HELLENIC REPUBLIC
National and Kapodistrian University of Athens
EST. 1837

School of Health Sciences
School of Medicine and Department of Pharmacy

Interdisciplinary M.Sc course in
"Nanomedicine"

Academic year 2018-2019

"Nanotechnology in Ophthalmology, new developments"

Andreas C. Dimakis

Supervisor: **Professor Efstathios Efstathopoulos**

Members of committee: **Associate Professor Maria Gazouli**
Post-Doc Ellas Spyratou

Date of Submission: February 2020

Acknowledgements

I would like to acknowledge everyone who played a role in my academic accomplishments. First of all, my family, who supported me with love and understanding. Without you, I could never have reached this current level of success. Secondly, my committee members, each of whom has provided patient advice and guidance throughout the work process. Thank you all for your unwavering support.

Abbreviations:

5-FU	Fluorouracil
ACV	Acyclovir
ACZ	Acetazolamide
ARE	Antioxidant Response Element
ARMD	Age-Related Macular Degeneration
AUC	Area Under the Curve
BAK	Benzalkonium Chloride
BIM	Bimatoprost
BT	Brimonidine tartrate
BZ	Brinzolamide
CH	Chitosan
CsA	Cyclosporin A
CT	Computerized Tomography
CNV	Choroidal Neovascularization
CPX	Ciprofloxacin
DDS	Drug Delivery Systems
DEX	Dexamethasone sodium phosphate
DME	Diabetic Macula Edema
DR	Diabetic Retinopathy
DSP	Dexamethasone Sodium Phosphate
DXR	Doxorubicin
DZ	Dorzolamide hydrochloride
ECM	Extracellular Matrix
EPR	Enhanced Permeability and Retention
ESCs	Embryonic Stem Cells
FA	Fluocinolone Acetonide
GDD	Glaucoma Drainage Device
GLA	Galucoma
CLS	Contact Lens Sensor
GNPs	Gold Nanoparticles
HA	Hyaluronic Acid
HEMA	Hydroxyethylmethacrylate
IPSCs	Induced Pluripotent Stem Cells
IOL	Intraocular Lens
IOP	Intraocular Pressure
IV	Intravitreal
KF	Ketotifen Fumarate
LE	Loteprednol Etabonate
MEMS	MicroElectroMechanical Systems
MIP	Molecular Imprinted Polymer technology
MMC	Mitomycin C
MN	Microneedles
MP	Microparticle
MPP	Mucus Penetrating Particles
MW	Molecular Weight

ND	Nanodiamonds
NHPs	Non-Human Primates
NiMs	Nanoparticles-in-Microparticles
NLC	Nanostructured Lipid Carriers
NPs	Nanoparticles
NTX	Naltrexone Hydrochloride
NV	Neovascularization
NW	Nanowafers
ODD	Ocular Drug Delivery
PAM	Photoacoustic Microscopy
PAMAM	Polyamidoamine
PC	Phosphatidylcholine
PCL	Polycaprolactone
PCO	Posterior Capsular Opacification
PCR	Polymerase Chain Reaction
PDGF	Platelet-Derived Growth Factor
PDMS	Polydimethylsiloxane
PDR	Proliferative Diabetic Retinopathy
PDT	Photodynamic Therapy
PEG	Polyethylene Glycol
PGT	Propoxylated Glyceryl Triacrylate
PLA	Polylactic Acid
PLGA	Polylactic-co-glycolic acid
PNPs	Polymeric nanoparticles
PVP	Polyvinylpyrrolidone
Qdots	Quantum dots
RB	Retinoblastoma
RPE	Retinal Pigment Epithelium
SCT	Subconjunctival
SLN	Solid Lipid Nanoparticles
SPIO	Superparamagnetic Iron Oxide
TA	Triamcinolone Acetonide
TEM	Transmission Electron Microscopy
TKI	Tyrosine Kinase Inhibitor
TM	Timolol Maleate
TMS	Trabecular Meshwork
TPGS	Tocopheryl Polyethylene Glycol Succinate
VEGF	Vascular Endothelial Growth Factor
VOR	Voriconazole

Contents

Abstract

Nanotechnology, initially described by Richard Feynman, is a promising multidisciplinary field of science that dealing with the design, development and manufacturing of structures in the dimensions of nanometers. M. Saladin El Naschie's in 2006 [1] defines nanotechnology *as the application of the technology in the gray area between the classical mechanism and quantum mechanics*. Nowadays the rapid evolution of nanotechnology is due to the development of novel technological procedures for detection, control and characterization of nanoparticles.

The application of nanotechnology in biology is loosely designated nanobiotechnology. Nanomedicine is a derivative of nanobiotechnology. Ophthalmology is a field of medicine that could really be benefit from nanotechnology's applications. The human eye is a very sophisticated small sensory organ that among others includes very specific barriers against the external environment. Drugs and pharmaceutical agents have to overcome these barriers in order to treat ocular diseases. Most of these diseases are chronic in their nature, need chronic expensive treatments and unfortunately commonly result to blindness. Chronic treatments besides of side effects engender issues in compliance and adherence to treatment.

Nanoparticles like liposomes, micelles, dendrimers, polymers, nanogels look very attractive as novel ocular drug delivery systems. They can overpass the ocular barriers and deliver their drug load with a very specific spatiotemporal controlled manner. Hence such NPs facilitate the sustained release and bioavailability of medicaments. Furthermore the coating of nanoparticles with surface targeting moieties makes possible the drug delivery precisely to the diseased cell. The contribution of nanotechnology in monitoring, diagnosis and imaging of ocular diseases is also significant. Indeed the combination of diagnostic and therapeutic properties in the same NP introduced the multimodality of theragnostics into the ophthalmology field. Regenerative ophthalmology is another area where the use of nanomaterials in the form of nano scaffolds and genes nanocarriers can conduce to significant improvements mainly in the treatment of degenerative ocular diseases. All the above very promising approaches lead to the era of personalized medicine.

Obstacles to be overcome are the scale production of nanostructures in a safety manner and in an economically advantageous way. Likewise nanotoxicity issues must be taken into account as nanoapplications in everyday practice will be increase.

Purpose

To review the implementations of nanotechnonology and nanomedicine in the field of ophthalmology.

Design

Perspective following literature review.

Methods

This review includes analysis of relevant publications in the peer-reviewed scientific literature. Research articles about nanotechnology-based treatments for particular eye diseases and diagnostic technologies were searched through PubMed and Google scholar and the most representative of them were reported.

1. Introduction

In 1959, Nobel laureate Richard Feynman gave his lecture, “There’s Plenty of Room at the Bottom”, to the annual meeting of the American Physical Society [2], introducing the concept of nanotechnology and miniaturization. Typically the term “nanotechnology” was first mentioned by Prof. Norio Taniguchi [3] in 1974.

Nanotechnology is an overarching multidisciplinary area of research that promotes the development of enabling materials and devices with at least one dimension of less than 100 nm. For comparison, an erythrocyte is 7 µm wide; a strand of deoxyribonucleic acid [DNA] is 2 nm wide. At its inception, nanoelectronics were the cynosure of nanotechnology. Of paramount importance in nanoelectronics was the minimization of the length scale of transistors, perpetuating Moore’s law [4] and forging on its ambitious technological and economic aspirations.

Nanoscale science and technology remained in a nascent stage until the last decade, largely because of the technological limitations involved in characterizing, manipulating, and building nanostructures. The advent of the scanning tunneling microscope (STM) [5] allowed visualization at the nanoscale level and ignited the nanotechnology acceleration. Subsequent iterations of STM include atomic force microscopy (AFM), scanning probe microscopy, and scanning acoustic microscopy. These tools and other readily accessible characterization tools, such as electron microscopy and X-ray diffraction (XRD) make characterization of nanostructures possible. On the other hand, semiconductor processing technologies, along with electron-beam lithography or dip-pen nanolithography [6], provide us with the necessary toolset to define and build nanostructures.

The current, widespread, mainstream commercialization of nanotechnology ranges from carbon nano-tube-embedded tennis rackets to nanoparticle-fortified beauty lotions, from atomic resolution imaging systems to molecular diagnostics, from magnetic storage devices to semiconductor microprocessor chips. The application of nanotechnology in biology is loosely designated nanobiotechnology. Nanomedicine is a derivative of nanobiotechnology.

The aim of nanomedicine is the comprehensive monitoring, control, construction, repair, defense, and improvement of human biological systems at the molecular level, using engineered nanodevices and nanostructures, operating massively in parallel at the

single cell level, ultimately to achieve medical benefit. [7] Individual atoms and molecules can be manipulated to form microscopic tubes, spheres, wires, and films for specific tasks, such as generating electricity or transporting drugs in the body. The application of nano-scale technologies to the practice of medicine will alter profoundly our approach to diagnosis, treatment, and prevention of disease. [8] Miniaturization of devices, chip-based technologies, and increasingly sophisticated novel nanosized materials and chemical assemblies already provide novel tools that are contributing to improved healthcare in the 21st century.

The application of nanotechnology platforms to the field of medicine is far reaching and too vast to cover in a single review article, but suffice it to say that nanotechnology has dramatically affected drug discovery, drug therapeutic efficacy, drug and gene delivery systems, biocompatible implant materials, regenerative medicine, and diagnostic procedures. Exciting new developments in the field of nanotechnology are now impinging directly on ophthalmology.

According to 2015 global estimates, 36 million people are blind, 217 million are moderately or severely vision impaired and 188 million have mild vision impairment. Of these cases, approximately 84% of the visual impairments result from chronic eye diseases [9] that demand frequent drug administration. For example it is well established [10, 11, 12] among ophthalmologists that at least the 50% of glaucoma patients don't comply with given therapy. The complex anatomy and physiology of the eye not only disallows permeation of the topically administered medicaments across various ocular tissues but also readily clears drugs from the tissues thereby resulting in sub-therapeutic levels [13]. Despite of the availability of numerous interventions, an effective carrier that can deliver therapeutic agents of any kind, to the tissue of interest in the eye in a spatially and temporally controlled manner for long durations remains an unmet need. Therefore, the design of an effective drug delivery carrier for the treatment of ocular diseases is a need of the hour.

The main objective of ocular drug delivery is to improve bioavailability and residence time of administered agents at ocular tissues so as to avail therapeutic benefits while reducing the frequency of administrations. Poor bioavailability can be partly addressed by administration of drugs in the form of solution/suspensions directly at the tissue site using a suitable invasive injection. However, the invasive routes of administration can be

associated with serious complications and discomfort to the patient which result in poor patient compliance [14].

Another important area of nanotechnology involvement in ophthalmology is the monitoring, diagnosis and imaging of ophthalmic diseases. Many of ocular diseases are chronic in their nature and given this, strictly monitoring of specific parameters like intraocular pressure (IOP) or macula edema, are of paramount importance for accurate therapeutic intervention. Also the delicate and special structures of the eye demands highly sophisticated diagnostic techniques and is expected that nanotechnology will be very beneficial in this field. The combination of diagnostic and therapeutic properties in the same nanostructure device leads to the introduction of the “theragnostics” probes. Obviously in this way we could diagnose but also cure the disease at the same time with multiple benefits for the patient and for the health system's costs.

In this review there will be mentioned the novel applications of nanotechnology in ophthalmology. In particular, important and characteristic interventions will be presented in the following areas: drug delivery, monitoring, diagnosis and imaging, surgery, tissue regeneration and prosthetics. Also it will be cited the toxicity and safety issues of nanomaterials as well as the future perspectives of "nano-ophthalmology".

1.1 Properties of nanomacines

A key property of nanomaterials is that they are surface-rich objects in relation to volume, in other words they exhibit high surface to volume ratio. At the nanolevel (i.e., the size scale of intracellular structures and molecules), materials acquire properties that seem surprising to us but that are predicted based on the principles of quantum physics. For instance, carbon becomes stronger than steel, gold melts at room temperature, and aluminum becomes highly explosive. Quantities such as weight and inertia are of relatively little importance. The strength of a given material is thus proportionately greater as the size diminishes.

The influence of gravity on the function of true nanomachines probably is negligible because their mass is that of atoms. On the other hand, the distance between the elements is in nanometers. Because of Van der Waals forces, parts of nanomachines might adhere to each other, which might not be desirable.

Concerning the manufacturing process of nanodevices, currently there are two synthesis approaches. Evidently, the top-down approach continues to be the quintessence of manufacturing industries. This approach is typified by the semiconductor industry practice, where the nanoscale features are defined and manufactured using processes such as lithography and etching with a lack of atomic-level control because the tools employed are larger than the smallest feature. In nanobiotechnology, the top-down approach includes disassembling the microscale materials to achieve the desired nanoscale structures (e.g., purification of mitochondria via the process of tissue isolation, homogenization, disruption, and centrifugation). The bottom-up approach is, in general, the process of self-assembly from atomic and molecular constituencies governed by molecular interactions, subatomic level forces, and the principles of thermodynamics. Using the self-assembly bottom-up approach, nature has devised and synthesized complex biosystems from basic building blocks for millennia. A generalized depiction is shown in figure 1.

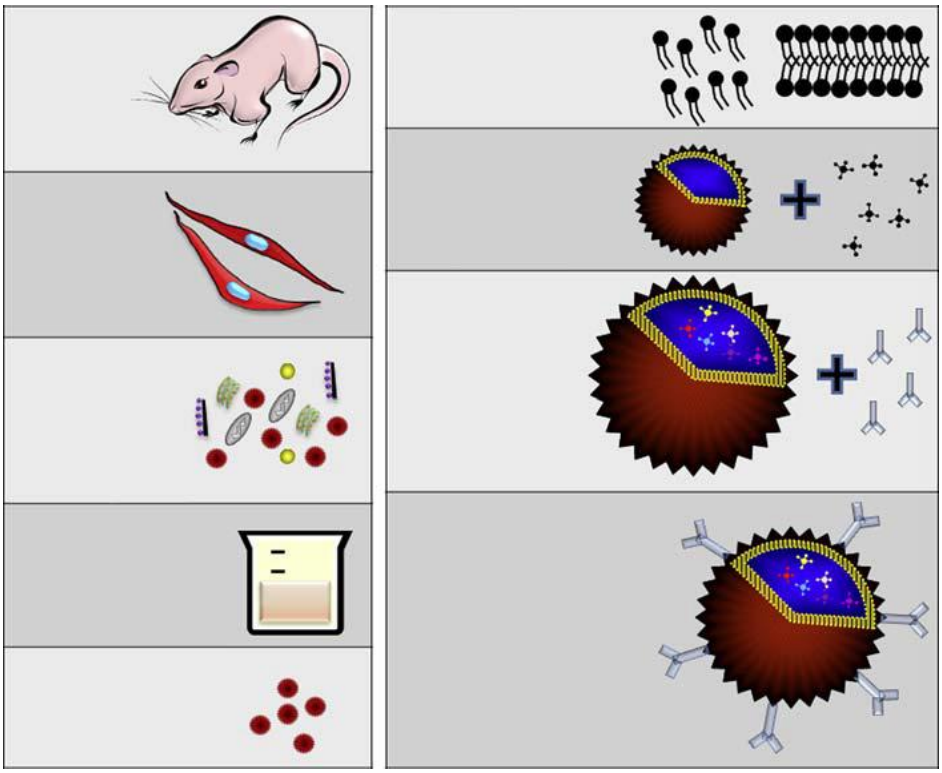

Figure 1: Top-down versus bottom-up processes *(Nguyen P et al., Ophthalmic Research, 2010; 44: 1-16).*

1.2 Nanotechnology and nanomedine

General principles of nanotechnology as applied to nanomedicine include the following [15]:

1. Biomimicry: directs molecules within a cell and (or) directs molecules/machines to the proper cells in the body using approaches copied from nature.
2. Size and location drive biocompatibility and biological efficacy. Size matters in the nanoworld. The same molecules or structures can have very different functions when located in different parts of the cell or different tissues or organs of the body.
3. Feedback control engineered into therapeutic systems (e.g., therapeutic gene synthesis) allows one to control dosage precisely at the single-cell level and will be an important part of regenerative medicine.
4. Molecules as machines: molecules engineered to perform specific physical tasks (e.g., open ion channels) to alter cell and organism behavior.
5. ''Pseudointelligence'' resulting from intelligent design, e.g., self-assembly of extracellular matrix (ECM) molecules.
6. Highly interdisciplinary undertaking: biology, engineering, chemistry, physics.

The functional properties of living systems arise not simply from their component parts. Rather, these properties are a function of how the component parts are assembled, which dictates interactions between the parts, the nature and flow of information within the system, and the outputs that the system produces. Thus, an important concept for the development of nanomedicine is that spatial control of the distribution of nanomachines directly affects the efficiency of the macromolecular assembly and nature of this assembly's work product. [16]

2. Anatomy of the eye and ocular barriers for drug delivery

The eye is a complex organ consisting of sensitive tissue structures arranged as compactly adjoined layers. The outer most layer consists of cornea, conjunctiva, and sclera; the middle vascular layer comprises of choroid, ciliary body, and iris; and the inner neural layer includes retina. The lens is attached to ciliary body via suspensory ligaments and is responsible for focusing light onto the retina. Retina consists of a variety of cell types including retinal pigmented epithelium (RPE), photoreceptors (rods and cones),

horizontal, bipolar, amacrine, Müller, and ganglionic cells. The optic nerve is composed of nerve fibers of ganglion cells and is responsible for conveying retinal impulses to the brain. Anatomically, the eye is divided into anterior and posterior segment by means of iris-lens diaphragm. The tissues that are present anteriorly such as cornea, conjunctiva, iris, ciliary body, and lens are classified as anterior segment tissues, whereas, the tissues present posteriorly such as sclera, posterior choroid, Bruch's membrane, vitreous body, RPE, and retina are classified as posterior segment tissues. The anterior chamber is filled with aqueous humor, whereas, the vitreous cavity is filled with vitreous body. The ciliary epithelium continuously secretes aqueous humor that is drained through trabeculum meshwork to the canal of Schlemm. The continuous secretion and drainage of aqueous humor serves two functions. The first is to provide oxygen and nutrients to the anterior eye tissues and the other is to maintain IOP of the eye. Together, the structures of the eye act in coordination with each other and are responsible for the physiology of vision [17]. In addition to their physiological role, these structures also act as strong barriers to limit entry of foreign substances into the eye including topically administered therapeutic agents.

2.1 Barrier properties of ocular layers

The therapeutic molecules used for the treatment of ocular diseases are either hydrophilic, hydrophobic, or amphiphilic in nature. Upon topical administration, the molecules encounter the following tissue barriers:

2.1.1 Tear film

This is the first and foremost ocular barrier that is encountered after topical administration of a therapeutic agent. Tear film is a precorneal film consists of three layers: an outer lipid layer, a middle aqueous layer, and an inner mucous layer. The meibomian glands and to a lesser extent the glands of Zeis and Moll secrete the lipids of the outer layer. The main lacrimal gland secretes the middle aqueous layer with an additional minor contribution from the accessory lacrimal glands (the glands of Kraus and Wolfring). The goblet cells and to some extent the crypts of Henle and the glands of Manz secrete the inner mucous layer. Tear film has high turnover rate and is continuously secreted and drained into the nasolacrimal duct. Its main function is to

moisten the superficial ocular tissues in order to prevent among others the ocular surface drying. The continuous secretion and drainage of tear fluid readily clear topically administered drugs from the ocular surface, resulting in low bioavailability (<5%) in the underlying ocular tissues [18]. Hence, the tear film is considered as a dynamic ocular barrier. The hydrophilicity/-phobicity of drug molecules governs their interaction with tear fluid [19]. Consequently physicochemical properties of drug molecules and delivery systems and their interaction with tear fluid, together govern the ability of the drug molecules to traverse through the tear film barrier and interact with the subsequent tissue, the cornea [20].

2.1.2 Cornea

The cornea is a transparent, five-layered avascular tissue composed of cellular and extracellular matrix (ECM) components. After tear film, the cornea is the next outermost layer of the eye which consists of five dinstict layers: epithelium, stroma, and endothelium and two interface layers consisting of Bowman's membrane and Descemet's membrane. Of these, corneal epithelium and endothelium are cellular in nature, whereas, others are majorly composed of ECM components. The cellular layers allow permeation of molecules through paracellular or transcellular routes based on the physicochemical characteristics of therapeutic molecules [21]. It has been speculated that low molecular weight hydrophilic molecules (up to 300 Da) traverse through the paracellular route. However, higher molecular weight hydrophilic molecules (>2000 Da) cannot traverse easily across cellular layers of the cornea [22]. Whereas, hydrophobic molecules (molecular weight up to 600 Da), due to their ability to partition into the cell membrane, can traverse through paracellular and/or transcellular route in order to get access to underlying tissues [21]. Furthermore, ECM layers of the cornea offer better interaction towards hydrophilic molecules compared to hydrophobic molecules. The stromal layer acts as a barrier for hydrophobic molecules, whereas, it allows permeation of hydrophilic molecules [21]. Hence, permeability characteristics of the cornea are majorly governed by molecular weight and hydrophilicity/-phobicity of drug molecules. An optimal combination of these characteristics is required for a drug molecule in order to obtain access to the underlying tissues [23]. In addition to barrier properties, corneal tissue also possesses transmembrane efflux pumps which cause efflux of drug molecules

from the cornea and thus further reducing the bioavailability of administered therapeutic molecules in the cornea [24, 25].

2.1.3 Conjunctiva

Conjunctiva is a transparent and vascularized tissue composed of epithelial cells, goblet cells, and basal lamina. Conjunctival epithelial cells contain tight intercellular junctional proteins that disallow free diffusion of high molecular weight molecules through paracellular route. The presence of such tight junctional proteins in conjunctiva causes hindrance for permeability of high molecular weight hydrophilic molecules compared to hydrophobic molecules. Since hydrophobic molecules traverse the transcellular route, their bioavailability at conjunctiva is relatively higher when compared to hydrophilic molecules [26]. Furthermore, low molecular weight hydrophilic (up to 1,500 Da) and hydrophobic (up to 5,000 Da; lower the molecular weight, higher the permeation) molecules can be transported through the paracellular pathway in the conjunctiva. The goblet cells and glandular cells present in conjunctiva secrete mucin and tear fluid, respectively, that play an important role in trapping and clearing of administered therapeutic molecules resulting in low bioavailability of administered drugs. It has been demonstrated that conjunctival epithelial cells, as the cornea, possess drug efflux pumps which further lower the bioavailability of topically administered drugs [27].

2.1.4 Sclera

The sclera is a largely collagenous structure and forms five-sixth of the outer covering of the eye. The scleral matrix consists of collagen and elastin fibrils and interfibrillar proteoglycans with a sparse distribution of scleral fibrocytes. The fibrous structure allows faster diffusion of low molecular weight (up to 4,000 Da) molecules, whereas, an increase in molecular weight, molecular radius, or hydrophobicity decreases permeability through the sclera [28].

2.1.5 Iris, ciliary body and choroid

These three structures together form the uveal tract, a dynamic vascular barrier of the eye responsible for dilution of the administered drugs in systemic blood circulation. Iris is located anteriorly to the lens and is responsible for controlling the entry of light into the lens. The ciliary body is located posterior to the iris and secretes aqueous humor which

nourishes interior ocular tissues. The secreted aqueous humor flows towards the cornea and through the trabecular meshwork is collected into the canal of Schlemm and ultimately drained into the episcleral blood veins. Hence, the aqueous humor acts as a dynamic barrier that clears therapeutic molecules from the anterior chamber. The choroid is the posterior continuation of the ciliary body which contains circulatory blood vessels that continuously supply oxygen and nutrients to the outer retinal tissue. Choroidal's blood flow is the higher among the human body tissues with estimates ranging from 500 to 2000 ml/min/100 g tissue [17]. A continuous flow of blood in choroid acts also as dynamic barrier and causes clearance of drugs from ocular tissues and dilution of drugs in the systemic circulation.

2.1.6 Lens

The lens is a transparent biconvex structure, majorly composed of different types of crystallin proteins embedded in a thin layer of acellular outer membrane called lens capsule which acts as a basement membrane for lens epithelial. Lens capsule is composed of laminins, collagens and proteoglycans that impart a negative (anionic) charge to it. Furthermore, it has been demonstrated that diffusion rate of neutral molecules across the lens capsule is relatively higher compared to anionic molecules [29]. Hence, the charge on a drug molecule affects its permeability across the lens tissue.

2.1.7 Retinal pigmented epithelium (RPE)

The RPE with Bruch's membrane separate the outer retina from choroidal capillaries. RPE forms the **outer blood–retinal barrier**. Due to the presence of tight intercellular junctional proteins, RPE disallows the entry of hydrophilic molecules into the retina, whereas, hydrophobic molecules can partition into the RPE [30]. RPE is also known to express drug efflux proteins that lower the bioavailability of therapeutic agents in the retina [31].

2.1.8 Vitreous body

The vitreous body is a transparent gelatinous viscous liquid that fills the space between the lens and the retina and is surrounded by a collagenous layer known as the vitreous membrane. The vitreous body is composed of hydrated molecules such as collagen and glucosaminoglycans. The net anionic charge of the vitreous body regulates

the diffusion of drug molecules. It has been demonstrated that negatively charged particles diffuse freely in the vitreous, whereas, positively charged particles get immobilized [32]. Hence, the charge of the administered drug molecule affects both vitreal drug distribution and retinal bioavailability.

3. Ocular diseases and their treatments

A large majority of the diseases that affect the structure and function of the eye demand therapeutic interventions. The diseases that commonly affect ocular tissues are classified based on the location as anterior or posterior ocular diseases and herein will be mentioned the most important of them. Potentially all of these diseases without treatment lead to blindness.

3.1 Diseases affecting anterior eye segment

Keratitis is an inflammatory disease that affects the cornea. Mainly it is caused by microorganisms such as bacteria, fungi, or viruses resulting in inflammation or injury to the cornea. Infectious keratitis is treated by topical administration of antibacterial, antifungal, or antiviral drugs as eye drops. Non infectious keratitis is related with autoimmune disorders, diabetes mellitus and neurotrophic lesions and also need to be treated, among others, by topical administration of anti-inflammatory drugs or artificial tears.

Conjunctivitis, dry eye disease (also known as kerato-conjunctivitis sicca) and Sjögren syndrome (an autoimmune disease) are inflammatory diseases that affect the conjunctiva and are treated by topical administration of anti-inflammatory drugs or artificial tears.

Cataract is a pathological condition characterized by clouding of the lens due to crosslinking of crystallin proteins present in the lens. The pathological condition is treated by a surgical method known as phacoemulsification that involves removal of the cloudy lens and replacing it with an artificial lens. An emerging therapeutic intervention for cataract is the topical administration of antioxidant eye drops which act by reducing and/or reversing crosslinking of crystallin proteins [33].

3.2 Diseases affecting both anterior and posterior eye tissues

Uveitis is an inflammatory disease, of infectious or non infectious origin, that affects the uveal tract. Uveitis is classified as anterior, intermediate or posterior uveitis based on the tissue being affected, that is, anterior, middle, or posterior uvea, respectively. Panuveitis affects all the three parts of the uvea and is treated by administration of anti-inflammatory or anti-microbial drugs either topically or via injections.

The Glaucomas are a group of optic neuropathies that cause degeneration of retinal ganglion cells due mainly to an elevation in IOP resulting in optic nerve damage. The available treatment options include administration of IOP lowering drugs via topical route.

3.3 Diseases affecting posterior eye segment

Age-related macular degeneration (ARMD) is an age-related pathological condition characterized by degeneration of the macula, the central retinal tissue responsible for sharp and central vision. The treatment strategies include, depends on the dry or wet form of the disease, supplementation with antioxidants and intravitreal administration of anti-vascular endothelial growth factor (anti-VEGF) agents respectively [34].

Retinitis is an inflammatory disease caused most commonly by infectious agents such as Toxoplasma gondii (protozoa), cytomegalovirus (virus) or candida (fungi). The treatment strategies include intravenous or intravitreal administration of antiviral, antiprotozoal, or antifungal drugs.

Diabetic retinopathy (DR) is a pathological condition characterized by retinal neovascularization (NV) and retinal damage. DR is treated by intravitreal administration of anti-VEGF drugs [35].

Diabetic macular edema (DME) is an inflammatory disease characterized by inflammation of the retina, exudation of blood from retinal capillaries ultimately leading to retinal NV and retinal damage. DME is treated by intravitreal administration of anti-inflammatory steroids and anti-VEGF drugs [35].

Endophthalmitis is a devastating inflammatory disease characterized by inflammation of interior ocular tissues, mainly the vitreous cavity. This pathological condition is frequently observed after intraocular surgeries or traumas due to the exogenous or

endogenous spread of infecting organisms such as bacteria, virus, or fungi. The treatment options include intravitreal administration of antibacterial, antiviral, or antifungal drugs.

Optic nerve neuritis is characterized by inflammation of optic nerve and is treated by intravenous administration of anti-inflammatory drugs.

Optic nerve neuropathies are characterized by degeneration of optic nerve. The most common causes are glaucoma and ischemic optic neuropathies. The choice of treatment depends on the underlying disease.

Proliferative vitreoretinopathy is characterized by proliferation of ectopic cell sheets in the vitreous and/or periretinal area. The treatment strategies include surgical removal of proliferated tissue and halting the proliferation by intravitreal application of laser, anti-inflammatory drugs and/or growth factor pathway inhibitors [36].

Retinitis pigmentosa represents a spectrum of hereditary retinal diseases characterized by degeneration of retinal rod and cone photoreceptor cells. The pathological manifestations can be reduced by dietary supplementation with antioxidants.

Uveal melanoma is a primary acquired malignant neoplasm of uveal melanocytes and is the most commonly form of eye cancer. The therapeutic approaches include radiotherapy, thermotherapy, brachytherapy and/or surgery.

Retinoblastoma is characterized by abnormal proliferation of retinal astrocytes/glial cells. It is a rare form of eye cancer that mostly affects children. The treatment strategies include surgical methods such as enucleation of the eye and therapies such as radiotherapy, thermotherapy, brachytherapy, and chemotherapy (intravitreal injection) with anticancer drugs.

Retinopathy of prematurity is characterized by the growth of abnormal blood vessels in the immature retinas of preterm neonates. The treatment strategies include surgical methods such as vitrectomy, scleral buckling, laser surgery and cryosurgery, and intravitreal administration of anti-VEGF drugs [37].

4. Routes of administration for the treatment of ocular diseases

The therapeutic drug molecules (hydrophilic, hydrophobic, or amphiphilic) are administered to ocular tissues by different routes so as to improve their bioavailability in

the tissue of interest in the eye. The most commonly employed routes for drug administration are as shown in figure 2.

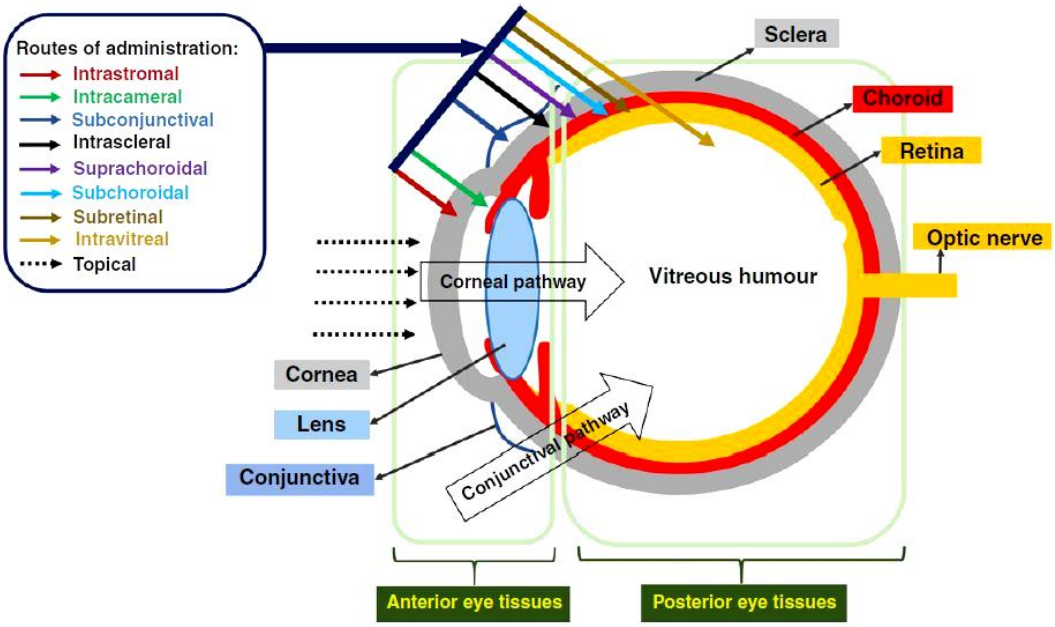

Figure 2: Schematic representation of human eye and routes of drug administration

Among all the routes of drug administration mentioned, the topical route is the only noninvasive way, whereas, all the other routes are invasive in nature. Therefore, the topical application is preferred due to safety reasons and also it improves patient compliance. Upon topical administration of a drug solution, a fraction of the administered dose is bioavailable at anterior eye tissues to enable therapeutics. However, the bioavailability of drugs at posterior eye tissues is negligible upon topical administration due to the presence of static and dynamic ocular barriers. To overcome this, drugs are injected into the eye via subretinal, subchoroidal, and intravitreal injections to achieve higher bioavailability at the tissue of interest [38]. Although these injections provide higher bioavailability at the tissue of interest, they can be associated with serious complications such as endophthalmitis, retinal detachment, IOP increase, intraocular bleeding, pain and discomfort to the patient, resulting in poor patient compliance. Therefore, if the bioavailability of drugs to both anterior and posterior eye tissues can be improved using the noninvasive route of administration (topical route), it would clearly

hold an advantage over the other ways for the treatment of ocular diseases. Thus, in the following sections, the topical route of administration has been emphasized.

