier for the entrance of active substances into the ocular tissues, it is able to provide an opposite permeation of the drug into the systemic circulation as well. Therefore, it also functions as an elimination tissue.

Molecular weight, lipophilicity, hydrophilicity and ionic charge can affect diffusion through the vitreous as well as blood–ocular barriers and the elimination rates of the drug delivered to the posterior segment.³¹

The vitreous is becoming more rigid with the aging of the organism and after vitrectomy, which leads to changes in a diffusion coefficient of administered drugs.²⁰

8 Posterior eye segments diseases

The most common diseases that affect posterior segments are **age related macular degeneration (AMD), retinitis pigmentosa, cytomegalovirus retinitis, retinal vein-occlusive diseases and diabetic retinopathy**, which causes neural changes.⁵

8.1 Age related macular degeneration

A disease process characterized as an abnormality of the retinal pigment epithelium (RPE), which leads to deterioration of the photoreceptor cells in the *macula lutea* and consequently loss of central vision.³²

This disease usually occurs in people over 55 years of age, and is the most common reason of vision loss in elderly patients. AMD is considered a multifactorial disease, which can be promoted by the environmental influence, genetic material and gender, the human race, cardiovascular illnesses, smoking, and UV radiation, light colour of the iris or several drugs, whose metabolites lead to the toxic side effects. The examples of these medicaments are antimalaric or antipsychotic drugs with the phenothiazine group.³³

AMD is a proliferative defect (considered even a cancer), which leads to damage of retina and optic nerve and eventually to blindness. As mentioned before, during this pathological state, the yellow spot (*macula lutea*), which permits the central sharp vision, is impaired.³²

Generally, AMD is classified into two forms-dry and wet.

Therefore, dry AMD, having generally a gradual development, is represented by drusen and hyper- or hypopigmentation of the RPE without loss of vision.³³ The wet type of AMD (exudative) has a very dangerous character with the detachment of the part of the RPE epithelium leading to loss of nutrition guaranteed by the associated capillaries. Consequently, RPE cells become atrophied. Abnormal growth of blood vessels leads to choroidal neovascularization or bleeding near the *macula lutea*.³³ Sometimes, the combination of both forms of ADM is also possible.³³

The cells of retinal pigment epithelium (RPE) are responsible for the right metabolism and phagocytosis in the posterior part of the eye. However, if the

function of RPE cells is damaged by the influence of the AMD, non-metabolized substances are accumulated in this area.³²

The best known of these substances is lipofuchsin, chemically a combination of proteins, lipids and carbohydrates. A large amount of lipofuchsin leads to tissue ageing.³⁴ Lipofuchsin is activated by oxygenous free radicals that are formed during angiogenesis, the process associated with AMD. The accumulation of lipofuchsin results in the apoptosis of the RPE cells leading to the reduction of permeability and accumulation of metabolic side toxic products.³⁴

Cells of the retinal epithelium produce VEGF (vascular endothelial growth factor), which is a crucial factor in the pathogenesis of AMD.³² Physiologically this factor is very important for the right nutrition and the exact throughput of choroid and protection of the retina. It stimulates endothelial cell growth, is responsible for better vascular permeability and causes dissociation of tight junctions' components. It is useful also for the other organs of human body like liver or kidney. Without that, the regeneration of musculoskeletal system, female reproductive cycle or vasorelaxation are not possible.³² In the case of wet AMD, it is considered a strong inductor of angiogenesis and neovascularization.³⁵ The other undesirable effect of VEGF is higher risk of inflammation because of increased permeability of capillaries. Inhibitors of VEGF, mainly monoclonal antibodies, impede the overproduction of this growth factor.^{32,35}

Histologically, AMD is characterized by accumulation of membranous debris under the RPE membrane forming drusen (Fig.4). Drusen are small, yellow extracellular structures composed of lipids, cellular debris and proteins, proteins of the immune system like complement structures, anaphylatoxins and modulators. The formation of drusen can be provoked by a dysfunction of RPE cells or changes in a permeability or structure of Bruch's membrane.³²

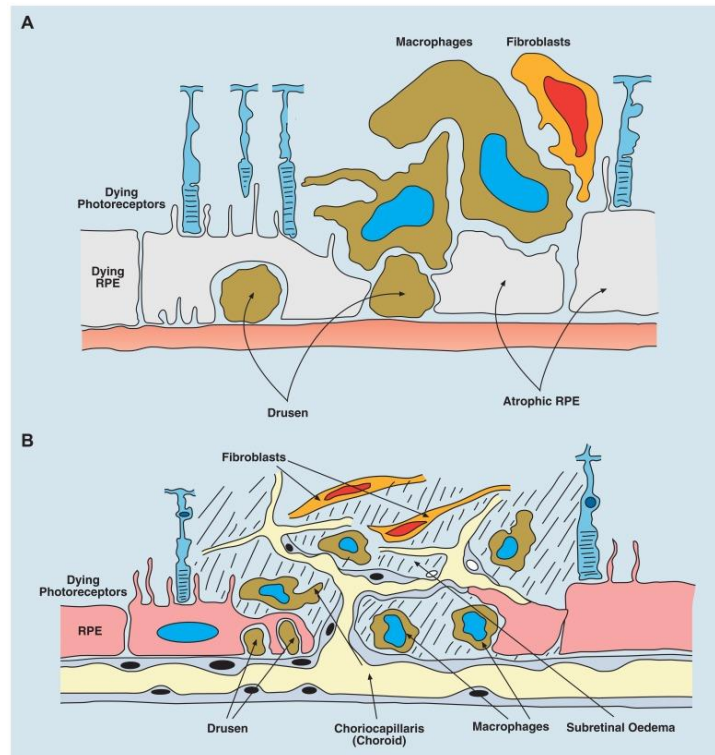


FIG. 4: Histological changes during AMD.³⁶

With increasing occurrence of macular degeneration, there is raised interest in the treatment of this disease. Nowadays there are several methods used in the treatment of this illness. Those are photodynamic therapy and VEGF inhibitors, which will be referred to below.³²

8.2 Other diseases of posterior segments

Retinitis pigmentosa (photoreceptor's degeneration) is an inherited disease, which leads to the retinal degeneration often leading to the blindness.³⁷ The patients usually experience night blindness followed by loss of vision and progressing toward the centre vision impairment. During this disease, mainly rod photoreceptor system is affected undergoing apoptosis. In a later phase, cone photoreceptors may be further affected leading to complete blindness.³⁸

For the treatment, intraocular injections of myriocin are used. It is an inhibitor of serine palmitoyl-CoA transferase, an enzyme important for ceramide biosynthesis.³⁷

Cytomegalovirus retinitis (CMV) is a disease occurring in cases of patients suffering from immunodeficiency, especially people infected by HIV virus and having AIDS. Retinal detachment that continues to the retinal necrosis leads to the blindness. For the treatment, intravitreal injections of ganciclovir are needed.³⁷

Diabetic retinopathy is the major cause of blindness and visual damage in working-age individuals.

Retinal vein-occlusive diseases comprise central retinal vein occlusions, hemiretinal vein occlusions and branch retinal vein occlusions.

Diabetic retinopathy and retinal vein-occlusive diseases leads to loss of vision through the following mechanisms: retinal vascular leakage and exudation, which results in macular oedema; and retinal ischaemia and secondary neovascularization, which can cause vitreous haemorrhage and detachment of the retina.^{32,39}

Current treatments for diabetic macular oedema or macular oedema derived from retinal vein-occlusive diseases include anti-VEGF therapies, focal laser therapy and steroids.³²

8.3 Active drugs in therapy of posterior segments

For the therapy of posterior segments, there are drugs from various Anatomical Therapeutic Chemical Classifications (ATC). Corticoids like dexamethasone, fluocinolacetonide and triamcinolone acetonide represent the main part of used drugs. However also drugs like antivirotics (ganciclovir), antibiotics (penicillin G) are frequently used. In addition, parasymptolytics (tropicamide) or parasymptomimetic alkaloids (pilocarpine) can be found in several dosage forms.⁴⁰ Monoclonal antibodies are the promising drugs in future for diseases affecting posterior part of the eye. They are the most common drugs used for the therapy of AMD even though they are used for short period.³²

Ranibizumab is the substance from the group of monoclonal antibodies used for the therapy of the wet form of macular degeneration.³⁵ It is present in medicine called Lucentis 10mg/ml. It is a humanized substance produced by the cells of bacterial

stem *E. Coli*. Consequently, it is modified by the technique of recombinant DNA technology for having needed therapeutic effect.⁴¹ Ranizumab, which is administered intravitreally, affects each isoform of VEGF-A.³⁵

Pegaptanib (Macugen) is an anti-angiogenic drug for the treatment of wet AMD and is applied intravitreally once every six weeks, because it is quickly metabolized by the enzymatic nucleases in the eye.³⁵ Pegaptanib is a PEGylated anti-vascular endothelial growth factor (VEGF) aptamer, which binds to the isoform VEGF-A16, causing the deactivation of this factor.³⁵ This drug has a high affinity to the neovascularized choroids without quite any effect on physiologic capillaries.⁴¹

Bevacizumab, which was initially approved for the treatment of certain types of cancers, is the other substance from monoclonal antibodies group.³² It is a human antibody used off-label for the treatment of wet AMD. However, the formulation of this drug is not suitable with the intraocular administration. In addition, several clinical trials observed that bevacizumab has more adverse effects than ranibizumab.^{42,43}

Aflibercept is a fusion protein that binds to all of isoforms of VEGF-A with high affinity. It is approved for the treatment of wet macular degeneration within the intravitreal administration.³²

TargeGen 801 VEGF receptor/Src kinase inhibitor is a prodrug, which has antipermeable, antiangiogenic and anti-inflammatory properties and acts against VEGF factor. For the administration of this substance, the transscleral route of application is being used. However, it is still in clinical trials.⁴⁴

9 Dosage forms in therapy of posterior segments

9.1 *Traditional ophthalmic preparations*

According to a Pharmacopoeia, eye preparations are sterile liquid, semisolid or solid preparations intended for administration upon the eyeball and/or to the conjunctiva, or for insertion in the conjunctival sac.¹

Eye preparations are classified into several categories such as eye drops, eye lotions, powders for eye drops and powders for eye lotions, semisolid eye preparations and ophthalmic inserts. Depending on whether the target is the anterior or posterior segment of the eye, different drug delivery systems are utilized.¹ In this thesis, eye drops, semisolid eye preparations and ophthalmic inserts are mentioned.

Eye drops are sterile aqueous or oily solutions, emulsions or suspensions of one or more active substances intended for instillation into the eye. They generally contain excipients to adjust the tonicity or the viscosity of the preparation or to adjust or stabilise the pH, to increase the solubility of the active drug or to stabilise the preparation. The excipients must not affect a medicinal action or cause a local irritation.¹

Preparation in multidose containers must involve a suitable antimicrobial preservative in a certain concentrations.¹ The exception is when a formulation has its own antimicrobial properties or a specific container, which protects the preparation from contamination, e. g. COMOD, supplies it.⁴⁵ A single-dose container as well as eye drops for a surgical use do not contain antimicrobial preservatives. Multidose preparations can contain only 10 ml of the preparation and the period of use generally does not exceed 4 weeks.¹

Eye drops are convenient dosage forms for the diagnosis of the ocular diseases, for the therapy of external inflammations and glaucoma. Therapy of posterior segments is very limited, because of a low bioavailability of eye drops in this part. Despite this, eye drops have been studying for the possible drug preferences to pass the

conjunctiva, which makes eye drops a potential drug formulation in therapy of back ocular parts.

The easiest possibility how to modulate biopharmaceutical aspects of eye drops is to enhance their viscosity by the addition of an appropriate viscosity ingredient. The use of semi-solid ocular formulation is the second alternative.^{46,47}

Formulations with *in-situ* forming polymers undergo phase transition forming a gel after a certain stimulus creating semi-solid structure or solid matrix, which gradually releases the drug. This phase transition can be mediated by changes in temperature, pH, and ionic composition.⁴⁸ The most common polymeric gelyfing systems involve chitosan, poloxamer, hydroxypropylmethylcellulose and polycaprolactone.

In general, these systems increase the contact time on the cornea. Therefore, the drug's bioavailability is enhanced. Although it cannot be used for macromolecules, because they have poor permeability through the cornea.³⁰

As they are administered in a form of low viscosity solutions, they allow easy administration to the desired site.¹⁰ These formulations are used in a liquid form for which it is easier to get to the target tissue and they are administered in a form of eye drops or intraocular injection.³⁰

Thanks to these properties, the concentration and absorption of drug increase. Moreover, these systems also reduce the frequency of application improving the compliance of the patients. Although the prolongation of time is only in hours, not months or years, this method represents promising change in the treatment of posterior segments.³⁰

Anionic gellan gum, an example of commercially utilized *in-situ* gel polymer, was accepted for the practical use in eye drop formulations with timolol (Timpoptic-XE).^{49,50} After a contact with surface of the eye and thanks to interaction with sodium ion from the tear fluid, gellan gum can transform the formulation to a gel state.⁵¹

Azasite is a drug formulation consisting of high molecular weight, non-toxic and biocompatible polymer of cross-linked polyacrylic acid and 1% azithromycin.