4.1 Transport of therapeutic molecules or DDS after the topical route of administration

Topically administered drug molecules or DDS first come in contact with tear film followed by transport across eye tissues. At this juncture, drug molecules can take two pathways which are classified as the corneal pathway and the conjunctival pathway. In the corneal pathway, drug molecules traverse through the cornea, aqueous humor, iris, lens, and vitreous body so as to reach the retina. In the conjunctival pathway, drug molecules traverse through conjunctiva, sclera, choroid, and RPE so as to reach the retina. The bioavailability of the drug is relatively higher at superficial tissues (cornea and conjunctiva) and relatively lower at the deep/underlying tissues due to the structure and physiology of the aforementioned ocular barriers [39]. This transport pathway can also be extrapolated to intraocularly administer therapeutic agents, that is, drug transport from the site of injection to the underlying ocular tissues. Furthermore, the permeation and extent of bioavailability for topically administered drug molecules is strongly governed by physicochemical properties of therapeutic agents such as molecular weight, molecular size, hydro-philicity/-phobicity and surface charge, etc. [28].

4.2 Influence of physicochemical properties of topically administered therapeutic molecules

Since ocular tissue layers are composed of dissimilar cellular and ECM components, they show differential permeability towards hydrophilic, hydrophobic, anionic, and cationic molecules. The tear film layer readily interacts with hydrophilic mucoadhesive molecules and presents them to the underlying corneal tissue for interaction [18]. Since cornea consists of cellular and ECM components, specific hydro-philicity/-phobicity is required for a molecule to traverse through corneal layers. The conjunctiva, due to its cellular composition, facilitates partitioning and permeation of hydrophobic molecules [22]. Sclera, due to its fibrous structure, causes higher partitioning of hydrophobic molecules [40] and higher penetration of hydrophilic molecules. The choroidal layer, due to the presence of fenestrations, does not exhibit strong barrier properties. However,

choroidal vascularization readily clears the drug from ocular tissues and dilutes it in the systemic circulation. The RPE shows high permeability for hydrophobic molecules compared to hydrophilic ones [30]. Furthermore, permeation across these ocular layers is inversely proportional to molecular weight or molecular size [28, 30]. It has been observed that increasing lipophilicity of β-blocker drugs decreases permeation across sclera–choroid–RPE layers of human, bovine, porcine, rabbit, and rat models [41] which demonstrate that an optimal hydro-philicity/-phobicity is required for therapeutic agents to cross the ocular barriers. It has also been demonstrated that anionic molecules cannot permeate across lens capsule, whereas, cationic molecules get immobilized in vitreous body which indicates that the net surface charge of a molecule also affects its tissue distribution in the eye. Similarly, the composition and physicochemical properties of a delivery system will also affect its tissue distribution [39]. Therefore, ocular tissues show differential drug penetration based on the physicochemical properties of therapeutic molecules/delivery systems being used.

5. Ocular drug delivery systems (DDS)

A delivery system is used as a vehicle to deliver its cargo to the tissue of interest in the body at the desired concentration, duration, and kinetics. For ocular drug delivery applications, a DDS plays an important role in transporting drugs across ocular tissues so as to make it bioavailable and enable therapeutics at specific tissues in the eye. Drug delivery to ocular tissues can be achieved by using conventional drug formulations as well as advanced DDS.

5.1 Conventional drug formulations for the treatment of ocular diseases

Conventional formulations such as drug solutions, suspensions, and ointments have been widely explored for drug administration to ocular tissues. Drug solutions and suspensions are administered via eye drops or injections, whereas ointment-based formulations are applied topically for the treatment of ocular diseases. The most commonly used conventional drug formulations are discussed below.

5.1.1 Ophthalmic solutions and suspensions

Ophthalmic solutions or suspensions are sterile, isotonic, aqueous, or oily preparations in which a drug is either dissolved or suspended in a solvent and administered in the form of eye drops or intraocular injections. When these formulations are administered topically, a substantial proportion of the drug is cleared off from the eye by tear fluid and the remaining drug (<5%) is bioavailable at anterior eye tissues [42]. Consequently, less than 0.02% of the drug will reach the anterior chamber and less than 0.0001% will reach the posterior chamber (vitreous body) [42]. When these formulations are injected intraocularly, a substantial proportion of the drug is bioavailable initially at the site of injection. However, due to the presence of dynamic ocular barriers (aqueous humor, choroidal, and lymphatic circulation), the bioavailable drug gets readily cleared from the eye and so demanded frequent drug administrations. A frequent drug administration may result in poor patient compliance. Furthermore, conventional formulations cannot control spatial distribution and temporal release of drug in order to elicit its desirable pharmacological effect.

5.1.2 Ophthalmic ointments

Ointments are semisolid dosage forms consisting of a base in which a drug is either dissolved or dispersed. There are four types of bases that are commonly used for preparation of ointments. These include oleaginous bases, absorption bases, emulsion bases and water soluble/water miscible bases. Upon topical application, the ointment melts at body temperature and as a consequence the drug is absorbed through ocular layers. The advantage of ointment systems over solution/suspension systems in topical drug applications is that ointments can resist clearance by tear fluid and thus they can offer a higher bioavailability at anterior eye tissues. However, ointments composed of oleaginous bases can transiently cause blurring of vision and discomfort to the patient. The disadvantages associated with such ointments can be overcome by using water soluble or water miscible bases that can form hydrogel-based delivery systems. Hydrogels due to their transparency and viscous nature are widely used for ocular drug delivery applications therefore have been discussed in advanced DDS.

5.2 Advanced nanoformulated ODD Systems

Nanomedicine has revolutionized the field of ocular drug delivery by improving the bioavailability of therapeutic molecules at ocular tissues. Ophthalmic drug delivery represents the lion's share in the ocular involvement of nanotechnology. Nanoparticles (NPs) one of the important carrier systems used in nanomedicine are colloidal drug delivery systems (colloidal DDS) with a high surface area to volume ratio (figure 3). Such colloidal systems (especially those with desired surface characteristics) get internalized by cells through different endocytosis pathways [44]. This, in turn, enables penetration of the entrapped therapeutic agents across ocular tissues. Furthermore, nanoparticles can provide sustained drug release and increased residence time of entrapped cargo molecules at ocular tissues which can help to reduce the frequency of drug administration. This has accelerated the exploration of nanoparticulate systems for sustained drug delivery applications in the treatment of ocular diseases through intravenous, periocular, subconjunctival, suprachoroidal or intravitreal injections and topical route of administration.

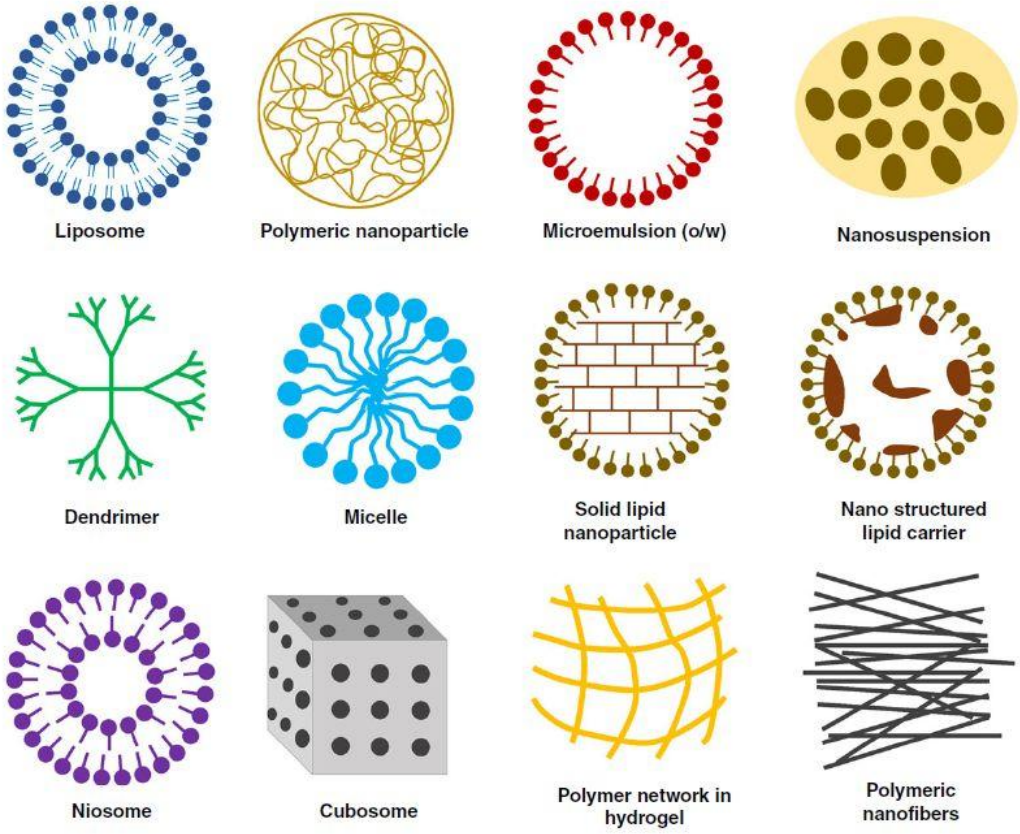

Figure 3: Schematic representation of various colloidal drug delivery systems (the relative dimensions of schematically represented colloidal carriers may differ from each other)

Subsequently will be mentioned the specific nanoparticles categories and some of their most representative applications as ocular drug delivery systems as they have been collected from international literature.

5.2.1 Lipidic Nanocarriers

Lipidic nanocarriers are considered the most beneficial and promising technological platforms for encapsulating bioactive molecules. Advantageous properties are the biocompatibility and biodegradability due to their lipidic nature and among others, exhibit high ability to entrap bioactive molecules with different solubilities. For that reason they have emerged as interesting nanosystem for delivering bioactive molecules to the target tissues. Their ability to improve the active and passive targeting mechanisms is of importance for exploring new disease targets.

According to their chemical structure lipid nanocarriers are categorized to the following categories:

a. Liposomes
b. Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC)
c. Nanoemulsions

5.2.1.1 Liposomes

Liposomes were firstly described by Alec Bangham in 1965 [45]. For decades, liposomes are one of the most promising bioactive molecules for drug delivery. Liposomes are basically artificial vesicles or colloidal particles, which are made up of natural and non-toxic cholesterol and phospholipids as the main ingredients for the formation vesicular bilayers of lipid, or lipid-drug complex, or a sheet-drug complex.

Most important parameters [46] studied in liposomal manufacturing are:

a. Liposome lipid composition. The predominant lipids are phospholipids and cholesterol.
b. Temperature, pressure, ionic strength, and presence of ions or macromolecules, e.g., proteins, enzymes, and bioactive molecules in the specific water environment.
c. Lipid membrane permeability, elasticity, width and shape, and its ability to interact with other cell membranes.

d. Lipid concentration in the final dispersion system.

According to size and number of bilayers the various liposomal types can be classified as following: multilamellar vesicles (MLVs), large unilamellar vesicles (LUVs), small unilamellar vesicles (SUVs) and multivesicular vesicles (MVVs).

The liposomes are very promising as DDS due to their size and amphiphilic properties. They can encapsulate hydrophilic drugs inside the aqueous core as well as hydrophobic drugs inside the lipid bilayers and also shows a very good biocompatibility with the ocular tissues. The liposomes that are approved for use in humans contain neutrally charged phosphatidylcholine (PC) with fatty acyl chain of variable degree of saturation and at different lengths. The properties shown by liposomes vary significantly with changes in the composition of lipid, its surface charge, the method that is used for its preparation and its size. For modulating the rigidity of the membrane, and also for reducing the instability caused due to serum-protein binding with the liposomal membrane, cholesterol is included in the formulation most of the time. The composition of the head group of the lipid and pH determines the surface charge of the liposomes, whether positive, negative, or neutral. It has been seen that the liposomes that bear a positive charge on their surface are favorably captured by the negatively charged corneal tissues in comparison with the negatively charged liposomes and the neutral ones. Furthermore, the density of these charges and their nature influences the characteristics of the formulation, such as its stability, kinetics, distribution in the body, and also determines how the target cells will interact with the liposomes. [47, 48]

On liposomal surface, small molecules or macromolecules can be connected; these include antibodies that change the physicochemical properties of their surface and also act as ligand moieties for specific cellular targets. This process is very important and specifies nanosystem functionality and its physical stability on time of production and over its use.

Liposomes have the ability of transferring bioactive molecules inside the cell through the following mechanisms:

a. Following administration, the liposomes on coming into contact with the cell may release its content onto the cell surface, which then enters the cytoplasm of the cell.

b. Liposomes that are loaded with drug may also get adsorbed onto the surface of the cell either specifically or non-specifically and release its content, after being destabilized by certain components of the cell membrane, and then enter the cell by the process of micropinocytosis.
c. Liposomes may also fuse with the membrane of the cell and deliver the therapeutic agent into the cytoplasm.
d. The drug-loaded liposomes may also get endocytosed either directly or indirectly, thereby being delivered into the lysosome by the endosome, and releasing the drug into the cytoplasm, following provocation of destabilization of the endosome by the liposome.
e. The liposomes also have the capability to undergo an exchange of its lipids with the lipids of the cellular membrane via the transfer-protein-mediated exchange [49].

Liposomal DDS have been widely explored for ophthalmic drug delivery applications to improve ocular bioavailability of administered drugs. Several research studies have also demonstrated that liposomal ocular delivery is effective for both anterior and posterior segments [50]. In the following paragraphs will be referred to existing commercial formulations, as well as on a few novel DDS that are under preclinical and clinical investigation.

Anterior segment ocular diseases

Ocular Surface Disease (OSD) or Dry Eye Disease (DED)

There are several liposomal products on the market for DED treatment, such as **Tears again®** and **Lacrisek®**. These liposomal formulations are composed of phospholipid PC that provides lubrication, prevents tear film evaporation and attenuates dry eye symptoms. Liposomal formulations have been demonstrated to provide improved efficacy compared with traditional eyedrops. Lacrisek® (the liposome formulation of vitamin A palmitate and vitamin E) and nonliposomal Artelac Rebalance® (the PEG and hyaluronic acid based aqueous formulation of vitamin B12) eyedrops were administrated to 15 dry eye patients separately. Both formulations improved patient blinking frequency and corneal exposure, and the effects from the liposomal formulation Lacrisek® appeared to be quicker, stronger and more stable over time than those of the nonliposomal Artelac

Rebalance® formulation. In particular, the liposomal eyedrops Lacrisek® provided progressive and sustained increase of the interblink interval up to 60 min after administration; while, Artelac Rebalance® showed protective effects only by 10 min. In comparison to the nonliposomal conventional eyedrops, liposomal eyedrops can better mimic the composition of tear film lipid layers and provide sustained integration with the lipid layers of the tear film and, thus, better efficacy. The liposomal formulations can also decrease tear film osmolarity and improve tear film stability [51]. Besides the liposomal eyedrops, liposomal sprays have also been developed, and have been shown to be more convenient for patients wearing contact lenses [52]. **Optrex™ ActiMist™** is a liposomal spray of vitamin A and E for DED treatment, which provides instant relief and longer effects for up to 4 h. However, none of these liposomal formulations was used to treat the disease itself but to provide relief of the discomfort caused by dry eye. The therapeutic role of the vitamins in all these liposomal formulations was not fully justified. The liposomes without vitamins could also generate a certain degree of relief in symptoms through the lipid materials. Therefore, the placebo liposomes without vitamins (or other actives) should be used as a control. The exact benefits and advantages from liposomes as topical eyedrops are still not fully established.

Ren *et al.* [53] investigated **azithromycin** liposomes for the treatment of dry eye disease. In vivo pharmacodynamic studies in rats showed a reduction in the symptoms of dry eye disease, and the azithromycin liposomal treatment had higher safety and efficacy as compared with hyaluronic acid sodium eye drops.

Corneal infections

A positively charged liposomal formulation for topical administration of **acyclovir** (ACV) was investigated by Chetoni *et al.* [54] in comparison with a commercial ACV ointment, by determining the pharmacokinetic profile of the drug in the aqueous humor of rabbits after topical administration. The ointment was tested at two different strengths: undiluted (3.0%) and diluted to the same ACV concentration as the liposomal vehicle (0.12%). A liquid formulation containing ACV plus “empty” liposomes and an isotonic aqueous ACV solution were also tested. The ACV liposomal dispersion (LIPO-ACV) produced a significantly higher drug concentration profile in the aqueous with respect the three reference formulations containing the same ACV concentration, and showed a 90-

minute plateau. Also the potential of liposomes, for carrying **acyclovir** (ACV) to eye was studied by Law *et al.* [55]

In another study, Taha *et al.* [56] formulated several liposomal formulations containing **ciprofloxacin** (CPX). The effect of formulation factors such as type of phospholipid, cholesterol content, incorporation of positively charging inducing agents and ultrasonication on the properties of the liposomal vesicles was studied. Bioavailability of selected liposomal formulations in rabbit eye aqueous humor has been investigated and compared with that of commercially available CPX eye drops (Ciproxin®). The investigated formulations showed more than three folds of improvement in CPX ocular bioavailability compared with the commercial product.

Topical **voriconazole** (VOR) liposomes were developed by De Sá *et al.* [57] for fungal keratitis treatment. Several types of liposomes were tested. Overall results suggest VOR can be effectively incorporated in liposomes for potential topical treatment of fungal keratitis.

Pre & Post – operative situations, inflamations

Li *et al.* [58] formulated a **diclofenac sodium**-loaded liposomal formulation with low molecular weight chitosan (CH) coating. Results demonstrated a slight increase in the particle size with prolonged release of drug. Stability test results were acceptable. CH-coated liposome showed an enhanced retention and penetration through the cornea. No cases of ocular irritancy or toxicity were observed.

Singh et al. [59] formulated liposomes containing **triamcinolone acetonide** (TA). They observed a twofold enhancement in drug concentrations in the cornea as well as in the aqueous humor, in rabbits, in comparison to the suspension formulation. The liposomes maintained the higher drug concentration in the aqueous humor for 5 h.

Sun *et al.* [60] entrapped short-chain-conjugated **ceramide** and C6-ceramide in liposomes and applied to the treatment of corneal inflammation in mice. Ceramides are known for their role as an antiproliferative and proapoptotic agents in sphingolipid metabolism. The C6-ceramide liposomal formulation demonstrated significant efficacy in corneal inflammation reduction in a murine model.

In another study Zhan *et al.* [61] observed that the presence of corneal surface binding ligand, succinyl-concanavalin A, on liposomal surfaces substantially enhanced the

duration of action of liposome entrapped **anesthetic drugs** (**tetrodotoxin** and **dexmedetomidine**) compared to unmodified liposomes.

The aforementioned studies demonstrated the ability of liposomal formulations and efficiency of surface modification in improving ocular retention, bioavailability and sustained release of entrapped therapeutic molecules. The bioavailability of liposomal drug carriers can be further improved using a gel-based hybrid system as a dispersion medium for the liposomes.

Glaucoma

Efficacy of such a **hybrid system** for ophthalmic drug delivery applications was described by Yu *et al.* [62] In this study, a hybrid formulation consisting of the PC-based liposomal system was incorporated into deacetylated gellan gum-based gel formulation for the delivery of **timolol maleate** (TM). Ex vivo permeation studies in isolated rabbit cornea showed a 1.93-fold increase in apparent permeability coefficient for the liposomal system. Furthermore, the hybrid system increased the retention time of liposomes on the corneal surface up to 10 min and showed a therapeutic effect in rabbits for a longer duration (double the time of solution-based formulations) along with better efficacy. Also Hathout *et al.* [63] showed that TM gelatinized liposome treatment resulted in lowering the IOP when evaluated *in vivo* on the eyes of glaucomatous rabbits. These studies demonstrate the efficacy of liposome-based hybrid DDS for the effective treatment of anterior eye diseases.

A study reported by Arroyo *et al.* [64] showed that topical ophthalmic administration of a 10-fold lower dose of conventional nanoliposomes elicited equivalent therapeutic effects (lowering IOP) as that commercially available eye drop formulations which demonstrates the ability of liposomal formulations to improve ocular bioavailability. The purpose of this study was to compare the *in vivo* efficacy of several **TM**-loaded liposomal formulations with current TM antiglaucoma treatment (aqueous 0.5% w/v eye drops). In addition to the IOP lowering the effective time was significantly longer and the formulations showed no irritant effects after instillation on the ocular surface. In addition to the delivery system per se, the surface properties and presence of ligands on the liposomal surface also play an important role in cellular interaction and subsequent

internalization thus influencing ocular residence time and bioavailability of the administered medicaments.

In a recent study Tan *et al.* [65] demonstrated the comparative efficacy of an unmodified liposomal system and a chitosan (CH) surface-modified liposomal system with respect to solution-based systems for the delivery of **TM** to anterior eye tissues. *In vivo*, pharmacokinetic studies demonstrated a higher bioavailability of CH-coated liposomes (3.9-fold) followed by unmodified liposomes (1.71-fold) when compared to drug solution. Furthermore, the liposomal systems showed sustained drug release in ocular tissues which led to an effective reduction of IOP in the treatment of glaucoma.

Quancheng *et al.* [66] developed a tocopheryl polyethylene glycol succinate (TPGS) modified liposome ocular DDS for **brinzolamide** (BZ) for the treatment of glaucoma. White New Zealand rabbits treated with BZ-liposomes maintained an effective reduction in IOP after drop instillation. Such results indicate a high potential for clinical translation for liposomal drug delivery of hydrophilic agents for the treatment of glaucoma.

Latanoprost, a lipophilic prostaglandin analogue, is a potent IOP lowering agent currently marketed as Xalatan® (Pfizer) for the treatment of glaucoma and ocular hypertension. In Xalatan®, the active ingredient, latanoprost 0.005%, is solubilized in water by 0.02% of benzalkonium chloride (BAK). Despite being the leading antiglaucoma medication, there are two drawbacks of Xalatan® that may have impacted its huge commercial success: a) the formulation was not stable at room temperature necessitating storage at 5◦C and b) BAK in the formulation as a preservative and solubilizing agent causes ocular surface toxicity which probably resulted in decreased compliance. As the patent protecting this molecule has been expired since 2011, there is an opportunity to improve upon the disadvantages of Xalatan®.

Subconjunctival injection of **latanoprost**-loaded liposomes demonstrated sustained IOP reduction in rabbits and non-human primates (NHPs) for up to 4 months [67]. Latanoprost was encapsulated into egg PC-liposomes with sizes of 100 nm and a drug:lipid ratio of 18.1%. The final drug loading was 1.18 mg/ml, which is 20-fold higher than the commercially available latanoprost eyedrops (50 µg/ml in Xalatan®) that need daily administration. The liposomal latanoprost formulation was reported to be stable for at least 6 months at 4℃ and at least 1 month below 25℃ without significant change in particle size. Latanoprost-loaded egg PC-liposomes demonstrated sustained release of

60% of drugs within 2 weeks and 100% of drugs within 60 days *in vitro*. A single SCT injection of egg PC liposomes when applied to NHPs provided sustained efficacy of lowering IOP for 120 days, much longer than the in vitro drug release, which was 60 days for complete release. The average IOP reduction of latanoprost liposomes (14.99 mmHg) was comparable to the daily administration of Xalatan® eyedrops (15.03 mmHg). The second SCT injection of liposomal latanoprost formulations in NHPs conducted at day 120 resulted in sustained IOP reduction in the following 180 days. It was explained that the longer IOP reduction in NHPs was caused by the interaction between latanoprost and the pigment melanin, which is not distributed in the eyes of New Zealand White rabbits. Because melanin is an important factor for ocular drug delivery, Dutch belted rabbits with pigmented eyes should be considered at the ocular pharmacokinetics and efficacy studies. Latanoprost is a hydrophobic drug, which demonstrated very low water solubility of 40 µg/ml in saline, and it would be very interesting to find out how the hydrophobic latanoprost was effectively transported into the eye. A pharmacokinetics study could be added to detect the drug levels in the anterior chamber and the total dose remaining in the conjunctiva injection site with time, which would greatly support the long-term efficacy at reducing IOP following one single SCT injection of the liposomal latanoprost formulation. Currently, the latanoprost-loaded liposomal formulation has finished its Phase II clinical trials (NCT02466399), demonstrating clinically significant IOP reduction in glaucoma patients 6 months after one single SCT injection [67].

Posterior segment ocular diseases

Topically administered liposomal carriers have also been shown to improve bioavailability in posterior eye segment tissues. The ability of liposomal carriers in offering a better therapeutic efficacy in retinal damage was demonstrated by Shimazaki *et al.* [68]. In this study, PC-based liposomal carriers when topically administered in mice, delivered **edavarone** (antioxidant) to retinal tissue in higher quantities when compared to edavarone solution. Hence, a higher therapeutic efficacy was observed with liposome-based DDS for the treatment of retinal damage. In another study, Sasaki *et al.* [69] have shown that surface modification of negatively charged liposomes using a cationic polymer poly-L-lysine enhanced retinal bioavailability of topically administered liposomes compared to unmodified liposomes.

Choroidal Neo – Vascularization (CNV)

Photodynamic therapy (PTT) is the only systemic therapy approved for wet AMD in which a photosensitizer is administered intravenously and after certain time, targeted site is illuminated with specific laser. It activates the photosensitizer liberating highly reactive short-lived singlet oxygen and other oxygen reactive radicals which cause death of local blood vessels. A liposome based formulation **verteporfin** (marketed as Visudyne® since 2002, Novartis AG) was a great achievement in ocular therapeutics but it required frequent visits of patients to the clinic and ample cautions to adopt, like patient should be in dark after treatment for some time to avoid side effects. It offered symptomatic relief as it was not targeting root of the diseases. After some time, patient may again develop NV. Frequent treatment with this may affect the quality of life. Like verteporfin, a new photosensitizer, **rostaporfin (Photrex®)** is under clinical trial for photodynamic therapy of macular degeneration [70].

Davis *et al.* [71] developed annexin A5 associated phospholipid vesicles. It has been suggested that the anionic phospholipid binding protein annexin A5, may have a role in drug delivery. In their study was demonstrated, using a combination of *in vitro* and *in vivo* assays, that the presence of annexin A5 can significantly enhance uptake and transcytosis of liposomal drug carrier systems across corneal epithelial barriers. This system is employed to deliver physiologically significant concentrations of **bevacizumab (Avastin®)** to the posterior segment of the rat eye (127 ng/g) and rabbit retina (18 ng/g) after topical application. Their observations provide evidence to suggest annexin A5 mediated endocytosis can enhance the delivery of associated lipidic drug delivery vehicles across biological barriers, which may have therapeutic implications.

In a study conducted by Zhang *et al.* [72], intravitreal delivery of anti-inflammatory antibody **infliximab (Remicade®)** using liposomal carriers showed long-lasting retention in retinal tissues, approximately three times higher, compared to infliximab solution. The authors also demonstrated that liposomes have a great affinity for retina and as a result efectively reduced inflammatory complications of autoimmune uveoretinitis in rats.

5.2.1.2 Solid Lipid Nanoparticles (SLNs) & Nanostructured Lipid Carriers (NLCs)

Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) are lipid-based colloidal systems which are formulated by emulsifying a mixture of molten lipid, drug and surfactant in aqueous media followed by cooling.

SLN have been widely used for drug delivery via oral, topical, ophthalmic, parenteral and other routes [73, 74]. SLN suffer from some disadvantages such as burst release with hydrophilic drugs and low drug loading owing to the solid crystalline state of the NPs. These shortcomings led to the development of second-generation lipid NPs, the NLC. NLC differ from SLN in that liquid lipid is mixed with solid lipid which prevents crystallization of lipids (Figure 4). This incorporation leads to the formation of one of the following: imperfect NLC, multiple types NLC or structureless NLC. The type of NLC formed depends on the concentration of liquid lipid utilized and method of preparation employed. Inclusion of the liquid lipid in the NLCs enhances drug solubility, thereby increasing drug loading significantly, and reduces the crystallinity of the solid lipid, consequently reducing the problem of drug expulsion. Recently, a few studies have demonstrated the ability of the SLN to permeate across the cornea into the aqueous humor.

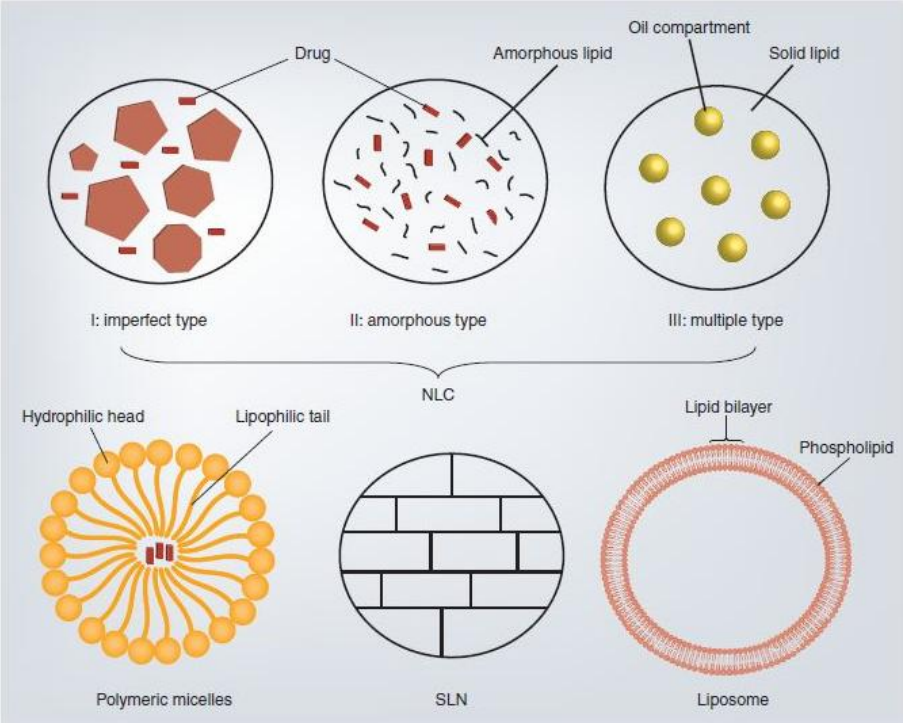

Figure 4: Illustration highlighting difference between polymeric micelles, liposome, solid lipid nanoparticle and nanostructured lipid carriers (NLC: Nanostructured lipid carriers; SLN: Solid lipid nanoparticle).

Anterior segment ocular diseases

Kumar *et al.* [75] formulated **VOR**-loaded SLN and studied ocular permeation of VOR, *ex vivo* (excised rabbit corneas) and *in vivo* in rabbits. The particle size of the formulation ranged from 234 to 343 nm. The area under the curve (AUC) for concentration in aqueous humor over the 12-h time period, was approximately twofold greater for SLN in comparison to drug suspension.

Similarly, in another study, Kalam et al. [76] showed that permeation of **gatifloxacin** was increased using SLN formulation, in vivo in rabbits. The gatifloxacin area under the aqueous humor concentration-time curve was approximately threefold higher in comparison to Gate® commercial eye drops. The authors ascribed this to the changes in the elimination from the aqueous humor (~half) and a significant increase in the Cmax.

In another study by Cavalli et al., **tobramycin** salt–ion pair complex-loaded SLNs were compared with solution formulations. Aqueous humor pharmacokinetic parameters were significantly better for the SLNs in comparison to tobramycin solution, in vivo in rabbits [77]. Tobramycin AUC was found to be significantly higher (~four-times) and Cmax was found to be about twofold higher for the SLNs in comparison to the solution. From the precorneal retention study, using a fluorescent probe tagged to the SLN, the residence time in the lower fornix of the eye (cul-de-sac) for SLN (90 min) was higher in comparison to solution (30 min).

Similarly, **baicalin, methazolamide** and **ibuprofen**-loaded SLNs showed significantly better transcorneal permeation and/or pharmacokinetic properties (AUC, Cmax) in comparison to the conventional dosage forms (suspensions, solutions) which were used as comparators [78].

In a recent study by Balguri *et al.* [74] demonstrated the advantages of SLNs and NLCs in terms of drug loading and ocular penetration. **Indomethacin** was used as the therapeutic candidate whose ocular distribution was evaluated following topical application. The studies revealed that the NLC formulations were capable of higher drug loading and achieved significantly higher concentrations in all the ocular tissues tested. The data also demonstrated that surface modification of the NLCs with PEG improved ocular penetration.

Other studies have also demonstrated that surface modification of the NLCs can improve in vivo biodistribution of the drugs. Shen *et al.* [79] formulated thiolated

nanostructured lipid carriers (Cys-NLCs) for ocular delivery of **cyclosporine A** (CsA). CsA is a very lipophilic immunosuppressant drug (calcineurin inhibitor) and is widely used by ophthalmologists due to its recognized therapeutic potential for the treatment of ocular diseases (dry eye, allergy, and inflammation) [80]. They compared Cys-NLCs, non-thiolated NLCs and oily solution of CsA in the pharmacokinetic study, by analyzing their tear fluid, cornea, iris ciliary and aqueous humor at various time points, in vivo in rabbits and thy found that Cys-NLCs demonstrated superior pharmacokinetic behavior in comparison to all other formulations. Also in a study conducted by Sandri *et al.* [81], surface modification of CsA-loaded SLN using chitosan improved cellular uptake and permeation of CsA across cornea.

The ocular bioavailability of SLN can also be improved using a gel-based system as a medium for SLN administration. Such **hybrid systems** are able to further reduce precorneal clearance of administered SLN and as a result, offer higher bioavailability and improved penetration in anterior tissues of the eye [82]. Development of lipid nanocarriers having optimal viscosity as well as mucoadhesion may together offer improved ocular bioavailability. The efficacy of such DDS was demonstrated by [83]. In this study, the corneal bioavailability of a fluorescent molecule, **coumarin-6** was enhanced upon topical administration using CH-N-acetylcysteine surface-modified nanostructured lipid carriers (CH-NAC-NLC) when compared to pristine NLC and CH-coated NLC. The designed CH-NAC-NLC offered dual advantage, that is, enhanced viscosity and enhanced mucoadhesion (due to the formation of ionic and disulfide bridges with mucus). This resulted in prolonged retention on the ocular surface that enabled transcorneal penetration of the nanocarrier when studied in rabbit eyes [74]. Based on the aforementioned studies, it is evident that SLN/NLC are a good choice for topical [74, 84] and intraocular drug delivery applications [85].

Posterior segment ocular diseases

Araujo *et al.* [86] formulated NLC for delivering **TA** to the posterior ocular segment. Their results demonstrated a particle size of 173.30 ± 0.32 nm, with a good encapsulation. Furthermore, *in vivo* studies in mice demonstrated that the system could effectively deliver lipophilic drugs to the posterior ocular segment. The formulations were also found to have acceptable stability.

5.2.1.3 Nanoemulsions

Nanoemulsions are clear, isotropic, thermodynamically stable colloidal DDS consisting of droplets (5–200 nm in size) dispersed in a medium. Nanoemulsions are formulated as w/o (water in oil) or o/w (oil in water) forms using water, oil, surfactant and cosurfactant. For ophthalmic drug delivery applications, o/w nanoemulsions are commonly used compared to w/o nanoemulsions because o/w nanooemulsions can readily mix with aqueous fluids of the eye and hence can improve bioavailability unlike w/o nanooemulsions. Hence, o/w-based nanoemulsions are widely used for the delivery of hydrophobic drugs across ocular tissues. Due to the small size and large surface area of the dispersed droplets, nanooemulsions have been demonstrated to exhibit a better interaction with ocular tissues and as a consequence improve permeation, retention, and bioavailability of topically administered medicaments.

An interesting approach of the above seems to be the Novasorb® technology, originated from work of Benita *et al.* [87] and developed by the Novagali Pharma SA. The Novasorb® technology platform is based on the cationic nanoemulsion approach. The overall Novasorb strategy exploits the fact that the corneal and conjunctival cells and the mucus layer of glycosyl amino glycans lining the ocular surface are negatively charged at a physiological pH [17]. When applying a positively charged formulation to the eye it is likely that an electrostatic attraction will occur prolonging the residence time of the formulation on the ocular surface (Figure 5). In addition, the nanosize of the oil droplets creates a huge contact surface with the ocular surface cells enabling enhanced absorption. The potential of cationic emulsions for ophthalmic drug delivery was rapidly seen to offer advantages over the existing topical drug delivery vehicles [88].

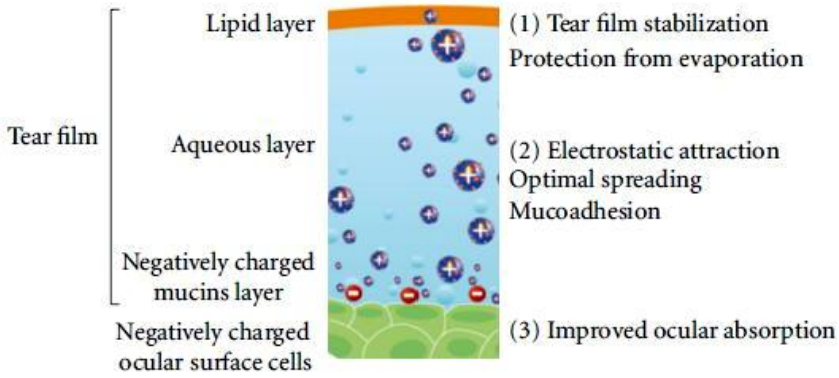

Figure 5: Cationic nanoemulsion interacting with negatively charged corneal cells. The effects of the cationic emulsion are (1) to bring lipids to stabilize the tear film, (2) to interact electrostatically with mucins, and (3) to improve ocular absorption [89].

The Novasorb® technology platform was ultimately designed to be loaded with active molecules. Even so, the cationic emulsion itself [89] with no active ingredient possesses beneficial properties. Its composition comprising oil, water, surfactants, and glycerol reduces evaporation of tears from the ocular surface while lubricating and moisturizing the eye. Altogether the components confer a protective effect by augmenting each layer of the tear film. Based on the inherent properties of theNovasorb® technology, restoring the deficient layers of the natural tear film, **Cationorm®**, a preservative-free cationic emulsion containing no active ingredient, has been commercialized globally for the relief of dry eye symptoms.

Nearly 40% of new chemical entities have a low aqueous solubility, therefore potential candidates to be loaded into Novasorb®. Novagali Pharma incorporated about 40 lipophilic active ingredients of various therapeutic classes (NSAID, SAID, antibiotics, antifungals, etc.) proving the versatility of this emulsion. Herein only the most advanced products will be mentioned. **CsA** was considered an excellent initial candidate to evaluate the potential of the Novasorb® cationic emulsion to improve the efficacy of an established drug. Therefore, the primary challenge in the development of a cationic emulsion containing CsA was to design the optimal formulation [80] for topical delivery. Today, Novagali Pharma has developed two products, with good results, based on the Novasorb technology loaded with CsA: Cyclokat® for the treatment of dry eye and Vekacia® for the treatment of vernal keratoconjunctivitis.

Given the disadvantages of Xalatan® (commercial Latanoprost 0,005%), as mentioned before, Novagali launched the development of Catioprost®, a preservative-free cationic emulsion loaded with latanoprost for the treatment of elevated IOP while protecting and improving the ocular surface.

In a study, Kalam *et al.* [89] demonstrated that topically administered nanoemulsion-based delivery system improved ocular permeation and retention of **gatifloxacin**, and as a consequence, improved the bioavailability by up to twofold in aqueous humor of rabbit eyes when compared to solution-based systems. Furthermore, the surface modification of microemulsion-based delivery systems has also been explored to improve the bioavailability of topically administered medicaments at anterior eye tissues.

In a study conducted by Kesavan *et al.* [91] an enhanced efficacy of topically administered CH-coated cationic nanoemulsions was observed for the treatment of

endotoxin induced uveitis in a rabbit model. The results demonstrated that the **dexamethasone** (DEX)-loaded nanoemulsion showed mucoadhesive characteristics and prolonged the release of DEX for the effective treatment of uveitis when compared to a commercially available drug solution.

Furthermore, the bioavailability of nanoemulsion-based DDS can also be improved using a gel-based system as a medium for topical ophthalmic delivery of nanoemulsion. Efficacy of such hybrid system for ophthalmic drug delivery applications was described by Gan L *et al.* [92]. In this study, a hybrid system consisting of a nanoemulsion dispersed in a gel system was explored for the delivery of **CsA**. The resultant system provided sustained CsA release and improved bioavailability of CsA (threefold compared to CsA emulsion) and hence presented therapeutic levels of CsA at corneal tissues even after 32 hr of administration. The results showed the ability of the hybrid formulation to offer better therapeutic effect with a reduced frequency of administrations. Other approaches that have been explored to enhance the ocular bioavailability of nanoemulsions include use of **permeation enhancers** that has been shown to enhance diffusion of the emulsified therapeutic molecules across ocular barriers [93]. Taken together, nanoemulsion-based delivery systems have great potential for use in ocular drug delivery applications.

Restasis® is a preservative-free 0.05% oil-in-water commercial emulsion of CsA and was the first CsA ophthalmic product approved by the FDA for DED treatment in 2002 [94]. In Restasis®, 0.5 mg/ml CsA was dissolved in castor oil with Polysorbate 80 as the emulsifying agent. The preservative-free emulsions (particle size 100–200 nm) overcame the toxicity shown in earlier preservative-contained oil-based formulations; however, Restasis® is still associated with side effects including ocular irritation, instillation pain and epiphora [95].

5.2.2 Polymers

Polymers are substances of high molecular weight, consisted of repeated units called monomers that are connected onto a long chain. Polymer molecules can be linear or branched, while the linear or branched chains can be linked with covalent bonds. Polymers that consisted of the same monomers are called homopolymers. Polymers that consisted of more than one type of monomers are called copolymers. [46]

Respectively to their structure polymeric ODD systems are categorized as following:

a. Polymeric nanoparticles (NPs)
b. Polymeric mycelles
c. Hydrogels / nanogels
d. Dendrimers
e. Polymeric nanofibers

5.2.2.1 Polymeric nanoparticles

Polymeric nanoparticles (PNPs) are colloidal NPs (10–1000 nm) where the drug is either uniformly distributed throughout the particle matrix (nanospheres) or encapsulated inside a polymer shell (nanocapsules). Polymeric NPs can be used to entrap either or both hydrophilic and hydrophobic drug molecules [96, 97]. The size of drug-loaded NPs can range from 50 to 500 nm to effectively overcome ocular barriers and to deliver the drug to the ocular tissue either by passive or active transport. A solution of NPs can be deposited in the cul-de-sac (lower fornix of conjunctiva) to attain sustained drug delivery over a prolonged period of time.

The composition and surface properties of polymers play an important role in governing drug release kinetics and particle–tissue interaction [39]. The surface charge of the NPs highly influences their efficient ocular absorption. The cornea and the conjunctival tissues have a negatively charged surface. It is observed that cationic NPs have a higher retention time on ocular surfaces as compared with anionic NPs. This can enhance the drug permeation into the ocular surfaces [98]. Colloidal NPs can also increase the solubility of highly hydrophobic drugs and increase the trans-corneal permeability of such agents. Various biodegradable and nonbiodegradable NPs for treating anterior and posterior segment ocular disorders have been developed. The commonly used polymers for ocular applications NPs are poly(lactic-*co*-glycolic acid) (PLGA), Polyethylene glycol (PEG), Polycaprolactone (PCL), polylactic acid (PLA), chitosan (CH), albumin, Eudragit® and gelation. Among these polymers, PLGA has been extensively studied for ocular drug delivery as it is approved by the Food and Drug Administration (FDA) due to its biodegradable, biocompatible and nonantigenic nature. PLGA undergoes hydrolysis in the body to produce the original monomers: lactic acid and glycolic acid. These two monomers under normal physiological conditions are by-products of various metabolic pathways in the body

Anterior segment ocular diseases

Corneal and ocular surface diseases

Gonzalez-Pizarro *et al.* [99] studied the development and optimization of **fluorometholone**-loaded PLGA NPs for the treatment of inflammatory conditions of the eye. The optimized formulation had significantly greater anti-inflammatory effects than the commercial formulation. In addition, NPs increased drug penetration toward the vitreous. Polymeric NPs of fluorometholone could provide a suitable alternative for the treatment of inflammatory disorders of the anterior and posterior segments of the eye against of conventional topical formulations.

Losa *et al.* [100] studied the enhancement of the corneal permeation of a hydrophilic drug (**amikacin sulfate**) entrapped in polycyanoacrylate NPs. They studied three NPs formulations varying in the type of the stabilizing. Amikacin concentration achieved in aqueous humor and cornea for the NPs with dextran was significantly higher in comparison to NPs with other stabilizing agents and control amikacin sulfate solution, in vivo in rabbits.

Among others approaches that have been explored for enhancing bioavailability of topically administered drugs is use of **mucus penetrating particles** (MMP). MMP are NPs with sizes smaller than the mucus meshwork and dense muco-inert coatings to avoid adhesive interactions with mucins of tear film, thus can rapidly penetrate the mucus network to reach the epithelium and avoid rapid tear clearance. MPP could potentially provide improved drug retention and distribution at the ocular surface [101]. The most commonly used molecule for NP fabrication/surface modification is the PEG. Dense surface coatings of PEG on NPs beyond a certain density threshold are crucial for MPP to achieve rapid penetration through mucus [102]. As the PEG density increased, the amount of bound mucin per nm^2 on the NP surface was decreased and eventually reached a plateau value when the PEG density was ~10 PEG chains per 100 nm^2. Therefore MPP eyedrops reduce particle adherence to mucins and lower particle elimination through tear clearance, thus improving drug ocular pharmacokinetics (Figure 6).

INVELTYS™ is a new MMP-formulation of **loteprednol etabonate** (LE) that was approved by the FDA in August 2018. With MPP, INVELTYS™ (MPP-LE 1% suspension) promotes LE ocular bioavailability and provides a twice-daily LE for the treatment of

inflammation and pain following ocular surgery, compared with other steroids only approved for 4-times-daily dosing [103]. MPP are expected to greatly improve patient compliance and convenience with less frequent dosing.

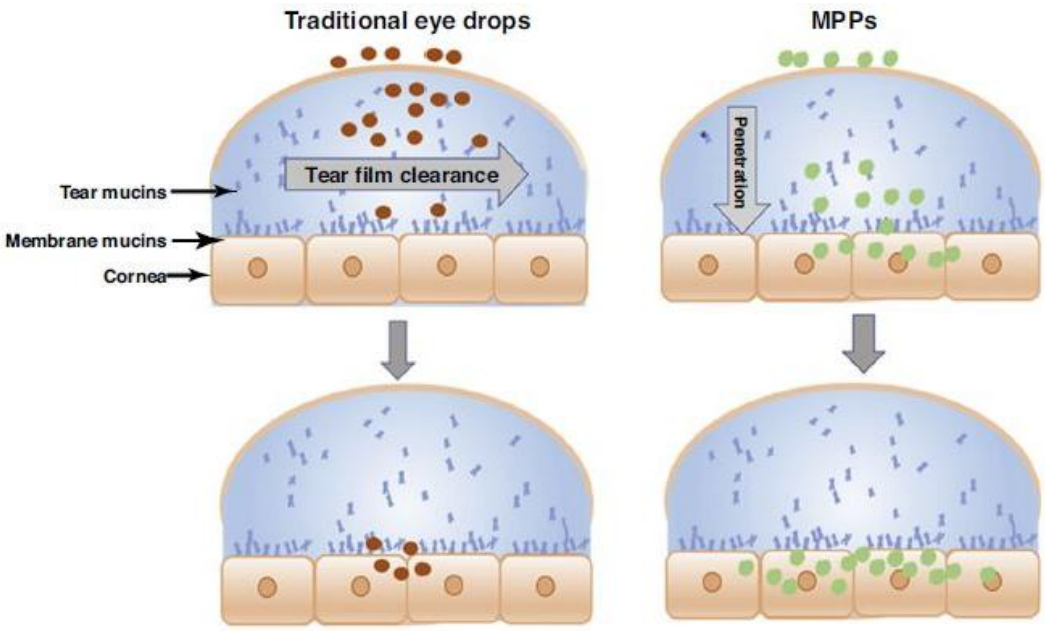

Figure 6: Traditional suspension eyedrops adhere to the mucins and are rapidly cleared from the tear film. MPP move through tear mucins to the epitheliumtethered mucins, allowing particle penetration to corneal epithelium *(Meng T et al., Drug Discovery Today, 2019; 24: 8).*

However, even with MPP technology, the retention time of nanomedicine following topical instillation is short and repeated dosing is still needed. Periocular injection (e.g., SCT and sub-tenon) of nanomedicine can achieve sustained drug delivery to the anterior segment, and even to the posterior segment of the eye [104]. It was thought that drugs administered by SCT injection and sub-tenon injection penetrate the eye mainly through transscleral diffusion, a process that is limited for drugs that are poorly water-soluble. However, the therapeutic effects of transscleral delivery of water-soluble drugs injected either SCT or sub-tenon are shortlived so frequent injections are required [105].

Pan *et al.* [106] developed a NP delivery system for **DEX** to treat corneal transplantation rejection using SCT injection without the use of frequent eyedrops. The clinical standard for preventing corneal graft rejection following surgery is to prescribe topical corticosteroid eyedrops for 4-6 times a day for at least 1 month. The requirement for frequent administration of corticosteroid eyedrops for extended periods of time is associated with poor patient compliance. Thus, there is a compelling need for a

formulation that provides sustained release of corticosteroids to the anterior segment of the eye. The water-soluble DEX was encapsulated into PLGA NPs. DEX-NPs provided delivery of DEX to the conjunctiva, aqueous humor and vitreous for >7 days, whereas DEX levels were nearly undetectable one day after soluble DEX solution injection. SCT injection of DEX-NPs also achieved high DEX levels in the vitreous, indicating that SCT injection can be used to treat posterior segment eye diseases. Observing the rat corneal allograft transplantation rejection model, when rats treated with weekly SCT injection of free DEX solution, saline or placebo NPs, their grafts were completely rejected in less than 4 weeks. By contrast, corneal grafts in rats treated with weekly SCT DEX-NPs (100 mg DEX) remained clear and healthy and maintained 100% survival throughout the 9-week study.

Treatment of corneal graft immunological rejection without the need for compliance with frequent eyedrop administration would be a great improvement in clinical management of corneal graft rejection. Achieving sustained, low but efficacious steroid levels in the eye could improve the treatment of various ocular disorders while removing the risk of noncompliance. Given the widespread application of corticosteroids, SCT DEX-NPs have the potential to treat many other ocular disorders, including corneal inflammation, corneal NV [107], noninfectious uveitis and post-surgical pain and inflammation. Weekly injection is still too often for clinical application, and long-lasting release of DEX up to 3–6 months can be potentially achieved through selecting slower degradable polymers such as more hydrophobic PLA polymers and higher molecular weight PLGA polymers. More standard pharmacokinetics and safety plus biocompatibility studies in larger animals, such as rabbits, need to be included as the next steps in development for DEX-NPs.

Glaucoma

Musumeci *et al.* [108] investigated PLGA and PLGA-PEG NPs (100-400 nm) for sustained release of **melatonin**. Melatonin-loaded PLGA-PEG NPs demonstrated extended precorneal residence of melatonin as evident from the lowering of the intraocular pressure in rabbit eye for up to 8 h, with a maximum IOP reduction of 5 mmHg. Aqueous solution demonstrated IOP-lowering effect for up to 4 h only. The duration of IOP reduction was, thus, significantly greater with the melatonin PLGA NPs than that observed with the solution formulation.

In another study, Warsi *et al.* [109] repared **dorzolamide (DZ)**-loaded PLGA NPs utilizing D-TPGS and polyvinyl alcohol as the emulsifiers. The NPs had significantly higher transcorneal permeation *in vitro*, approximately twofold for NPs with TPGS and approximately 2.5-fold for NPs with polyvinyl alcohol, in comparison to DZ solution. The three DZ formulations – solution, NP with TPGS and NP with polyvinyl alcohol, showed IOP reduction of 15.8%, 22.75% and 16%, respectively. The authors hypothesize that the increase in permeation and IOP reduction could be attributed to the capability of TPGS to inhibit P-glycoprotein.

In another study, Nagarwal et al. [110] demonstrated the effect of surface composition on the ocular bioavailability of polymeric NPs. In this study, the bioavailability of topically administered **5-fluorouracil** (5-FU, is an antimetabolite that is used as antifibrotic agent in glaucoma surgery) loaded sodium alginate-CH NPs, CH-coated sodium alginate-CH NPs and drug solution was evaluated in aqueous humor of rabbits. The CH-coated sodium alginate-CH NPs improved bioavailability up to fourfold, whereas, sodium alginate-CH NPs improved bioavailability up to twofold in aqueous humor when compared to fluorouracil solution. This study demonstrated the importance of surface properties in influencing the bioavailability of topically administered drugs at ocular tissues.

Posterior segment ocular diseases

As mentioned above MPP technology surprisingly was shown to increase LE concentration in the iris, ciliary body and retina in the rabbit pharmacokinetics study. Furthermore, the *in vivo* efficacy of PEG-based (MPP) was demonstrated by Schopf *et al.* [111] in animal models such as rabbit and mini-pigs for ocular drug delivery applications. These results demonstrated that MPP not only prolonged the residence time of therapeutic agents on the ocular surface but also enhanced bioavailability in posterior eye tissues when administered topically. However, it remains unknown how MPP improved the drug penetration through so many ocular barriers to reach the posterior segment of the eye. It should be noted that the detectable drug levels in the retina were measured in rabbits, which have smaller eyes than humans. MPP formulations provide a big hope for using noninvasive eyedrops to treat the most challenging posterior eye

diseases, and the future pharmacokinetic studies in human eyes following topical administration of MPP eyedrops would be the key.

A combined effect of sustained release and higher bioavailability can be achieved using a nanoparticulate system composed of a slow degrading polymer along with optimal surface characteristics. Polymeric NPs with an optimal surface composition not only improve the bioavailability of topically administered NPs at anterior eye tissues but also enhances bioavailability at posterior tissues such as the retina. In a study, Mahaling *et al.* [112] fabricated a core-shell nanoparticulate delivery system having a slow degrading PCL core and a mucoadhesive pluronic F68 shell for the noninvasive delivery of **TA** to the retina. The results demonstrated that core-shell poly-NPs DDS not only showed sustained drug release properties but also enhanced the transport of the drug across ocular tissues so as to enhance bioavailability at retinal tissue for the noninvasive treatment of DR.

Doxorubicin (DXR), a known antineoplastic agent, is a HIF-1(Hypoxia inducible factor) inhibitor that inhibits HIF-1 transcriptional activity and downregulates expression of proangiogenic factors causing strong suppression and regression of ocular NV. However, free DXR is rapidly cleared following intraocular injection, so the effects are too short-lived to be a viable alternative to current frontline therapeutics. Also, DXR caused reductions in b-wave amplitudes on electroretinograms (ERGs) and damaged the retina when doses were increased to elongate the duration of the activity [113]. In a study, Iwase *et al.* [114] fabricated a slow degradable copolymeric nanoparticulate system composed of PEG and poly-(sebacic acid) (PSA) for the treatment of chronic retinal diseases (choroidal and retinal NV). The intravitreal administration of DXR NPs to rabbits provided sustained release of DXR in aqueous and vitreous humors for 105 days, which significantly inhibited NV without showing obvious retinal toxicity. However, the toxicity was only evaluated on mice for 2 weeks, and a detailed long-term dose-dependent ocular toxicity study of DXR NPs in large animals would be helpful to address the safety issues as the next steps of development.

Fenofibrate is a peroxisome proliferator activated receptor-a (PPAR-a) agonist and has shown its therapeutic potential in PDR and wet AMD. Fenofibrate may offer some advantages over antiVEGF agents such as low-cost, fewer side effects, and neuroprotective effects but it suffers from poor water-solubility and low ocular

bioavailability following systemic administration, and can be quickly cleared from rat eyes after IVT injection (only lasts 4 days). Qiu *et al.* [115] developed fenofibrate-loaded PLGA NPs (Feno-NPs) to provide sustained release of fenofibrate for treating ocular NV. Feno-NPs provided sustained drug release for 2 months in vitro. A single IVT injection of 5 µl Feno-NPs (30 µg fenofibrate) exhibited sustained levels of fenofibric acid (the parent drug of fenofibrate) in rat eye for 2 months. Feno-NPs significantly attenuated retinal vascular leakage and retinal edema in streptozotocin (STZ)-induced diabetic rats and laser-induced CNV in rats for at least 8 weeks. The drug loading and drug release periods can be further increased by using polymers with different compositions and molecular weights, or through constructing larger particles.

Sunitinib malate is a multiple receptor tyrosine kinase inhibitor (TKI) that selectively inhibits VEGF receptors, PDGF receptors, colony-stimulating factor receptors and Fms-related TKI. TKI inhibition blocks multiple NV processes associated with wet AMD, DR and provides better effects than the selective blockage of VEGF alone. Sunitinib malate is an FDA-approved anticancer drug and can provide neuroprotective effects to retina ganglion cells in CNV [116]. GB102 [117] (GRAYBUG Vision) is a sunitinib-malate-loaded PLGA-PEG microparticle delivery system, which aggregates into a biodegradable depot and localizes at the periphery of the inferior vitreous without visual interference after IVT injection. The hydrophilic coatings avoided the toxicity and inflammatory reactions caused by IVT injection of PLGA particles. IVT injection of GB102 provided sustained release of sunitinib in the retina and RPE up to 4 months, which were 10 and 1000-fold higher than the plasma level of free sunitinib needed for anticancer therapy [118]. Preclinical studies have already proven the long-term efficacy of sunitinib release from PLGA-PEG microparticles following IVT injection in rabbit CNV models. The injectable particulate depot provided sustained CNV inhibition in Dutch belted rabbits for 6 months. GB102 is in a Phase I/IIa clinical trial (NCT03249740) as a potential therapy for twice-yearly IVT injections, thus reducing the burden of repeated IVT injection to patients. Currently, the second-generation formulation GB103 which is expected to provide once-yearly treatment is under preclinical evaluation. The technical details about GB102 and GB103 PLGA-PEG formulations are not fully available to the public. However, it should appreciated the strength of using PLGA and PLGA-PEG polymers with higher MW and a higher percentage of lactic acid to adjust sustained drug release profiles. The wide spectrum of available

PLGA and PLGA-PEG polymers gives the field a lot of freedom to develop more-sophisticated formulations with desired in vitro and in vivo properties. If GB102 and GB103 become successful through clinical trials, it would be very promising to use similar platforms to sustain release of many other drugs for treating various posterior eye segment diseases.

5.2.2.2 Polymeric micelles

Nanomicelles are colloidal DDS that self-assemble in a solution and can entrap therapeutic agents at their core. Their size ranges from 10 to 200 nm, and they are consisting of amphiphilic surfactants or block copolymers. Nanomicelles are formed instantaneously in a solution when the concentration of the polymers is above a specific concentration called the *critical micellar concentration* (CMC). Nanomicelles have the capacity to encapsulate hydrophobic drugs in the hydrophobic core of the micelles due to hydrophobic interactions. The hydrophilic corona interacts with the external aqueous fluid, increasing the solubility of a relatively lipophilic drug. This colloidal dosage form has the ability to form clear aqueous solutions which can be used as topical eye drops.

Nanomicelles can be broadly classified into three categories: surfactant, polymeric and polyionic complex nanomicelles. Polyionic complex nanomicelles, composed of polyion copolymers with neutral and ionic segments, have been investigated as nanocarriers for gene and antisense oligonucleotide ocular delivery, as electrostatic interactions between the core forming blocks and the oppositely charged drugs are responsible for nanomicelle formation and drug encapsulation [119]. Surfactant nanomicelles, made from low molecular weight surfactants, such as sodium dodecyl sulphate, generally show high CMC and thus physical instability. To overcome these limitations, polymeric micelles, i.e. micelles formed by amphiphilic copolymers, have been designed to exhibit lower CMC and better stability against dilution. Polymeric non-ionic surfactants are the major type of surface active polymers used in ophthalmic delivery systems since their advantages with respect to compatibility, stability and toxicity are significant compared to the cationic, anionic, or amphoteric polymers. PLGA, PEG, PCL, and polylactide are the most commonly used block copolymers on the micelles construction. The polymers can be conjugated to form diblock (A-B type), triblock (AB-A), or pentablock (A-B-C-B-A) copolymers.

Nanomicelles can be particularly useful for the formulation of hydrophobic drugs, leading to the possibility to apply a clear aqueous solution in the conjunctival sac. This feature avoids sticky feeling and blurred vision, commonly associated to the use of ointments. Additionally, lipidic excipients might cause ocular burning, conjunctival hyperemia, discharge, epiphora, eye pain, foreign body sensation, pruritus and stinging (these adverse effects are more or less pronounced depending on the lipid used). Furthermore, amphiphilic excipients used in nanomicelles can facilitate the spreading of the formulation on the ocular surface.

Anterior segment ocular diseases

Dry Eye Disease (DED) or Ocular Surface Disease (OSD)

CsA eyedrops have been used for the treatment of ocular inflammatory diseases including uveitis, keratoconjunctivitis and dry DED. The hydrophobic nature (solubility 10 mg/ml) and large molecular weight (1202.6 Da) of CsA as we mentioned before, rendered the development of aqueous eyedrops challenging. **Cecua®** (Sun Pharmaceuticals Inc.) is a nanomicellar formulation of 0.09% CsA recently approved by the FDA for DED. Cequa®'s NCELL™ technology platform demonstrate improved rapid onset of action as early as 4 weeks and improvement in tear production as compared with CsA commercial emulsion Restasis® [120]. The nanomicellar system was prepared from a polymeric mixture of two low-molecular-weight surfactants, with micelles particle size of 12-20 nm, which resulted in formation of a clear solution of CsA. The *in vivo* studies of the formulation of CsA conducted in rabbits demonstrated enhanced bioavailability in the anterior ocular tissues as compared with CsA emulsion with no ocular adverse effects. The Cequa® formulation was approved by the FDA for DED treatment in 2018.

Inflammations of Anterior Segment

Gupta *et al.* [121] synthesized polymeric micelles (<100 nm particle diameter) composed of the co-polymers of N-isopropylacrylamide (NIPAAM), vinyl pyrrolidone and acrylic acid crosslinked with N, N'-methylene-bisacrylamide (MBA) to deliver **ketorolac** (NSAID) to the eye. The authors reported a twofold increase in transcorneal permeability of ketorolac over an aqueous ketorolac suspension at the same concentration [90]. The micellar formulation was observed to yield better therapeutic response, compared with

the suspension, in terms of eyelid closure and polymorphonuclear leukocytes migration in the tear fluid.

A study reported by Li *et al.* [122] demonstrated enhanced corneal permeation (17-fold higher) and higher bioavailability (twofold higher) of topically administered **diclofenac** (NSAID) using a (MPEG–PCL)-based micelle delivery system when compared to diclofenac solution in rabbit eyes.

Tacrolimus, an immunosuppressive drug, has been proposed as an effective alternative to CsA for the treatment of different ocular surface diseases as it prevents the activation of several cytokines, such as IL-2 and IL-4. Tacrolimus loaded micelles (size of ~45 nm) were investigated by Kutlehria *et al.* [123] in a BAK induced ocular inflammation model in BALB/c mice. Micellar formulation significantly reduced the corneal fluorescence (inflammation index) for comparison with the drug suspension. Additionally, hematoxylin eosin staining demonstrated a better restoring of corneal epithelial thickness and the reduction of both stromal edema and number of inflammatory cells.

Corneal diseases

Taha *et al.* [124] reported enhanced bioavailability of **CPX** using polymeric micelles of PluronicR F127. They observed a statistically significant increase in the area under the aqueous humor concentration versus time curve in comparison to the commercial formulation.

Liaw *et al*. [125] demonstrated the use of Pluronic® P105 nonionic copolymeric micelles of hydrodynamic diameter <160 nm for efficient delivery of stable plasmid DNA with **lacZ gene**, *in vivo*. *In vivo* gene transfer to the different layers of the cornea from the micellar ocular drops was achieved in mice and male albino New Zealand rabbits, which highlighted the potential of the block copolymer for DNA delivery. The DNA delivery efficiency was enhanced using the penetration enhancers, EDTA and cytochalasin B.

Gene delivery using topical eye drops was also reported by Tong *et al.* [126]. Two cornea-specific promoters: ketatin 12 and keratocan were formulated using Pluronic® P105 polymeric micelles. These proteins play a crucial role in developing and maintaining corneal transparency. β-Gal activity was the biomarker for transgene expression in the ocular tissues. The hydrodynamic diameter for the micelles ranged from 150 to 200 nm with unimodal distribution. The formulations were tested on both mice and rabbit animal

models. From the *in vivo* tests, the authors concluded that the corneal epithelium and stroma-specific gene expression can be achieved with the help of cornea-specific promoters.

Farthermore, the ocular drug delivery efficiency of topically administered polymeric micelles can be further improved by minimizing precorneal clearance and premature drug release from micelles using gel-based systems as a medium for micelle delivery. In a study, Jaiswal *et al.* [127] demonstrated the enhanced bioavailability of such hybrid delivery system *in situ* gel-based micellar formulation. In this study, the hybrid formulation showed superior *ex vivo* transcorneal permeation of poorly water-soluble drug, **itraconazole** (across goat cornea) when compared to a commercial eye drop formulation Itral® (Jawa pharmaceuticals pvt ltd., India) and pure drug suspension.