Specific Dura site technology, where drug particles are suspended in a polymeric matrix represents the main advantage. In the aqueous environment of the eye, particles of azithromycin are released into the tear film. Unused polymer with drug particles is washed away through the lachrymal sac without a block of tear drainage.⁵²

Usage of eye drops with new generation of NSAID or depot eye forms in therapy of macular degeneration is also perspective. It could simplify the treatment because of the drug modified liberation.⁵³

Semisolid eye preparations are sterile ointments, creams or gels intended for application to the conjunctiva or to the eyelids. They contain one or more active substances dissolved or dispersed in a suitable basis having a homogenous appearance. The advantage of semisolid eye preparation is a low irritation of the conjunctiva.¹

Semi-solid eye preparations are packed in small, sterilised collapsible tubes or provided with a sterilised cannula, which can contain at maximum 10 g of the preparation. The tubes have to be closed carefully to prevent microbial contamination. Single-dose containers facilitate the administration without contamination. The period of use generally does not exceed 4 weeks after opening the multidose container.¹

However, they showed several side effects, like blurred vision or matted eyelids, which led to low patient compliance.⁷

Polymeric nanogels and hydrogels are composed of hydrophilic polymers conjugated with the drug. Polymeric nanogels and hydrogels can be used for controlled release of both hydrophilic and hydrophobic substances; it depends on the choice of polymer, which forms the major part. The properties vary from their composition and conformation and the degree of crosslinking. Thanks to the possibility of usage of several polymers, time of degradation can be different. In

addition, external sources like temperature and pH provoke a degradation of a polymer.⁵⁴

Pilogel was an ophthalmic gel indicated for the control of IOP in patients with chronic open-angle glaucoma. Pilogel contained pilocarpine and more than 90% of water and utilized Carbopol 940 (a synthetic high molecular weight polymer of acrylic acid) to enhance a viscosity providing a prolonged retention time in the eye. Unfortunately, it is not manufactured anymore.⁵⁵

Over the years, various approaches have been investigated to enhance the bioavailability of topically administered ocular drugs. The use of suspensions, in situ gelling polymers and semisolid preparations represent the easiest way. Drug delivery systems are examples of the other possibility how increase the effect of ophthalmic drugs.⁵⁶

Ophthalmic inserts are sterile, solid or semi-solid preparations of suitable size and shape, designed to be inserted in the conjunctival sac, to produce an ocular effect. They generally consist of a reservoir of active substance embedded in a matrix or bounded by a rate-controlling membrane. The active substance, which is more or less soluble in lachrymal liquid, is released over a determined period. Ophthalmic inserts are individually distributed into sterile containers.¹

The biggest advantage of these formulations is the opportunity of prolonged and sustained, controlled release of drug from several days to months.⁵⁷

Ocusert was the first insert, which released active substance pilocarpine. It was a type of membrane insert, where the polysaccharide alginate with a drug formed the matrix. Sustained release was possible thanks to EVA membrane (ethylvinylacetate). Nowadays it is not manufactured anymore.

Lacrisert is the insert used for the treatment of dry eye syndrome. Lacrisert is composed of a soluble matrix of hydroxypropyl cellulose. When inserted into the conjunctival sac, it stabilizes the lachrymal film.

Unfortunately, this type of drug system is not very popular, because of the application, which is quite complicated, especially for old people

The other disadvantage is possible loss from the conjunctiva sac during the sleep cycle.³⁰

Minitablets are promising dosage forms with a reduced diameter (2-3 mm). For comparison to classic tablets, see Fig. 5. They can be coated repeatedly, with a small amount of substance. The main advantages of mini-tablets are precise doses, better patient compliance owing to absence of continual instillation, longer residence time in cul de sac, less influence by the nasolachrymal drainage system, reliable drug release, and lower incidences of visual and systemic side effects, cost-effectiveness and ease of production.⁵⁶

They contain bioadhesive polymers, which promote increased contact time with the cornea. Especially biocompatible, biodegradable, non-toxic and inexpensive polymers should be used. Polymers used in mini-tablets include poly (acrylic acids) and carbomers, celluloses (methylcellulose, hydroxypropyl methylcellulose, ethyl (hydroxyethyl) cellulose), chitosan and drum-dried waxy maize starch. Drum-dried waxy maize starches are high-molecular weight, hydrophilic, swellable polymers, which contain chemical groups like hydroxyl, amine and carboxyl, which provide better bioadhesion to the cornea.⁵⁶

The example of tested mini-tablets is a sponge-like minitables containing acyclovir formulated for the treatment of herpes infection. Tested polymers included celluloses and chitosan. Thanks to the ability of sustained drug release and mucoadhesive properties, chitosan showed better properties. The tablets coated with chitosan penetrated across the cornea leading to a complete recovery within 2 days.⁵⁸

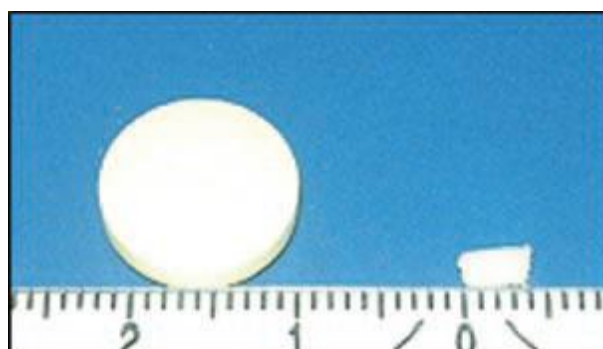


FIG. 5: Comparison of the size of mini tablets and classic tablets.⁵⁸

Nowadays, there are approaches to develop fast-dissolving minitables, which will disintegrate quickly, in a time of minutes or seconds.⁵⁶

9.2 Routes of application to posterior segments

The therapy of the posterior segments is very complicated due to the fact, that there are several barriers in the eye, which represent obstacles for the drug delivery to the target tissues.⁵⁴ There are various routes of drug delivery into the ocular tissues (see Fig. 6). The selection of the route of administration depends on the target tissue. Topical ocular and subconjunctival administrations are often utilized for the treatment of anterior segments and intravitreal administration for the posterior part of the eye. Applied dosage forms have considerable influence on the final drug concentrations and on the duration of drug action.⁵

- subconjunctival
- intravitreal
- suprachoroidal
- sub-Tenon
- sub retinal
- posterior juxtasceral

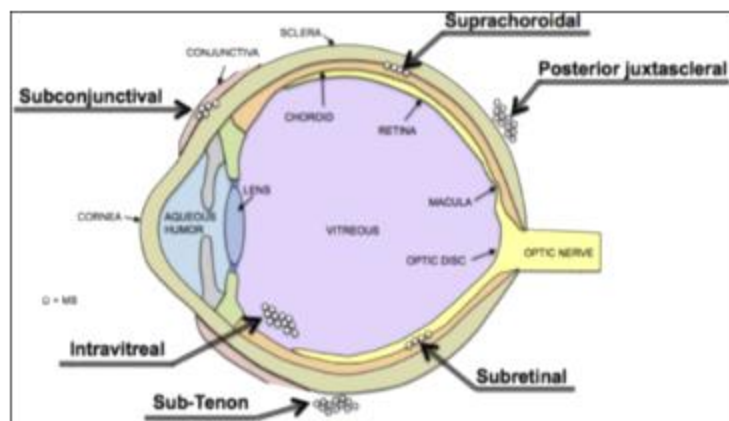


FIG. 6: Injectable routes of application.⁵⁹

Drugs can penetrate into the posterior segment of the eye by: (1) conjunctiva or sclera after topical application avoiding the flow of aqueous humour and lens barrier, (2) from the cornea and aqueous humour after topical application, (3) through the systemic circulation after topical, parenteral, oral and other administrations.^{30,60}

9.2.1 Topical and systemic route of application

Topical application is often connected with the eye drop form, which is being administered into conjunctival sac and is suitable for external treatment anterior segments.¹⁰ Corneal pathway is related with the entry into the cornea following to the interior tissues like iris, aqueous humour, lens, iris and ciliary body. On the contrary, conjunctival pathway is associated with the drug permeability across the conjunctiva followed by entrance into the sclera, choroid, retinal pigment epithelium, and retina.⁵⁴

Topical application is a non-invasive method, which is easy for the patient to use. As mentioned before, the main problem of the topical administration is the low bioavailability that is caused by ocular barriers.¹¹ Apart from this, the penetration to the posterior segment is limited also by the size of macromolecules like nucleic acids.³⁸ A detailed analysis of entry pathways for a drug administered as an eye drop to reach the posterior segment has been tested by Maurice *et.al.*⁶¹ They described that a TNF (tumour necrosis factor) -an inhibitory single-chain antibody fragment (scFv), was delivered to the vitreous, neural retina, and choroid-RPE of New Zealand white rabbits, after repeated topical application of eye drops every hour up to 10 h. The conjunctival pathway was determined as the basic pathway for delivering the TNF-an antibody fragment to the back of the eye

Systemic route (parenteral and less oral) can be used to treat ocular conditions, but the small size of the eye and ocular barriers, blood dilution of the drug, and gastrointestinal barriers in the case of oral route prevent the favourable passage of drugs into the eye. Therefore, large doses must be used to overcome these challenges, which can lead to systemic side effects and possible toxicity.^{10,11,54}

9.2.2 Intraocular application

The aim of intraocular application is to deposit the therapeutic formulation into the eye and in several cases to target directly at the site of action, which may cut the distance the drug needs to pass to increase necessary drug concentration. Further, the

targeting can reduce drug delivery to other sites, reduce side effects, and avoid ocular epithelial and other barriers to increase the bioavailability.¹⁰

Ocular formulation administered by an **injection**, provides the highest bioavailability of the drug to the retina because of the proximity of the vitreous to the retina. However, retinal haemorrhage, retinal detachment, endophthalmitis, and cataracts are the complications associated with intravitreal injections.⁶²

Intravitreal application involves direct injection of the formulation in a form of solutions, particles, suspensions, depot forms or implants into the vitreous cavity through *pars plana* (Fig. 6).²⁰ This application provides high bioavailability in the posterior segments resulting in an approach to the retina, RPE and choroid and thus minimizing systemic side effects.^{20,54} With higher hydrophilicity and greater size of drug molecule, half time of drug in vitreous is longer.¹¹

Intravitreal injection is the main modality to deliver macromolecules to the posterior segment.¹⁰ Prolonged forms like liposomes, microspheres and implants are often applied via intravitreal route.⁵ Transport of drugs and/or drug carriers depends on the structure and charge on the surface of administered particles.¹¹

Nowadays there are new trials to develop the administration of such drugs that would show lower incidence of side effects.³¹

To overcome low penetration of topically applied drug, **intrastromal injections** can be utilized. Furthermore, the cornea can serve as a reservoir for large molecular weight drugs, thanks to its structure composed of stromal proteoglycans, which can delay the diffusion of macromolecules inside the stroma, increasing their half-life.¹⁰

Anti-VEGF drug (bevacizumab) has been used to treat corneal neovascularization utilizing intrastromal route of application. Bevacizumab showed a significant regression of corneal neovascularization and increase in visual acuity.⁶³

Intracameral administration is provided by the injection of a drug into the anterior chamber of the eye. Although intracameral injections are not able to deliver

significant concentrations of drugs to the posterior segments, they should improve low bioavailability of several drugs. Antibiotics and antifungal drugs has been used to treat deep corneal infections via intracameral route. Intracameral injections can be used to deliver drugs into the anterior segment, such as prophylaxis after cataract surgery to prevent endophthalmitis. However, to resist the rapid turnover of fluid in the anterior chamber, repeated intracameral injections are needed to maintain therapeutic concentrations of the drug, which brings an increased risk of side effects.¹⁰

Intrascleral drug delivery has been explored as a possible delivery route to the back of the eye, because sclera is permeable to many drugs, including macromolecules and can potentially act as a drug reservoir for extended-release delivery. Unfortunately, results did not show significant chorioretinal targeting.¹⁰

9.2.3 Periocular application

Periocular application is provided out of the eye globe and is considered the least painful and the most efficient route of drug application. This route reduces complications associated with intravitreal administration.^{20,54} Moreover, it reaches also high drug concentrations in comparison to eye drops.⁶⁴

The periocular route includes **subconjunctival, subtenon, retrobulbar, posterior juxtascleral and peribulbar** administration by injection preparation.³⁸

Periocular route uses permeability of sclera to reach retina tissue exploiting passive diffusion to pass through sclera, choroid and RPE. In addition, physiological barriers like scleral thickness, choroidal blood, and blood-retinal barriers lower drug's bioavailability.^{54, 65}

Although the bioavailability is lower than in the case of intravitreal application, periocular route is a promising alternative thanks to the large surface area and the relatively high scleral permeability.^{20,54}

Subconjunctival route serves to install drug formulation ahead of the conjunctival membrane that covers the sclera; therefore, the drug can bypass the conjunctival–corneal barrier.

In addition, this route offers localized, sustained delivery in the treatment of diseases affecting the posterior segments.⁶⁶ In conjunctiva, there is the conjunctival epithelium, which can be overcome thanks to this route of application.¹¹

Larger surface area, easy accessibility, possibility to apply small molecules and macromolecules and relatively high permeability of sclera are main benefits of subconjunctival administration.⁶⁶ However, blood and lymphatic supply lower the bioavailability of drug washing away applied drug formulation.⁶⁶

Relatively non-invasive **sub-Tenon** (Fig. 6) injection provides the application of the drug formulation between the sclera and Tenon's capsule, which is an avascular membrane that envelops the eyeball from the optic nerve to the limbus. This technique contributes to high vitreal drug concentrations, prolonged contact time between the drug and the sclera, and fewer complications compared to intravitreal route. Although sub-Tenon administration can cause adverse effects like subconjunctival haemorrhage and oedema, it is one of the most promising routes for targeting the posterior segment.^{11,20}

The **subretinal** route is an invasive method utilizing space between photoreceptors and the RPE layer. This mode of administration is often used for targeting drugs into the retinal cells, especially macromolecules during the gene therapy, which is useful in the treatment of the diseases connected with the mutation of gene in photoreceptors or RPE.³⁸ Subretinal injections are applied via transcorneal or transscleral routes. Injection through the transcorneal route infiltrates the iris, lens and vitreous, while the transscleral route passes through the *pars plana* and vitreous.¹⁰

Subretinal injection can cause photoreceptor's detachment from the RPE, leading to irreversible death of photoreceptors if not reversed quickly. Therefore, it is a risky method of administration without a confirmation of long-term safety.¹⁰

9.2.4 Suprachoroidal application

Suprachoroidal application is a potential drug administration site to the posterior segment of the eye.