Chronic infections and ulcers may also be induced by delayed corneal wound healing. This condition may be caused by the binding of glucocorticoids to the mineralocorticoid receptor. Thus, the co-administration of mineralocorticoid receptor antagonists (MC) could be successful for overcoming the negative effects of glucocorticoids. Dahmana *et al.* [128] studied a topical micellar formulation of **spironolactone** prepared mPEG-dihexPLA diblock copolymer for preventing glucocorticoid-induced delayed corneal wound healing. *In vivo* efficacy studies were carried out in New Zealand rabbits. The study demonstrated that the spironolactone micelles were superior in re-epithelialization by comparison to the control groups. This indicating the potentiality of the micellar formulation to reduce the negative effects of glucocorticoids agent on the corneal wound healing.

As discussed earlier, the contact time of the formulation with the surface ocular tissues plays a critical role in ocular drug bioavailability. Residence time can be prolonged by various means, one of them being the use of cationic polymer/s possessing mucoadhesive properties. One such naturally occurring cationic polymer which can improve ocular bioavailability is CH. CH is reported to have mucoadhesive property and acts as a permeation enhancer by reversibly opening epithelial tight junctions. Pepic *et al.* [129] formulated a CH–Pluronic® F127 mixedmicellar system with particle size ranging from 25 to 29 nm and and was loaded with **DEX**. *In vivo* PK studies in male albino New Zealand rabbits produced 2.4 and 1.4-folds increase in ocular bioavailability with Pluronic® F127-CH micelles, compared with DEX ocular suspension and Pluronic® F127 micelles (CH free), respectively.

Glaucoma

Another study that demonstrates the beneficial role of CH as surface coating comes from Lin *et al*. [130]. They have developed poloxamer 407 micelles modified by chitosan for the delivery of **metipranolol**, a non selective beta blocker that used to reduce the IOP. The *in vivo* studies on New Zealand rabbits were performed comparing the commercial ophthalmic solution (OptiPranolol), poloxamer micelles and poloxamer micelles modified with 0.5% w/w CH; drug loading was 0.3% w/w in all cases. CH-micelles gave the best results: the AUC of IOP versus time (up to 360 min) for the nanomicelles of poloxamer 407/chitosan was 1.67- and 1.88-fold higher than OptiPranolol and poloxamer micelles, respectively. IOP variation values showed that poloxamer 407 micelles do not have enough residence time on ocular surface, while the presence of CH increased mucoadhesion, leading to a higher pharmacological response.

Xu *et al.* [131] developed micelles-laden contact lenses (CLs-M) that could achieve the sustained release of **TM** and **latanoprost** simultaneously for the treatment of glaucoma. CLs-M were obtained by free radical polymerization of HEMA monomer with TM and latanoprost loaded mPEG-PLA micelles. The prepared CLs-M had a minimal impact on critical CLs properties, and could release TM and latanoprost in simulated tear fluid for 144h and 120h individually, which is promising for sustained drugs release applications. The *in vivo* PK study on rabbit eyes showed sustained TM and latanoprost release for up to 120h and 96h in tear fluid, respectively. There was significant improvement of the mean residence time (79.6-fold and 122.2-fold) and bioavailability (2.2-fold and 7.3-fold) for both TM and latanoprost delivered by CLs-M compared with commercial eye drops formulations. An *in vivo* PD study in a rabbit model with high IOP showed sustained reduction in the IOP for over 168h. The relative pharmacological availability (PA) of CLs-M was 9.8 times as high as the eye drops. The protein adsorption, ocular irritation study and histological examination study indicated the safety of CLs-M. Therefore, this work has demonstrated the promising potential of micelles-laden CLs to co-deliver TM and latanoprost for an extended period of time to treat glaucoma.

Posterior segment ocular diseases

Mandal *et al.* [132] demonstrated the entrapment of hydrophobic drug and hydrophilic peptides within the core of nanomicelles for ocular drug delivery. A lipid

prodrug of cyclic **cidofovir** (B-C12-cCDF) was encapsulated within surfactant-based nanomicelles for antiviral drug delivery for cytomegalovirus (CMV) retinitis, and multilayered nanomicelles were developed for the delivery of octeriotide peptide to the anterior segment of the eye. The researchers also demonstrated that a mixed micellar structure designed from a fixed ratio of low-molecular surfactants had a lower critical micellar concentration. This indicates that the nanomicellar structure is stable over dilution in the systemic fluids and will not result in premature drug release. These highly lipophilic agents form a clear solution when encapsulated in the nanomicelles. Also, nanomicelle aid is sustained, and release of the drug to the ocular tissue is controlled [133].

Although still a few, *in vivo* experiments have clearly demonstrated the capability of polymeric nanomicelles to overcome a variety of hurdles associated to ocular drug delivery, notably increasing drug bioavailability. However, there are still some very important issues to be solved, such as tolerability and stability; additionally, the role of micelles in drug uptake by the ocular tissues and their potential for the treatment of posterior eye diseases still need to be clarified/verified.

5.2.2.3 Hydrogels /Nanogels

Nanogels are nanosized hydrogel networks consisted of hydrophilic or amphiphilic cross-linked polymer chains. They can be fabricated using various natural/synthetic hydrophilic polymers [134, 133]. The type of polymer employed for fabrication of nanogels defines its gel structure. The gel structure is maintained via physical (hydrophobic interactions, charge-based interactions, hydrogen bonding, etc.), chemical (covalent bonding) or enzymatic crosslinks [135, 136]. Hydrogels have been explored for ophthalmic drug delivery applications because their viscous nature offers a relatively longer retention of entrapped medicaments at the site of application while their transparency reduces blurring of vision. For topical ophthalmic drug delivery, nanogels composed of mucoadhesive (bioadhesive) polymers that have shown promising results in improving bioavailability of the administered therapeutic agent. Furthermore, *in situ* forming hydrogels have been reported to offer additional advantages as they undergo sol to gel transformation *in situ* upon receiving the desired stimulus [137, 138].

They swell into a considerable volume when dispersed in aqueous medium. Nanogels may be loaded with therapeutic agents by interaction between agent and functional group present in the polymer either physically or chemically. Nanogels are highly biocompatible, versatile, show a controlled release of drug, and are also able to protect biodegradable molecules from degrading inside the body [139]. NP size offers high special surface that is available for bioconjugation with moieties targeting special macromolecular targets on damaged tissue surface.

Hydrogels, due to their gel structure, allow slow diffusion of entrapped therapeutic agents thereby offering sustained drug release characteristics. For example, in a study conducted by Liu *et al.* [140] alginate/hydroxypropylmethyl cellulose-based *in situ* gel system reduced precorneal clearance of topically administered **gatifloxacin** in rabbits and enabled sustained release of gatifloxacin for 8 hrs.

The hydrogels can be fabricated using stimuli-responsive polymers so as to offer on-demand drug delivery. Physical stimuli such as light and temperature are commonly used for ophthalmic drug delivery applications. A thermoresponsive gel exhibits low viscosity at reduced temperature and high viscosity at body temperature. Such systems offer ease of administration while maintaining gel consistency upon contact with body fluids.

Cao *et al.* [138] demonstrated the efficacy of a thermosensitive *in situ* gelling system that had a low critical solution temperature of 32°C. The fabricated system was a liquid at room temperature and could undergo a phase change to form a gel at 32°C (surface temperature of the eye). The gel system was fabricated using poly-(N-isopropylacrylamide)-CH copolymer and loaded with **TM**. A comparative study between the fabricated gel system and a conventional eye drop formulation, that was conducted in rabbits for treating glaucoma showed a twofold increase in bioavailability of the gel system along with a sustained therapeutic effect over a period of 12 hrs. This study showed the efficiency of thermoresponsive delivery systems for ocular drug delivery applications.

Light-responsive gel systems have also been explored for on-demand delivery of therapeutic agents at the site of interest in the eye for longer durations. Tyagi *et al.* [137] demonstrated the fabrication of a light-responsive PCL dimethacrylate and hydroxyethyl methacrylate-based hydrogel system for sustained drug delivery. The gel system was loaded with Alexa Fluor 488 dye conjugated **bevacizumab**, injected into the

suprachoroidal space (a potential space between the sclera and choroid) of Sprague-Dawley rats and evaluated for its efficiency to release the drug when provided with a light stimulus (wavelength: 365 nm). The results showed that the fabricated gel system provided a sustained release of bevacizumab for 4 months in intraocular tissues. This demonstrated the ability of light-responsive gel-based DDS to provide sustained drug release in intraocular tissues.

Furthermore, as discussed elsewhere in this review, the consistency and gel structure of hydrogels enables entrapment of other nanoparticulate delivery systems. The resultant hybrid systems can potentially improve ocular bioavailability when compared to hydrogels without nanoparticles [141, 142]. Consequently, hydrogels can be used for topical or intraocular drug administration in order to reduce drug clearance by dynamic ocular barriers. Because of their properties hydrogels have great potential for sustained and stimuli-responsive drug delivery applications in the treatment of anterior and posterior eye diseases.

5.2.2.4 Dendrimers

Dendrimers are polymeric nanocarriers sized about 10nm having a branched star-shaped structure. In contrast with other polymers, the critical parameters in nanoscale like the size and shape of the dendrimer can be accurately controlled and customized during the synthesis to form a dendrimer with specific functional groups and a specific architecture. These properties are not the sum of the monomer properties but are completely different and follow different rules. These nanoconstructs have unique physiochemical properties such as high drug encapsulation and conjugation ability, high water solubility, and a plethora of functional groups on the surface for chemical modification. Dendrimers also have lower polydispersity index that reducing their uptake by the RES and enhancing their tissue permeability. Hydrophilic and lipophilic drugs can either be conjugated to the surface of the dendrimer or be encapsulated by caging in the internal structure of the dendrimer [143, 144]. Upon topical or intraocular administration, dendrimers have the potential to show an enhanced transport of therapeutic molecules across ocular tissues and prolonged ocular retention of the resultant transported. A polyamidoamine (PAMAM) polymer having carboxylic and hydroxyl functional groups, is the most commonly used dendrimer for ocular drug delivery. High branching of the

PAMAM polymer can lead to primary, secondary, and tertiary generations of the dendrimer nanocarrier.

Anterior segment ocular diseases

Soiberman *et al.* [142] designed a **hybrid** gel formulation system of the G4-PAMAM dendrimer with cross-linked hyaluronic acid, entrapping **DEX** intended for the treatment of corneal inflammation. The results demonstrated that the SCT injection of the dendrimer-based gel formulation provided sustained DEX release and reduced inflammatory complications compared to free DEX. Really important was the remark that the drug induced increase in IOP was not observed in animals that received dendrimer-based gel formulation, whereas, free DEX administered animals showed an increase in IOP. This study demonstrated that control on spatial distribution and/or temporal release of DEX can be achieved using a dendrimer-based gel formulation. The dendrimer formulation led to reduction in central corneal thickness and improved corneal clarity in an alkali burn rat model, which was highly clinically relevant.

Matrix metalloproteinases-9 (MMP-9) can trigger corneal damage and result in DED. Cerofolini *et al.* [145] synthesized an **MMP-9 inhibitor** and solubilized with PAMAM dendrimers. The synthesized inhibitor had high binding affinity to MMP-9 and can be used for the treatment of corneal inflammation and DED.

Vandamme *et al.* [146] entrapped **tropicamide and pilocarpine nitrate** in PAMAM dendrimers to study the effect of drug-release kinetics after altering the size, molecular weight, carboxylate and hydroxyl surface groups, and total number of amines present in the PAMAM dendrimer. *In vivo* results in New Zealand albino rabbits revealed higher drug residence time of dendrimers functionalized with carboxylic and hydroxyl functional groups.

Posterior segment ocular diseases

Yavuz *et al.* [147] evaluated the potential of **DEX**-PAMAM dendrimers for the delivery to the posterior segment of the eye for the treatment of diseases such as DR and AMD. In vivo studies in rats showed that the drug-loaded dendrimers enhanced the ocular permeability of DEX after SCT injection as compared with the free drug.

In yet another study by Iezzi *et al.* [148] a prolonged retention of **fluocinolone acetonide** (FA)-conjugated PAMAM dendrimers in rat retina was observed after intravitreal injection. The PAMAM dendrimer delivery system localized in activated microglia and as a result delivered FA intracellularly for 1 month. Hence, the developed system effectively attenuated neuro-inflammation for prolonged durations in a rat retinal degeneration model.

Kambhampati *et al.* [149] studied the potential of **TA** given as a complex with PAMAM dendrimer for ocular delivery. Results demonstrated that the conjugates of dendrimer with the TA showed a better profile of toxicity in comparison with normal TA at the target site.

Another exciting application of dendrimers is noninvasive **photodynamic therapy (PPT)** as a treatment for pathologic retinal NV. The majority of conventional photosensitizers used in PTT easily form aggregates, causing a self-quenching effect and diminishing the quantity of reactive oxygen species generated by photoirradiation. Treatment efficacy is not optimized. A new generation of photosensitizers has been introduced, based on a dendrimer with a central core of porphyrin surrounded by the third generation of poly(benzylether) dendrons. Dispersion is presumably assisted by dendrimeric steric hindrance and electrostatic repulsion. Using an *in vitro* Lewis lung cell model with micelle as the delivery vehicle, Ideta *et al.* [150] reported a remarkable 280-fold increase in phototoxicity when the dendrimers are encapsulated into the micelle. In an *in vivo* rat model of CNV, the authors showed rapid accumulation and sustained release of the dendrimers in the lesions, likely via the enhanced permeability and retention (EPR) effect. No microscopic evidence of long-term phototoxicity was observed with the dendrimer-loaded micelle group compared to the group injected with Photofrin, a commercial photosensitizer. Similar results had been duplicated by Sugisaki *et al.* [151] using an in vivo mouse model of trauma-induced corneal NV. Naphthalocyanine dendrimers for photodynamic applications were recently developed by Jang *et al.* [152].

To suppress CNV other groups explore the use of dendrimer macromolecules as a gene therapy modality, rather than PTT. Marano *et al.* [153, 154] successfully introduced a sense oligonucleotide sequence with anti-VEGF activity into a rat CNV model. The deliver instrument was a lipid-lysine dendrimer construction. The transfective capability positively correlates with the number of positive charges on the dendrites. The authors

observed a significant reduction in VEGF expression in the dendrimer/oligonucleotide group compared to the naked oligonucleotide group. A follow-up longterm study by the same group determined that the effective life span and treatment interval of the dendrimer/oligonucleotide complex is approximately 4–6 months. No observable toxicity was noted.

Mastorakos *et al.* [155] developed hydroxyl- and amine-functionalized PAMAM dendrimer-based gene vectors for transgene delivery to human RPE cells. The colloidal stability was improved via PEGylation of the gene vector surface and complexation with **TA** as a nuclear localization enhancer resulted in a significant improvement in cellular uptake and transfection of human RPE cells.

Tamaki *et al.* [156] introduced an interesting delivery process wherein the actual offloading was triggered by laser irradiation. The DNA sequence was packaged with cationic peptides and enveloped in the photosensitive phthalocyanine dendrimer. SCT injection followed by laser irradiation demonstrated site-specific transgene expression in a rat model. This laser-induced delivery system presents another option for the management of ophthalmic diseases.

Kang et al. [157] reported on **carboplatin**-loaded half generation PAMAM dendrimers for the management of murine retinoblastoma. Each mouse received a single SCT injection in one eye, and the opposite eye was left untreated as a control. The mean tumor burden in the treated eyes was significantly lowered compared to untreated eyes and no toxic effects were observed in any of the groups. However, dendrimers (258 nm average particle size) used in this study are unlikely to cross the sclera-choroid-RPE in their original form.

Furthermore, control on spatiotemporal drug release from dendrimer-based delivery systems can be achieved by fabrication of stimuli-responsive or bioreducible dendrimers [158, 159]. Although such on-demand DDS have not been explored for ophthalmic applications, they can be useful for the effective treatment of specific ocular diseases.

So according to the above, dendrimer-based DDS can be used as promising carriers to enhance ocular permeability, intraocular/cellular retention, and to enable controlled drug release of topically or intraocularly administered agents.

5.2.2.5 Polymeric nanofibers

Polymeric nanofibers are fibrous systems that are fabricated using natural or synthetic hydrophilic/-phobic polymers by employing a simple and versatile technique called electrospinning. Based on the polymer employed for fabrication, nanofibers can entrap hydrophilic or hydrophobic drugs and can deliver the entrapped drugs in a sustained manner. Furthermore, nanofibers possess high surface area that may enable adequate tissue contact which can further be improved by surface modification. An optimal tissue–fiber interaction/adhesion can facilitate drug delivery at the tissue of interest. Nanofibers have been explored for drug delivery and tissue engineering applications [160] for treatment of anterior as well as posterior segment eye diseases [161]. It has been demonstrated that delivery of **CsA** to the ocular surface using a PLA nanofiber-based delivery system enhanced CsA delivery to cornea and as a result, effectively reduced inflammation and NV when tested in an alkali-injured rabbit corneal model [162]. It has also been demonstrated that insertion of drug-loaded (**TM** and **DZ**) polymeric (polyvinyl alcohol or PCL) nanofiber patches in the cul-de-sac of glaucoma-induced rabbits effectively reduced IOP when compared to commercial eye drops [163]. These studies infer that nanofiber-based delivery systems can reduce clearance of administered drugs from ocular tissues and present therapeutic concentration of drug for longer duration so as to treat ocular complications.

The duration of drug release/delivery from the nanofibers play an important role in the treatment of chronic eye diseases. Nanofibers composed of slow degrading polymers are being explored for the treatment of chronic pathological complications [162]. The duration of drug release can be substantially enhanced by further improvements in existing nanofiber systems. In a study, Yavuz *et al.* [164] designed a hybrid system composed of **CsA**–PLGA NPs impregnated thin films and nanofibers for the long-lasting delivery of CsA for the treatment of DED. The fabricated delivery system showed a sustained CsA release at cornea, sclera, and lens of mice even after 90 days of SCT implantation and was effective for the treatment of DED. Furthermore, a hybrid system consisting of PAMAM dendrimer-based nanofibers (DNFs) has been investigated as a topical drug delivery vehicle for the delivery of anti-glaucoma drug, **brimonidine tartrate** (BT) (alpha-2 adrenergic agonist for IOP reduction). The results demonstrated that

delivery of BT in DNF is efficacious compared to solution-based systems for the treatment of glaucoma [165].

These studies demonstrate that nanofiber-based delivery systems can be applied at the ocular surface for sustained drug delivery applications for the treatment of chronic ocular complications.

5.2.3 Niosomes

Niosomes are surfactant-based vesicular bilayered systems formed by the self-assembly of **nonionic** amphiphiles/surfactants that expose their hydrophilic ends on the outer and inner side of the vesicle, while, the hydrophobic chains face each other within the bilayer [166]. Due to their hydrophilic surface, niosomes can easily interact and cross the tear film barrier and as a result, reach the corneal/conjunctival tissue to undergo internalization. The small size and the presence of a surfactant layer help niosomes to entrap both hydrophilic/-phobic drugs and transport them across cellular layers via paracellular and/or transcellular routes thereby enhancing the bioavailability of topically administered drugs [167].

Niosomes are preferred over liposomes due to their enhanced chemical stability. They also have low toxicity because of their nonionic nature. Since surfactants are used, special precautions and conditions are not required like phospholipids (i.e. liposomes). Therefore, niosomes are flexible in their structural composition, fluidity, and size. Niosomes are biodegradable, biocompatible, and non-immunogenic and increase therapeutic effect of the drug by better bioavailability and controlled delivery to the particular site.

Discomes are a modified nonconventional form of niosomes, made with the addition of nonionic surfactant like Solulan [168]. They are disk shaped and larger in size than conventional niosomes. Discomes are reported to provide several advantages over niosomes, such as better fit in the cul-de-sac of the conjunctiva and improved ocular drug bioavailability due to slower nasolacrimal drainage. Also, entrapment efficiency and bioavailability of discomes are better compared with niosomes [169]. The increased corneal uptake by niosomes and discomes is attributed to many factors like their ability to disrupt the tight junctions of corneal epithelium, better spread ability on the lipophilic corneal surface, and suitable rheological properties. There are many reports of enhanced

ocular drug absorption due to higher viscosity (leading to increased pre-corneal residence time) of ophthalmic solution [170]. However, this increase in bioavailability by increasing viscosity has its limitation. Application of conventional viscous formulation leads to blurry vision and poor patient compliance. Also, the drug bioavailability does not increase proportionally to the increase in viscosity, it reaches a plateau, and further increase in viscosity yields insignificant increase in the ocular bioavailability. Thus, niosomes and discomes are proposed as alternative vesicular carrier for ocular delivery.

Spanlastics are another interesting category of modified niosomes. These are Span sorbitan)-based elastic vesicular drug delivery systems patented by Kaur *et al.* [171]. Spanlastics have been shown to be superior to niosomes in terms of enhanced deformability and thus bear the potential to deliver drugs to the posterior segment of the eye.

Anterior segment ocular diseases

Zeng *et al.* [172] evaluated corneal permeation of poloxamer-based niosomal formulation, hyaluronatecoated poloxamer-based niosomal formulation and **tacrolimus** suspension. The results demonstrated that hyaluronate-coated niosomal formulation significantly enhanced tacrolimus permeation across rabbit cornea compared to plain niosomes and suspension system. From this study, it can be inferred that surface modification using hyaluronate played an important role in improving the bioavailability of niosomal delivery systems.

Abdelkader *et al.* [168, 170] have reported development of niosomes and discomes for **naltrexone hydrochloride** (NTX), an opioid antagonist for treatment of the diabetic complications affecting the cornea. They suggest that the developed formulation provided better wetting properties, higher viscosity, and better protection to NTX against light-induced degradation and oxidizing agent than aqueous formulation. Akhtar *et al.* [173] have reviewed a wide variety of drug molecules delivered to the eye through niosomal vesicular carrier.

Khalil *et al.* [174] studied novel proniosomal gel as carriers for delivery of **lomefloxacin** HCl (LXN) into the eye. It showed a controlled in vitro release of LXN for over 12 h, acceptable stability, and optimum pH. Transmission electron microscopy (TEM) images showed distinct spheres with smooth surface morphology. The formulation was

non-irritant when applied topically and showed better bacterial inhibition than the commercial LXN eye drops Orchacin®.

Glaucoma

Prabhu *et al.* [175] have reported formulation and evaluation of niosomes containing anti-glaucoma drug **BT**. They demonstrated significant reduction in intraocular pressure by this niosomal preparation on male albino rabbits. Similarly, Sathyavathi *et al.* [176] have described improved bioavailability and increased pre-corneal residence time of **BT** in niosomal gel compared to marketed drops.

Aggarwal *et al.* [177] used spanlastics niosomes using CH or carbopol for delivery of **TM**. The resultant surface-modified niosomal formulation exhibited equal therapeutic efficacy at half the dose of commercial solution-based formulation. This study demonstrated the potential of surface-modified noisome-based formulations for the effective treatment of anterior eye diseases.

Guinedi *et al.* [178] studied multilamellar niosomes as a promising carrier for ophthalmic delivery of **ACZ**. Results showed that the entrapment efficiency (EE %) of ACZ into the niosomes increased with only upto a certain concentration of cholesterol. TEM showed multilamellar niosomes with absence of aqueous interior, but a greater particle size than others. The formulations also showed a good stability at normal and refrigerated storage. *In vivo* studies revealed that IOP was decreased considerably using the ACZ-loaded niosomes, in comparison with the plain niosomes as well as the simple solution of ACZ.

Posterior segment ocular diseases

Spanlastics nanovesicles have been studied [179] for the delivery of **fluconazole**, a well known antifungal drug, as treatment of ocular mycoses. The fluconazole-containing spanlastics were compared to commercial fluconazole (Zocon®) eye drops using *ex vivo* corneal permeability studies. Considering the hydrophilicity of fluconazole, drug permeation was least in case of Zocon® eye drops when compared to different types of spanlastic vesicles. Drug delivery to the posterior eye is limited to intravitreal injections, periocular routes, and systemic dosing. The use of spanlastics provides a unique drug delivery system that can be instilled topically as an eye drop and deliver drugs to the

posterior eye segment. Several diseases such as fungal infections, uveitis, and ARMD can benefit from such delivery systems. Spanlastics DDS also could be beneficial considering patient compliance as they could replace intravitreal injections.

Niosomes have shown significant promise for effective delivery of administered therapeutic agents to ocular tissues.

5.2.4 Cubosomes

Cubosomes are self-assembled, hydrated surfactant nanostructures containing a liquid crystalline phase with cubic crystallographic symmetry. Cubosomes are formed by the self-assembly of surfactant-like molecules [180]. Monoglyceride glycerol monoolein is a well-known surfactant useful to make cubosomes. Due to the presence of cubic phases, cubosomes are able to solubilize hydrophilic, hydrophobic, and amphiphilic molecules thereby improving drug stability and exhibiting sustained drug release properties [181]. The structure and surface properties of surfactants used in cubosome formulation affect solubility, mucoadhesivity, drug release kinetics and rheological properties of the formulation so as to improve precorneal and intraocular retention of administered therapeutic agents [182]. Cubosomes have been explored in ODD applications to improve the bioavailability of topically administered therapeutic agents. Generally, cubosomes have biodegradable and bioadhesive constituents and can provide sustained release of a drug and also work as a penetration enhancer.

Han *et al.* [183] developed **flurbiprofen** cubosomes with main emphasis on reducing ocular irritation and increasing drug bioavailability. *In vitro* corneal permeation using modified Franz-type cells revealed up to twofold increase in permeation with these cubosomes. These were further evaluated for ocular irritation using Draize method and histological examination. Cubosomes exhibited excellent ocular tolerance with improved AUC as measured by microdialysis. These studies suggested cubosomes as promising delivery vehicles.

In the same year, Gan *et al.* [184] reported cubosomes as delivery vehicles for **DEX**. In vitro studies showed up to 3.5-fold increase in permeability with DEX cubosomes, when compared to solution and gel-based systems. Additionally, these cubosomes showed longer retention and up to 1.8-fold increase in AUC as measured by microdialysis. These

studies further emphasize that these highly biocompatible, biodegradable delivery vehicles can be an effective approach for certain ocular therapies.

Li *et al.* [185] formulated cubosome of glycerol monoolein and poloxamer 407 loaded with **pilocarpine nitrate** for glaucoma therapy. In comparison to the marketed eye drops they observed that the cubosomes had approximately two-times higher transcorneal flux across the rabbit corneas, had prolonged the retention time and also they had significantly higher effect on IOP reduction (~50% IOP reduction). In the same way, Huang *et al.* [186] formulated cubosomes of glycerol monoolein and poloxamer 407 loaded with **TM**. They observed similar results as the previous study. Cubosomes were deemed to be safe in the above reports by the authors on the basis of draize test, corneal hydration levels and corneal histology.

Cubosomes have also been explored for on-demand drug delivery mediated by a stimuli-responsive system, however, such [187] stimuli-responsive systems have not been explored till date for ODD applications. In future, such systems can be explored for on-demand sustained ODD applications for the treatment of ocular diseases. In conclusion, cubosomes may serve as a potential drug delivery carrier for topical and intraocular administration for the treatment of ocular diseases.

5.2.5 "Trojan" Microparticles

The trojan systems can be defined as microparticles that are loaded with nanoparticles, which, in turns carry the active compound. Ideally, once administered, the microparticles will slowly release the NPs for long periods, protecting the unreleased NPs from external environment. Once released, NPs will be then directed to the target cells, internalized and will release the active compound into the cell. This would allow maximizing the efficacy of the administered active molecule, by minimizing their clearance before reaching the target cell.

Gómez-Gaete *et al*. [188], developed an interesting work proposing PLGA NPs loaded with **DEX** included in microparticles composed by a combination of a phospholipid with hyaluronic acid (HA). Unfortunately authors have not published at the moment any *in vivo* study to confirm the real potential of the interesting trojan systems proposed, however, they claimed that "the *in situ* release of drug loaded nanoparticles should favor their

internalization within retinal pigmented epithelial cells and might, therefore, increase the drug efficacy".

Yandrapu *et al.* [189], elaborated porous PLGA microparticles incuding **bevacizumab** (Avastin®)-loaded PLA NPs. Following *in vitro* studies trojan system containing 10% of NPs and 90% of microparticles was selected for *in vivo* studies. It is interesting to remark that in this NPs-in-Microparticles (NiMs) approach, the use of NPs is neither focused on their cell internalization properties nor on their ability to protect the active compound from the external environment, so once the NP is released from the porous microparticles, the adsorbed protein is rapidly released. The use of NPs in this case simply ensures the progressive release of the compound. Two months post-dosing, rat's eyes were enucleated and bevacizumab was detected by ELISA in vitreous, retina and choroid-RPE, but only in eyes treated with microspheres.

In a similar way Elsaid *et al.* [190], elaborated PLGA microparticles including **ranibizumab** (Lucentis®)-loaded CH and N-acetyl-cys-chitosan NPs. Interestingly, CH has demonstrated to have antiangiogenic activity itself. Sadly, no tube length inhibition studies were performed using the trojan systems. In any case, authors demonstrated that the systems created might be considered interesting platforms for the delivery of active anti-VEGF proteins after intravitreal administration.

All these results confirmed the high potential of the new methodology proposed for microencapsulation of active proteins system for vitreoretinal diseases.

5.2.6 Nanobubbles

As mentioned before the intravitreal route faces many challenges in rapidly and effectively reaching posterior eye pathology, with administered therapeutics experiencing non-specific distribution around and premature clearance from ocular tissues. Nanobubbles and ultrasound may improve outcomes of intravitreally administered drugs by influencing the directionality of drug-containing particle migration. Thakur *et al.* [191] assessed the impact of trans-scleral or corneal ultrasound application on the distribution of intravitreally-injected nanobubbles (Z-average size of 205.0 ± 35.3 nm). Rhodamine-tagged gas entrapped nanobubble formulations were prepared and injected into *ex vivo* bovine and porcine eyes and subjected to ultrasound (1 MHz, 0–2.5 W/cm2, 50–100% duty, 60s). Bovine eyes were partially dissected to visualize the vitreous body and particle

migration was evaluated via optical fluorescence spectroscopy. Administration of ultrasound significantly enhanced the directional migration of nanobubbles in both *ex vivo* models, with multiple corneal ultrasound cycles promoting greater migration of dye-filled nanobubbles to posterior regions of the vitreous. Moreover, particles moved in a directional manner away from the ultrasound wave source demonstrating an ability to effectively control the rate and path of nanobubble migration. These findings establish an encouraging new and safe modality enabling rapid distribution of intravitreally-injected therapeutics where expeditious therapeutic intervention is warranted.

5.2.7 Gold nanoparticles (GNPs)

Due to their unique properties, GNPs are used more and more in the biomedical field, especially for drug delivery, gene delivery, and thermal treatment of cancer. GNPs are stabilized by a wide variety of ligands that influence their size and properties. According to the experimental conditions of their synthesis, their diameter range from 1 to more than 120 nm. Different shapes can be obtained, including coreshell NPs, nanocages, or nanorods whose aspect ratios (length divided by width) modify their optical properties. The excellent stability of GNPs permits chemical reactions directly on their surface. The ligands used to stabilize GNPs may be specifically chosen for drug encapsulation and release, or to target specific tissues, such as tumors. The ligands' size, charge, length, and polarity influence the properties of the GNPs and play a major role in drug-release efficiency. Drugs can be either covalently bound to GNPs, or encapsulated via supramolecular interactions.

The delivery of **bevacizumab** was tested via the use of an agarose hydrogel containing GNPs [192]. The system was photo-modulated and several cycles of illumination did not alter the structural integrity of the drug that remained above 80%. Moreover, its *in vitro* biological activity was retained. These data suggest that GNPs can be combined with hydrogels to couple their properties, leading to a biocompatible controlled drug delivery system.

Also GNPs have been tested for gene delivery. Sharma et al. topically administered 2-kDa polyethylenimine conjugated to GNPs (PEI2-GNP) containing green fluorescent protein (GFP) gene on rabbit corneas [193]. In another study the topical administration of

the same GNPs delivery system containing BMP7 gene, was demonstrated a significantly reduced postsurgery laser fibrosis in the rabbit cornea [194].

Several nanodelivery systems are developed for retinoblastoma (RB) therapy. Mitra *et al.* [195] elaborated GNPs for the targeted delivery of small interfering ribonucleic acid (si-RNA) able to knockdown the epithelial cell adhesion molecule (EpCAM) gene in RB cells. These GNPs were capped with polyethylenimine (PEI) ligands and their biocompatibility was confirmed on RB Y79 RB cells. *In vitro* experiments on these cells led to a significant downregulation of the EpCAM expression levels when GNPs were combined with an EpCAM antibody, highlighting the importance of a targeted therapy.