Suprachoroidal space, which has 30 μm in thickness, lies internal to the sclera and external to the choroid. It is composed of collagen and elastic fibres, fibrocytes, melanocytes, ganglion cells and nerve plexi. The drug is injected across the sclera to flow rapidly along the inner surface of the eye and consequently into the posterior part.⁶⁷ It is interesting that tested contrast agent injected into the suprachoroidal space did not reach the anterior part-lens, cornea or conjunctiva.⁶⁵ Another study executed in rabbits *ex vivo*, demonstrated, that molecular size and lipophilicity of the drug have an effect on suprachoroidal delivery and potential passage into the vitreous.⁶⁸

The manner of application, no interference with optical pathways, safety profile and pharmacokinetics of suprachoroidal route have been studied in rabbit and pig models for treatment of macular diseases.^{38,67} This route has shown safety and tolerability profiles, therefore it can be studied also in humans.

Several strategies have been studied to promote drug delivery to the posterior segment of the eye via the suprachoroidal route; these included polymeric nanoparticles, microcannulation, light-activated in situ gels and microneedles.⁶⁷

Because of a scleral barrier, the most severe disadvantage of this route is poor bioavailability of drugs.⁶⁷ Particularly, macromolecules are quickly cleared and thus sustained release formulation is necessary for longer duration.³⁸ Microneedles has been investigated for overcome this obstacle.⁶⁷ Application of **microneedle** is a quite novel concept, which has been tested by few studies, but it could be a minimally invasive method of injecting drug solutions or particulate systems into the suprachoroidal space. In addition, microneedles could improve drug bioavailability and lower the risk of infection and mechanical damage to the retina connected with the intravitreal route. However, the efficacy of microneedles has been investigated only in *ex vivo* and *in vivo* studies.⁶⁷ This application route will also be describe in Chapter 9.3.

Touchard *et al.* have developed a transfection method called suprachoroidal electrotransfer, which combines the application of a non-viral plasmid DNA into the suprachoroidal space utilizing an electrical field. The electric field is important for driving plasmid DNA into cells. The non-viral plasmid DNA efficiently reached choroidal cells, RPE, and the outer segment of photoreceptors in rat eyes for at least 1 month.⁶⁹

9.2.5 Surgical route of application

In addition to the topical and injectable applications, traditional ophthalmic preparations involve also implantation via the surgical route. Implants, independently, are discussed in Chapter 9.5.

9.3 Physical methods in therapy of posterior segments

Iontophoresis is a non-invasive method of delivering ionized drugs through membranes with low electrical current.⁷⁰ The drugs are transferred across the membranes by two mechanisms: migration and electro-osmosis.

According to the localization of application, iontophoresis can be classified into trans-corneal, corneoscleral or trans-scleral, which is very interesting option, because sclera is very permeable structure, even for macromolecules.³⁰ Thanks to this method, RNA and DNA molecules up to 8 million Da can be delivered across the sclera.²⁰

Iontophoresis is effective, easy to use and well tolerated and either less invasive than intraocular application. This method was executed with corticoids, immunosuppressive drugs and oligonucleotides decreasing also elimination through conjunctival or blood flow thanks to the contemporal use of vasoconstrictor-oxymethazolin.

According to Halhal ET AL,³⁰ several ocular systems were developed, e.g. **Eyegate** (dexamethasone phosphate), **Ocuphor** (diclofenac), and **Visulex1**, (dexamethasone phosphate). They are used for the treatment of anterior uveitis, dry eye, and ocular inflammation after cataract surgery and for the treatment of posterior.⁷

Thanks to low current, appearance of side effects is reduced. However, adverse effects include epithelial oedema, decrease in endothelial cells, the inflammation, infiltration and burns can lead to a detachment of retina and choroid.²⁰ Nonetheless, iontophoresis is a promising technique; duration of the drug action is less prolonged than with the controlled release systems.³⁰

Other promising physical method is the usage of solid or hollow drug coated **micro needles**, which are 500-750 μm long. Microneedles are applied into intraocular regions (it can be inserted into the vitreous), mainly for the treatment of posterior segment diseases.¹¹ For targeting the posterior segments solid metal has been covered with a drug. The drug is released from the metal very quickly. After a time the drug is fully released, the micro needle has to be removed. It is minimally invasive method with a lower potential of infections, toxicity and mechanical damage. It could provide also better bioavailability.⁶⁸

In the first study of ocular drug delivery, coated microneedles were used to administer pilocarpine to the corneal stroma, achieving a bioavailability almost two orders of magnitude greater than topical delivery and inducing constriction of the pupil in the rabbit eye.⁷¹

Another study showed that hollow microneedles could be used to make minimally invasive injections of soluble molecules, as well as nano- and microparticles, into the sclera.⁷² Intrasceral injection was successful thanks to scleral thickness, infusion pressure, retraction depth of microneedle, and contemporary use of enzymes like hyaluronidase and collagenase, which hydrolysed quickly the extracellular matrix and collagen of vitreous, enhancing the drug passage. By inserting hollow microneedles into the sclera, fluid injection could be targeted to the suprachoroidal space, a potential space between the sclera and choroid of the eye.⁷³ Fluid was able to flow around the eye and reached the macula after injection. Microneedle length, intraocular pressure, infusion pressure, and particle size influenced behaviour of soluble molecules, as well as nano- and microparticles that were injected.⁷⁴

Ultrasound is a method, which uses microbubbles to potentiate pore formation in cells or polymeric membranes. Higher drug bioavailability after crossing the sclera is possible thanks to the use of polymeric microbubbles as carriers and ultrasound as the stimulus, which leads to more effective therapy of the macular disorders.⁷⁵

9.4 Photodynamic therapy

The photodynamic therapy (PDT) is a method based on the use of combination of three basic elements—a photosensitizer, light and oxygen. The active substance, a photosensitizer, is activated by light in a specific wavelength (between 630 and 820 nm).⁷⁵ The irradiation wavelength required for drug release is given by the absorption spectrum of the liposome-entrapped sensitizer. If an appropriate sensitizer is chosen, liposome oxidation can occur at wavelengths that are non-destructive to the drug or bordering tissues.³⁵

Consequently, photosensitizer gets excited and it transfers the energy from its excited form to oxygen, forming reactive oxygen species (ROS), which attack surrounding molecules and inhibits their biological function leading to a cell death. The complete destruction of CNV is provided by nourishment and oxygen depletion needed for rapidly dividing cells and molecules, and the activation of immune system cells and immunological processes. The result of this mechanism is a formation of free radicals, which leads to oxidative stress of CNV. It consequently leads to damage of neovascular capillaries.⁷⁶

Nowadays verteporfin (Visudyne) is the only used drug from this category.³⁰

The mechanism of drug is based on drug's affinity to the lipoproteins that are accumulated in neovascular capillaries during AMD.³⁵ Therefore, the drug is also attracted into this area. Especially lipophilic drugs are attracted there, because of a low expression of LDL receptors in neovascular tissue.⁷⁵

Photodynamic therapy with Visudyne starts with an intravenous infusion of the preparation, which consist of liposomes for 10 minutes. Liposomes in Visudyne therapy are primarily the vesicles composed of phospholipids and cholesterol.

Consequently, after 15 minutes, light activation at wavelengths between 630 and 820 nm is applied. In response to photosensitization (Fig. 7), reactive oxygen species attack the plasmenychole vinyl–ether bond, generating single chain surfactants, such as lysolipids and fatty aldehydes, which results in defects in vesicle lipid bilayer, leading to liposome leakage. Upon light exposure, thus, the light sensitive liposomes release their content (verteporfin).

It is possible to repeat this process four times a year. However, patients who receive Visudyne will become photosensitive for 48 hours after the infusion. The most frequently reported adverse reactions to Visudyne (verteporfin for infusion) are injection site reactions (including pain, oedema, inflammation, extravasation, rashes, haemorrhage, and discolouration) and visual impairment (including blurred vision, photopsia, reduced visual acuity and visual field defects, including scotoma and black spots).⁷⁷

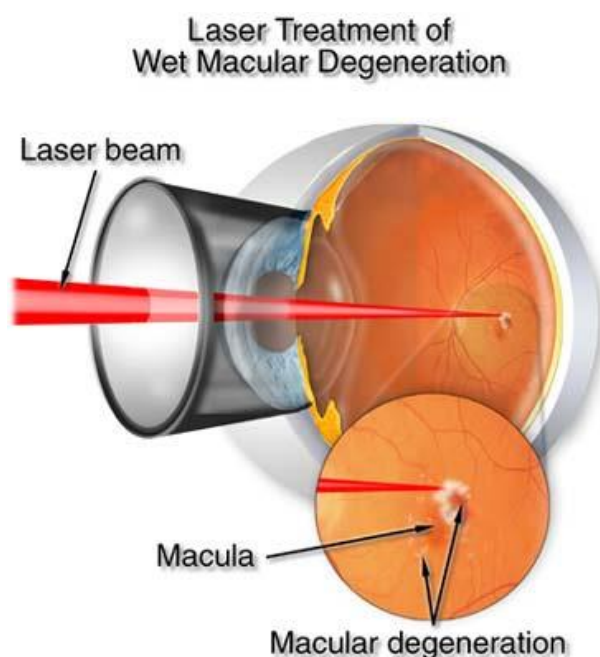


FIG. 7: Photosensitization of the eye during PDT.⁷⁸

Experimental use of **gold nanoparticles** that were incorporated into liposomes was carried out. These nanoparticles (2–3 nm) were embedded in the inner and outer layers of liposomes, and they were loaded with a marker, calcein. Heat absorbance by the incorporated gold nanoparticles was followed by a transfer to the surrounding

microenvironment.²⁰ That led to its increased permeability and the drug could cross the liposomal layers.

9.5 Polymer implants

Implants are dosage forms implanted directly into the ocular globe. Implantable devices are used for sustained and controlled drug delivery to the posterior segment, lowering frequency of needed applications. Implants are applied inside the sclera or they remain within the deeper ocular structures to deliver drugs for an extended period, sometimes many years, minimizing the need for repeat injections.⁷⁹

Although this is an invasive method, implants have the advantage of by passing the blood–ocular barriers to deliver constant therapeutic levels of drug directly to the site of action, need of smaller quantity of drug during the treatment, and avoidance of the side effects associated with frequent systemic and intravitreal injections.³⁰

Biodegradable and non-biodegradable drug depot devices have been developed and tested for the treatment of posterior segments.⁷⁹

Non-biodegradable implants have ability of better control of drug release and longer releasing time.³⁰ They are usually called reservoir type, because they contain drug core surrounded by a semipermeable membrane. That allows steady release of the drug from months to years.¹⁰ They are surgically placed at the *pars plana* or sutured on the sclera, which leads to a risk of retinal detachment, chronic irritation or scar formation.¹⁰ The only disadvantage is necessary surgical removal, which can bring complications.³⁰

Ocular controlled release system **Vitrasert** was the first one from the group of non-biodegradable implants. It is a polymeric implant of ganciclovir used mainly for the patients suffering from AIDS. Vitrasert has a shape of tablet formed by insoluble polymer and is coated by a control membrane of polymer EVA (ethylvinylalcohol) and PVA (polyvinyl alcohol) which regulated permeability of the drug. It requires

chirurgic application to *pars plana*.⁴⁴ The effect is up to 6-8 months. After this period, Vitrasert has to be removed. Due to the fact, that ganciclovir is mutagen; the manipulation with Vitrasert requires careful handling.³⁰

Retisert is also non-biodegradable implant, which releases fluocinoloneacetonide for the therapy of chronic uveitis, and it is applied locally to posterior segments of the eye. Retisert contains a reservoir in a shape of tablet composed of microcrystalline cellulose, magnesium stearate and PVA (polyvinyl alcohol) in a silicone capsule with a hole. A control membrane of PVA, which regulates releasing of drug for 30 months, coats the hole. The effect of Retisert was compared to systemic corticoids in the matter of strength of effectivity, but it has not equally big potential to increase infections as the systemic ones.³¹ It provides side effects like cataract and increase of intraocular pressure and strong ocular pain in 50-90% of patients.³⁰

Iluvien (Fig. 8) is an implant, which consists of a narrow, 3.5 mm long, cylindrical polyimide tube loaded with fluocinolone acetonide, a corticosteroid with a history of treating ocular diseases, and PVA based end caps, which provides rate limiting drug delivery. The implant is applied through a 25-g needle under local anaesthesia and it releases the drug for up to 36 months directly into the retina. Thanks to a special Medidur technology, it creates a self-healing wound, which eliminates the need for surgery.^{30,31}

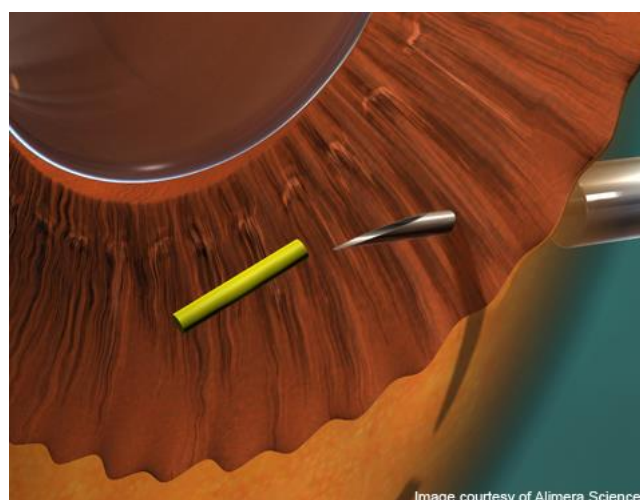


FIG. 8: Injectable application of Iluvien.⁸⁰

I-vation sustained drug delivery system incorporates 5 mm long titanium helical structure (Fig. 9), which is injected into the sclera, leaving the coil end, coated with drug and a polymer matrix, sitting in the vitreous, but is available for removable when necessary.⁴⁴ It is implanted with a 0.5-mm diameter needle stick. This implant utilizes special technology of polymers. Urethane-linked multi-block copolymer incorporates PLA, PGA or poly (caprolactone). Thanks to a helical structure, a fixation into sclera is allowed and also area for drug release is bigger, thus the implant liberates triamcinolone acetonide for 2 years.²⁰

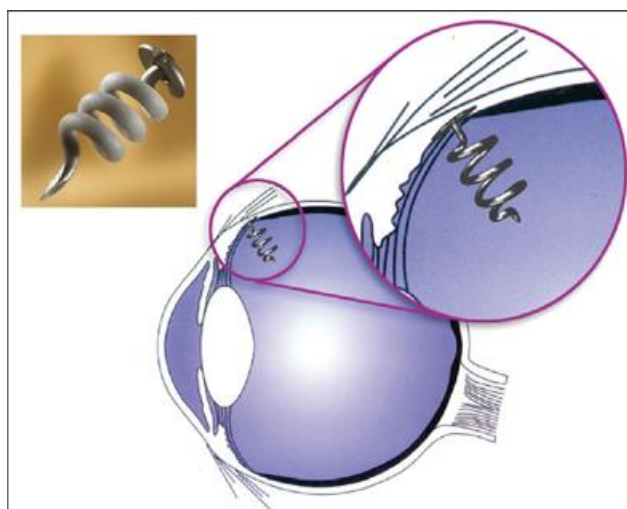


FIG. 9: Helical structure of I-vation.⁸¹

Biodegradable implants are safely cleared by the body. The most common used polymers are polylactic-co-glycolic acid (PLGA), (PLA) or (PGA). Small particles can be encapsulated into these polymers for sustained drug release. They can be also mixed with a fluid carrier. The degradation rate of these polymers depends on the molecular weight, conformation, copolymer constitution, total surface area and the environment in which the system is placed. Modifying these factors, the degradation rate can be varied from weeks to months.^{82,88} The polymer and its biodegradation products has to be safe. The stability and control of release rate are other important characteristics of biodegradable polymers.¹⁰ Drug loading, surface area and volume of implant, polymer composition, molecular weight and solubility of the drug affect the drug release profile.

The biggest advantage of biodegradable polymers is larger drug loading, thanks to the size of drug formulation, and smaller surface-to-volume ration of the polymer; thus higher prolongation is allowed.¹⁰

The disadvantages of biodegradable polymers are unstable concentrations of the drug and shorter time for sustained release.⁶²

Ozurdex is a rod-shaped implant, which contains dexamethasone and PLGA. The intravitreal route with a pre-filled injection applies this implant to the posterior segments. This drug system is being used for the treatment of posterior uveitis, DME, retinal vein occlusion and macula oedema.³¹ Ozurdex releases the drug in three phases: first, burst effect on a surface of the eye, second a diffusion directed by an osmotic pressure and degradation of polymer, third final burst during matrix disintegration. Due to a presence of corticoid, increase of intraocular pressure, as a side effect, was observed.⁷⁹

Surodex is a rod-shaped device to be inserted in the anterior segment at time of cataract surgery to deliver dexamethasone for up to 10 days. Biodegradable Surodex matrix consists of PLGA and hydroxypropyl methylcellulose. Surodex does not require suture fixation and is well tolerated.^{83,79}

Implantable reservoirs with micropumps allow controlled release of drug. Replenish MicroPump releases nanoliter doses in a programmed interval of release. The advantage is that the reservoir can be refilled by the subconjunctival application and it does not need to be removed. For the illustration of a MicroPump, see Fig. 10.

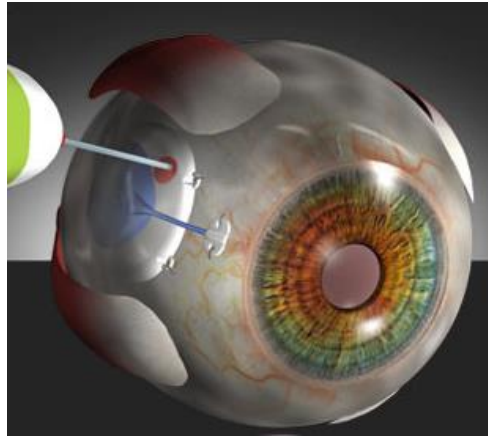


FIG. 10: Replenish MicroPump.⁸⁴

Port Delivery system, ranizumab, is the other refillable drug delivery device. Nowadays it is in phase 1 of clinical trials.³¹

9.6 Modern drug delivery systems

In recent years, a great development of modern drug delivery systems can be observed. This section describes the types of modern drug delivery systems that have been investigated for posterior segment diseases and interprets their features.

There are different kinds of drug delivery technologies that are designed to serve as drug delivery systems including, **nano- and microparticles**, which are constituted of very small particles of an appropriate polymer and active drug, **micelles and niosomes**, which include suitable surfactant, and **liposomes**, that are composed of phospholipids. In addition, **dendrimers and cubosomes** are promising delivery systems with specific properties. For illustration see Fig. 11.

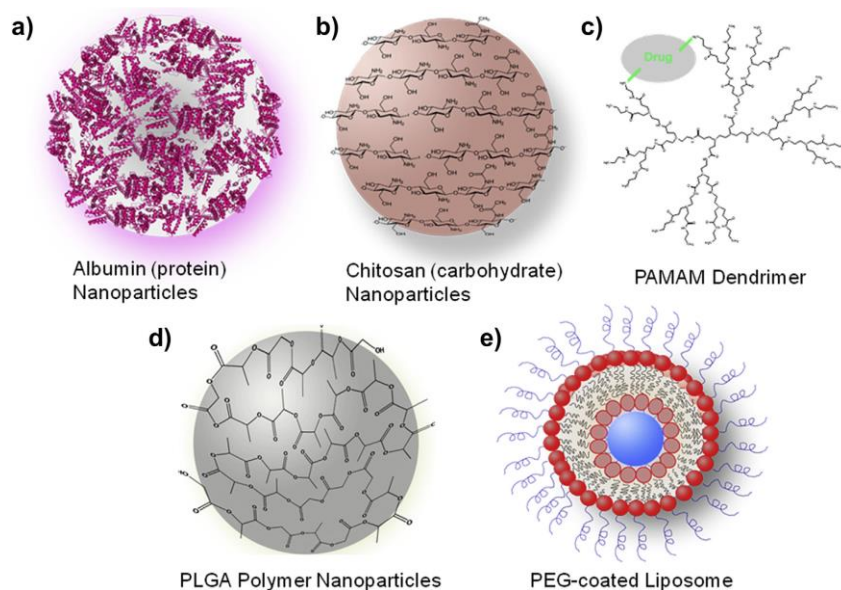


FIG. 11: Modern drug delivery systems.⁵⁴

These drug delivery systems differ in size and their properties. Especially size of particles in drug delivery systems is very important, because too large particles increase surface for drug release, allow encapsulation, enhance stability of drug formulation and protect drug from the biodegradation. Otherwise, too small particles are washed away from the ocular surface due to tear drainage. On the contrary, bigger particles can cause a mechanical irritation in the eye.⁶²

In general, these systems were developed for these main reasons: they allow better permeation of the drug through barriers, they can overcome specifically the blood-ocular one and efflux transporters, controlled release is possible, and drug targeting is desirable too.⁶² The other advantages include decrease in the frequency of administration, lower fluctuation of the drug's concentration level and therefore reduction of side effects. These benefits contribute to better patient compliance.

Modern drug delivery systems alleviated problems associated with poorly soluble drugs, increasing their bioavailability while decreasing their administered dose and toxicity.⁸⁵ Moreover, colloidal and particulate drug delivery systems can be utilized for topical administration as eye drops, sub-conjunctival, periorbital, and intraocular injections.⁸⁶ Liposomes, micelles, nanoparticles and vectosomes are suitable for intravitreal injection. In addition, they provide drug availability generally from days

to several weeks.⁷⁹ The basic requirements for polymer, which forms a major part of these systems, are its compatibility with a drug and delivery system, sterilisability, no irritation and no toxic properties.⁶²

9.6.1 Particulate carriers

Nanocarriers, such as **microparticles** (1–1000 μm) and **nanoparticles** (1–1000 nm) are able to deliver ocular drugs to specific target sites.⁵⁴

Polymeric nanoparticles can be divided into two subtypes, **nanospheres**, where the drug is uniformly dispersed in the matrix or adsorbed on the surface of the nanoparticle, and **nanocapsules**, which are small capsules with a central cavity where the drug is dissolved or dispersed and surrounded by a polymeric membrane.⁸⁷ In nanocapsules, the active drug substance can also be adsorbed on the capsule's surface.⁸⁸

Micro- and nanospheres are usually applied as a suspension using conventional needles for intravitreal injection, which is less invasive than the surgical implantation.^{87,88} After injection, microparticles remain in the administrated site proximities, where they can release the loaded drugs, differentiating them from smaller particles (nanoparticles) which are mainly cleared by the systemic and lymphatic systems.⁸⁸

These particulate systems include several advantages as a small particle size with adhesive properties, better bioavailability of poorly water-soluble drugs, protection of sensitive drug molecules, biodegradable, biocompatible and non-irritant properties, better pre-ocular retention, which leads to a better absorption, and targeted and controlled release.⁸⁷

The main disadvantages reveal to a certain particle size, difficulty of production, standardization of product (encapsulation), stability during their storage, sterilization, aggregation, and complexity of administration faster release rates associated with nanoparticles when compared to microspheres. It is also important to choose right polymer, surface charge and modification.⁸⁶

The most important properties required for ophthalmic nanoparticles are the ability for retention in the ocular tissues, a nanosize diameter and the use of polymers with mucoadhesive properties. For example, polyacrylates, polyalkylcyanoacrylates, poly (lactide-coglycolide), poly (lactide), poly ϵ -caprolactone, albumin, dextran, gelatine, alginate, collagen, hyaluronic acid and chitosan can be used.⁶²

Poly (lactide) microspheres coated with gelatine, applied by the injection, were phagocytosed by RPE cells. Macrophages, loaded with microspheres, may act as a carrier of a drug to target posterior segments; especially microspheres are able to react with specific cells. The tissue distribution of microspheres depends on the diameter of particles. The microspheres with lower diameter were found also in the retina. On the other hand, the bigger ones were detectable only in intravitreal cavity and trabecula.⁶²

Microspheres, given periocularly in a porcine model of laser induced choroidal neovascularisation to provide transscleral drug delivery of active substance PKC412, were tested during clinical trials. PKC412 is an inhibitor of protein kinase C and receptors for VEGF, which was proved for the treatment of CNV. It was approved that, the inhibitor penetrated through the sclera and suppressed choroidal neovascularization.⁸⁹

Andrieu-Soler *et al.*⁹⁰ developed PLGA microspheres loaded with a proteic neurotrophic factor (glial-cell-line-derived neurothropic factor; GDNF). After intraocular administration, microparticles were able to control protein release for three months according to in vitro studies, and resulted efficient at preventing the retinal degeneration in a mouse model for 17 days.

For topical administration, **chitosan nanoparticles** were tested. Chitosan is a natural cationic polysaccharide that comes from chitin. Positively charged surface leads to mucoadhesion to biological tissue, which has negative charge on its surface.⁹¹ Chitosan hydroxyl groups compete with water on the surface and increase bioadhesion.⁵⁴ Chitosan is degraded by enzyme lysozyme, which is concentrated in mucosal surfaces.⁹²

Chitosan is also an interesting material to coat nanoparticles, liposomes or niosomes, improving their mucoadhesive properties on the ocular surface and drug-corneal permeation.⁸⁸ Various authors have observed a significant improvement of the bioavailability of several drugs administered with nanoparticles coated with chitosan, compared to the uncoated vehicles.⁹²

Hybrid PLGA-chitosan nanoparticles loaded with a plasmid encoding a specific plasminogen with antiangiogenic activity, were able to deliver the plasmid to the retina after intravitreal injection. The expression of plasminogen was found in the retina up to 2 weeks after injection.⁵⁴

Interesting properties have been observed with the incorporation of a di-block copolymer of ethylene oxide and propylene oxide (PEO-PPO), which led to a significant increase in the release rate of the encapsulated protein bovine serum albumin, thus confirming the possibility of modulating.⁹³

Albumin offers advantages of endogenous protein.⁵⁴ If nanoparticle is composed of this protein, safe metabolites will be formed.³⁰ Human serum albumin increases the solubility in water, lowers the toxicity of the drug. On the other hand, it can protect a drug against oxidation processes and prolongs the half-life of the drug. It is the main protein of aqueous humour that helps keeping lens epithelial healthy.⁵⁴ Protein nanoparticles are used also for non-viral gene therapy, which will be mentioned later.

Lipid nanoparticles consist of aqueous dispersions of two different types of particles: the solid lipid nanoparticles (SLNs) and the nanostructured lipid carriers (NLCs) with sizes from 150 to 300 nm.⁹⁴

SLNs are similar in composition to nanoemulsions, but they replace the inner liquid lipid with a solid lipid, where active molecules are stabilized and surrounded by a layer of surfactants in an aqueous dispersion. SLNs are built from triglycerides (tricaprin, trilaurin, trimyristin) in a specific orientation, which consists of a polar core with polar heads facing towards the aqueous phase.^{95,96,97} Triglycerides, hard fat types, partial glycerides, steroids, and waxes) and all classes of emulsifiers (ionic and

non-ionic) are used to prepare SLNs.⁹⁸ Tween-80, sodium lauryl sulphate, poloxamer 188 and saponins are the examples of used emulsifiers.