Moreover, GNPs can also be considered as drugs because of their intrinsic therapeutic properties. Indeed, their anti-inflammatory and antiangiogenic properties make them ideal drugs for ocular inflammations [196] and ocular NV [197], which is the primary cause of visual deficiency in industrialized countries.

5.3 Other applications of nanotechnology for ODD:

5.3.1 Soft Contact lenses (SCLs)

Besides their application as corrective lenses, SCLs may serve as ODD system for the anterior segment of the eye [198, 199], or as protective device (bandage lenses) for the treatment of corneal injuries. Although the use of SCLs as DDS is relatively an old idea there are currently no approved products on the market. However it is still an interesting and challenging approach. The main concept is that the SCL acts as drug reservoir increasing the contact time of the drug and thus its bioavailability. Nevertheless, these systems cannot sustain drug release beyond 1 day and require high levels of drug to ensure adequate loading, which can lead to an unwanted burst release. In order to overcome this problem researchers examined the use of nanotechnology. They developed two different aproaches. The first is soft contact lenses loaded with drug-loaded nanoparticles and the second one is molecular imprinted soft contact lenses (molecular imprinted polymer technology – MIP).

The first technique uses known nanosystems such as polymers, liposomes, micelles and microemulsions to capture the drug and then disperse the DDS in the material which constitutes the contact lens. Other techniques include piggybacking of drug–polymer

films on SCL, and the use of ionic ligands. Also using variables such as temperature or pH the engineered particles may undergo reactions leading to the release of their content only in the ocular milieu, avoiding the prior issues.

Jung *et al.* [200] designed temperature-sensitive contact lenses to induce ophthalmic drug delivery. In particular, they prepared **TM**-loaded polymeric NPs and dispersed them in polymer gels. *In vitro* release experiments evidenced that NPs loaded gels can be used as contact lenses that are able to release the drug upon insertion in the eye with minimal impact on other physical properties.

Ho-Joong *et al.* produced lysozyme-degredable, **TM** loaded nanodiamonds (ND)-nanogels. The ND-embedded contact lens had great mechanical properties and appropriate water content values without significant change in optical clarity. After triggering by lysozyme, the ND-nanogels lenses released cumulative drug of 9.5 µg over 24 h [201]. Behl *et al.* produced **DEX**-laden CH NPs incorporated HEMA contact lenses. The obtained HEMA contact lens showed approximately 56% drug release in 22 days, with 10% initial burst and 98% transmittance relative to the blank contact lens without the NPs [203].

Besides the above mentioned drug-laden NPs that can be dispersed into contact lenses, pure NPs without drug can also be embedded into contact lenses. **Silver** NPs demonstrate good anti-bacterial activity through enzyme inactivation and interference of DNA replication, and show an anti-bacterial effect when loaded within contact lenses as well [203].

Several studies also have been done concerning the use of liposomes loaded SCLs. In addition to liposomes embedded in the lens matrix, liposomes can be also immobilized on the contact lens surface for drug loading. This is beneficial, as liposomes on the lens surface have been shown to improve tear-film stability and lipid-layer thickness, without affecting visual ability and lipid deposition of the contact lens surface [204].

Micelles and microemulsions are the two others kind of NPs that have been used in similar studies. Polymer micelles can be used to extend the duration of drugs and to maintain the desired physical properties of the contact lens. HEMA hydrogels containing **CsA** polymer micelles achieved drug release for more than 60 days, without compromising the mechanical and optical properties [205]. Microemulsions have advantages for ocular delivery such as their simple preparation and sterilization. The

release kinetics of drugs from contact lenses correlate with the stability of microemulsions.

The second technique, MIP technology, is an approach for loading drug templates into contact lenses, by creating cavities in the contact lens that have a high affinity for drug molecules during the polymerization reaction. After production, drugs can then be reloaded into the lens through soaking due to the high affinity cavities. The molecular imprinting process is presented in Figure 7. The composition of the imprinted contact lens plays a key role, as the functional monomer and backbone monomer determines the polymeric matrix and the cavity affinity for the template molecules.

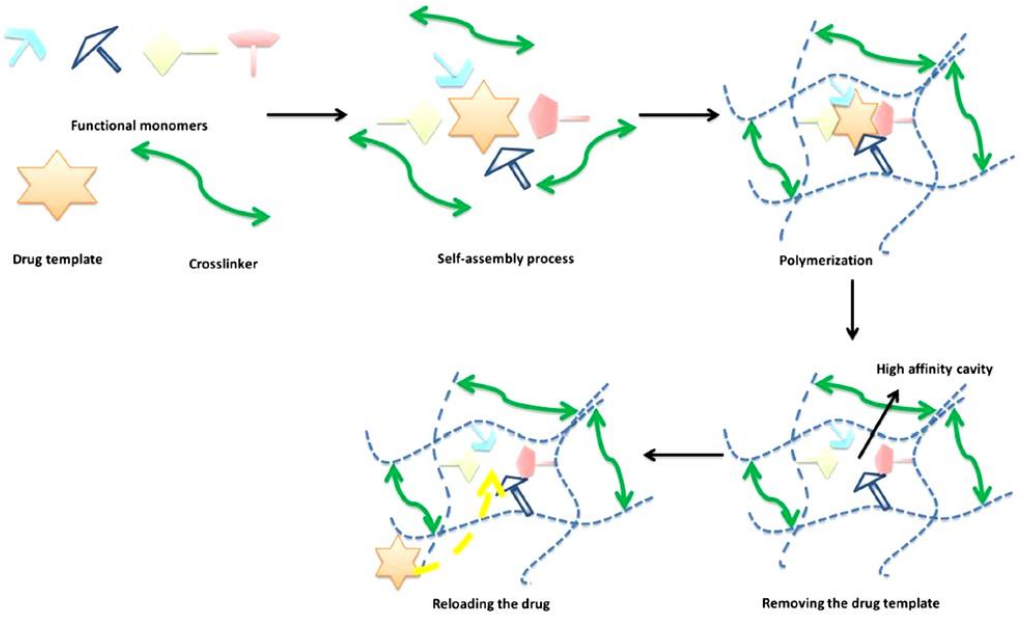

Figure 7: Molecular imprinting process (*Xu J et al., J. of Contr. Release, 2018; 281: 97–118).*

Studies that used MIP contact lenses technology for delivery of **TM** [206] or **ketotifen fumarate** revealed a 1.6-times higher capacity to encapsulate the drug and also a higher bioavailability in comparison to the usual process of soaking lenses or even to commonly-used eye drops. The relation between the parameter of bioavailability between the MIP lenses and the non-printed lenses was 3-fold higher for the first referred. Such results clearly demonstrate the capacity of the drug-stamped lenses to be much more effective in the delivery of drug to the desired tissue in relation to the other two referred formulations.

The largest drawback to contact lenses for drug delivery is the potential for low patient compliance rates, estimated to be as low as 53 % for replacement of lenses and

45 % for proper handling. This tendency could be problematic for drug delivery applications, as lenses would need to be changed at the appropriate intervals to ensure that therapeutic drug levels are being delivered. Additionally, improper handling and poor compliance could lead to additional complications such as contact lens-related dry eye and ocular surface infections. Also another disadvantage of NPs-delivered contact lenses that need to be overcome is the natural aggregation of the NPs that cause a decrease in transparency, and ubiquitous burst release caused by drug diffusion from the NPs during storage periods.

5.3.2 Micro devices and Microelectromechanical systems (MEMS) for ODD

The ultimate objective of intraocular drug delivery is to deliver drugs more than two years with improved ocular drug bioavailability, minimal complications, and maximal patient compliance. These unmet needs can be fulfilled by microtechnology which enables miniaturization of biocompatible materials and integration of active control components. Thus, microtechnology-based intraocular drug delivery systems, such as minimally invasive microneedle systems, MEMS-based drug delivery systems with on-demand drug release, and advanced bioresponsive polymeric devices for self-regulated drug delivery, are actively researched. Design factors for intraocular drug delivery systems include volume of the implants, site of implantation, materials (biodegradability), and actuation methods (passive or active), and reliability.

5.3.2.1 Ocular implants and inserts

Ocular implants are devices prepared using biodegradable or non-biodegradable materials and need to be surgically inserted in the vitreal compartment. Implants provide several advantages over conventional ocular drug delivery systems in that they reduce potential for systemic side effects, avoid restrictive blood-ocular barrier, and provide a sustained release of medications. This makes them immensely useful for use in chronic diseases such as uveitis, cytomegalovirus retinitis, and ARMD.

Ocular inserts are intended to conjunctiva. They are solid and semisolid polymeric mass that are placed in the lower fornix (cul-de-sac) of the eye. Ocular inserts are used to overcome the problems related to the conventional eye drops as mentioned before. They are sterile solid devices made either of thin discs or cylinders of variable size and shape

composed of polymer which may contain drug or may be devoid of it. The drug can be later included into the polymeric material as a solution or dispersion.

Based on the physicochemical property of solubility, the inserts are classified as insoluble, soluble and bioerodible. The insoluble ocular inserts can further be divided into groups such as diffusion system, osmosis system and contact lenses. Soluble inserts are classified based on the type of polymer. Bioerodible inserts can be categorized as insoluble ocular inserts or soluble ocular inserts.

Anterior segment ocular implants/inserts

In a remarkable study Di Trani *et al.* [207], introduced a new intraocularly implantable device called the nanofluidic Vitreal System for Therapeutic Administration (**nViSTA®**, figure 8) for continuous and controlled drug release based on a nanochannel membrane (which measures from 2.5 to 250 nm) that obviates the need for pumps or actuation. In vitro release analysis demonstrated that this device achieves sustained release of **bimatoprost** (BIM) and **DEX** at concentrations within clinically relevant therapeutic window. In this proof of concept study, the investigators constructed an anatomically similar *in silico* human eye model to simulate DEX release from the implant and gain insight into intraocular pharmacokinetics profile. The DEX-loaded nViSTA maintained a constant release of approximately 20 µg over 20 days, while BIM demonstrated a burst of approximately 40 µg over the first 2 days then a linear release of 37 µg over the next 18 days. The release mechanism of the nViSTA is drug-agnostic and can be leveraged as potentially viable platform for long-term treatment of various chronic ocular diseases.

Fulgêncio *et al.* [208] developed a CH-coated **TM** hydrogel mucoadhesive film for the treatment of glaucoma. In vitro study showed that 85% of TM was released from the mucoadhesive films in 2 weeks, and the total content was released within 4 weeks. IOP-lowering efficacy of the 0.5% TM commercial ophthalmic solution was similar to the films. However, animals that received TM-loaded CH films kept their IOP at lower levels over a 10-week period, while the effect of the TM commercial eye drops group was maintained for 12 days.

Another notable IOP-lowering device, a fornix insert, developed by Jung *et al.* [209] studied the continuous release of **TM** *in vitro*. TM was crosslinked to propoxylated glyceryl triacrylate NPs by an ester bond. The NPs were then mixed with hydroxylethyl

methacrylate, and the extended release of TM could therefore be dispensed over a range of 10–30 days by adjusting the fraction of the particles in the insert or the amount of drug loaded in the insert.

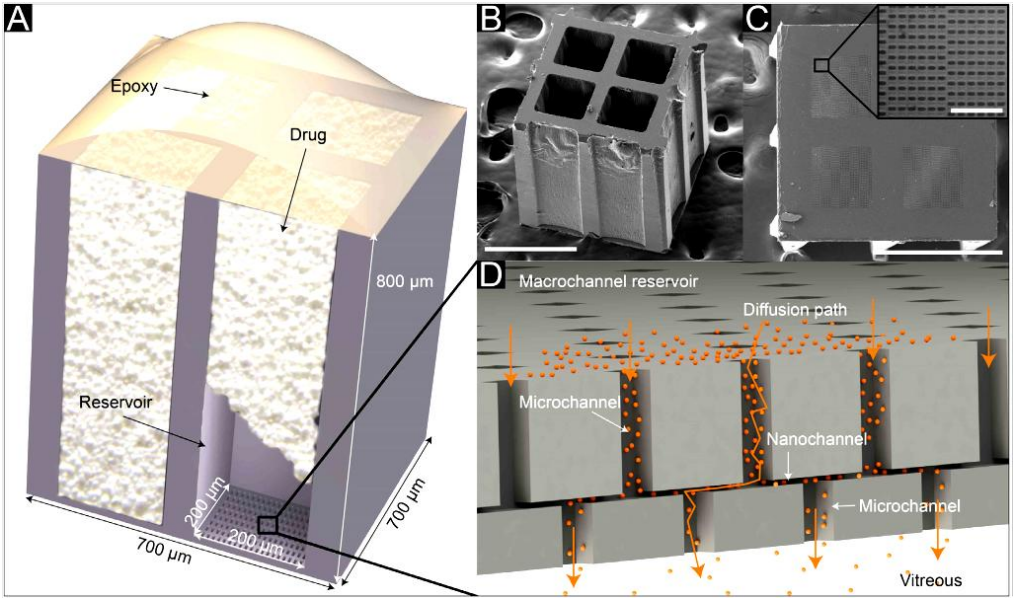

Figure 8: nViSTA®: (A) Rendering of the device showing dimensions and device compartments. The drug is loaded in the macrochannel reservoirs then sealed with epoxy. (B) Scanning electron microscopy (SEM) of diced 20 nm nanochannel 2 × 2 silicon membrane. (C) SEM bottom view of membrane showing microchannels. Scale bars in SEM images represent 400 µm. Scale bar in inset is 25 µm. (D) Side view rendering of microchannels showing the nanochannelorientation and drug diffusion path (not to scale) [207].

Also Rathod *et al*. [210], dedscribed Eudragit® NPs of **ACZ** incorporated into an ocular insert. Ex vivo transcorneal permeation study showed that ACZ-loaded Eudragit NPs displayed better permeability and flow across corneal tissue than the drug suspension. In vivo studies demonstrated substantial IOP lowering and improved ocular tolerability of ACZ inserts when compared to ACZ suspension.

In another study Franca *et al.* [211], prepared CH **BIM**-loaded hydrogel inserts. Biodistribution studies showed that a higher amount of 99mTc-BIM remained in the eye after CH insert implantation compared to eye drop instillation. BIM-loaded inserts were able to lower IOP for 4 weeks after one application, whereas marketed eye drop could only lower IOP for 1 day.

De Souza *et al.* [212] recently developed a mucoadhesive insert of CH polymer for the sustained release treatment of glaucoma by incorporating **BT**. CH polymer was able to adhere on to the conjunctival area and it was mucoadhesive in nature. The cell study showed that insert had no burst release of the drug and was biocompatible in nature.

Inserts showed remarkable *in-vivo* results in lowering IOP by providing sustained release of drug up to 30 days.

Posterior segment ocular implants

Particle Replication in Non-wetting Templates **(PRINT®**, figure 9**)** is a new very promising technology from Envisia Therapeutics (nowadays acquired by Aerie Pharmaceuticals Inc.) established to rationally design and manufacture micro- and NP systems. Unlike self-assembled particle systems or harsh micronization techniques, the PRINT® technology offers the unique ability to rationally design precise particles of virtually any size, shape and chemistry. Because each particle is manufactured with precision, the PRINT particles and implants have been designed to have unparalleled lot-to-lot and dose-to-dose consistency. Envisia has developed the ENV515 and ENV705 products for delivering prostaglandin analogues (GLA) and anti-VEGF agents (ARMD) respectively with good results. [213, 214, 215]

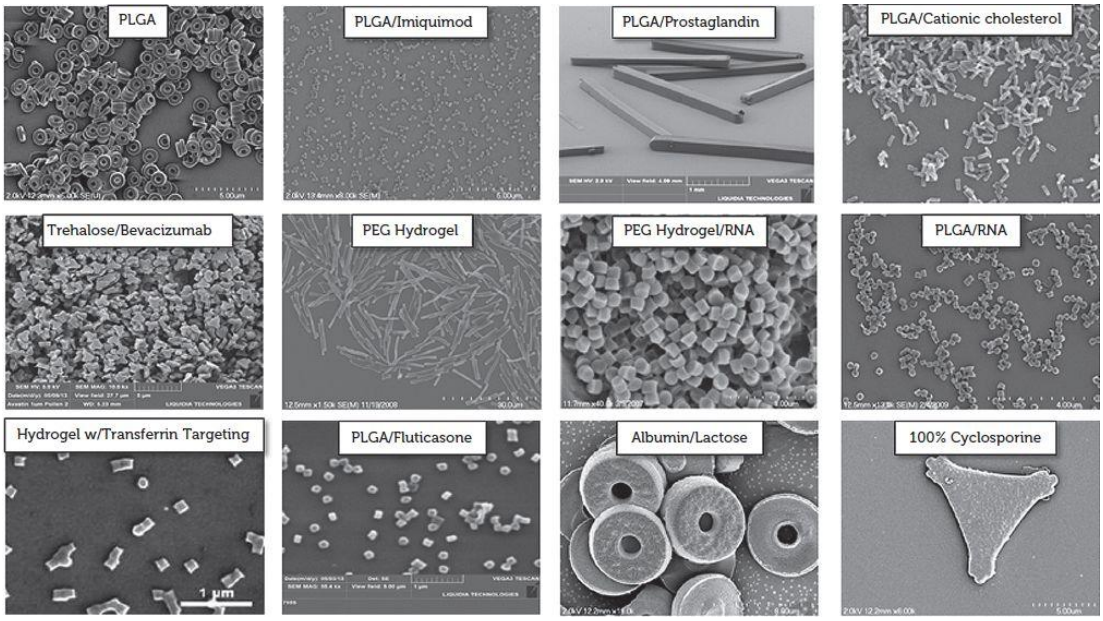

Figure 9: PRINT® technology is compatible with small molecules and biologics and allows for precise control of size, shape and chemistry [213].

Instead of providing cytokines/neurotrophic factors to ocular cells, scientists are developing systems that incorporates cells within it which can secret those bio-chemicals. NT501 (Renexus®, Neurotech Pharmaceuticals, figure 10) and NT503 are **Encapsulated Cell Therapy (ECT)** based IVT implants made of 1.5mm long AN69 copolymer

(polyacrylonitrile-methalylsulfonate) microcapsules loaded with genetically modified cell line that secretes ciliary neurotrophic factor (CNTF) and soluble VEGF receptor. The implanted cells survived for at least 90 days and their IVT release of cellular factors induced a delay of photoreceptor cells degeneration in the treated eyes. The procedure was associated with changes of the retinal architecture in a few eyes. However, no rejections or factor induced tumor formation were observed in any of the treated eyes. ECT device made of polyether sulfone (PES, N99%), caprolactame (plasticizer) and polyvinyl-pyrrolidone (antifouling) was developed and then fixed in the vitreous cavity by a titanium anchor [216, 217]. Neurotech Pharmaceuticals has received Orphan Drug designation for NT-501 ECT for the treatment of Macular Telangiectasia II from the FDA.

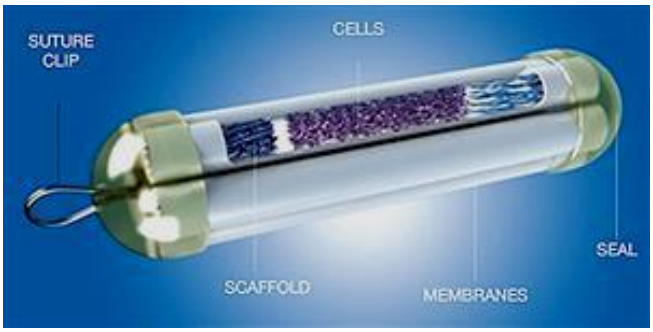

Figure 10: Renexus® device, Neurotech Pharmaceuticals

5.3.2.2 Minimally invasive Microneedles

Microneedles are solid or hollow needles of micron measurements. As drug-delivery technology was originally used for overcoming the stratum corneum and was used for transdermal drug delivery [218]. In case of transdermal delivery, they form very small but numerous pores in the skin through which absorption/penetration of drug/microparticle/NPs increases giving higher bioavailability without any wound or foreign body response. The effectiveness of microneedles for transdermal drug-delivery systems inspired researchers to investigate their potential to treat anterior and posterior segment ocular disorders. This minimally invasive technique can also be applied for ODD of hydrophilic and hydrophobic drugs.

Solid stainlesssteel microneedles (MNs) coated with drugs such as **sunitinib malate** and **pilocarpine** resulted in higher drug bioavailability in the anterior ocular segment compared with topical drop applications in vivo [219, 220]. Microneedles can also be used to deliver therapeutic agents for the posterior ocular segment. Microneedle NP and MP

suspension can be delivered to the suprachoroidal space. Than *et al*. [221] have shown a polymeric eye patch consisting of an array of detachable and biodegradable MN for controlled and localized ODD. These MN could penetrate into the corneal layers and deliver antiangiogenic monoclonal antibody (DC101) for the treatment of CNV. The MNs were double layered with DC101 to provide biphasic drug-release kinetics to enhance the therapeutic efficacy of the MNs. The DC101 MN eye patch produced approximately 90% reduction in CNV in a CNV disease mouse model as compared with a topical eye drop. The researchers also suggested that the MN patch is minimally invasive and can be self-applied by patients on their corneas.

Thakur *et al.* [222] developed a dissolving polymeric MN based on a (PVP)/hydrogel formulation. Drug-loaded PVP rapidly dissolved in biological tissues due to its highwater solubility. In addition to the fact that no additional surgery is required for the MN system, dissolvable polymeric MN systems offer an advantage of enhanced safety; a broken small part of the rigid MNs during implantation could lead to a serious complication (figure 11).

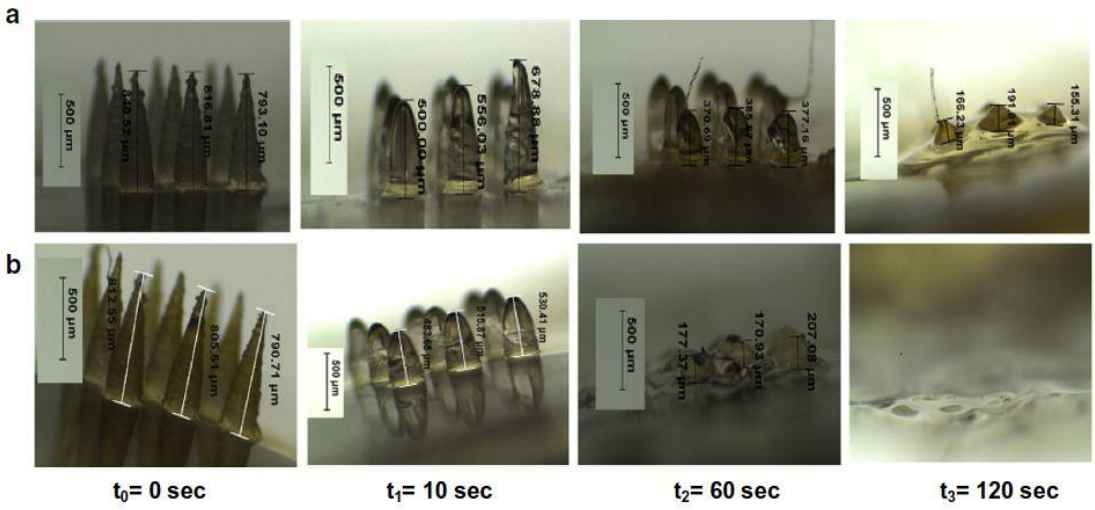

Figure 11: 7 Representative digital images of PVP MN arrays before and after insertion into porcine **a** the corneal and **b** the scleral tissues for predefined times; t_0 = 0 s, t_1 = 10 s, t_2 = 60 s, t_3 = 120 s [222].

Use of MN in the suprachoroidal space or scleral region allows easy penetration of drug/MP/NP into the above. As suprachoroidal space is empty space between sclera and choroid, it acts as reservoir from where drug releases slowly to choroid/retina. There are investigations going on human cadaver eye, and rabbit eye showing their higher effectiveness in terms of tolerance, compliance and bioavailability. These studies show that delivery of drug/nanotechnology based DDS with the help of hollow and/or coated

MN are very promising [223]. It is minimally invasive and effective technique for delivering drugs also to posterior segment [224, 225].

MN can greatly aid in increasing the bioavailability of a certain drug in a particular tissue by localizing the DDS. MN can be a paradigm shift for the way ocular formulations are administered, but their current limitations demand further research in the field for desired clinical translation.

5.3.2.3 Nanowafers

Nanowafers are small, transparent, rectangular membranes or circular discs containing drug loaded into nanoreservoirs which can be smeared on the ocular surface using a fingertip. Controlled drug release from the nanowafer can increase the residence and contact time of the drug with the corneal and conjunctival surfaces. This can aid in higher drug absorption into anterior ocular tissues. The nanowafer not only enhances the drug bioavailability but also acts as a protective polymer membrane to heal injured and abraded corneal surfaces commonly found in corneal NV and DED. This novel nanocarrier is designed from biodegradable and biocompatible polymers which can be eliminated over a period of time.

Coursey *et al.* [226] and Bian *et al.* [227] developed a **DEX**-loaded nanowafer (Dex-NW) for the treatment of DED. The nanowafer was fabricated using carboxymethyl cellulose polymer and consisted of an array of nano drug reservoirs filled with DEX. The in vivo efficacy of Dex-NW was tested in a dry eye disease mouse model. Dex-NW was administered as once-a-day treatment on alternating days for a 5-day period of time. After the treatment duration, it was observed that Dex-NW was able to restore the corneal barrier function along with a healthy ocular surface which was similar to twice-a-day treatment of topically applied DEX eye drops. Yet another interesting finding the scientists reported was that Dex-NW was effective in lowering the overexpression of inflammatory cytokines such as tumor necrosis factor-a, interferon-g, interleukin-1b, and interleukin-6. Also, the expression of inflammatory chemokines such as CXCL-10, CCL-5, and MMP-3 and MMP-9 was lowered.

Axitinib (a tyrosine kinase inhibitor used as anti-VEGF agent, Pfizer Inc.) loaded NW were developed by Yuan *et al*. [228] for the treatment of CNV. A murine ocular burn model was used to evaluate the in vivo efficacy of axitinib-loaded NW. The laser-scanning

confocal imaging and reverse-transcription PCR results revealed that the once-a-day axitinib-loaded nanowafer was twice as effective as compared with axitinib daily topical eye drops. These findings have shown the potential of NW for further evaluation in clinical trials.

5.3.2.4 MEMS

Micropump™ (Replenish Inc., Pasadena, CA) is a small, refillable, non-biodegradable implantable microelectro-mechanical system (MEMS) which is placed in the suprachoroidal space to dispense nanolitre sized doses in predefined rate every hour, day or month. It was proved safe and functional till 12 months in small animals [229]. It is refillable, can be replenished when needed with the help of disposable proprietary 31 gauge needles. The Drug Refill System™ (figure 12) is a separate console unit used to fill and refill these with drug. It eliminates the need of frequent surgery for replacement and refilling [230].

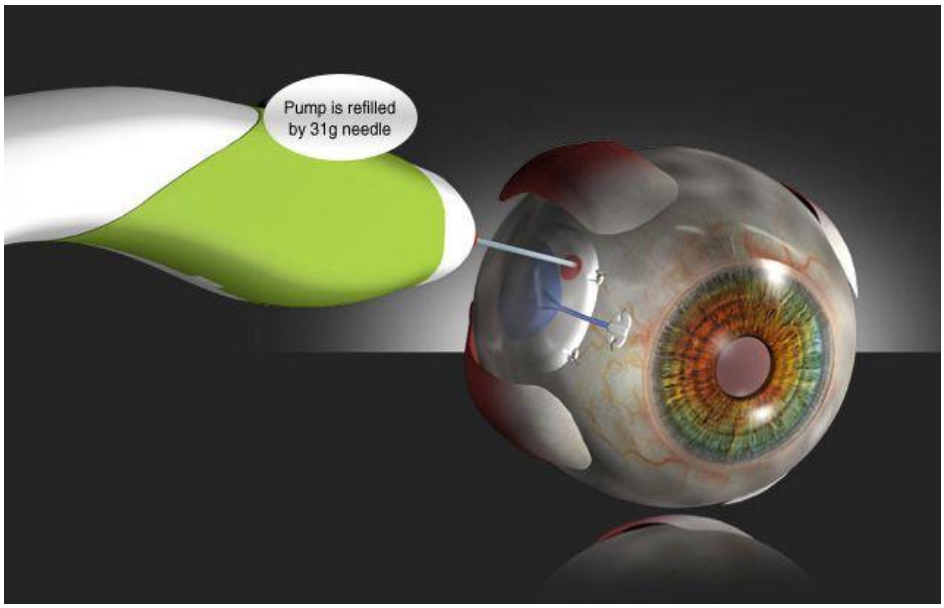

Figure 12: Schematic representation of the MicroPump™ implanted at the pars plana position with the Drug Refill System™. The Replenish MicroPump™ System is for investigational use only and is not yet approved for commercial distribution *(Replenish Inc., Pasadena, CA).*

Song *et al.* [231] developed a triboelectric nanogenerator which harvested energy from human body movements and interfaced the nanogenerator to an electrochemical pressure pump embedded in an implantable intraocular DDS. A PDMS microtube was implanted at the sclera and allowed for successful delivery of 50 µL of microparticles to

the anterior segment of porcine eyes *ex vivo*. However, because the self-powered triboelectric nanogenerator must be attached to the body with largest movements and because it does not generate high enough power for wireless transmission, this unit was connected to the implant through long wiring. For clinical applications, a more practical solution for the connection should be proposed.

A very new but less explored approach is use of magnetic field for drug delivery to posterior segment. Pirmoradi *et al.* [232] showed that a device basically MEMS, consisting of reservoir system of drug (docetaxel), polymer (EVA) with small magnetic beads is placed in the targeted site and external magnetic field is applied which causes movement in magnetic beads leading to breakdown of polymer, intake of solvent/surrounding media, swelling of polymer and then release of drug from reservoir. The limitation of this system is rapid depletion of the magnetic response of the small magnets and thus chronic functionality must be further investigated. In addition, magnetic actuation is not compatible with two major medical imaging modalities: CT and MRI. This system was biocompatible and quite effective but needs further investigation.

6. Nanotechnology augmented Diagnosis and imaging of ocular diseases

6.1 Imaging

A number of diagnostic imaging applications for the eye are anticipated based on advances in nanotechnology and nanomedicine. Light signals from the NPs and changes in emission properties (e.g. fluorescence lifetime) as a function of the environment are useful measures that can be used to non-invasively assess biomarkers of disease [233].

One imaging based application is **polychromatic angiography**. Current diagnostic imaging for detection of retinal vascular leakage and NV is conducted by intravenously administering a fluorescent molecule, fluorescein, (Fluorescein angiography, FA) which permeates across the blood retinal barrier in diseased eye, but not in a normal healthy eye. This diagnostic technique cannot differentiate early from late stage disease and is an “all or none” diagnostic of neovascular diseases. Therefore, a method that can distinguish early from late stage vascular diseases would allow for a better assessment of disease progression, improve ability to choose appropriate treatment and dose, and allow for monitoring effects of treatment and dose. Tari *et al.* [234] propose a polychromatic

angiography diagnostic that will be able to distinguish early from moderate and late stages of disease and hence, offers potentially several advantages in the treatment and management of retinal vascular diseases. The idea is that small particles extravasate through leaky vasculature or compromised blood barriers in early stage disease, but not larger particles. However, in late stage disease, large and small particles alike will leak through vasculature or permeate across blood barriers and colocalize in the tissue. If the small particles contain a different colored dye than large particles, then the color can be tracked to monitor the stage of disease. The detection of both large and small particles indicates severe breakdown or dysfunction of blood barriers, whereas the detection of only small particles indicates that lesser breakdown or dysfunction exists.

Polychromatic angiography can also be applied to detect vasculature blockage; for instance, both large and small particles will travel through normal vessels, but when blockage occurs, only the small particles or no particles will travel across the blockage. Therefore, immediately after the blockage either no fluorescence or only fluorescence from small particles will be detected and fluorescence of large and small particles will be detected before the blockage.

Other inorganic NPs made of noble metals, such as gold or silver are generating much interest due to their small size, about 20 nm, and their great potential of crossing the blood-retina barrier. Also functionalized NPs could be bound to specific target. The NP coat creates enhanced signaling by producing additional contrast, allowing identification of isolated defects. With this technology, neuroectodermal cells can be imaged using iron oxide NPs. Functionalization of iron oxide NPs with PEG, chlorotoxin and the near-infrared fluorescing molecule Cy5.5 enables high-affinity binding to and endocytosis by neuroectoderm-derived cells [235]. Enhanced visualization via magnetic resonance imaging and confocal fluorescence microscopy was observed. Thus, detection of RPE pathologies or location of choroidal or optic nerve invasion by retinoblastoma may be facilitated using this imaging technology. It is also interesting to investigate long-term migration pathways of these iron oxide nanoparticles, as compared to the DNA NP.