Drug like timolol, cyclosporine-A, diclofenac and tobramycin have been entrapped in SLNc.⁸⁶ Absence of toxicity due to the use of physiological lipids, possibility of sterilization by autoclaving, and easy large-scale production are the main advantages of SLNs.⁹⁸

On the other hand, the NLCs are produced by addition of a spatially incompatible liquid lipid to the solid lipid, leading to special nanostructures with a capacity to contain larger quantity of drugs and release properties. Lipid choice is essential for the stability of the drug in NLCs.⁹⁹

Araújo *et al.* developed a formulation based on NLCs, which contain triamcinolone acetone for topical ocular instillation. They used a solid lipid, a liquid lipid, and hydrophilic surfactant. The carriers showed a particle size range of 100–300 nm. When the formulation was administered into the conjunctival sac of male albino New Zealand rabbits, no irritation was detected. After this preliminary study, they administered the formulation in mice onto the eye surface; NLCs were detected in the retina 40 minutes after administration and almost disappearing 160 min after administration.¹⁰⁰

9.6.2 Liposomes

Liposomes are small vesicles composed of a bi-layer of phospholipids and aqueous core.⁶² They can be uni- or multilamellar, which differ from each other also in their size (small unilamellar 10-100 nm; large unilamellar 0.1-10 μm), charge, fluidity of bi-layers, mode of production, stability and pharmacokinetics.^{30,54} Because of liposomes unique structure, hydrophilic, hydrophobic and amphiphilic substances can be incorporated within these vesicles.³⁰ They are capable of holding several types of solutes, and ionic drugs that can be incorporated into liposomes by the use of cationic or anionic lipids.⁶² The most common ways of drug release from liposome, are passive diffusion, vesicle fusion or vesicle disruption.⁵⁴

The advantage of liposomes is the biocompatibility, degradability, and the possibility to use natural lipids.³⁰

The common problems associated with liposomes are that they are in a liquid form, which limits their formulation feasibility. Moreover, most methods of sterilization are considered unsuitable for liposomes.¹⁰¹

They have also limited capacity as a storage. However, it depends how the drug and liposomes are constituted. It is poorly effective when hydrophilic drugs are encapsulated. They are mainly used cationic liposomes in gene delivery. The examples of utilized drugs are penicillin G or tropicamide. Liposomes have also the possible proinflammatory effect.⁶² The disadvantage of liposomes is possible intraocular clouding after intravitreal application.³⁰

Positively charged (cationic) liposomes have a high efficiency for entrapping DNA or RNA thanks to electrostatic interactions. These complexes formed by DNA and positively charged liposomes are called lipoplexes, and they are used usually as gene carriers to treat ocular diseases.⁸⁸

The surface of liposomes can be modified e.g. with polyethyleglycol to maintain better stability of the drug; a fixed aqueous layer is formed on the surface, thus it is sterically stabilized.⁶²

Liposomes that are suitable for delivery to the posterior segment of the eye were tested by Sasaki *et al.*¹⁰² Importantly, liposome's surface modification with water-soluble cationic polymer poly-L-lysine significantly increased delivery of the marker coumarin-6, biocompatibility and it had low toxicity in cells of the anterior segment.¹⁰²

A sub-micron sized liposomal drug delivery system capable of delivering hydrophilic drugs via the non-corneal pathway to the posterior segment of the eye was proved by Hironaka *et.al.*¹⁰³

Liposomes were also used in the case of Visudyne used for PDT as mentioned above.³⁰ In this case, the active drug verteporfin is incorporated into the phospholipid bi-layer.⁶²

9.6.3 Other particulate systems

Cubosomes are self-assembled nanostructured particles, which are formed from amphiphilic lipid molecules. The surfactants help to form a structure of honeycomb (Fig.12). Into this structure, water-soluble, lipid-soluble and amphiphilic-soluble molecules can be embedded. The microstructure of cubosomes is similar to that of biological membranes, which enables a fusion of lipid carriers with the lipid bilayers of the corneal epithelial cells after a topical application. With this fusion, cubosomes become a drug reservoir, which is able to release the drug through their bicontinuous cubic phase constituted of monoolein, phytanetriol and Poloxamer 407.¹⁰⁴

A cubosome loaded with dexamethasone was provided by fragmenting a cubic crystalline phase of monoolein and water in the presence of stabilizer Poloxamer 407. The retention of cubosomes was significantly longer in preocular space than that of solution and Carbopol gels (Pilogel, mentioned above).¹⁰⁵

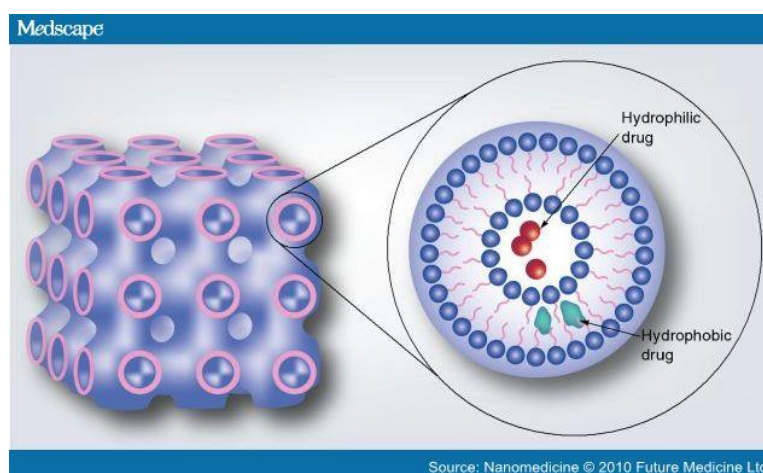


FIG.12: Honeycomb structure of cubosome.¹⁰⁶

Niosomes are a self-assembly of non-ionic surfactant bilayer vesicles (polysorbate, sorbitan esters, polyoxyethylenes, etc.) of 10-0.5 μm in size.⁸⁸ As well as liposomes, they have bilayer structure in aqueous media, which is able to entrap both hydrophilic and lipophilic drugs. They are administered topically, but also intraocular and periocular administrations were tested. The advantage of niosomes regard to the chemical stability, low toxicity, high compatibility, lower cost and

availability of materials. A combination with chitosan provides good ocular tolerance, better penetration and improved corneal residence time.^{107,108}

Vectosomes are other promising delivery systems that was tested for delivering antisense oligonucleotide ODNs to posterior tissues of the eye.⁷⁵ They are vesicles constituted of VP22, a structural protein of herpes simplex virus. Antisense oligonucleotide is bonded to c-terminal amino acids of the purified VP22 forming spherical particles of 0.3-1 μ m in diameter.²⁰

Vectosomes are stable in the retinal layers for several weeks. They can be destabilized by the illumination of a laser light, which leads to a release of the incorporated ODN.^{75,20}

However, limitations related with the preparation of vectosomes (poor stability, limited drug loading, and sterilization problem) and usage (intraocular clouding, retinal abnormalities and transient impaired vitreous) need to be overcome before clinical acceptance of vectosomes for ocular delivery.⁷⁵

Micelles

Micelles are nanosized particles (10-100 nm), which have an amphiphilic character.¹¹⁰ Therefore, they contain both hydrophilic shell and hydrophobic core. From the chemical point of view, they are often composed from polymers or surfactants.³⁰ They are similar to liposomes, but they exhibit higher ordered structure composed of monolayers.⁵⁴ The shell is responsible for micelle's stabilization and it can interact with biomembranes too.

Amphiphilic character could prolong the stability of micelles in the eye fluids, to induce bioadhesion or to modify release of the drug.¹¹⁰

The advantages of micelles are their ability to enhance solubility of hydrophobic drugs, their nanosize, and thermodynamic stability, presentation as an aqueous dispersion, prevention or minimization of drug degradation, lower side effects and better drug permeation through the eye with minimal irritation.^{54,110} They are generally applied topically or intravitreally, and further they were tested in a

treatment of choroidal neovascularization (PDT).^{30,110} Polymeric micelles usually contain poly (ethylene glycol) that prevents aggregation and protects micelles from immune system cells, like macrophages, and thus from phagocytosis.¹¹⁰

9.6.4 Micro- and nanoemulsions

Microemulsions and nanoemulsions are new promising dispersion systems. According to IUPAC, microemulsions are dispersions made of water, oil, surfactant(s) and co-surfactants (glycols, short chain amines and low molecular weight alcohols).⁸⁸ Emulsions vary approximately from 1 to 100 nm, usually 10 to 50 nm in their size.¹⁰⁹ Alternative names, for these systems, such as transparent emulsion, swollen micelle, micellar solution, and solubilized oil are often used.⁶²

They are thermodynamically stable systems that are easily prepared and sterilized and they present the advantage of the incorporation of water-soluble and lipophilic drugs.⁸⁸ They are effective delivery systems for anti-glaucoma, anti-viral, anti-allergic, and anti-inflammatory drugs.²⁵

Nanoemulsions are capable of sustained drug release, high penetration to the eye's layers and easy way of sterilization.¹¹⁰ However, compared to microspheres and liposomes, microemulsions are also unsuitable for long-term sustained drug release.⁶² They are especially applied by a topical route and thanks to the small size, they are able to permeate well.⁶² In addition, co-surfactants in nanoemulsions act as permeability enhancers and certainly, they lower the interfacial tension and simplify a dispersion of liquids.¹¹⁰

The choice of all components of nanoemulsions is important because these ingredients need to be non-irritating and nontoxic to the corneal surface and other ocular tissues.¹¹⁰ However, ionic surfactants are toxic to the ocular surface, so their use in ocular formulations is very restricted.⁸⁸ The non-ionic surfactants such as polyoxypropylene/polyoxyethylene block copolymers (poloxamers), polysorbates, polyethylene glycol and tyloxapol and amphiphilic surfactants like lecithin are the most frequently used.⁸⁸

9.6.5 Dendrimers

Dendrimers are the structures of synthetic macromolecules that have branched, three-dimensional functions that are similar to the architecture of a tree. Despite their large molecular size (5000-500 000 Da), dendrimers are structurally well defined and they have a low polydispersity.⁸⁸

Dendrimer consists of three main structural components: a multifunctional central core, branched units and surface groups. The branched units are organized in layers called “generations”, which represent the repeating monomer. On a molecular level, the dendritic branching has semi-globular to globular structure. These groups can be easily modified chemically. This fact can be used to adapt the dendrimer with targeting ligands able to direct it to the desired tissue.⁸⁸ Dendrimer or drug complexes are able to cross cell membranes and resist to premature clearance after intravitreal injection.^{54,62}

Hydrophobic drugs can be incorporated into the core thanks to hydrogen bonds, hydrophobic and ionic interactions or covalent bonds.^{54,62} However, they can possess the smallest amount of the drug in comparison with the other drug delivery systems. In addition, safety of dendrimers in ocular delivery has not yet been approved *in vivo*.⁶² Polyamidoamine dendrimers are the most investigated dendrimers for drug and gene delivery.⁵⁴

Marano *et. al.*¹¹¹ tested lipid-lysine dendrimers to deliver a sense oligonucleotide ODN-1 into cells that would reduce the VEGF expression after intravitreal injection in choroidal neovascularisation animal models (rats). The results showed that dendrimer/ODN-1 dendriplexes suppressed VEGF expression in cells during the first 24 hours (40-60 %); some of them even remained active for up to 2 months after injection, verifying that these lipid-lysine dendrimers could protect the ODN-1 from nucleases and prolong delivery *in vivo*. In 2006, a new family of lipid-lysine dendrimers were presented with galactose on their surface. The authors observed that this modification increased the solubility of the nanosystems and the transfection of oligonucleotides in RPE.¹¹²

Izze *et. al.*¹¹³ showed that hydroxyl-terminated polyamidoamine (PAMAM) dendrimers have an intrinsic ability to localize selectively microglia, and they can deliver drugs inside these cells for a sustained period. The authors prepared conjugates of PAMAM with fluocinolone acetonide, a neuroant inflammatory drug. The conjugate released the drug over 90 days. *In vivo* efficacy studies demonstrated that just one intravitreal injection was able to block retinal degeneration for an entire month in a rat retinal degeneration model.

9.6.6 Encapsulated cell technology and gene therapy

Encapsulated cell technology and gene therapy are very promising methods for delivering drugs in the posterior segments. They utilize biological material, which is able to ensure a release of needed protein to the targeted tissue with low immune response.³⁰

Encapsulated cell technology (ECT) is a method that requires genetic engineering and allows the use of substances with bigger volume and concentration.³¹

Specific cells are immunologically isolated with microcapsules or hollow fibres before application into the eye.³⁰ It is important to choose cells able to produce and release needed protein; e.g. immortalized human RPE cells.^{7,31} Successively the cells are encapsulated contemporary with collagen and hydrogel based on hyaluronic acid. The fact that cells are able to survive also without encapsulated matrix is very important. They should be able to produce protein and not affect immune system too much.³¹ This method certainly has a great potential to be a great way for treatment of chronic diseases.³⁰

The example of ECT is an implant **NT501**, Neurotech (Fig. 13), which contains genetically modified human RPE cells. Thus, they secrete ciliary neurotrophic factor (CNTF), a growth factor that inhibits photoreceptor degradation.^{20,30} The administration of Neurotech is executed with a small cut and the implant is sewn to this location and if needed it is able to produce protein.³⁰ It is sutured onto the sclera through a titanium loop.²⁰ Due to the semi-permeable membrane, the influx of nutrients and oxygen is provided.²⁰ Thanks to the nutrients the diffusion and

secretion of a certain protein is allowed.⁷ A big advantage of ECT is low immune response of the body to the modified cells.^{20,31}

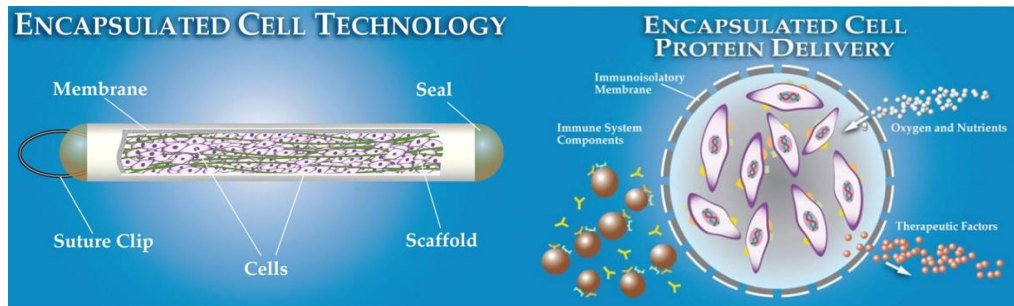


FIG. 13: Encapsulated cell technology–Neurotech.¹¹⁴

Gene therapy is based on specific nucleotide sequence of DNA, RNA or their modification. The aim is the expression of gene that would lead to the translation of certain mRNA (antisense, oligonucleotides, ribosomes), or bind to a certain protein.¹¹⁵ For gene therapy they are often used hydrophilic larger molecules. Compounds are administered intravitreally with saline solvent.

Easier foundation of nucleotide sequence compared to small molecular size in drug delivery systems mentioned before is a big advantage of gene therapy. Gene expression can last for a certain time and thus, continual synthesis of needed protein is secured. The release of protein is controlled specifically, or thanks to the promoters. For delivering a specific sequence of DNA or RNA, viral or non-viral vectors are utilized. **Viral** vectors are more effective, but their usage is limited because of the toxicity, immunogenicity, possible genomic integration, restricted size of inserted DNA and difficult preparative processes.¹¹⁵

On the other hand, **non-viral** vectors promote weaker immune response, easy formulation and unlimited size of inserted particle.³⁰

As an example of gene delivery method Pegaptanib introduced in Chapter 8.3. can be mentioned.³⁰

10 Conclusions

Therapy of the ocular diseases represents the serious problem for necessity to overcome the protection mechanisms of the eye. Particularly, the back segment treatment is still challenging. Recently, a lot of promising dosage forms occurred either being still investigated, used in clinical trials or successfully introduced in praxis. This review showed the most important ones. In conclusion, the summary information is presented below in form of the original Tables 1-3.

Table 1: Advantages and disadvantages of different types of nanocarriers.(Modified according to ⁶²)

Advantages		Disadvantages
Liposomes	<ul style="list-style-type: none"> • Low toxicity and antigenicity • Biodegradable and metabolized in vivo • Can prolong the drug half-life in the vitreous • Reduces drug toxicity 	<ul style="list-style-type: none"> • Blurring of vision after intravitreal injection • Limited storage conditions
Micro/nanospheres	<ul style="list-style-type: none"> • Drug release is stable for a long time (nondegradable) 	<ul style="list-style-type: none"> • Need to be removed surgically (nondegradable) • Sustained release period is short (biodegradable)
Microemulsions	<ul style="list-style-type: none"> • Good tissue permeability 	<ul style="list-style-type: none"> • Unsuitable for long-term sustained drug release
Dendrimers	<ul style="list-style-type: none"> • Physical properties and size can be controlled at the molecular level 	<ul style="list-style-type: none"> • Conjugates low amount of drugs • Safety is unclear in vivo

Table 2: Resume of drug delivery systems

Drug delivery system	Advantages	Disadvantages
Eye drops	<ul style="list-style-type: none"> ✓ Easy to apply ✓ The least invasive ✓ Compliance 	<ul style="list-style-type: none"> • Poor ocular BAV • Ineffective treatment of the posterior segments • Needed high concentrations or frequent instillations
Systemic administration	<ul style="list-style-type: none"> ✓ More effective for the treatment of posterior segments 	<ul style="list-style-type: none"> • Difficult to pass blood – ocular barriers • Systemic toxicity
Intravitreal, periocular, subconjunctival routes	<ul style="list-style-type: none"> ✓ Better drug absorption ✓ Safer than systemic administration ✓ Drug delivery to the target parts of the eye 	<ul style="list-style-type: none"> • Short half- life • Frequent applications • Poor compliance • Side effects
Implants	<ul style="list-style-type: none"> ✓ Longer half – life of the drug ✓ Better compliance ✓ Stabilization of the drug ✓ Non – biodegradable – controllable delivery features, longer periods of drug release ✓ Biodegradable – do not need to be removed 	<ul style="list-style-type: none"> • Side effects – retinal detachment, intravitreal haemorrhage • Non – biodegradable – required surgery • Biodegradable – uncontrollable release profile
Microparticles, nanoparticles, liposomes	<ul style="list-style-type: none"> ✓ Stabilization of the drug ✓ Increase drug's half- life ✓ Lower toxicity ✓ Targeting (smaller toxicity) ✓ Better compliance 	<ul style="list-style-type: none"> • Side effects – injections and vitreous clouding
Cell encapsulation	<ul style="list-style-type: none"> ✓ Long- lasting of the given protein ✓ Targeting (smaller toxicity) ✓ Better compliance 	<ul style="list-style-type: none"> • Side effects – invasive method
Iontophoresis	<ul style="list-style-type: none"> ✓ Non – invasive method ✓ Easy to use ✓ Possible combination with other drug delivery systems ✓ Good drug penetration to both segments of the eye ✓ Good compliance 	<ul style="list-style-type: none"> • Required frequent administrations • Side effects – mild pain • Risk of low compliance

Table 3: Promising drugs in clinical trials (in 2016)

Name	Mechanism of action/Drug delivery system	Phase of clinical trials
AGN - 150998	Intravitreal injection	Phase 2
AL – 78898a	Intravitreal injection	Phase 2
BEVASIRANIB + LUCENTIS	Intravitreal injection	Phase 3 (terminated)
CGC - 11047	Subconjunctival injection	Phase 1
ECULIZUMAB	Intravenous infusion	Phase 2
ESBA1008	Intravitreal injection (biological solution)	Phase 1
LHA510	Topcially (ophthalmic suspension)	Phase 1
OT - 551	Topcially (eye drops)	Phase 2
PKC412	Microspheres	
RN6G	Intravenous single dose	Phase 1
SIRGA2 (Sirolimus)	Intravitreal injection	Phase 1

11 References

- ¹ *Český lékopis 2009*, Doplněk 2015, Grada: Praha, CD ROM.
- ² RATHBONE, Michael J., HADGRAFT, Jonathan and ROBERTS, Michael S.: *Modified-release drug delivery technology*. 2nd Ed.. New York: Informa Healthcare, 2008, s. 962. ISBN 9781420044355.
- ³ ALLEN, L. V., POPOVICH, N. G., ANSEL, H. C.: *Ansel's pharmaceutical dosage forms and drug delivery systems*. 9th Ed.. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins, 2011, s. 710. ISBN 9780781779340.
- ⁴ ANCHOURI, Djamila, ALHANOUT, Kamel, PICCERELLE, Philippe, ANDRIEU, Véronique: Recent advances in ocular drug delivery. *Drug Development and Industrial Pharmacy*, 2013, 39 (11), s. 1599-1617. ISSN 0363-9045. DOI: 10.3109/03639045.2012.736515.
- ⁵ URTTI, Arto: Challenges and obstacles of ocular pharmacokinetics and drug delivery. *Advanced Drug Delivery Reviews*, 2006, 58 (11), s. 1131-1135. ISSN 0169-409X. DOI: 10.1016/j.addr.2006.07.027.
- ⁶ CHADER, Gerald J., TAYLOR, A.: Preface: The Aging Eye: Normal Changes, Age-Related Diseases, and Sight-Saving Approaches. *Investigative Ophthalmology & Visual Science*, 2013, 54 (14), s. SI. ISSN 0146-0404. DOI: 10.1167/iovs.13-12993.
- ⁷ CHEN, Hongming, TAYLOR, Allen: Recent developments in ocular drug delivery. *Journal of Drug Targeting*, 2015, 23 (7-8), s. 597-604. ISSN 1061-186x. DOI: 10.3109/1061186X.2015.1052073.

⁸ BASPINAR, Yücel, BORCHERT, Hans-Hubert, PARENKY, Ashwin and MITRA, Ashim K.: Penetration and release studies of positively and negatively charged nanoemulsions-Is there a benefit of the positive charge?. *The AAPS Journal*, 2010, 12 (3), s. 348-360. ISSN 1550-7416. DOI: 10.1016/j.ijpharm.2012.03.040.

⁹ KUCHYNKA, Pavel a KOLEKTIV: *Oční lékařství*. 1.vyd., 2007. Praha: Grada, 812 s., ISBN 802471163x.

¹⁰ KIM, Yoo Chun, CHIANG, Bryce, WU, Xianggen, PRAUSNITZ, Mark R.: Ocular delivery of macromolecules. *Journal of Controlled Release*, 2014, 190, s. 172-181. ISSN 01683659. DOI: 10.1016/j.jconrel.2014.06.043.

¹¹ GAUDANA, Ripal, ANANTHULA, Hari Krishna, PARENKY, Ashwin and MITRA, Ashim K.: Ocular Drug Delivery. *The AAPS Journal*, 2010, 12 (3), s. 348-360. ISSN 1550-7416. DOI: 10.1208/s12248-010-9183-3.

¹² ROZSÍVAL, Pavel, et al.: *Oční lékařství*. 1.vyd., 2006. Praha: Galén, 373 s., ISBN 80-7262-404-0.

¹³ ZHOU, Lei, BEUERMAN, Roger W.: Tear analysis in ocular surface diseases. *Progress in retinal and eye research*, 2012, 31, s. 527-550. ISSN 1350-9462. DOI: 10.1016/j.preteyeres.2012.06.002.

¹⁴ CORFIELD, A.P., CARRINGTON, S.D., HICKS, S.J., BERRY, M., ELLINGHAM, R.: Ocular mucins: purification, metabolism and functions. *Prog.Ret.Eye Res.*, 1997, 16 (4), s. 627-656. ISSN 1350-9462. DOI: 10.1016/S1350-9462(96)00039-0.

¹⁵ TSUBOTA, K.: Tear dynamics and dry eye. *Prog.Ret.Eye Res.*, 1998, 17 (4), s. 565-596. ISSN 1350-9462. DOI: 10.1016/S1350-9462(98)00004-4.

-
- ¹⁶ Lee, V.H.L., Robinson, J.R.: Review: Topical ocular drug delivery. Recent advances and future challenges. *Journal of Ocular Pharmacology and Therapeutics*, 2009, 2 (1), s. 67-108. ISSN 1080-7683. DOI: 10.1089/jop.1986.2.67.
- ¹⁷ ABRÀMOFF, MD., GARVIN, MK., SONKA, M.: Retinal Imaging and Image Analysis. *IEEE Rev Biomed Eng.*, 2010; 3, s. 169-208. ISSN 1937-3333. DOI:10.1109/RBME.2010.2084567.
- ¹⁸ IMAMOTO, Yasushi and SHICHIDA, Yoshinori: Cone visual pigments. *Biochimica et Biophysica Acta-Bioenergetics*, 2014, 1837 (5), s. 664-673. ISSN 00052728. DOI: 10.1016/j.bbabi.2013.08.009.
- ¹⁹ KOMPELLA, U. B., KADAM, R. S., LEE, V. H.: Recent advances in ophthalmic drug delivery. *Therapeutic Delivery*, 2010, 1 (3), s. 435–456. ISSN 2041-6008. DOI: 10.4155/TDE.10.40.
- ²⁰ THRIMAWITHANA, ThiliniRasika, YOUNG, Simon, BUNT, Craig Robert, GREEN, Colin, ALANY, Raid Ghassan: Drug delivery to the posterior segment of the eye. *Drug Discovery Today*, 2011, 16 (5-6), s. 270-277. ISSN 13596446. DOI: 10.1016/j.drudis.2010.12.004.
- ²¹ LEE, V.H.L., ROBINSON, J.R.: Mechanistic and quantitative evaluation of precorneal pilocarpine disposition in albino rabbits. *Journal of Pharmaceutical Sciences*, 1979, 68 (6), s. 673–684. ISSN 00223549. DOI: 10.1002/jps.2600680606.
- ²² JARVINEN, K., JARVINEN T., URTI A.: Ocular absorption following topical delivery. *Advanced drug delivery reviews*, 1995, 16 (1), s. 3-19. ISSN 0169409X. DOI: 10.1016/0169-409X(95)00010-5.