NPs also offer a unique imaging modality that could further elucidate our understanding of biological pathways, for example via fluorescence imaging in animal models. Tam *et al.* [236] used Qdots, such as CdSe/ZnS core/shell NPs, to trace live images of lymphatic drainage from mouse eyes. Qdots, unlike conventional methods for

observing the lymphatic network, are ideal for non-invasive in vivo imaging due to their broad excitation spectra, narrow and tunable emission spectra and high photobleaching threshold. By changing the diameter of the Qdots, the emission spectra may increase or decrease appropriately. This allows for a level of modification to adapt to the Qdots in different situations. In this mouse study, the Qdots were found to show lymphatic drainage from the skin, peritoneal and pleural spaces. They also enabled the visualisation of a newly described lymphatic drainage route for fluid exiting the eye.

Bioinert GNPs of various shapes and functionalities are widely accepted as contrast agents (CAs) for several modalities of imaging, electron microscopy, CT, X-ray and photoacoustic (PA) imaging. Sreejith *et al.* [237] were successfully demonstrated gold nanocages (AuNcgs) for high-contrast PA ocular imaging for the first time. PA and ultrasound (US) images were acquired using a commercial US imaging scanner integrated with a tunable nanosecond pulsed laser. This integrated hybrid-modality system is a combined PA and US platform for imaging which enabled acquiring of complementary structural and optical absorption-based information simultaneously. Initial experiments were conducted using tubings filled with solutions of different concentrations of quickly synthesized AuNcgs. Biological PA and US imagings were demonstrated using enucleated porcine eye samples. Based on the acquired results, it is envisaged that AuNcgs can be employed as a high strength PA CA to potentially diagnose ocular disease like uveal melanoma in the near future.

Interestingly a recent study from Nguyen *et al.* [238] showed that multimodal imaging with photoacoustic microscopy (PAM) and optical coherence tomography (OCT) can be an effective method to evaluate the choroidal and retinal microvasculature. To improve the efficiency for visualizing capillaries, colloidal GNPs have been applied as a multimodal contrast agent for both OCT and PAM by taking advantage of the strong optical scattering and the strong optical absorption of GNPs due to their surface plasmon resonance. Ultrapure GNPs were fabricated by femtosecond laser ablation, capped with PEG, and administered to 13 New Zealand white rabbits and 3 Dutch Belted pigmented rabbits. The synthesized PEG-GNPs (20.0 ± 1.5 nm) were demonstrated to be excellent contrast agents for PAM and OCT, and do not demonstrate cytotoxicity to bovine retinal endothelial cells in cell studies. The image signal from the retinal and choroidal vessels in living rabbits was enhanced by up to 82% for PAM and up to 45% for OCT, respectively, by the administered PEG-GNPs, which enables detection of individual blood vessels by both imaging

modalities. The biodistribution study demonstrated the GNPs accumulated primarily in the liver and spleen. Histology and TUNEL staining did not indicate cell injury or death in the lung, liver, kidney, spleen, heart, or eyes up to seven days after GNPs administration. PEG-GNPs offer an efficient and safe contrast agent for multimodal ocular imaging to achieve improved characterization of microvasculature.

Nanowires are another kind of nanomaterials that could be defined as the structures possessing a diameter of less than 100 nm and the length that is not definite. Semiconductor nanowires exhibit peculiar electronic features and sizes similar to biological structures included in cellular communication. That consequently makes them suitable nanostructures for producing active communications with biological systems [239]. These techniques should be applicable to measuring functions such as electrical activity and contractility in neurons. Micro- and nano-electromechanical systems (M/NEMS)-based engineering permits the construction of small diagnostic devices.

6.2 Biosensors

A very interesting use among others of GNPs due to their special property of plasmon resonance is that of biosensors. Kim *et al.* [240] functionalized biosensing paper strips with GNPs for early detection of infectious keratoconjunctivitis. This approach was based on the surface-enhanced Raman scattering (SERS) effect [241]. Raman spectroscopy is a powerful technique allowing the identification of specific molecules. Here, the localized surface plasmon resonance of the GNPs produces the SERS effect, greatly increasing the Raman signal [242]. Human tears were analyzed and the Raman spectral data were studied through the comparison of different principal component (PC) scores. The analysis not only allowed the differentiation between normal and infectious eyes, but also the determination of the infection type (viral, bacterial, or allergic).

Verma *et al.* [243] developed a “chemical nose” based on gold nanostars for detection of deferent ocular pathogens. When bacteria were added to CTAB–gold nanostars, the absorbance tuning allowed the identification of the different species with 99% success.

In another study Wang *et al.* [244] designed a biosensor based on GNPs or gold nanorods and fluorophore-labeled locked nucleic acid probes (LNA) for dynamic gene expression profiling in native tissue microenvironments. The GNPs and gold nanorods were functionalized with specific chemical groups. This system was then tested with

mouse cornea and retina tissues and was able to follow the regulation of a single gene. This probe, which has excellent stability and low toxicity in living cells, is a promising approach for dynamic cell gene expression analysis in tissue morphogenesis and regeneration.

Interestingly, other biosensors based on gold wire or gold layer, whose properties differ from those of GNPs, were also developed for the classification of human lachrymal liquid [245] or human aqueous humor [246]. These new platforms could be developed for point-of-care diagnosis of ocular pathologies.

Also other sensors are being developed, that allow direct identification on the molecular-level, for therapeutic purposes. An excellent example of this has been worked on for directly detecting retinoblastoma (RB) [247]. RB has been linked to the inactivation of the RB gene (Rb), which is a tumor suppressor gene. The promoter region of the Rb gene is methylated in a significant number of primary RB tumors. A direct electrochemical detection of DNA methylation for RB has been devised using a 40-nm-thick nanocarbon film electrode that has a nanocrystalline sp2 and sp3 hybrid structure, providing a wide potential window, low background current, little surface fouling, and high electrode activity compared with boron-doped diamond and glassy carbon electrodes. Through these unique properties of nanocarbon, it is possible to detect biomolecules with slower electron transfer rates, such as nicotinamide adenine dinucleotide phosphate and pyrimidine bases, allowing direct detection of single-nucleotide polymorphisms and consequently, the oxidization of all DNA bases individually, enabling the identification of DNA bases and their derivatives [247, 248] These nanocarbon films have been used to characterize DNA methylation directly from a 6mer oligonucleotide without any treatment, and 24mer oligomers containing methylation sites for RB (RB1) gene fragments and the CpG (cytosinephosphoguanosine) repeat sequences (60mers) could be detected using nanocarbon film electrodes. Moreover, electrochemical experiments using the nanocarbon film electrode provided quantitative detection of DNA methylation ratios solely by measuring methylated 5’-CpG repetition oligonucleotides (60mers) with different methylation ratios [249].

The process of coupling superparamagnetic iron oxide (SPIO) NPs or Qdots to antibodies that identify molecules as the elements of the complement system or molecular constituents connected with ARMD caused alterations in Bruch membrane.

That could serve as a vehicle to image the biochemical and/or structural changes accompanying ARMD [250]. This ability might be essential for the improvement of ARMD treatments that are aimed at early molecular alterations and early stages of the disease.

7. Nanotechnology augmented Diagnosis and Therapy of ocular diseases (Theranostics)

Theragnostics refers to a process in which diagnosis of a disease state, individualized for a particular patient (even to particular cells within a patient), is coupled with therapy that is targeted precisely in its nature, amount, and location.

Prow et al. [251] have developed a biosensor DNA tethered to a magnetic NP. The biosensor is based on an enhanced green fluorescent protein (EGFP) reporter gene driven by an antioxidant response element (ARE), which normally is activated by oxidative stress and enhances the expression of genes downstream in its sequence. This engineered NP penetrates endothelial cells (preferentially dividing cells), and exposure of the cells to hyperoxia drives the expression of EGFP. After subretinal injection, these biosensor NPs report the activation of the ARE in diabetic rat RPE. The antioxidant biosensor could provide a means for clinicians to identify patients likely to need therapy (e.g., babies with retinopathy of prematurity who will need treatment) at a time before clinical manifestations of severe disease are evident. By coupling a therapeutic gene (e.g., catalase, peroxidase, superoxide dismutase) to the ARE (in addition to a reporter gene such as EGFP), one creates a combined diagnostic–therapeutic device that enables endothelial cells (or any cell that takes up the NP) to ‘‘treat themselves’’ in the setting of oxidative damage.

Concerning the polychromatic angiography that was described earlier, it could also potentially serve as a therapeutic means wherein drugs are loaded into the particles and are delivered to the damaged tissue. Different therapeutics can be loaded into the particles such that small particles will contain a different therapeutic than larger particles in order to specialize therapy for each stage of disease or blood vessel type and hence, provide more appropriate and effective treatment for the disease.

As mentioned in previous paragraphs ocular NV can result in devastating diseases that lead to marked vision impairment and eventual visual loss. In clinical implementation,

neovascular eye diseases are first diagnosed by fluorescein angiography and then treated by multiple intravitreal injections, which nevertheless involves vision-threatening complications, as well as lack of real-time monitoring disease progression and timely assessment of therapeutic outcomes. To address this critical issue Tang *et al.* [252] introduced a peptide-functionalized NP SiNPs-RGD as a new kind of theranostic probe for the combined imaging and treatment of ocular NV. In addition to negligible toxicity and high specific binding ability to human retinal microvascular endothelial cells tube formation, the SiNPs-RGD features efficacious antiangiogenic ability in wound healing migration, transwell migration, transwell invasion, and tube formation assays. Taking advantage of these unique merits, the as-prepared SiNPs-RGD is further employed for concurrent angiogenic blood vessels imaging and treatment of corneal NV. These results indicate that the photostable and biocompatible SiNPs-based theranostic probes are highly promising for simultaneous diagnosis and treatment of NV eye diseases.

Another interesting approach against oxidative stress was introduced by Chen *et al.* [253]. It is known as a fundamental property of nanomaterials that their surface area/volume ratio is relatively high. Vacancy-engineered, mixed-valence state cerium oxide (CeO_2) NPs (‘‘nanoceria’’ particles) illustrate the biological utility of this property. Alteration in the oxidation state of CeO2 creates defects in its lattice structure through loss of oxygen or its electrons. As their size decreases, nanoceria particles demonstrate formation of more oxygen vacancies in the crystal structure. Engineered nanoceria particles can scavenge reactive oxygen intermediates because the large surface area/volume ratio at 5 nm diameter enables CeO_2 to regenerate its activity and thereby act catalytically. Unlike nanoceria particles, most free-radical scavengers require repetitive dosing. Chen demonstrated that intravitreal injection of nanoceria particles prevents light-induced photoreceptor damage in rodents, even if injected after the initiation of light damage. Vacancy-engineered nanoceria particles may function as a highly effective treatment for conditions associated with oxidative damage, such as age-related macular degeneration (AMD) and diabetic retinopathy.

In a similar study Yurui *et al.* [254] was used a kind of autoregenerative redox CeO_2 NPs coated with PEG-PLGA (PCNPs) against oxidative damage correlated with diabetic cataract. They found that PCNPs could work not only as an antioxidant to protect lens epithelial cells from oxidative stress based on the repetitive elimination of reactive

oxygen species (ROS), but also as a glycation inhibitor effectively restraining α-crystallin glycation and crosslinking, thereby keeping the lens transparent and alleviating DCs. Experimental results successfully validated the fact that the PCNPs were able to operate in eyes for a long time to attenuate lens opacity expecting that this strategy will provide promising potential for the treatment of DCs.

7.1 Photodynamic therapy (phototherapy)

Photodynamic therapy (PDT) is a form of phototherapy involving light and a photosensitizing chemical substance, used in conjunction with molecular oxygen to elicit cell death (phototoxicity). It used mainly in cancer therapy. NPs have unique advantages for PDT as they can act as a transducer for converting light with deep penetration ability into light within visible wavelength. It can also be used to carry photosensitizers for treating tumors. It has been reported that the use photosensitizer loaded-magnetic NPs and/or polyethyleneimine are novel attempts to improve PDT to treat uveal melanoma. As single excitation wavelength has multicolor-emission capability, multifunctional nanoplatform as potential dual carrier system has been developed.

Makky *et al.* [255] have developed prophyrin-based glycodendrimers with the mannose-specific ligand protein concanavalin A conjugated on to their surface, to specifically target the tumor cells in the retina. These hybrid dendrimers are designed as photosensitizers for preferential accumulation in malignant ocular tissue, for enhancing the effectiveness of PDT. The mannosylated dendrimers demonstrated specific interactions with the receptors in the lipid bilayer inducing protein channel rearrangement favoring the entry of the dendrimers into the cell. Wang *et al.* [256] synthesized α-mannosyl dendrimeric porphyrins, which exhibited good photo efficiency, superior cellular uptake, and significant photo toxicity in retinoblastoma cells.

7.2 Brachytherapy

Brachytherapy is a form of radiotherapy where a sealed radiation source is placed inside or next to the area requiring treatment. Brachytherapy is commonly used as an effective treatment, among others tumors, also for uveal melanoma. However, as the energy absorption dose of normal and tumor tissue are quite similar, the maximum radiation dose is limited according to the normal tissue which surrounds the tumor. The

use of radiosensitizing agents may address this problem and overcome hypoxia mediated heterogeneity response of the tumor [257]. Fullerene and lipid NPs have been explored as strategies to deliver effective brachytherapy and longitudinal imaging in brain tumors. More commonly, GNPs have been applied as radiosensitizing agents due to their high atomic number and strong photoelectric absorption coefficient. Studies have shown by combining brachytherapy with GNPs, it could induce apoptosis of uveal melanoma *in vitro* and *in vivo*.

Chang *et al.* [258] revealed that GNPs can sensitize melanoma B16F10 cells to radiation and showed that the NPs can accumulate within the tumor cells. The radiation and NPs combo treatment strategy has also significantly prolonged mice survival while potently inhibiting tumor growth in a B16F10 mice model. Further evidence of apoptosis induction was found in the combination group *vs* control. Also, GNPs has additional vasculature disruption properties when combined with brachytherapy. Berbeco *et al.* [259] found that even low concentrations of GNPs have vasculature disruption effects to the tumor endothelial cells. As the NPs can target both induce apoptosis of the tumor cells and disrupt its supporting vasculature when combined with radiation, it could potentially be used to support brachytherapy for treating uveal melanoma.

8. Nanotechnology augmented monitoring of ophthalmic diseases

In addition to developing nanomaterials for drug delivery, nanotechnology has given rise to MEMS that can track biophysical measurements such as IOP non-invasively. Corneal thickness, corneal curvature and the fluctuating nature of IOP complicate Goldmann tonometer accuracy at any given moment. Consequently, a single tonometric evaluation may be inadequate to evaluate IOP risk. A minimally invasive monitor to track the changes of continuous IOP precisely over a period of time would therefore be invaluable to clinicians.

Leonardi *et al.* [260] developed a non-invasive, wireless, and soft silicone contact lens sensor (CLS) capable of detecting changes in IOP. A gauge sensor of platinum-titanium which was 170 nm thick and embedded in two layers was used. In order to power the sensor, a gold antenna, microprocessor, and an integrated circuit were fixed in the lens, this also aided in wireless communication via an external recording unit. As the stress in the gauge increased, other mechanical forces compressed and altered the electrical

forces present in the gauge. They observed that the contact lens exhibited linear and reproducible IOP in the range (15–30 mm Hg) in enucleated pig eyes. The device that Leonardi *et al.* commercialized has obtained the CE mark in Europe (SENSIMED Triggerfish; Sensimed AG) and regulatory approval in other regions, but is not FDA-approved in the USA (figure 13).

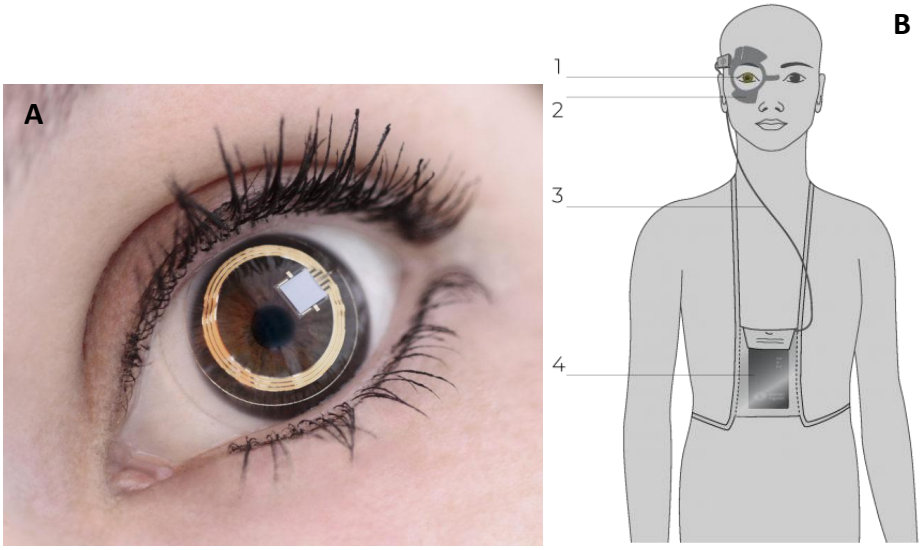

Figure 13: (**A**) Sensimed Triggerfish® mounted on the eye as contact lens, (**B**) 1: the sensor, 2: the adhesive antenna, 3: data is transmitted through a thin flexible cable 4: recorder *(Sensimed AG, Switzerland)*

A piezoresistive sensor capable of measuring intraocular pressure was engineered by Rizq *et al.* [261]. The sensor is capable of detecting changes in electrical resistance when stress is applied and had a radio frequency powering reverse telemetry as well as an interface circuit. In addition, Dresher *et al.* [262] developed a compact circuit with low power which can be fixed in a wireless intraocular pressure monitoring system. Applying the Bourdon tube technique, i.e., using a thin-walled, curved and hollow tube, other devices have been produced and are capable of measuring IOP. Chen *et al.* [263] used the Bourbon tube technique in developing a sensor that had a protective parylene membrane. Extraordinarily, the device is capable of measuring IOP without depending on an external energy source. Though these sensors have an advantage, it should be noted that using the Bourbon tube is an invasive procedure and may uncomfortable for some patients. All this research and work done serves as a good platform for wireless and implantable devices to be employed in patients with glaucoma and ocular hypertension in the future.

Also **nanoindentation** has gained a lot of interest in recent years due to its ability to determine the effective modulus and hydraulic conductivity of human ocular surface [264]. Through indentations of various regions of the ocular surface, could be design novel drug delivery models to penetrate different barriers of the eye through different mechanisms such as transscleral transmission. In this manner, nanotechnology can help for noninvasively measure the mechanical properties of soft biological tissue such as sclera, which due to its large surface and high permeability, would be an ideal route of administration for NPs.

9. Nanotechnology augmented ophthalmic surgery

Albert R. Hibbs suggested the notion of doing surgery by "swallowing the surgeon." He proposed that this nanosurgeon could travel in blood vessels, identify areas of damaged tissue, and remove or repair them. The nanosurgical ophthalmic operating theater is in its infancy. Sretavan *et al.* [265] introduced a multifunctional axon surgery platform, sized about 1 mm^3 that could repair a single axon using dielectrophoresis and electrofusion. While peripheral nerve repair may be its first clinical application, the availability of a microsurgical operating platform may render its introduction into ophthalmology more quickly than into intracranial surgery.

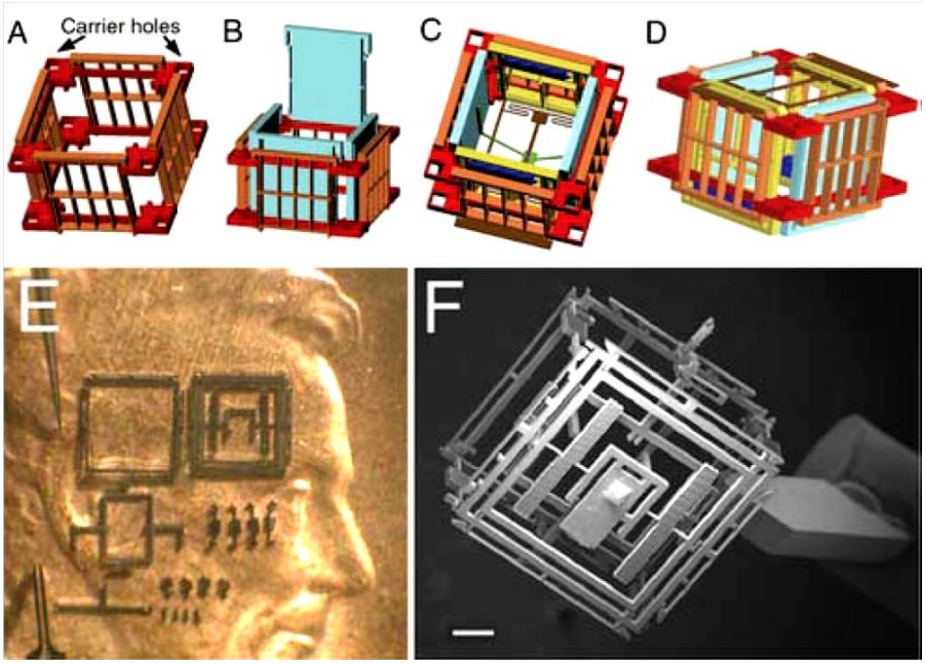

Figure 14: Diagrams showing the design and assembly of prototypemultifunctional axon surgery platform, Scale = 200 µm [265]

Kim *et al.* [266] developed nanotweezers for the interrogation and manipulation of nanostructures. Electrically conducting, mechanically robust carbon nanotubes were attached to electrodes fabricated on fine glass micropipettes. The free ends of the nanotubes came into apposition as increasing voltage was applied across the electrodes. The use of electric current to close the tweezers might cause tissue coagulation, however, and the presence of a polar fluid environment might alter the properties of the tweezers. An alternative approach, employed by Bhisitkul *et al.* [267] is the use of MEMS technology to synthesize micromechanical forceps. One can anticipate that robotic surgical manipulators would be needed to perform fine movements with true nanotweezers (humans have minimally a 50-µm tremor amplitude), and imaging systems providing very high magnification would be needed to enable the surgeon to visualize the target tissue and the instruments themselves. Nanosurgical devices will enable one to perform procedures such as internal recanalization of retinal artery and vein occlusions, dissection of complex epiretinal membranes, repair of retinal breaks (through tissue regeneration), and re-anastomosis of severed optic nerves.

Given that the main reason for the overall success decrease of glaucoma drainage devices (GDD) is the postoperative fibrosis, Ponnusamy et al. [268] successfully manufactured a film of PLGA with two layers loaded with 5-FU and MMC for sustained drug release. The film was developed to cover the surgical implant. The studies conducted *in vitro* reported MMC was only stable on the surface, so 5-FU was loaded at the bottom. 5-FU indicated to be released after three to five days continuously until day 28. After five days, the information obtained showed cell proliferation was inhibited by a COS-I cell culture model. This gives a promising use of glaucoma drainage devices in conjunction with anti-inflammation and anti-fibrosis properties.

In the same direction Shaunak *et al.* [269] created carboxylated PAMAM generation 3.5 dendrimer conjugates with D(+)-glucosamine and D(+)-glucosamine 6-sulphate to control scar tissue formation after glaucoma filtration surgery in a rabbit model. The glucosamine dendrimer inhibited pro-inflammatory chemokines and cytokines, while the glucosamine 6-sulfate dendrimer prevented fibroblast growth factor and NV. The dendrimers also increased the long-term success of the surgery from 30% to 80%.

Likewise, Ye *et al.* [270] developed cationic nanocopolymers, named CS-g-(PEI-b-mPEG), that mediate IκBkinase β (IKKβ). These NPs successfully target siRNA to reduce

scarring locally by selectively suppressing inflammation-induced nuclear factor κ-light-chain-enhancer of activated B cell activity. Rhesus monkeys underwent trabeculectomy and were randomly distributed among three types of subconjunctival injections: CS-g-(PEI-b-mPEG)/IKKβ-siRNA, mitomycin C and phosphate-buffered saline (PBS). Ye *et al.* determined that the siRNA and mitomycin C exhibited significantly prolonged bleb survival compared to the PBS group. There were no significant differences in IOP readings in the three groups after surgery. A histologic examination showed that the siRNA group had a marked reduction of subconjunctival scar tissue compared to the PBS group, and the conjunctival epithelium did not show the acellularity that was present in the mitomycin C group.

Pan *et al.* [271] engineered an artificial nano-drainage system that can be implanted in the trabeculum of the eye and guide flow of aqueous humor on the adjacent subconjunctival space. The nano-drainage implant known as ANDI is composed of an integrated polymeric shaft and a nanoporous membrane (mimicking the drainage function of the trabecular meshwork). There was clogging of proteins in the pores of the nanofiltration membrane, though the membrane was able to give the designed flow resistance. This promising nano-drainage system may be further improved by altering the surface chemistry.

A glaucoma valve that contains ferrofluid nanoparticles was developed by Paschalis *et al.* [272]. The ferrofluid was 10–100 nm in size and was magnetic in nature, had inert properties, and showed supramagnetic behavior. Until the magnetic pressure was lower than the liquid flow pressure, the ferrofluid served as a valve. Using X-ray diffraction, the device's stability was confirmed as it was not oxidized after exposure to air and water. *In vivo* studies showed an IOP decrease after two weeks in comparison with the contralateral control rabbit eye.

Maleki *et al.* [273], in order to overcome the postoperative hypotony in non-valved GDD developed a cone-shaped polymer biodegradable plug filter. The plug disappears once wound healing and bleb formation has progressed past the stage where hypotony from overfiltration may cause complications in the eye. The biodegradable nature of device eliminates the risks associated with permanent valves that may become blocked or influence the aqueous fluid flow rate in the long term. The plug-filter geometry simplifies its integration with commercial shunts. Aqueous humor outflow regulation is achieved by

controlling the diameter of a laser-drilled through-hole. The developed plug filter is 500µm long with base and apex plane diameters of 500 and 300µm, respectively, and incorporates a laser-drilled through-hole with 44-µm effective diameter in the center.

Contemporary aqueous shunts although initially effective at delaying glaucoma progression, often lead to numerous complications and only 50% of implanted devices remain functional after 5 years. In a remarkable work reported by Harake *et al.* [274] was introduced a novel micro-device which provides an innovative platform for IOP reduction in glaucoma patients. The device design features an array of parallel micro-channels to provide precision aqueous outflow resistance control (figure 14). Additionally, the device's microfluidic channels are composed of a unique combination of PEG materials in order to provide enhanced biocompatibility and resistance to problematic channel clogging from biofouling of aqueous proteins. The microfabrication process employed to produce the devices results in additional advantages such as enhanced device uniformity and increased manufacturing throughput. Surface characterization experimental results show the device's surfaces exhibit significantly less non-specific protein adsorption compared to traditional implant materials. Results of *in vitro* flow experiments verify the device's ability to provide aqueous resistance control, continuous long-term stability through 10-day protein flow testing, and safety from risk of infection due to bacterial ingression.

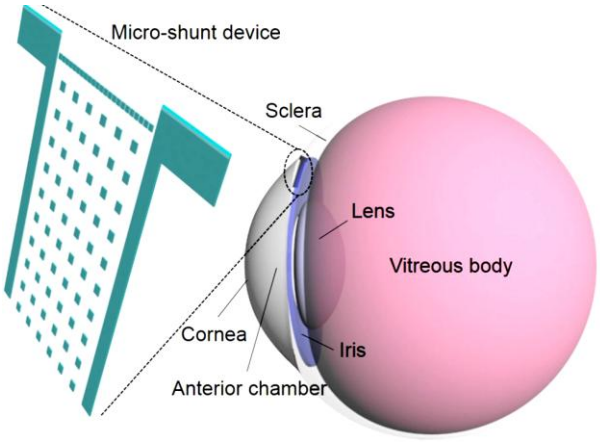

Figure 15: Illustration showing micro-shunt device concept and trans-scleral implantation placement into anterior chamber of the eye [274].

It is well known that cataract surgery often leads to a posterior capsule opacification (PCO). An intraocular lens (IOL) used for the cataract was improved by the use of gold

nanorods (GNR) and their photothermal properties [275]. Commercial IOL were functionalized with GNR previously coated with silica. After an irradiation by 808 nm light, photons penetrated into rabbit model eyes, resulting in a photothermal effect by focused laser surgery. This treatment allowed reducing the PCO occurrence by about 60% after 30 days.

Concerning the field of vitreoretinal surgery Yamamoto *et al.* [276] used a new quantum dot based method to detect vitreous lesions and even demonstrated that this nanotechnology method to stain the vitreous can guide surgeons to do vitrectomies with more convenience and safeness.

10. Nanotechnology in retinal prosthetics

Current concepts of retinal prostheses have demonstrated partial recovery of visual loss caused by degeneration of the outer retina, whole-thickness retina, optic nerve, or even the eye or diseases of the central nervous system. Detailed discussions of intraocular retinal prostheses based on integrated-circuit devices are widely disseminated. Many challenges remain, prior to successful implementation of high-resolution retinal prosthesis, notably implant substance toxicity, implant corrosion and degradation, tissue reaction and inflammation, meningeal irritation, electrical stimulation-induced neural injury, and heat dissipation and thermal damage.

Innovations in nanotechnology enable fabrication of vertical arrays of conducting and semiconducting nanostructures for applications in nanoelectronics, imaging and biosensors. The advantages of nanoelectrode arrays include a high density of stimulation locations, decreased resistive heat generation, lower power consumption, highly compact integration of sensor, processor, and communication element, as well as possible independent fuel cell. The diminutive size may enable integration with chemical storage systems as well. Such nanoelectrode arrays, i.e., nanoelectrochemicomechanical (NEMS) stimulators, can, not only be implanted anywhere along the visual pathway, but can also be implanted anywhere in the central and peripheral nervous systems. Disadvantages of such NEMS systems at present include immature manufacturing process, difficulty in integrating nanoelectrodes to the current semiconductor processing technology, specialized surgical techniques, and limited study on biocompatibility.

Carbon nanotubes have high tensile strength, are ultralightweight, have excellent chemical and thermal stability, and have electrical conductivity on a par with metal. Investigations into the application of conducting nanowires and nanotubes in retinal, neural, and cortical prostheses are ongoing. In a theoretical visual prosthesis model based on the vertical nanoelectrode array [277] the external sensor unit captures the visual input, which is processed and wirelessly transferred to the implantable visual prosthesis. The visual prosthesis receives the telemetry data, processes them, and transmits appropriate signals to the nanoelectrode arrays, which will stimulate the retina, optic nerve, or visual cortex. The fact that the array density, the nanoelectrode cluster density, and the nanoelectrode length and diameter can be controlled by controlling the manufacturing variables make these arrays much more enticing for neurostimulation applications. Good biocompatibility of the carbon nanotube meshwork substrate has been demonstrated in an *in vivo* study.

Other materials that could be used for visual prostheses are under active investigation. A nanoscale light-harvesting device has been successfully fabricated using zinc (II) metalloporphyrin-coated, single-walled, carbon nanotube field effect transistors by Hecht *et al.* [278]. The single-walled carbon nanotubes have an average tube diameter of 1.5 nm and an average tube length of 3 μm. When illuminated with a 420-nm light-emitting diode, an electronic current is conducted across the carbon nanotube. The current is a function of illumination intensity. The authors are optimistic about future prospects of such devices in the field of artificial eye or a photovoltaic device.

Bacteriorhodopsin is a nanoscale photoactive transmembrane protein of the *Halobacterium salinarium* microorganism. The ability of this protein molecule to switch back and forth between its purple and yellow forms using laser light of different frequencies has promoted its application as an artificial retina or as an ultrahigh-density nanostorage or computational device. Frydrych *et al.* [279] presented design principles for the application of bacteriorhodopsin as a sensor material for a color-sensitive artificial retina with an artificial neural network learning algorithm. Using these design principles, the authors have built a simple artificial retina using the bacteriorhodopsin protein and demonstrated that it is possible to produce an artificial color-sensitive retina based on protein sensors. Photoelectric response was observed for bacteriorhodopsin-based

optoelectronic membrane sensors, suggesting the feasibility of such a bacteriorhodopsin-based artificial retina or volumetric optical storage device.

11. Nanotechnology in regenerative ophthalmology

Regenerative medicine is the branch of medicine that deals with repairing or replacing tissues and organs by using advanced materials and methodologies and in recent years is gaining momentum. In the ophthalmology field, various types of allogenic and autologous stem cells have been investigated to treat some ocular diseases due to age-related macular degeneration, glaucoma, retinitis pigmentosa, diabetic retinopathy, and corneal and lens traumas. Nanotechnology is serving as a catalyst.

Different types of **nanoscaffolds** including electrospun nanofibers, self-assembled peptide nanofibers and nanotopographies have been designed and investigated for retinal [280, 281], corneal [282, 283], and lens [284, 285] tissue regeneration. Generally, unlike traditional scaffolds, nanoscaffolds provide highly porous 3D frameworks that facilitate oxygen and nutrient transport and cellular waste removal and promote cellular attachment, proliferation and differentiation. By modifying their content including surface coating and geometry including nanofiber length, diameter, arrangement and alignment, nanoscaffolds can be engineered to have appropriate chemical, physical, biological and mechanical properties mimicking the natural retinal and corneal microenvironments for ocular and stem cell growth and differentiation to regenerate ocular tissues. Usually, natural polymers including self-assembled peptides are more suitable for promoting cell attachment and biological activity by closely imitating the native extracellular matrix than synthetic polymers. However, they have less mechanical strength and shorter half-life than synthetic polymers. As a result, a combination of natural and synthetic polymers has been used to form transparent and biocompatible corneal nanoscaffolds and biomimetic retinal nanoscaffolds.

The eyes are special sites where foreign antigens are tolerated instead of rejected, making it a great place for taking advantage of gene therapy. The other advantage for gene therapy for the eye is that the eye is a closed organ with limited space. This would limit the local delivered drug diffusing into the body blood circulation because of the physical barrier structures. Therefore, more and more experiments with NP gene therapy

focusing on treating eye diseases are conducted. Non-viral gene nanocarriers, including lipoplexes, polyplexes, mesoporous NPs, organic-inorganic hybrid nanocarriers, NanoScripts, self-assembled DNA nanostructures and magnetic NPs, are emerging as promising tools for cell reprogramming for ocular tissue regeneration. They were utilized to deliver pluripotency genes including Oct3/OCT4, SOX2, KLF4 and c-Myc or OCT4, SOX2, Nanog and Lin14 that reprogram specialized cells into induced pluripotent stem cells (IPSCs). They have also been used to deliver genes that initiate or enhance differentiation of embryonic stem cells (ESCs) and IPSCs into specialized ocular cells including RPE cells and photoreceptors. In addition, nanocarriers have also been investigated as delivery devices for delivering specific genes including p53 gene, Otx2, Crx and Nrl expressing genes, or CHX10VP16, OTX2, and CRX to non-neuronal retina cells, mainly Müller glia cells and stem cells located in the ciliary margin of the eye, and reprograming them into photoreceptors and retinal ganglion cells. If this approach is successful, the impact will be significant, because it will allow utilization of intrinsic cells for retinal tissue regeneration and stop the progression of some hereditary ocular degenerative diseases. Therefore, continuous efforts should be devoted to optimize nanotechnology to reprogram somatic cells into IPSCs and stem cells that can be differentiated into specialized ocular cell lineages for successful ocular tissue regeneration.

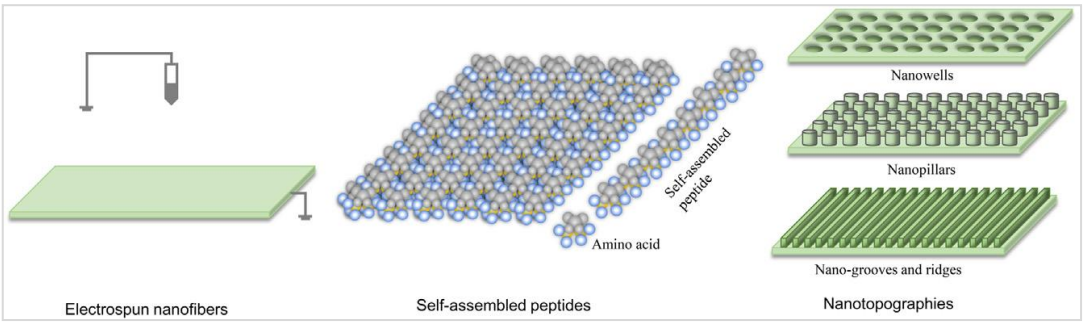

Figure 16: Schematic representations of nanoscaffolds including electrospun nanofibers, self-assembled peptides and nanotopographies used for ocular regeneration (*Sahle FF et al., Adv.Drug Delivery Reviews, 2019; 148: 290–307).*

During the past 10 years, nanotechnology has been used to control release of cytokines and immunosuppressants to prevent corneal tissue rejection and increase corneal graft survival rate. In the future, deeper studies of the effects of the size, shape, surface feature and elastic property of NPs on the phagocytic clearance of the NPs in the

eye are expected. Combination of nanotechnology and immunoengineering to modulate both innate and adaptive immune systems to promote a pro-regenerative microenvironment at the defect/injury site will be critical for ocular healing and regeneration.

The trabecular meshwork (TMS) is the primary drainage mechanism for aqueous humor and therefore the main structure controlling IOP. Gene therapy is one of the suggested treatment strategies for TMS-focused treatments. In recent years, the generation of recombinant adenoviral (Ad) vectors has made gene transduction possible for this tissue [286]. The Ad vector carrying the dominant-negative RhoA gene was successful in inactivating RhoA and reducing IOP in organ cultures. Cell-based regeneration of TMS tissue can also be considered as a treatment strategy because of the disease-related decrease of TMS cells. In this regard, TMS stem cells have been investigated in terms of their localization into the TMS and differentiation into functional TMS cells [287] Whole tissue regeneration should also be considered as a future treatment strategy. Thereby, TMS could be replaced with regenerative tissue before trabeculectomy where all structural changes could be reversed and postoperative complications due to TMS opening could be overcome.

Moreover, artificial TMS tissues form a suitable base for testing biomechanical functions of tissue and the effect of various therapeutic agents. The first attempt in that regard was the construction of micropatterned porous SU-8 scaffolds [288].

Successful ocular tissue engineering and regeneration research and application need an appropriate method to track the proliferation and migration of transplanted cells *in vivo*. Different NPs such as magnetic and GNPs and Qdots have been used as cell-tagging agents for repeated or continuous tracking of cells in vivo in combination with magnetic resonance imaging (MRI), photoacoustic imaging, and optical imaging, respectively. In this process, nanomaterials that can be imaged are first incorporated into stem cells by endocytosis. Afterwards, the nanomaterials-containing stemcells are implanted at the target site and their migration and differentiation are monitored using the corresponding scanning device. However, the studies of nanomaterials as tagging agents for tracking cells in the eye are scarce and limited to the toxicity of SPIONs and gold NPs to rabbit corneal endothelial cells and MSCs, respectively, and tracking MSCs in the subretinal layer

of the rat's eye. More development of nanotechnology for live cell imaging and tracking for disease diagnosis and tissue regeneration in the eye is expected in the future.

Nanotechnology has been revolutionizing tissue regeneration to restore function of diseased, damaged and aged tissues in the eye. However, the research in nanotechnology for regenerative ophthalmology is still at its early stage, and there are very limited numbers of *in vivo* experiments and to date, based on reports on clinicaltrial.gov, no clinical trial has been reported in this area.

12. Nanotoxicity

Nanotechnology has been a rapidly expanding field and is continuously being explored in the field of pharmaceutical and biotechnology sciences to achieve the desired product or therapeutic outcomes. Nanotoxicology is a branch of nanoscience that deals with the safety and toxicity issues of nanomaterials and evaluating their interactions with biological systems.

The nanotechnology industry is a rapidly emerging industry generating numerous engineered nanomaterials that are incorporated into medical, cosmetic and consumer products. So far (2016) [289] more than 3000 nanomaterials have been used in pharmaceutical companies, but toxicity data are sparse [290].

Nanomaterials can exhibit altered physical or chemical properties or biological effects (dimension-dependent properties or phenomena) in comparison to their corresponding large-scale materials with the same chemical composition. It is necessary to assess the toxicity of nanomaterials even though the utilized/matrix is considered safe in its bulk form. Each class of nanoparticles is unique, and “FDA believes that evaluations of safety, effectiveness, public health impact, or regulatory status of nanotechnology products should consider any unique properties and behaviors that the application of nanotechnology may impart” [291, 292]. There are three major considerations related to the products based on nanotechnology, which includes characterization, safety, and environmental impact. Various essential parameters that need to be considered during the characterization of NPs are: critical physical and chemical properties and their impact on quality and performance of the product, reliable and reproducible in vitro and in vivo characterization tools or techniques and their sensitivity, short/longterm stability issues in various environments, critical quality attributes, critical processing variables, overall

chemistry, manufacturing, and controls (CMC), and the forms in which they were presented to the host [293].

The important parameters that need to be considered for evaluating the *in vivo* safety of NPs are: tissue penetration/distribution and pharmacokinetic parameters (ADME profile) of the nanomaterials compared to their large-scale counterparts, tissue retention, transient and/or permanent bioeffects in vivo, clearance mechanisms, and potential variation in effects in different tissues. The major parameters that need to be considered for assessing the environmental impact of the nanomaterials are: potential implications of the releasing of these materials into the environment post use, and methodologies to detect and quantify the environmental effects. In June 2014, FDA released the final guidance for industry related to nanomaterials – "Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology" [292]. Currently, FDA addresses the issues related to the safety, effectiveness, and public health impact of nanotechnology products on a case by case basis, and advises industry to consult with the agency in the early development process [292]. Therefore, any entity that plans to develop a nanoparticle product for ophthalmic applications should follow the FDA guidelines and communicate with FDA as early as possible when it is feasible to learn the regulatory requirements and implement appropriate steps for bringing the product into the market.

The toxicity of NPs in ocular tissues can be influenced by many factors, including, but not limited to the solubility, dose, size, shape, surface charge and chemical groups, time of assessment, and the biodistribution pattern of the particles in the eye. Size and concentration of NPs have been suggested as the most important factors in determining the toxicological effects of NPs on the nervous system. Investigating microglial responses, for example, it has been shown that larger silica NPs (200 nm) would not cause any cytotoxicity even at relatively high concentrations (292 µg/mL), while smaller NPs (50 nm) would increase cell death in a dose-dependent manner [293]. The similar response was observed is a case of copper NPs, where 40 nm NPs exerted the maximum toxic effect when compared with 60 and 80 nm NPs. The antigenicity of NPs, however, is assumed to depend on particle size, in which small-sized NPs (25 nm or smaller) that can improve lymphatic uptake and may have a surface charge are more probable to be antigenic compared to bigger NPs. In another study, compared to micro-sized particles, NPs expressed more cytotoxicity and necrosis induction in human skin organ cultures [294].

These data might originate from the fact that smaller NPs have larger surface area per unit mass, thereby be able to interact with surrounding structures more efficiently, simultaneously demonstrating higher toxicity. However, some NPs show their toxicity independent from casual size-dependent manners. For instance, using 10, 30, 60, and 200 nm sized zinc oxide (ZnO), these NPs have been suggested to manifest their toxicity on neural stem cells merely based on their concentration rather than their size.

Charge and coating can also play important roles regarding the effects of the surface chemistry of NPs on their toxicity and the integrity of BRB. Thanks to the slightly negatively charged intercellular membranes, positively charged NPs are more likely to have higher cellular uptake and biointeractions with cellular structures, which may be accompanied with more impairments in the membrane function and faster clearance from biological systems. In cellular viability, hemolysis, and bacterial viability assays, anionic gold NPs demonstrated less toxicity than cationic ones. Some evidence shows that time might be another important factor in neuronal toxicity.

Increased biodistribution and prolonged bioavailability of NPs in the retina and the brain may cause more toxicity. The delivery of genes or drugs to the eye for therapeutic purposes by intravitreal injection requires fewer dose units in comparison to systemic route and thus could compromise the toxicity of injected NPs to other organs. Taken together, the best option to help decrease the toxicity of NPs is to put more emphasis on the design of the NP chemistry, size, and surface modification. Therefore, once NPs are considered as therapeutic agents, their size and concentration should be carefully determined to effectively maximize bioavailability and minimize the possible toxicity of these two-edged sword particles.

Biodegradable polyesters (e.g., PLGA, PLA) have been depicted as safe polymers for drug delivery. After being injected into the eye, PLGA undergoes degradation and forms lactic acid and glycolic acid, and eventually gets removed from the body via the Krebs cycle by conversion to carbon dioxide and water. PLGA has been applied to deliver dexamethasone (Ozurdex® implant) for the treatment of DME, noninfectious posterior uveitis and wet AMD. It has been observed that PLGA implant materials at some rare cases could remain after the complete release of drugs in patient eyes [86], which poses the concerns of potential material buildup, especially for repeated dosing. Many other factors also need to be addressed in detail, including the polymer purity, NP

manufacturing technology, solvent residue, potential local acidic environment during polymer degradation, material buildup in the eye after repeated dosing, foreign-body reactions and the potential snowball effects in the vitreous to disturb the visual axis. Success in the translation of nanomedicine would require a careful risk/benefit analysis, which is often skewed toward risk when it comes to novel therapeutics.

The selection of a suitable animal model in assessment of ocular toxicity of the nanomaterials and the product is important for the development of safe and effective ocular formulations. The rabbit eye is more sensitive and susceptible to irritant nanomaterials than the human eye and is considered to be a suitable model. Toxicity of the nanomaterials-based ophthalmic formulation can be best evaluated by corneal opacity, eye irritation (Draize eye test), hypersensitivity, LVET, and ocular and non-ocular organotypic methods. The corneal opacity test is generally performed on the cornea of rabbits and it is most widely used in the assessment of corneal toxicity. A comprehensive review on current techniques for ocular toxicity was presented by Wilson *et al.* [295].

13. Obstacles need to be overcome

In all circumstances, prior to translation of nanomedicines and nanosystems for treatment and management of ocular diseases, a number of obstacles need to be overcome. It is believed that the following aspects need to be addressed in future [15].

Persistence of NPs despite immune surveillance: the biodistribution of NPs and their persistence in tissues and organs is still not well known. The major obstacle is trying to locate and visualize suboptical NPs in large areas of tissues—an extremely difficult task and a variation of the proverbial problem of finding the needle in the haystack. The ultimate confirmation of NP presence in organs and tissues is transmission electron microscopy, but it is impossible to scan large areas to find out where to look at this suboptical level.

Safe manufacturing techniques: safe bionanomanufacturing is still a largely unexplored area since it requires not only the clean-room processes similar to that of the manufacture of semiconductor devices, but also makes extreme demands on the manufacturing of biological components and their attachment to the NPs.

Cell-by-cell dose delivery and control: as described earlier, delivery of a precise amount

of drug to individual cells *in vivo* is an extremely difficult task. One way of approaching this problem namely *in situ* production of therapeutic genes under the control of molecular biosensors that can regulate the amount of drug per cell according to what is needed as detected in a feedback loop with an upstream molecular biosensor.

Unintended biological consequences: the biggest advantage of nanomedical approaches is that one can at least minimize unintended biological consequences by using highly targeted nano drug delivery systems.

Use of appropriate experimental models: murine, rats, rabbits are widely used as experimental eye models. In nano-levels some variations compare to human eye (e.g. tissue behavior, tear fluid production and turnover) are of paramount importance in order to extract safe conclusions. Hence it has to be invented an experimental eye model (*ex* or *in vivo*) more compatible with human eye anatomy and physiology.

NPs aggregation*:* It is routine for several investigators to report a presumptive particle size for various NPs based on their molecular size or based on electron microscopy measurements. While these measures sometimes tend to be of a very small dimension less than 100 nm, there is evidence that, even such small materials tend to form lose aggregates in aqueous media, with measured dimensions larger than 100 nm. Thus, the aqueous environment dramatically influences the size of particles. Further, NPs can grow in size during storage. Future studies will understand the fundamentals of NPs aggregation and devise approaches to minimize such aggregation in aqueous and biological media as well as the shelf. Once a NP is administered into a tissue, the particles can interact with each other, forming larger aggregates. NPs administered in the periocular space, when retained over a few hours, tend to aggregate. Such aggregates form implant like structures which can persist for prolonged periods. Future studies will investigate the influence of vitreous humor, aqueous humor, blood, and other tissues on the properties of NPs.

A major but essential barrier to the translation of nanomedicines [296] is regulatory approval, largely led in North America by the United States FDA. Nanomedicines present challenges for the FDA, because any single “nanomedicine” may contain many distinct biologically active compounds, each with its own characteristics and composing an entirely distinct compound with its own properties. Nanoscale multifunctionality maximizes the regulatory barriers presented to nanomedicine translation. Each added

component to the nanoformulation requires its own individual justification and testing (possibly including clinical trials), particularly if the components are compounds not previously approved; ultimately, the complete particle requires the same. Thus, a nanomedicine containing three novel components requires preclinical and clinical evidence for each component, with an additional trial for the complete nanomedicine before approval is granted.

In addition, typical modern multifunctional nanomedicines with "targeting" functions are considered as being at "high risk to exhibit clinically significant changes in exposure, safety, and/or effectiveness relative to the referenced product", even in cases where the individual components are previously approved and are subject to greater regulatory scrutiny. Greater scrutiny is also placed on the nanomedicine for each additional claimed clinical target (e.g., each diagnostic or therapeutic application), each of which require individual evidence of efficacy. These regulatory barriers bring with them increased cost and time spent in the regulatory phase, thereby presenting additional barriers to clinical translation. Abraxane™ is a good example of this notion, as it made a small modification to paclitaxel (a known oncologic drug) by conjugating it to albumin, two approved drugs, to make a third drug that required only the further testing that would be required by any new drug requesting regulatory approval. Thus, multifunctionality should be balanced carefully against the ultimate clinical function each element will contribute.

The goal of nanomedicine design should be to create a tailored solution to address a specific clinical problem optimally and to change clinical practice. Rather than designing a NP with multiple functions and struggling to adapt those functions to clinical use, clinicians should be involved in the development at early stages to ensure that nanomedicines are designed as solutions to identifiable problems.

14. Future perspectives

Nanomedicine is a promising platform for ocular disease treatment; but it is still challenging for translation from the bench into clinical products. There are several aspects associated with nanomedicine development: to prepare uniform NPs with reproducible characteristics on a large scale and control potential toxicity and safety issues associated with ocular nanomedicine.

During future NP development, several key areas of study need to be addressed, including active targeting, pharmacokinetic/pharmacodynamics (PK/PD), safety and toxicology, stability, and production scale-up. Active targeting based on the binding affinity between a targeting ligand attached on the NP surface and the targeted units of diseased tissue (receptors/antigens) has been considered as an effective approach for improving the efficacy of NP-based delivery systems. Since NPs can offer many reactive functional groups, active targeting to specific tissues in the anterior eye segment is expected to be developed by conjugating targeting moieties onto the NP surface in the near future. However, as the active targeting is a complex process, the following factors need to be considered thoroughly during the NP development for active targeting: targeted unit localization and expression in the diseased site, targeting ligand selection, conjugation chemistry, ligand density, NP size and architecture, accessibility of the NPs to the target site, non-specific binding, and in vivo stability of the ligand-conjugated NPs.

As NPs can alter the PK/PD properties of a free drug and subsequently the therapeutic effect and toxicity of the drug, there is an unmet need to critically assess the PK and local and systemic biodistribution of the NPs after administration into the eye. It is also in need to study how ocular diseases affect anterior and posterior segment nanodelivery. In addition to the PK/PD studies, more focused emphasis should be given to study the acute and long-term toxicity of the NPs and the ultimate fate of the NPs or their degradants after the release of the drug at the intended site. More deeply, it is important to understand and measure the effects of nanoparticulate parameters (size, charge, architecture, surface chemistry, active targeting) on the *in vivo* fate including clearance, and the long-term toxicity of the NPs. Future studies can also be performed in evaluating the specific interactions of the NPs with cells, tissues and/or human organs, and the mechanisms for the enhancement of drug bioavailability in the eye.

Physical and chemical stability is another key aspect that needs to be considered during the pharmaceutical development of drug nanoparticulate systems to ensure the safety, efficacy, and quality of the products. Currently, there are scarce studies about the stability of NPs for ocular applications. In the future development, the physical, chemical, biological, and microbiological stability of NPs should be evaluated according to regulatory guidelines. Most of the reported methods for fabricating NPs for ocular applications are at lab scale and not suitable for industry mass production. In the future,

cost-effective technology needs to be developed to scale up NPs production for clinical translation. During scale up, special consideration should also be given to the techniques for sterilizing the NPs such as aseptic processing/sterile filtration or terminal sterilization by autoclaving. Besides the above discussed key areas of future studies, the evaluation of the environmental impacts of the nanomaterials needs to be carried out in the future. With the further development of NPs for drug delivery and other treatments for ocular diseases, appropriately designed clinical trials for the NP systems are expected to be conducted in the future.

In the near future nanomedicine is expected to contribute significantly to personalized medicine. Personalized medicine includes tailoring of disease treatment to the individual person based on that person's genetics or severity of disease to achieve better patient outcomes and is an emerging and growing field within ophthalmology. The use of nanotechnology can allow for personalization of therapeutics and optimization of drug dosages for a more precise and efficient treatment of ophthalmic diseases. Fortunately the multifunctional potential of nanomedicines makes them ideal to fill this clinical niche. To date, most studies have focused on the use of nanotechnology in the sustained release of drugs to treat ophthalmic conditions in a personalized manner. In the future, nanotechnology is expected to be applied to personalize regenerative medicine using human stem cells from the same patient and delivering therapeutics to maintain a healthy environment for stem cells to grow and mature in the location of disease, such as using plasma rich in growth factors from the same patient.

15. Conclusions

Nanotechnology involves the creation and use of materials and devices at the size scale of intracellular structures and molecules and involves systems and constructs on the order of ~100 nm. Nanomedicine involves the comprehensive monitoring, control, construction, repair, defense, and improvement of human biological systems at the molecular level, using engineered nanodevices and nanostructures, operating massively in parallel at the single cell level, ultimately to achieve medical benefit. Individual atoms and molecules can be manipulated to form microscopic tubes, spheres, wires, and films for specific tasks, such as generating electricity or transporting drugs in the body. The

incorporation of nanotechnology in clinical medicine is a *translational research* endeavor. The earliest impact of nanomedicine is likely to involve the areas of biopharmaceuticals (eg, drug delivery, drug discovery), implantable materials (eg, tissue regeneration scaffolds, bioresorbable materials), implantable devices (eg, intraocular pressure monitors, glaucoma drainage valves), and diagnostic tools (eg, genetic testing, imaging, intraocular pressure monitoring). Nanotechnology will bring about the development of regenerative medicine (i.e., replacement and improvement of cells, tissues, and organs), ultrahigh-resolution in vivo imaging, microsensors and feedback devices, and artificial vision. The examples of nanotechnology in ophthalmology cited in this perspective demonstrate that nanotechnology will play an important role in both early and late-stage intervention in the management of blinding diseases. “Regenerative nanomedicine,” a new subfield of nanomedicine, uses NPs containing gene transcription factors and other modulating molecules that allow the reprogramming of cells *in vivo*. Nanotechnology already has been applied to the measurement and treatment of different disease states in ophthalmology, and many additional innovations will occur during the next decades.

References

1. Saladin El, Naschie M: Nanotechnology for the development word. *Chaos Solitons Fractals* (2006); 30(4):769–773
2. Feynman RP: There's plenty of room at the bottom: an invitation to enter a new field of physics. *Eng Sci* (1960); 23: 22–36.
3. Taniguchi N: On the Basic Concept of 'Nanotechnology'. *Meeting of the Japan Society of Precision Engineering in* 1974.
4. Moore GE: Cramming more components onto integrated circuits. *Electronics* (1965); 38114–117.
5. Binning G, Rohrer H, Gerber C, Weibel E: Surface studies by scanning tunneling microscopy. *Phys Rev Lett* (1982); 49: 57–61.
6. Ginger DS, Zhang H, Mirkin CA: The evolution of dip-pen nanolithography. *Angew Chem Int Ed Engl* (2004); 43: 30–45.
7. Freitas RA: What is nanomedicine? *Nanomedicine* (2005); 1:2–9.
8. Morrow KJ, Bawa R, Wei C: Recent advances in basic and clinical nanomedicine. *Med Clin North Am* (2007); 91: 805-843.
9. Bourne RR, Flaxman SR, Braithwaite T, Cicinelli MV, Das A, Jonas JB, … Limburg H: Magnitude, temporal trends, and projections of the global prevalence of blindness and distance and near vision impairment: A systematic review and meta-analysis. *Lancet Global Health*, (2017); 5, 888–897.
10. Schwartz GF, Quigley HA: Adherence and Persistence with Glaucoma Therapy, *Survey of Ophthalmology,* (2008); 53: supl. 1.
11. Marcum ZA, Sevick MA, Handler SM: Medication Nonadherence: A Diagnosable and Treatable Medical Condition. *JAMA,* (2013) May 22; 309(20): 2105–2106.
12. Bansal RK, Tsai JC: Compliance/Adherence to Glaucoma Medications - A Challenge. *Journal of Current Glaucoma Practice,* (2007);1(2):22-25.
13. Barar J, Javadzadeh AR, Omidi Y: Ocular novel drug delivery: Impacts of membranes and barriers. *Expert Opinion on Drug Delivery*, (2008); 5: 567–581.
14. Sampat KM, Garg SJ: Complications of intravitreal injections. *Current Opinion in Ophthalmology*, (2010); 21, 178–183.
15. Zarbin MA, Leary JF, Montemagno C, Ritch R, Humayun MS: Nanomedicine in Ophthalmology, "Retina" Ryan SJ, extbook, Elsevier, (2013)
16. Montemagno CD: Integrative technology for the twenty-first century. *Ann N Y Acad Sci* (2004); 1013: 38–49.
17. Kaufman PL, Adler FH, Levin LA, Alm A: Adler's physiology of the eye. Philadelphia: *Elsevier Health Sciences*. (2011).
18. Mishima S: Some physiological aspects of the precorneal tear film. *Archives of Ophthalmology*, (1965); 73, 233–241.
19. Cheng L, Muller SJ, Radke CJ: Wettability of silicone-hydrogel contact lenses in the presence of tear-film components. *Current Eye Research*, (2004); 28, 93–108.
20. Ali Y, Lehmussaari K: Industrial perspective in ocular drug delivery. *Advanced Drug Delivery Reviews*, (2006); 58, 1258–1268.
21. Grass GM, Robinson JR: Mechanisms of corneal drug penetration I: In vivo and in vitro kinetics. *Journal of Pharmaceutical Sciences*, (1988); 77, 3–14.
22. Hämäläinen K, Kananen K, Auriola S, Kontturi K, Urtti A: Characterization of paracellular and aqueous penetration routes in cornea, conjunctiva, and sclera. *IOVS*, (1997);38, 627–634.
23. Schoenwald RD, Huang HS: Corneal penetration behavior of β-blocking agents I: Physicochemical factors. *Journal of Pharmaceutical Sciences*, (1983); 72, 1266–1272.
24. Karla PK, Pal D, Mitra AK: Molecular evidence and functional expression of multidrug resistance associated protein (MRP) in rabbit corneal epithelial cells. *Experimental Eye Research*, (2007); 84, 53–60.
25. Vellonen KS, Mannermaa E, Turner H, Hakli M, Wolosin JM, Tervo T, … Urtti A: Effluxing ABC transporters in human corneal epithelium. *Journal of Pharmaceutical Sciences*, (2010); 99, 1087–1098.
26. Wang W, Sasaki H, Chien DS, Lee VH: Lipophilicity influence on conjunctival drug penetration in the pigmented rabbit: A comparison with corneal penetration. *Current Eye Research*, (1991); 10, 571–579.
27. Saha P, Yang JJ, Lee V: Existence of a p-glycoprotein drug efflux pump in cultured rabbit conjunctival epithelial cells. *IOVS*, (1998); 39, 1221–1226.
28. Ambati J, Canakis CS, Miller JW, Gragoudas ES, Edwards A, Weissgold DJ, … Adamis AP: Diffusion of high molecular weight compounds through sclera. *IOVS* (2000); 41, 1181–1185.
29. Winkler J, Wirbelauer C, Frank V, Laqua H: Quantitative distribution of glycosaminoglycans in young and senile (cataractous) anterior lens capsules. *Experimental Eye Research*, (2001); 72, 311–318.
30. Pitkänen L, Ranta VP, Moilanen H, Urtti A: Permeability of retinal pigment epithelium: Effects of permeant molecular weight and lipophilicity. *IOVS*, (2005); 46, 641–646.
31. Mannermaa E, Vellonen KS, Ryhanen T, Kokkonen K, Ranta VP, Kaarniranta K, Urtti A: Efflux protein expression in human retinal pigment epithelium cell lines. Pharmaceutical Research, (2009); 26, 785–1791.
32. Xu Q, Boylan NJ, Suk JS, Wang YY, Nance EA, Yang JC, … Hanes J:Nanoparticle diffusion in, and microrheology of, the bovine vitreous ex vivo. *Journal of Controlled Release*, (2013); 167, 76–84.
33. Zhao L, Chen XJ, Zhu J, Xi YB, Yang X, Hu LD, … Lin D: Lanosterol reverses protein aggregation in cataracts. *Nature*, (2015); 523, 607–611.
34. AREDS Research Group: A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for agerelated macular degeneration and vision loss: AREDS report no. 8. *Archives of Ophthalmology*, (2001); 119, 1417–1436.
35. Ciulla TA, Amador AG, Zinman B: Diabetic retinopathy and diabetic macular edema: Pathophysiology, screening, and novel therapies. *Diabetes Care*, (2003); 26, 2653–2664.
36. Khan MA, Brady CJ, Kaiser RS: Clinical management of proliferative vitreoretinopathy: An update. *Retina*, (2015); 35, 165–175.
37. Hellström A, Smith LE, Dammann O: Retinopathy of prematurity. *Lancet*, (2013); 382, 1445–1457.
38. Peng Y, Tang L, Zhou Y: Subretinal injection: A review on the novel route of therapeutic delivery for vitreoretinal diseases. *Ophthalmic Research*, (2017); 58, 217–226.
39. Mahaling B, Katti DS: Physicochemical properties of core-shell type nanoparticles govern their spatiotemporal biodistribution in the eye. *Nanomedicine*, (2016); 12, 2149–2160.
40. Wen H, Hao J, Li SK: Influence of permeant lipophilicity on permeation across human sclera. *Pharmaceutical Research*,(2010); 27, 2446–2456.
41. Kadam RS, Cheruvu NP, Edelhauser HF, Kompella UB: Sclera-choroid-RPE transport of eight beta-blockers in human, bovine, porcine, rabbit, and rat models. *IOVS*, (2011); 52, 5387–5399.