-
- ²³ DARTT, Darlene A.: Tear Lipocalin: Structure and Function. *Ocular surface*, 2011, 9 (3), s. 126-138. ISSN 1542-0124.
- ²⁴ CHEN, Y., MEHTA, G., VASILIOU, V.: Antioxidant Defenses in the Ocular Surface. *Ocular surface*, 2009; 7 (4), s.176-185. ISSN 1542-0124.
- ²⁵ AKO-ADOUNVO, Ann-Marie, et. al.: Recent Patents on Ophthalmic Nanoformulations and Therapeutic Implications. *Recent Patents on Drug Delivery & Formulation*, 2014, 8 (3), s.193-201. ISSN 2212-4039.
- ²⁶ DUXFIELD, L., SULTANA, R., WANG, R., ENGLEBRETSSEN, V., DEO, S., RUPENTHAL, I. D., AL-KASSAS, R.: Ocular delivery systems for topical application of anti-infective agents. *Drug Dev Ind Pharm*, 2015, 1, s. 1-11. ISSN 1520-5762. DOI: 10.3109/03639045.2015.1070171.
- ²⁷ MALHOTRA, M., MAJUMDAR, DK.: Permeation through cornea. *Indian journal of experimental biology*, 2001, 39 (1), S. 11-24. ISSN 0975-1009.
- ²⁸ SHIRASAKI, Yoshihisa: Molecular Design for Enhancement of Ocular Penetration. *Journal of Pharmaceutical Sciences*, 2008, 97 (7), s. 2462-2496. ISSN 00223549. DOI: 10.1002/jps.21200.
- ²⁹ CUNHAVAZ, José: Blood-ocular barriers. *Survey of ophthalmology*, 1979, 23 (5), s. 279-296. ISSN 0039-6257. DOI: 10.1016/0039-6257(79)90158-9.
- ³⁰ DEL AMO, E., URTTI, A.: Current and future ophthalmic drug delivery systems. A shift to the posterior segment. *Drug Discovery Today*, 2008, 13 (3-4), s. 135-143. ISSN 1359-6446. DOI: 10.1016/j.drudis.2007.11.002.
- ³¹ PEARCE, William, HSU, Jason, YEH, Steven: Advances in drug delivery to the posterior segment. *Current Opinion in Ophthalmology*, 2015, 26 (3), s. 233-239. ISSN 1040-8738. DOI: 10.1097/ICU.0000000000000143.

³² ZHANG, K., ZHANG, L., WEINREB, R. N.: Ophthalmic drug discovery: novel targets and mechanisms for retinal diseases and glaucoma. *Nat Rev Drug Discov*, 2012, 11 (7), s. 541-59. ISSN 1474-1776. DOI: 10.1038/nrd3745.

³³ ŠTROFOVÁ, H., SEDLÁČEK, K., JAROŠOVÁ, A., DUBSKÁ, Z.: Věkem podmíněná makulární degenerace. *Klin. Farmakol. Farm.*, 2013, 27 (2), s. 68-74. ISSN 1803-5353.

³⁴ BRUNK, U.T., TERMAN, A.: Lipofuscin: mechanisms of age-related accumulation and influence on cell function. *Free radical biology and medicine*, 2002, 33 (5), s. 611-619. ISSN 0891-5849. DOI: 10.1016/S0891-5849(02)00959-0.

³⁵ CHRAPEK, O.: Současné možnosti léčby vlhké formy věkem podmíněné makulární degenerace. *Remedia*, 2007, 17, s. 610-616. ISSN 0862-8947.

³⁶ FORRESTER, J. V., XU, HP.: Good news–bad news: the Yin and Yang of immune privilege in the eye. *Frontiers in Immunology*, 2012; 3, 338. ISSN 1664-3224. DOI: 10.3389/fimmu.2012.00338.

³⁷ HERRERO-VANRELL, R., BRAVO-OSUNA, I., ANDRÉS-GUERRERO, V., VICARIO-DE-LA-TORRE, M., MOLINA-MARTÍNEZ, IT: The potential of using biodegradable microspheres in retinal diseases and other intraocular pathologies. *Progress in Retinal and Eye Research*, 2014, 42, s. 27-43. ISSN 13509462. DOI: 10.1016/j.preteyeres.2014.04.002.

³⁸ ANGELES SOLINÍS, M., DEL POZO-RODRÍGUEZ, A., APAOLAZA, PS., RODRÍGUEZ-GASCÓN, A.: Treatment of ocular disorders by gene therapy. *European Journal of Pharmaceutics and Biopharmaceutics*, 2015, 95, s. 331-342. ISSN 09396411. DOI: 10.1016/j.ejpb.2014.12.022.

-
- ³⁹ ADAMIS, AP., et al.: Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. *American Journal of Ophthalmology*, 1994, 118 (4), s. 445–450. ISSN 0002-9394.
- ⁴⁰ GAUDANA, R., JWALA, J., BODDU, SHS.: Recent Perspectives in Ocular Drug Delivery. *Pharmaceutical Research*, 2009, 26 (5), s. 1197-1216. ISSN 07248741. DOI: 10.1007/s11095-008-9694-0.
- ⁴¹ Mikro-verze AISLP–Automatizovaný Informační Systém Léčivých Přípravků, k 1.5.2016
- ⁴² EL SANHARAWI, M., KOWALCZUK, L., TOUCHARD, E., OMRI, S., DE KOZAK, Y., BEHAR-COHEN, F.: Protein delivery for retinal diseases: From basic considerations to clinical applications. *Progress in Retinal and Eye Research*, 2010, 29 (6), s. 443-465. ISSN 13509462. DOI: 10.1016/j.preteyeres.2010.04.001.
- ⁴³ MARTIN, D.F., et al.: Ranibizumab and bevacizumab for neovascular age-related macular degeneration. *The New England Journal of Medicine*, 2011, 364 (20), s. 1897–1908. ISSN 1533-4406. DOI: 10.1056/NEJMoa1102673.
- ⁴⁴ FISCHER, N., NARAYANAN, R., LOEWENSTEIN, A., KUPPERMANN BD.: Drug delivery to the posterior segment of the eye. *European Journal of Ophthalmology*, 2011, 21 (6), s. 20-26. ISSN 1120-6721. DOI: 10.5301/EJO.2010.6051.
- ⁴⁵ BAUDOIN, C., LABBE A., LIANG, H., PAULY, A., BRINGOLE-BAUDOIN, F.: Preservatives in eyedrops: The good, the bad and the ugly. *Progress in Retinal and Eye Research*, 2010, 29 (4), s. 312-334. ISSN 13509462. DOI: 10.1016/j.preteyeres.2010.03.001.

-
46. ZHU, H., CHAUHAN, A.: Effect of viscosity on tear drainage and ocular residence time. *Optometry and Vision Science*, 2008, 85 (8), s. 715-724. ISSN 1040-5488.
47. LUDWIG, A.: The use of mucoadhesive polymers in ocular drug delivery. *Adv. Drug Del. Rev.*, 2005, 57 (11), s. 1595-1639. ISSN 0169-409X. DOI 10.1016/j.addr.2005.07.005.
48. MUNDADA, A. S., AVARI, J. G.: In situ gelling polymers in ocular drug delivery systems: A review. *Critical Review in therapeutic drug carrier systems*, 2009, 26 (1), s. 85-118. ISSN 0743-4863.
49. ROZIER, A., MAZUEL, C., GROVE, J., PLAZONNET, B.: Gelrite-a novel, ion-activated, in-situ gelling polymer for ophthalmic vehicles-Effect on bioavailability of timolol. *International journal of pharmaceutics*, 1989, 57 (2), s. 163-168. ISSN 0378-5173. DOI: 10.1016/0378-5173(89)90305-0.
50. DING, S.: Recent developments in ophthalmic drug delivery. *Pharmaceutical Science & Technology Today*, 1998, 1 (8), s. 328-335. ISSN 1461-5347. DOI: 10.1016/S1461-5347(98)00087-X.
51. PAULSSON, M., HÄGERSTRÖM, H., EDSMAN, K.: Rheological studies of the gelation of deacetylated gellan gum (Gelrite) in physiological conditions. *European Journal of Pharmaceutical Sciences*, 1999, 9 (1), s. 99-105. ISSN 0928-0987. DOI: 10.1016/S0928-0987(99)00051-2.
- 52 OPITZ, DL., HARTHAN, JS.: Review of Azithromycin Ophthalmic 1% Solution (AzaSite®) for the Treatment of Ocular Infections. *Ophthalmology and eye diseases*, 2012, 23 (4), s. 1-14. ISSN 11791721. DOI: 10.4137/OED.S7791.

⁵³ BARANOWSKI, P., KAROLEWICZ, B., GAJDA, M., PLUTA, J.: Ophthalmic Drug Dosage Forms: Characterisation and Research Methods. *Scientific World Journal*, 2014, s. 1-14. ISSN 2356-6140. DOI: 10.1155/2014/861904.

⁵⁴ KOMPELLA, UB., AMRITE, AC., PACHA RAVI R., DURAZO SA.: Nanomedicines for back of the eye drug delivery, gene delivery, and imaging. *Progress in Retinal and Eye Research*, 2013, 36, s. 172-198. ISSN 13509462. DOI: 10.1016/j.preteyeres.2013.04.001.

⁵⁵ LOGO, Alcon, *PILOGEL® ophthalmic gel*, k 20.12.1983

<http://home.intekom.com/pharm/alcon/pilogel.html>

⁵⁶ MOOSA RM. et. al.: A review of topically administered mini-tablets for drug delivery to the anterior segment of the eye. *Journal of pharmacy and pharmacology*, 2013, 66 (4), s. 490-506. ISSN 0022-3573. DOI: 10.1111/jphp.12131.

⁵⁷ FLORENCE, A.T., SIEPMANN, J.: *Modern Pharmaceutics*, 5th Ed.. Infoma Healthcare USA, Inc., New York, 2009, s. 537. ISBN 978-142006566-4.

⁵⁸ REFAI, Hanan, TAG, Randa: Development and characterization of sponge-like acyclovir ocular minitablets. *Drug delivery*, 2011, 18 (1), s. 38-45. ISSN 10717544. DOI: 10.3109/10717544.2010.509364.

⁵⁹ HERRERO-VANRELL, Rocío, BRAVO-OSUNA, Irene, ANDRÉS-GUERRERO, Vanessa, VICARIO-DE-LA-TORRE, Marta and MOLINA-MARTÍNEZ, Irene Teresa: The potential of using biodegradable microspheres in retinal diseases and other intraocular pathologies. *Progress in Retinal and Eye Research*, 2014, 42, s. 27-43. ISSN 13509462. DOI: 10.1016/j.preteyeres.2014.04.002.

⁶⁰ AL-HALAFI, Ali M.: Nanocarriers of nanotechnology in retinal diseases. *Saudi Journal of Ophthalmology*, 2014, 28 (4), s. 304-309. ISSN 13194534. DOI: 10.1016/j.sjopt.2014.02.009.

⁶¹ MAURICE, DM.: Drug delivery to the posterior segment from drops. *Survey of Ophthalmology*, 2002, 47, s. 41-52. ISSN 0039-6257. DOI: 10.1016/S0039-6257(02)00326-0.

⁶² HONDA, M., ASAI, T., OKU, N., ARAKI, Y., TANAKA, M., EBIHARA, N.: Liposomes and nanotechnology in drug development: focus on ocular targets. *International Journal of Nanomedicine*, 2013, 8, s. 495-503. ISSN 1178-2013. DOI: 10.2147/IJN.S30725.

⁶³ HASHEMIAN, MN., ZARE, M.A., RAHIMI, F., MOHAMMADPOUR, M.: Deep intrastromal bevacizumab injection for management of corneal stromal vascularization after deep anterior lamellar keratoplasty, a novel technique. *Cornea*, 2011, 30 (2), s. 215–218. ISSN 0277-3740. DOI: 10.1097/ICO.0b013e3181e291a6.

⁶⁴ RANTA, Veli-Pekka, MANNERMAA, Eliisa, LUMMEPURO, Kirsi, et al.: Barrier analysis of periocular drug delivery to the posterior segment. *Journal of Controlled Release*, 2010, 148 (1), s. 42-48. ISSN 01683659. DOI: 10.1016/j.jconrel.2010.08.028.

⁶⁵ Seiler, G.S. et al.: Effect and distribution of contrast medium after injection into the anterior suprachoroidal space in ex vivo eyes. *Invest. Ophthalmol. Vis. Sci.*, 2011, 52 (8), s. 5730–5736. ISSN 0146-0404. DOI: 10.1167/iovs.11-7525.

⁶⁶ CHAWLA, Bhavna, JAIN, Amit, AZAD, Rajvardhan: Conservative treatment modalities in retinoblastoma. *Indian journal of ophthalmology*, 2013, 61 (9), s. 479-485. DOI: 10.4103/0301-4738.119424. ISSN 03014738.

⁶⁷ RAI, Uma Do J.P., YOUNG, Simon A., THRIMAWITHANA, Thilini R., ABDELKADER, Hamdy, ALANI, Adam W.G., PIERSCIONEK, Barbara and ALANY, Raid G.: The suprachoroidal pathway: a new drug delivery route to the back of the eye. *Drug Discovery Today*, 2015, 20 (4), s. 491-495. ISSN 13596446. DOI: 10.1016/j.drudis.2014.10.010.

⁶⁸ KADAM, R.S. et al.: Suprachoroidal delivery in a rabbit ex vivo eye model: influence of drug properties, regional differences in delivery, and comparison with intravitreal and intracameral routes. *Molecular vision*, 2013, 19, s. 1198–1210. ISSN 1090-0535.

⁶⁹ TOUCHARD, E., BERDUGO, M., BIGEY, P., EL SANHARAWI, M., SAVOLDELLI, M., NAUD, M.C., et al.: Suprachoroidal electrotransfer: a nonviral gene delivery method to transfect the choroid and the retina without detaching the retina. *Molecular therapy*, 2012, 20 (8), s. 1559–1570. ISSN 1525-0016. DOI: 10.1038/mt.2011.304.

⁷⁰ MYLES, M.E., et al.: Recent progress in ocular drug delivery for posterior segment disease: Emphasis on transscleral iontophoresis. *Advanced Drug Delivery Reviews*, 2005, 57 (14), s. 2063-2079. ISSN 0169-409X. DOI: 10.1016/j.addr.2005.08.006d.

⁷¹ JIANG, J, GILL, HS., GHATE, D., MCCAREY, BE., PATEL, SR., EDELHAUSER, HF., PRAUSNITZ, MR.: Coated microneedles for drug delivery to the eye. *Invest Ophthalmol Vis Sci.*, 2007, 48 (9), s. 4038–4043. ISSN 0146-0404. DOI: 10.1167/iovs.07-0066.

⁷² JIANG, J., MOORE, JS., EDELHAUSER, HF., PRAUSNITZ, MR.: Intrasccleral drug delivery to the eye using hollow microneedles. *Pharmaceutical Research*, 2009, 26 (2), s. 395–403. ISSN 0724-8741. DOI: 10.1007/s11095-008-9756-3.

⁷³ PATEL, SR., LIN, ASP., EDELHAUSER, HF., PRAUSNITZ, MR.: Suprachoroidal drug delivery to the back of the eye using hollow microneedles. *Pharmaceutical Research*, 2011, 28 (1), s. 166–176. ISSN 0724-8741. DOI: 10.1007/s11095-010-0271-y.

⁷⁴ PALAKURTHI, NK., CORREA, ZM., AUGSBURGER, JJ., BANERJEE, RK.: Toxicity of a biodegradable microneedle implant loaded with methotrexate as a sustained release device in normal rabbit eye: a pilot study. *Journal of Ocular Pharmacology and Therapeutics*, 2011, 27 (2), s. 151–156. ISSN 1080-7683. DOI: 10.1089/jop.2010.0037.

⁷⁵ JAIN, Gaurav, et al.: Therapeutic stratagems for vascular degenerative disorders of the posterior eye. *Drug discovery today*, 2012, 17 (13-14), s. 748-759. ISSN 13596446. DOI: 10.1016/j.drudis.2012.03.006.

⁷⁶ ZIMČÍK, P., MILETÍN, M.: Fotodynamická terapie, jako nová perspektivní metoda léčby nádorových onemocnění. *Historie, základní princip. Česká a slovenská farmacie*, 2004, 53 (5), s. 219-224. ISSN 1210-7816.

⁷⁷ TÜRKÇÜOĞLU, Peykan, İLHAN, Nevin, KURT, Julide, AYDEMİR, Orhan, ÇELIKER, Ülk: The protective role of Visudyne eyeglass® against VEGF synthesis after photodynamic therapy with verteporfin. *Acta Ophthalmologica*, 2009, 87 (8), s. 871-874. ISSN 1755375X. DOI: 10.1111/j.1755-3768.2008.01368.x.

⁷⁸ Cornell University: *Age related macular degeneration: An epidemic of blindness*, k 1.5.2016

http://atlaseye.sg/wp-content/uploads/focal_laser_amd.jpg.

⁷⁹ MORRISON, Peter WJ and KHUTORYANSKIY, Vitaliy V.: Advances in ophthalmic drug delivery. *Therapeutic Delivery*, 2014, 5 (12), s. 1297-1315. ISSN 2041-5990. DOI: 10.4155/tde.14.75.

⁸⁰ Drugdevelopment-technology.com: *Iluvien for the Treatment of Diabetic Macular Oedema, United States of America.*

<http://www.drugdevelopment.technology.com/projects/iluvienfordiabeticma/iluvienfordiabeticma4.html>

⁸¹ PERSICO, Jack, MATHIS, Andrew: *RPS: From the Podium to the Practice*, k 5.1.2009.

<http://www.retinalphysician.com/articleviewer.aspx?articleID=102989>

⁸² BEJJANI, Riad, et al.: Polymeric Nanoparticles for Drug Delivery to the Posterior Segment of the Eye. *Chimia*, 2005, 59 (6), s. 344-347. ISSN 00094293. DOI: 10.2533/000942905777676281.

⁸³ LEE, Susan, et al.: Biodegradable Implants for Sustained Drug Release in the Eye. *Pharmaceutical Research*, 2010, 27 (10), s. 2043-2053. ISSN 07248741. DOI: 10.1007/s11095-010-0159-x.

⁸⁴ Farmacologica oculare.com.: *Replenish MicroPump™: un dispositivo "intelligente" per il rilascio di farmaci*, k 11.10.2011.

<http://www.farmacologiaoculare.com/2011/10/11/replenish-micropump%E2%84%A2-un-dispositivo-%E2%80%9Cintelligente%E2%80%9D-per-il-rilascio-di-farmaci/>

⁸⁵ YASUKAWA, Tsutomu, et al.: Drug delivery systems for vitreoretinal diseases. *Progress in Retinal and Eye Research*, 2004, 23 (3), s. 253-281. ISSN 13509462. DOI: 10.1016/j.preteyeres.2004.02.003.

⁸⁶ SEYFODDIN, Ali, SHAW, John and AL-KASSAS, Raida: Solid lipid nanoparticles for ocular drug delivery. *Drug Deliver*, 2010, 17 (7), s. 467-489. ISSN 1071-7544. DOI: 10.3109/10717544.2010.483257.

-
- ⁸⁷ ALMEIDAA, H., AMARALA, MH., LOBÃO, P., SILVAA, AC., LOBO, JMS.: Applications of Polymeric and Lipid Nanoparticles in Ophthalmic Pharmaceutical Formulations: Present and Future Considerations. *Journal of pharmacy and pharmaceutical sciences*, 2014, 17 (3), s. 278-293. ISSN 1482-1826.
- ⁸⁸ HERRERO VANRELL, R., et al.: Nano and microtechnologies for ophthalmic administration, an overview. *Journal of drug delivery science and technology*, 2013, 23 (2), s. 75-102. ISSN 17732247.
- ⁸⁹ HERRERO VANRELL, Rocío, et al.: The potential of using biodegradable microspheres in retinal diseases and other intraocular pathologies. *Progress in Retinal and Eye Research*, 2014, 42, s. 27-43. ISSN 13509462. DOI: 10.1016/j.preteyeres.2014.04.002.
- ⁹⁰ ANDRIEU-SOLER, C., et al.: Intravitreal injection of PLGA microspheres encapsulating GDNF promotes the survival of photoreceptors in the rd1/rd1 mouse. *Molecular Vision*, 2005, 11 (118-20). ISSN 1090-0535.
- ⁹¹ AHUJA, Alka, KHAR, Roop K., and ALI, Javed: Mucoadhesive drug delivery systems. *Drug Development and Industrial Pharmacy*, 1997, 23 (5), s. 489-515. ISSN 1520-5762. DOI: 10.3109/03639049709148498.
- ⁹² PAOLICELLI, Patrizia, DE LA FUENTE, Maria, SÁNCHEZ, Alejandro, SEJO, Begoña and ALONSO, María José: Chitosan nanoparticles for drug delivery to the eye. *Expert Opinion on Drug Delivery*, 2009, 6 (3), s. 239-253. ISSN 1742-5247. DOI: 10.1517/17425240902762818.
- ⁹³ CALVO, P., REMUÑÁN-LÓPEZ, C., VILA-JATO, JL., ALONSO, MJ.: Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers. *Journal of Applied Polymer Sciences*, 1997, 63 (1), s.125-132. ISSN 0021-8995. DOI: 10.1002/(SICI)1097-4628(19970103)63:1<125::AID-APP13>3.0.CO;2-4.

-
- ⁹⁴ SAWANT, K.K., DODIYA, S.S.: Recent advances and patents on solid lipid nanoparticles. *Recent Patents on Drug Delivery & Formulation*, 2008, 2 (2), s. 120–135. ISSN 2212-4039.
- ⁹⁵ WADHWA, S., PALIWAL, R., PALIWAL, SR.: Nanocarriers in ocular drug delivery: An update review. *Current Pharmaceutical Design*, 2009, 15 (23), s. 2724–2750. ISSN 1381-6128.
- ⁹⁶ DOMB, AJ.: Long acting injectable oxytetracycline-liposphere formulations. *International Journal of Pharmaceutics*, 1995, 124 (2), s. 271–278. ISSN 0378-5173. DOI: 10.1016/0378-5173(95)00098-4.
- ⁹⁷ WESTESEN, K., BUNJES, H.: Do nanoparticles prepared from lipids solid at room temperature always possess a solid lipid matrix? *International Journal of Pharmaceutics*, 1995, 115 (1), s.129–131. ISSN 0378-5173. DOI: 10.1016/0378-5173(94)00347-8.
- ⁹⁸ MEHNERT, W., MÄDER, K.: Solid lipid nanoparticles production, characterization and applications. *Adv Drug Deliv Rev*, 2001, 64, s.83-101. ISSN 0169-409X. DOI: 10.1016/j.addr.2012.09.021.
- ⁹⁹ DEL POZO-RODRÍGUEZ, Ana, et al.: Lipid Nanoparticles as Drug/Gene Delivery Systems to the Retina. *Journal of ocular pharmacology and therapeutics*, 2013, 29 (2), s. 173-188. ISSN 10807683. DOI: 10.1089/jop.2012.0128.
- ¹⁰⁰ ARAUJO, Joana, et.al.: Nanostructured lipid carriers for triamcinolone acetonide delivery to the posterior segment of the eye. *Colloids and surfaces. B-Biointerfaces*, 2011, 88 (1), s. 150-157. ISSN 09277765. DOI: 10.1016/j.colsurfb.2011.06.025.
- ¹⁰¹ MEISNER, D., MEZEL, M.: Liposome ocular delivery systems. *Adv Drug Deliv Rev.*, 1995, 16 (1), s. 75–93. ISSN 0169-409X. DOI: 10.1016/0169-409X(95)00016-Z.

¹⁰² SASAKI, Hitoshi, et al.: Retinal drug delivery using eye drop preparations of poly-L-lysine-modified liposomes. *European Journal of Pharmaceutics and Biopharmaceutics*, 2013, 83 (3), s. 364–369. ISSN 0939-6411. DOI:10.1016/j.ejpb.2012.10.014.

¹⁰³ HIRONAKA, K., INOKUCHI, Y., TOZUKA, Y., SHIMAZAWA, M., HARA, H., TAKEUCHI, H.: Design and evaluation of a liposomal delivery system targeting the posterior segment of the eye. *Journal of Controlled Release*, 2009, 136 (3), s. 247–253. ISSN 0168-3659. DOI: 10.1016/j.jconrel.2009.02.020.

¹⁰⁴ GAN, Li, et al.: Recent advances in topical ophthalmic drug delivery with lipid-based nanocarriers. *Drug discovery today*, 2013, 18 (5-6), s. 290-297. ISSN 13596446. DOI: 10.1016/j.drudis.2012.10.005.

¹⁰⁵ GAN, L., et al.: Self-assembled liquid crystalline nanoparticles as a novel ophthalmic delivery system for dexamethasone: improving preocular retention and ocular bioavailability. *International Journal of Pharmaceutics*, 2010, 396 (1-2), s. 179–187. ISSN 0378-5173. DOI: 10.1016/j.ijpharm.2010.06.015.

¹⁰⁶ DI, Bei, JIANING, Meng, BI-BOTTI, C. Youan: *Engineering Nanomedicines for Improved Melanoma Therapy: Progress and Promises*, k 30.4.2016.

http://www.medscape.com/viewarticle/734055_5

¹⁰⁷ DU TOIT, Lisa C., PILLAY, Viness, CHOONARA, Yahya E., GOVENDER, Thirumala and CARMICHAEL, Trevor: Ocular drug delivery—a look towards nanobioadhesives. *Expert Opinion on Drug Delivery*, 2010, 8 (1), s. 71-94. ISSN 1742-5247. DOI: 10.1517/17425247.2011.542142.

¹⁰⁸ ZENG, W., et al.: Hyaluronic acid-coated niosomes facilitate tacrolimus ocular delivery: Mucoadhesion, precorneal retention, aqueous humour pharmacokinetics,

and transcorneal permeability. *Colloids and Surfaces B: Biointerfaces*, 2016, 141, s. 28-35. ISSN 09277765. DOI: 10.1016/j.colsurfb.2016.01.014.

¹⁰⁹ FIALHO, SL., DA SILVA-CUNHA, Armando: New vehicle based on a microemulsion for topical ocular administration of dexamethasone. *Clinical & experimental ophthalmology*, 2004, 32 (6), s. 626-632. ISSN 14426404. DOI: 10.1111/j.1442-9071.2004.00914.x.

¹¹⁰ KAUR, Indu Pal and KAKKAR, Shilpa: Nanotherapy for posterior eye diseases. *Journal of Controlled Release*, 2014, 193, s. 100-112. ISSN 01683659. DOI: 10.1016/j.jconrel.2014.05.031.

¹¹¹ MARANO, RJ., et al.: Inhibition of in vitro VEGF expression and choroidal neovascularization by synthetic dendrimer peptide mediated delivery of a sense oligonucleotide. *Experimental Eye Research*, 2004, 79 (4), s. 525-535. ISSN 0014-4835. DOI: 10.1016/j.exer.2004.06.023.

¹¹² PAREKH, HS., MARANO, RJ., RAKOCZY, EP., BLANCHFIELD, J., TOTH I.: Synthesis of a library of polycationic lipid core dendrimers and their evaluation in the delivery of an oligonucleotide with hVEGF inhibition. *Bioorganic & Medicinal Chemistry*, 2006, 14 (14), s. 4775-4780. ISSN 0968-0896. DOI: 10.1016/j.bmc.2006.03.029.

¹¹³ IEZZI, R., GURU, B.R., GLYBINA, I.V., MISHRA, M.K., KENNEDY, A., KANNAN, R.M.: Dendrimer-based targeted intravitreal therapy for sustained attenuation of neuroinflammation in retinal degeneration. *Biomaterials*, 2012, 33 (3), s. 979-988. ISSN 0142-9612. DOI: 10.1016/j.biomaterials.2011.10.010.

¹¹⁴ REEMA, Syed, JACQUE, L. Duncan: *Growth Factor Therapy for Retinal Neuroprotection*, k 10.1.2011.

<http://www.retinalphysician.com/articleviewer.aspx?articleID=106241>

¹¹⁵ NAIK, Rangeetha, MUKHOPADHYAY, Arijit and GANGULI Munia: Gene delivery to the retina: focus on non-viral approaches. *Drug Discovery Today*, 2009, 14 (5-6), s. 306-315. ISSN 13596446. DOI: 10.1016/j.drudis.2008.09.012.