42. Le Bourlais C, Acar L, Zia H, Sado PA, Needham T, Leverge R: Ophthalmic drug delivery systems-recent advances. Progress in Retinal and Eye Research, (1998); 17, 33–58.
43. Maurice DM: Drug delivery to the posterior segment from drops. *Surv Ophthalmol*. (2002); 47(Suppl 1):S41–S52.
44. Albanese A, Tang PS, Chan WC: The effect of nanoparticle size, shape, and surface chemistry on biological systems. *Annual Review of Biomedical Engineering*, (2012); 14, 1–16.
45. Bangham AD, Standish MM, Watkins JC: Diffusion of univalent ions across the lamellae of swollen phospholipids. *J Mol Biol,* (1965); 13:238–252.
46. Demetzos C: Pharmaceutical Nanotechnology Fundamentals and Practical Applications, *Springer Science + Business Media* Singapore (2016).
47. Sahoo SK, Dilnawaz F, Krishnakumar S: Nanotechnology in ocular drug delivery. *Drug Discov, Today* (2008); 13(3–4): 144–151.
48. Lian T, Ho RJY: Trends and developments in liposome drug delivery systems. *J Pharm Sci,* (2001); 90: 667–680.
49. Torchlin VP: Recent advances with liposomes as pharmaceutical carriers. *Nat Rev Drug Discov, (*2005); 4(2): 145–160.
50. Patel A, Cholkar K, Agrahari V, et al.: Ocular drug delivery systems: an overview. *World J Pharmacol* (2013); 2(2): 47–64.
51. Garrigue JS, Amrane M, Faure MO, Holopainen JM, Tong L: Relevance of lipid-based products in the management of dry eye disease. *J. Ocul. Pharmacol. Ther.* (2017); 33, 647–661.
52. Khan W, Aldouby YH, Avramoff A, Domb AJ: Cyclosporin nanosphere formulation for ophthalmic administration. *Int. J. Pharm.* (2012); 437, 275–276.
53. Ren T, Lin X, Zhang Q, You D, Liu X, Tao X, Gou J, Zhang Y, Yin T, He H: Encapsulation of azithromycin ion pair in liposome for enhancing ocular delivery and therapeutic efficacy on dry eye. *Mol Pharm* (2018); 15:4862–4871.
54. Chetoni P, Rossi S, Burgalassi S, Monti D, Mariotti S, Saettone F: Comparison of liposome-encapsulated acyclovir with acyclovir ointment: ocular Pharmacokinetics in rabbits. *J. of Ocular Pharm. and Therapeutics* (2004); (20); 2.
55. Law SL, Hung HY: Properties of acyclovir-containing liposomes for potential ocular delivery. *Int J Pharm* (1998); 161: 253–259.
56. Taha EI, El-Anazi MH, El-Bagory IM, Bayomi MA: Design of liposomal colloidal systems for ocular delivery of ciprofloxacin. *Saudi Pharmaceutical Journal* (2014); 22, 231–239.
57. Pires de Sáa FA, Taveirab SF, Gelfusoa GM, Limab EM, Gratieri T: Liposomal voriconazole (VOR) formulation for improved ocular delivery. *Colloids and Surfaces B: Biointerfaces* (2015);133: 331–338.
58. Li N, Zhuang C, Wang M: Liposome coated with low molecular weight chitosan and its potential use in ocular drug delivery. *Int J Pharm* (2009); 379: 131–138.
59. Singh K, Mezei M: Liposomal ophthalmic drug delivery system I. Triamcinolone acetonide. *Int. J. Pharm.* (1983); 16(3), 339–344.
60. Sun Y, Fox T, Adhikary G, Kester M, and Pearlman E: Inhibition of corneal inflammation by liposomal delivery of short-chain, C-6 ceramide. *J Leukoc Biol* (2008); 83: 1512–1521.
61. Zhan C, Santamaria CM, Wang W, McAlvin JB, Kohane DS: Long-acting liposomal corneal anesthetics. Biomaterials, (2018); 181, 372–377.
62. Yu S, Wang QM, Wang X, Liu D, Zhang W, Ye T, … Pan W: Liposome incorporated ion sensitive in situ gels for opthalmic delivery of timolol maleate. *International Journal of Pharmaceutics*, (2015); 480, 128–136.
63. Hathout RM, Gad HA, Abdel-Hafez SM, Nasser N, Khalil N, Ateyya T, Amr A, Yasser N, Nasr S, Metwally AA: Gelatinized core liposomes: a new Trojan horse for the development of a novel timolol maleate glaucoma medication. *Int J Pharm* (20109); 556:192–199.
64. Arroyo C, Quinteros D, Cózar-Bernal M, Palma S, Rabasco A, González-Rodríguez M: Ophthalmic administration of a 10-fold-lower dose of conventional nanoliposome formulations caused levels of intraocular pressure similar to those induced by marketed eye drops. *European Journal of Pharmaceutical Sciences,* (2018); 111, 186–194.
65. Tan G, Yu S, Pan H, Li J, Liu D, Yuan K, … Pan W: Bioadhesive chitosan-loaded liposomes: A more efficient and higher permeable ocular delivery platform for timolol maleate. *International Journal of Biological Macromolecules*, (2017); 94, 355–363.
66. Quansheng J, Lia H, Jina Z, Huanga L, Wanga F, Zhoua Y, Liua, Jianga C, Oswaldb J, Wua J, Songa X: TPGS modified nanoliposomes as an effective ocular delivery system to treat Glaucoma. *International Journal of Pharmaceutics,* (2018); 55321–28.
67. Natarajan, Ang M, Darwitan, Chattopadhyay S, Wong T, Venkatraman S: Nanomedicine for glaucoma: liposomes provide sustained release of latanoprost in the eye. *Int. J. Nanomed*., (2012); 7, 123–131.
68. Shimazaki H, Hironaka K, Fujisawa T, Tsuruma K, Tozuka Y, Shimazawa M, … Hara H: Edaravone-loaded liposome eyedrops protect against lightinduced retinal damage in mice. *IOVS*, (2011); 52: 7289–7297.
69. Sasaki H, Karasawa K, Hironaka K, Tahara K, Tozuka Y, Takeuchi H: Retinal drug delivery using eyedrop preparations of poly-L-lysine-modified liposomes. *European Journal of Pharmaceutics and Biopharmaceutics,* (2013); 83, 364–369.
70. ARVO Annual Meeting Abstract, May 2005.
71. Davis BM, Normando EM, Guo L, Turner LA, Nizari S, O'Shea P, Moss SE, Somavarapu S, Cordeiro MF: Topical delivery of avastin to the posterior segment of the eye in vivo using annexin A5-associated liposomes, *Small,* (2014); 10 1575–1584.
72. Zhang R, Qian J, Li X, Yuan Y: Treatment of experimental autoimmune uveoretinitis with intravitreal injection of infliximab encapsulated in liposomes. *British Journal of Ophthalmology*, (2017); 101, 1731–1738.
73. Bahari ASL, Hamishehkar H: The impact of variables on particle size of solid lipid nanoparticles and nanostructured lipid carriers; a comparative literature review. *Adv. Pharm. Bull.* (2016); 6(2), 143–151.
74. Balguri SP, Adelli GR, Majumdar S: Topical ophthalmic lipid nanoparticle formulations (SLN, NLC) of indomethacin for delivery to the posterior segment ocular tissues. *Eur. J. Pharm. Biopharm.* (2016); 109, 224–235.
75. Kumar R, Sinha VR: Solid lipid nanoparticle: an efficient carrier for improved ocular permeation of voriconazole. *Drug Dev. Ind.Pharm.* (2016); 42(12), 1956–1967.
76. Kalam A, Sultana Y, Ali A *et al.*: Part II: Enhancement of transcorneal delivery of gatifloxacin by solid lipid nanoparticles in comparison to commercial aqueous eye drops. *J. Biomed. Mater. Res. A*(2013); 101(6), 1828–1836.
77. Cavalli R, Gasco MR, Chetoni P, Burgalassi S, Saettone MF: Solid lipid nanoparticles (SLN) as ocular delivery system for tobramycin. *Int. J. Pharm.* (2002); 238(1), 241–245.
78. Wang F, Chen L, Zhang D *et al.*: Methazolamide-loaded solid lipid nanoparticles modified with low-molecular weight chitosan for the treatment of glaucoma: vitro and vivo study. *J. Drug Target.* (2014); 22(9), 849–858.
79. Shen J, Deng Y, Jin X, Ping Q, Su Z, Li L: Thiolated nanostructured lipid carriers as a potential ocular drug delivery system for cyclosporine A: improving *in vivo* ocular distribution. *Int. J. Pharm.* (2010); 402(1), 248–253.

80. Lallemand F, Felt-Baeyens O, Besseghir K, Behar-Cohen F, Gurny R: Cyclosporine A delivery to the eye: a pharmaceutical challenge, *European Journal of Pharmaceutics andBiopharmaceutics*, (2003); 56(3), 307-318.
81. Sandri G, Bonferoni MC, Gökçe EH, Ferrari F, Rossi S, Patrini M, Caramella C: Chitosan-associated SLN: In vitro and ex vivo characterization of cyclosporine a loaded ophthalmic systems. *Journal of Microencapsulation*, (2010); 27, 735–746.
82. Hao J, Wang X, Bi Y, Teng Y, Wang J, Li F, … Liu J: Fabrication of a composite system combining solid lipid nanoparticles and thermosensitive hydrogel for challenging ophthalmic drug delivery. *Colloids and Surfaces B: Biointerfaces*, (2014); 114, 111–120.
83. Liu D, Li J, Cheng B, Wu Q, Pan H: Ex vivo and in vivo evaluation of the effect of coating a coumarin-6-labeled nanostructured lipid carrier with chitosan-N-acetylcysteine on rabbit ocular distribution. *Molecular Pharmaceutics*, (2017); 14, 2639–2648.
84. Wang J, Zhao F, Liu R, Chen J, Zhang Q, Lao R, … Liu C: Novel cationic lipid nanoparticles as an ophthalmic delivery system for multicomponent drugs: Development, characterization, in vitro permeation, in vivo pharmacokinetic, and molecular dynamics studies. *International Journal of Nanomedicine*, (2017); 12, 8115–8127.
85. Abrishami M, Abrishami M, Mahmoudi A, Mosallaei N, Vakili Ahrari Roodi M, Malaekeh-Nikouei B: Solid lipid nanoparticles improve the diclofenac availability in vitreous after intraocular injection. *J of Drug Delivery*, (2016); 1368481.
86. Araujo J, Nikolic S, Egea MA, Souto EB, Garcia ML: Nanostructured lipid carriers for triamcinolone acetonide delivery to the posterior segment of the eye. *Colloids Surf. B* (2011); 88: 150–157.
87. Benita S, Elbaz E: "Oil-in-water emulsions of positively charged particles," US patent 6,007,826, Yisum Research Development Company of the Hebrew University of Jerusalem, Jerusalem, Israel, (1999).
88. Tamilvanan S, Benita S: The potential of lipid emulsion for ocular delivery of lipophilic drugs. *European Journal of Pharmaceutics and Biopharmaceutics*, (2004); 58(2), 357–368.
89. Lallemand F, Daull P, Benita S, Buggage R, Garrigue JS: Successfully Improving Ocular Drug Delivery Using the Cationic Nanoemulsion, Novasorb. *J of Drug Delivery* (2012); Article ID 604204
90. Kalam MA, Alshamsan A, Aljuffali IA, Mishra AK, Sultana Y: Delivery of gatifloxacin using microemulsion as vehicle: Formulation, evaluation, transcorneal permeation and aqueous humor drug determination. *Drug Delivery*, (2016); 23, 886–897.
91. Kesavan K, Kant S, Singh PN, Pandit JK: Mucoadhesive chitosan-coated cationic microemulsion of dexamethasone for ocular delivery: In vitro and in vivo evaluation. *Current Eye Research*, (2013); 38, 342–352.
92. Gan L, Gan Y, Zhu C, Zhang X, Zhu J: Novel microemulsion in situ electrolyte-triggered gelling system for ophthalmic delivery of lipophilic cyclosporine a: In vitro and in vivo results. *Int J of Pharmaceutics*, (2009); 365, 143–149.
93. Raval N, Khunt D, Misra M: Microemulsion-based delivery of triamcinolone acetonide to posterior segment of eye using chitosan and butter oil as permeation enhancer: An in vitro and in vivo investigation. *Journal of Microencapsulation*, (2018); 35, 62–77.
94. Ames P, Galor A: Cyclosporine ophthalmic emulsions for the treatment of dry eye: a review of the clinical evidence. *Clin. Invest*.(2015); 5, 267–285.
95. Boujnah Y, Mouchel R, El-Chehab H, Dot C, Burillon C, Kocaba V: [Prospective, monocentric, uncontrolled study of efficacy, tolerance and adherence of cyclosporin 0.1 % for severe dry eye syndrome]. *J. Fr. Ophtalmol*. (2018); 41, 129–135.
96. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V: PLGA-based nanoparticles: An overview of biomedical applications. *J of Controlled Release*, (2012); 161, 505–522.
97. Ahamad N, Rao DAS, Katti DS: Overview of methods of making polyester nano- and microparticulate systems for drug delivery. *Handbook of Polyester Drug Delivery Systems*. Singapore: Pan Stanford, (2016); 81–124.
98. Akhter S, Anwar M, Siddiqui MA, Ahmad I, Ahmad J, Ahmad MZ, Bhatnagar A, Ahmad FJ: Improving the topical ocular pharmacokinetics of an immunosuppressant agent with mucoadhesive nanoemulsions: formulation development, in-vitro and in-vivo studies. *Colloids Surf B Biointerfaces,* (2016); 148:19–29.
99. Gonzalez-Pizarro R, Silva-Abreu M, Calpena AC, Egea MA, Espina M, Garcia ML: Development of fluorometholone-loaded PLGA nanoparticles for treatment of inflammatory disorders of anterior and posterior segments of the eye. *Int J Pharm*, (2018); 25; 547(1-2):338-346.
100. Losa C, Calvo P, Castro E, Vila-Jato JL, Alonso MJ: Improvement of ocular penetration of amikacin sulphate by association to poly(butylcyanoacrylate) nanoparticles. *J. Pharm. Pharmacol.,* (1991); 43(8), 548–552.
101. Lai SK, Wang YY, Hanes J: Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. *Adv. Drug Deliv. Rev*. (2009); 61, 158–171.
102. Suk JS, Xu Q, Kim N, Hanes J, Ensign LM: PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. *Adv. Drug Deliv*. Rev.(2016); 99, 28–51.
103. Schopf L, Enlow E. Popov A, Bourassa J, Chen H: Ocular pharmacokinetics of a novel loteprednol etabonate 0.4% ophthalmic formulation. *Ophthalmol. Ther*. (2014); 3, 63–72.
104. Huang, D, Chen YS, Rupenthal ID: Overcoming ocular drug delivery barriers through the use of physical forces. *Adv. Drug Deliv. Rev*. (2018); 126, 96–112.
105. Prausnitz MR, Noonan JS: Permeability of cornea, sclera, and conjunctiva: a literature analysis for drug delivery to the eye. *J. Pharm. Sci.* (1998); 87, 1479– 1488.
106. Pan Q, Xu Q, Boylan NJ, Lamb NW, Emmert DG, Yang JC, Tang L, Heflin T, Alwadani S, Eberhart CG, Stark WJ, Hanes J: Corticosteroid-loaded biodegradable nanoparticles for prevention of corneal allograft rejection in rats. *J Control Release,* (2015); 201, 32–40.
107. Wang, B, Tang Y, Oh Y, Lamb NW, … Xu Q: Controlled release of dexamethasone sodium phosphate with biodegradable nanoparticles for preventing experimental corneal neovascularization. Nanomedicine (2019); 17, 119–123.
108. Musumeci T, Bucolo C, Carbone C, Pignatello R, Drago F, Puglisi G: Polymeric nanoparticles augment the ocular hypotensive effect of melatonin in rabbits. *Int. J. Pharm.,* (2013); 440(2), 135–140.
109. Warsi MH, Anwar M, Garg V, Jain GK, Telegaonkar S, Ahmad FJ, Khar RK: Dorzolamide-loaded PLGA⁄vitamin E TPGS nanoparticles for glaucoma therapy: pharmacoscintigraphy study and evaluation of extended ocular hypotensive effect in rabbits. *Colloids Surf. B Biointerfaces*(2014); 122(122), 423–431.
110. Nagarwal RC, Kumar R, Pandit J: Chitosan coated sodium alginate–chitosan nanoparticles loaded with 5-FU for ocular delivery: In vitro characterization and in vivo study in rabbit eye. *European J. of Pharmaceutical Sciences*, (2012); 47, 678–685.
111. Schopf LR, Popov A, Enlow EM, Bourassa JL, Ong WZ, Nowak P, Chen H: Topical ocular drug delivery to the back of the eye by mucus-penetrating particles. *Transl. Vis. Sci. Technol*.(2015); 4, 11.
112. Mahaling B, Srinivasarao DA, Raghu G, Kasam RK, Reddy G, Katti D: A non-invasive nanoparticle mediated delivery of triamcinolone acetonide ameliorates diabetic retinopathy in rats. *Nanoscale*, (2018); 10, 16485–16498.

113. Curran CF, Luce JK: Ocular adverse reactions associated with adriamycin (doxorubicin). *Am. J. Ophthalmol.* (1989); 108, 709–711.
114. Iwase T, Fu J, Yoshida T, Muramatsu D, Miki A, Hashida N, … Seidel C: Sustained delivery of a HIF-1 antagonist for ocular neovascularization. *Journal of Controlled Release*, (2013); 172, 625–633.
115. Qiu F, Meng T, Chen Q, Zhou K, Shao Y, Matlock G, Ma X, Wu W, Du Y, Wang X, Deng G, Ma JX, Xu Q: Fenofibrate-loaded biodegradable nanoparticles for the treatment of experimental diabetic retinopathy and neovascular age-related macular degeneration. *Mol. Pharm*. (2019); 16, 1958–1970.
116. Takahashi H, Obata R, Tamaki Y: A novel vascular endothelial growth factor receptor 2 inhibitor, SU11248, suppresses choroidal neovascularization in vivo. *J. Ocul. Pharmacol. Ther*. (2006); 22, 213–218.
117. Graybug Vision Presents Top Line Results of Phase 1/2a ADAGIO Study at Hawaiian Eye & Retina 2019
118. Yang M, Peterson WM, … Cleland JL: Modifications of sunitinib-loaded GB-102 microparticles that lengthen drug release: 9-months ocular tolerability and PK in rabbit following IVT dosing. *IOVS,* (2017); 58, 4115.
119. Vaishya RD, Khurana V, Patel S, Mitra AK: *Controlled ocular drug deliverywith nanomicelles* Wiley Interdisciplinary Reviews: *Nanomedicine and Nanobiotechnology*, (2014); 6(5), 422-37.
120. Mandal A, Gote V, Pal D, Ogundele A, Mitra AK: Ocular pharmacokinetics of a topical ophthalmic nanomicellar solution of cyclosporine (Cequa®) for dry eye disease. *Pharm Res*, (2019); 36:36.
121. Gupta AK, Madan S, Majumdar DK, Maitra A: Ketorolac entrapped in polymeric micelles: preparation, characterisation and ocular anti-inflammatory studies. *Int J Pharm,* 92000); 209(1–2), 1–14.
122. Li X, Zhang Z, Li J, Sun S, Weng Y, Chen H: Diclofenac/biodegradable polymer micelles for ocular applications. *Nanoscale*, (2012); 4, 4667–4673.
123. Kutlehria S, Vhora I, Bagde A, Chowdhury N, Behl G, Patel K, Singh M: Tacrolimus Loaded PEG-Cholecalciferol Based Micelles for Treatment of Ocular Inflammation. *Pharm Res* (2018); 35(6), 117.
124. Taha EI, Badran MM, El-Anazi MH, Bayomi MA, El-Bagory IM: Role of Pluronic F127 micelles in enhancing ocular delivery of ciprofloxacin. *J Mol Liq,* (2014); 199, 251–256.
125. Liaw J, Chang SF, Hsiao FC: *In vivo* gene delivery into ocular tissues by eye drops of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) polymeric micelles. *Gene Ther.* (2001); 8(13), 999–1004.
126. Tong YC, Chang SF, Liu CY, Kao WW, Huang CH, Liaw J: Eye drop delivery of nano-polymeric micelle formulated genes with cornea-specific promoters. *J Gene Med* (2007); 9(11), 956–966.
127. Jaiswal M, Kumar M, Pathak K: Zero order delivery of itraconazole via polymeric micelles incorporated in situ ocular gel for the management of fungal keratitis. *Colloids and Surfaces B: Biointerfaces*, (2015); 130, 23–30.
128. Dahmana N, Mugnier T, Gabriel D, Kaltsatos V, Bertaim T, Behar-Cohen F, Gurny R, Kalia YN: Topical Administration of Spironolactone-Loaded Nanomicelles Prevents Glucocorticoid-Induced Delayed Corneal Wound Healing in Rabbits. *Mol Pharm*, (2018); 15(3), 1192-1202 (2018).
129. Pepic I, Hafner A, Lovric J, Pirkic B, Filipovic-Grcic J: A nonionic surfactant/chitosan micelle system in an innovative eye drop formulation. *J Pharm Sci*, (2010); 99(10), 4317–4325.
130. Lin HR, Chang PC: Novel pluronic-chitosan micelle as an ocular delivery system. *J Biomed Mater Res B Appl Biomater*, (2013); 101(5), 689-99 (2013).
131. Xu J, Ge Y, Bu R, Zhang A, Feng S, Wang J, Gou J, Yin T, He H, Zhang Y, Tang X: Co-delivery of latanoprost and timolol from micelles-laden contact lenses for the treatment of glaucoma. *J Control Disease*, (2019); 305:18-28.
132. Mandal A, Cholkar K, Khurana V, Shah A, Agrahari V, Bisht R, Pal D, Mitra AK: Topical formulation of self-assembled antiviral prodrug nanomicelles for targeted retinal delivery. *Mol Pharm* (2017); 14:2056–2069.
133. Mandal A, Patel P, Pal D, Mitra AK: Multi-layered nanomicelles as selfassembled nanocarrier systems for ocular peptide delivery. *AAPS PharmSciTech*, (2019); 20:66.
134. Wang K., Han Z: Injectable hydrogels for ophthalmic applications. Journal of Controlled Release, (2017); 268, 212–224.
135. Li J, Mooney DJ: Designing hydrogels for controlled drug delivery. *Nature Reviews Materials*, (2016); 1, 16071.
136. Egbu R, Brocchini S, Khaw PT, Awwad S: Antibody loaded collapsible hyaluronic acid hydrogels for intraocular delivery. *European J of Pharm and Biopharmaceutics*, (2018); 124, 95–103.
137. Tyagi P, Barros M, Stansbury JW, Kompella UB: Light-activated, in situ forming gel for sustained suprachoroidal delivery of bevacizumab. *Molecular Pharmaceutics*, (2013); 10, 2858–2867.
138. Cao Y, Zhang C, Shen W, Cheng Z, Yu LL, Ping Q: Poly (N-isopropylacrylamide)–chitosan as thermosensitive in situ gel-forming system for ocular drug delivery. *Journal of Controlled Release*, (2007); 120, 186–194.
139. Sivaram AJ, Rajitha P, Maya S, Jayakumar R, Sabitha M: Nanogels for delivery, imaging and therapy. *Wiley Interdiscip Rev Nanomed Nanobiotechnol,* (2015); 7: 509–533.
140. Liu Z, Li J, Nie S, Liu H, Ding P, Pan W: Study of an alginate/HPMC-based in situ gelling ophthalmic delivery system for gatifloxacin. *International Journal of Pharmaceutics*, (2006); 315, 12–17.
141. Pachis K, Blazaki S, Tzatzarakis M, Klepetsanis P, Naoumidi E, Tsilimbaris M, Antimisiaris S: Sustained release of intravitreal flurbiprofen from a novel drug-in-liposome-in-hydrogel formulation. *European Journal of Pharmaceutical Sciences*, (2017); 109, 324–333.
142. Soiberman U, Kambhampati SP, Wu T, Mishra MK, Oh Y, Sharma R, … Stark WJ: Subconjunctival injectable dendrimer-dexamethasone gel for the treatment of corneal inflammation. *Biomaterials*, (2017); 125, 38–53.
143. Kalomiraki M, Thermos K, Chaniotakis NA: Dendrimers as tunable vectors of drug delivery systems and biomedical and ocular applications. *Int J Nanomedicine* (2015); 11:1–12.
144. Lancina MG, Yang H: Dendrimers for ocular drug delivery. *Can J Chem* (2017); 95:897–902.
145. Cerofolini L, Baldoneschi V, Dragoni E, Storai A, Mamusa M, Berti D, Fragai M, Richichi B, Nativi C: Synthesis and binding monitoring of a new nanomolar PAMAM-based matrix metalloproteinases inhibitor (MMPIs). *Bioorg Med Chem*, (2017); 25:523–527.
146. Vandamme TF, Brobeck L: Poly(amidoamine) dendrimers as ophthalmic vehicles for ocular delivery of pilocarpine nitrate and tropicamide. *J Control Release*, (2005); 102:23–38.
147. Yavuz B, Bozda_g Pehlivan S, Sümer Bolu B, Nomak Sanyal R, Vural _I, Ünlü N: Dexamethasone - PAMAM dendrimer conjugates for retinal delivery: preparation, characterization and in vivo evaluation. J Pharm Pharmacol (2016); 68:1010–1020.
148. Iezzi R, Guru BR, Glybina IV, Mishra MK, Kennedy A, Kannan RM: Dendrimer-based targeted intravitreal therapy for sustained attenuation of neuroinflammation in retinal degeneration. *Biomaterials*, (2012); 33, 979–988.

149. Kambhampati SP, Mishra MK, Mastorakos P, Oh Y, Lutty GA, Kannan RM: Intracellular delivery of dendrimer triamcinolone acetonide conjugates into microglial and human retinal pigment epithelial cells. *Eur J Pharm Biopharm,* (2015); 95(Pt B): 239–249.
150. Ideta R, Tasaka F, Jang WD, Nishiyama N, Zhang GD, Harada A, Yanagi Y, Tamaki Y, Aida T, Kataoka K: Nanotechnology-based photodynamic therapy for neovascular disease using a supramolecular nanocarrier loaded with a dendritic photosensitizer. *Nano Lett* (2005); 5: 2426–2431.
151. Sugisaki K, Usui T, Nishiyama N, Jang WD, Yanagi Y, Yamagami S, Amano S, Kataoka K: Photodynamic therapy for corneal neovascularization using polymeric micelles encapsulating dendrimer porphyrins. *IOVS*, (2008); 49:894–899.
152. Jang WD, Nishiyama N, Kataoka K: Preparation of naphthalocyanine dendrimer loaded polyion complex micelle for photodynamic therapy. *Key Eng Mater,* (2007); 342/343: 465–468.
153. Marano RJ, Wimmer N, Kearns PS, Thomas BG, Toth I, Brankov M, Rakoczy PE: Inhibition of in vitro VEGF expression and choroidal neovascularization by synthetic dendrimer peptide mediated delivery of a sense oligonucleotide. *Exp Eye Res,* (2004); 79: 525–535.
154. Marano RJ, Toth I, Wimmer N, Brankov M, Rakoczy PE: Dendrimer delivery of an anti- VEGF oligonucleotide into the eye: a longterm study into inhibition of laser-induced CNV, distribution, uptake and toxicity. *Gene Ther,* (2005); 12: 1544–1550.
155. Mastorakos P, Kambhampati SP, Mishra MK, Wu T, Song E, Hanes J, Kannan RM. Hydroxyl PAMAM dendrimer-based gene vectors for transgene delivery to human retinal pigment epithelial cells. *Nanoscale*, (2015); 7:3845–3856.
156. Tamaki Y: Novel approach for management of age-related macular degeneration – antiangiogenic therapy and retinal regenerative therapy (in Japanese). *Nippon Ganka Gakkai Zasshi* (2007); 111: 232–268.
157. Kang SJ, Durairaj C, Kompella UB, O'Brien JM, Grossniklaus HE: Subconjunctival nanoparticle carboplatin in the treatment of murine retinoblastoma. *Arch Ophthalmol*, (2009); 127:1043–1047.
158. Eghtesadi SA, Kashfipour MA, Sun X, Zhang W, Lillard RS, Cheng SZ, Liu T: Hierarchical self-assembly of zwitterionic dendrimer–anionic surfactant complexes into multiple stimuli-responsive dynamic nanotubes. *Nanoscale*, (2018); 10, 1411–1419.
159. Zhong D, Tu Z, Zhang X, Li Y, Xu X, Gu Z: Bioreducible peptide-dendrimeric nanogels with abundant expanded voids for efficient drug entrapment and delivery. *Biomacromolecules*, (2017); 18, 3498–3505.
160. Vasita R, Katti DS: Nanofibers and their applications in tissue engineering. *International Journal of Nanomedicine*, (2006); 1, 15–30.
161. Hotaling NA, Khristov V, Wan Q, Sharma R, Jha BS, Lotfi M, … Bharti K: Nanofiber scaffold-based tissue-engineered retinal pigment epithelium to treat degenerative eye diseases. *Journal of Ocular Pharmacology and Therapeutics*, (2016); 32, 272–285.
162. Cejkova J, Cejka C, Trosan P, Zajicova A, Sykova E, Holan V: Treatment of alkali-injured cornea by cyclosporine A-loaded electrospun nanofibers - An alternative mode of therapy. *Experimental Eye Research*, (2016); 147, 128–137.
163. Garg T, Malik B, Rath G, Goyal AK: Development and characterization of nano-fiber patch for the treatment of glaucoma. *European Journal of Pharmaceutical Sciences*, (2014); 53, 10–16.
164. Yavuz B, Bozda G, Pehlivan S, Kaffashi A, Çalamak S, Ulubayram K, Palaska E, … Ünlü N: In vivo tissue distribution and efficacy studies for cyclosporine a loaded nano-decorated subconjunctival implants. *Drug Delivery*, (2016); 23, 3279–3284.
165. Lancina MG, Singh S, Kompella UB, Husain S, Yang H: Fast dissolving dendrimer nanofiber mats as alternative to eye drops for more efficient antiglaucoma drug delivery. *ACS Biomaterials Science & Engineering*, (2017); 3, 1861–1868.
166. Uchegbu IF, Vyas SP: Non-ionic surfactant based vesicles (niosomes) in drug delivery. *International Journal of Pharmaceutics*, (1998); 172, 33–70.
167. Moghassemi S, Hadjizadeh A: Nano-niosomes as nanoscale drug delivery systems: An illustrated review. *Journal of Controlled Release*, (2014); 185, 22–36.
168. Abdelkader H, Ismail S, Kamal A, Alany RG: Design and evaluation of controlledrelease niosomes and discomes for naltrexone hydrochloride ocular delivery. *J Pharm Sci*, (2011); 100:1833–1846.
169. Vyas SP, Mysore N, Jaitely V, Venkatesan N: Discoidal niosome based controlled ocular delivery of timolol maleate. *Pharmazie*, (1998); 53:466–469.
170. Abdelkader H, Wu Z, Al-Kassas R, Alany RG: Niosomes and discomes for ocular delivery of naltrexone hydrochloride: morphological, rheological, spreading properties and photoprotective effects. *Int J Pharm* (2012); 433:142–148.
171. Kakkar S, Kaur IP: Spanlastics--a novel nanovesicular carrier system for ocular delivery. *Int J Pharm*, (2011); 413(1-2):202-10.
172. Zeng W, Li Q, Wan T, Liu C, Pan W, Wu Z, … Lin Y: Hyaluronic acid-coated niosomes facilitate tacrolimus ocular delivery: Mucoadhesion, precorneal retention, aqueous humor pharmacokinetics, and transcorneal permeability. *Colloids and Surfaces B: Biointerfaces*, (2016); 141, 28–35.
173. Akhtar N: Vesicular ocular drug delivery system: preclinical and clinical perspective of drugs delivered via niosomes. *Int J Biopharm* (2013); 4:11.
174. Khalil RM, Abdelbary GA, Basha M, Awad GE, El-Hashemy H: Design and evaluation of proniosomes as a carrier for ocular delivery of lomefloxacin HCl. *J Liposome Res,* 2016; 27(2): 118–129.
175. Prabhu P, Nitish KR, Koland M, Harish N, Vijayanarayan K, Dhondge G, Charyulu R: Preparation and evaluation of nano-vesicles of brimonidine tartrate as an ocular drug delivery system. *J Young Pharm* (2010); 2:356–361.
176. Sathyavathi V, Hasansathali AA, Sangeetha RI: Formulation and evaluation of niosomal *in situ* gel ocular delivery system of brimonidine tartrate. Int. Journal of life Sc. & Pharma Res, (2012); 2, 1.
177. Aggarwal D, Kaur IP: Improved pharmacodynamics of timolol maleate from a mucoadhesive niosomal ophthalmic drug delivery system. International Journal of Pharmaceutics, (2005); 90, 155–159.
178. Guinedi AS, Mortada ND, Mansour S, Hathout RM : Preparation and evaluation of reverse-phase evaporation and multilamellar niosomes as ophthalmic carriers of acetazolamide. *Int J Pharm,* (2005); 306: 71–82.
179. Kaur IP, Rana C, Singh M, Bhushan S, Singh H, Kakkar S: Development and evaluation of novel surfactant-based elastic vesicular system for ocular delivery of fluconazole. *J Ocul Pharmacol Ther*, (2012); 28(5):484-96.
180. Spicer PT: Progress in liquid crystalline dispersions: Cubosomes. Current Opinion in Colloid & Interface Science, (2005); 10, 274–279.
181. Gan L, Han S, Shen J, Zhu J, Zhu C, Zhang X, Gan Y: 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, 179–187.
182. Souza C, Watanabe E, Borgheti-Cardoso LN, Fantini MCDA, Lara MG: Mucoadhesive system formed by liquid crystals for buccal administration of poly (hexamethylene biguanide) hydrochloride. *Journal of Pharmaceutical Sciences*, (2014); 103, 3914–3923.
183. Han S, Shen JQ, Gan Y, Geng HM, Zhang XX, Zhu CL, Gan L: Novel vehicle based on cubosomes for ophthalmic delivery of flurbiprofen with low irritancy and high bioavailability. *Acta Pharmacol Sin*, (2010); 31:990–998.

184. Gan L, Han S, Shen J, Zhu J, Zhu C, Zhang X, Gan Y: Self-assembled liquid crystalline nanoparticles as a novel ophthalmic delivery system for dexamethasone: Improving preocular retention and ocular bioavailability. *Int J Pharm,* (2010); 396:179–187.
185. Li J, Wu L, Wu W, Wang B, Wang Z, Xin H, Xu Q: A potential carrier based on liquid crystal nanoparticles for ophthalmic delivery of pilocarpine nitrate. *Int. J.Pharm.* (2013); 455(1–2), 75–84.
186. Huang J, Peng T, Li Y, Zhan Z, Zeng Y, Huang Y, Pan X, Wu CY, Wu C: Ocular cubosome drug delivery system for timolol maleate: preparation, characterization, cytotoxicity, *ex vivo*, and *in vivo* evaluation. *AAPS PharmSciTech*, (2017); 18(8), 2919–2926.
187. Bukusoglu E, Wang X, Martinez-Gonzalez JA, de Pablo JJ, Abbott NL: Stimuli-responsive cubosomes formed from blue phase liquid crystals. *Advanced Materials*, (2015); 27, 6892–6898.
188. Gómez-Gaete C, Fattal E, Silva L, Besnard M, Tsapis N: Dexamethasone acetate encapsulation into Trojan particles. *J. Control. Release,* (2008); *128*(1), 41-49.
189. Yandrapu SK, Upadhyay AK, Petrash JM, Kompella UB: Nanoparticles in porous microparticles prepared by supercritical infusion and pressure quench technology for sustained delivery of bevacizumab. *Mol. Pharm.,* (2013), *10*(12), 4676-4686.
190. Elsaid N, Jackson TL, Elsaid Z, Alqathama A, Somavarapu S: PLGA Microparticles Entrapping Chitosan-Based Nanoparticles for the Ocular Delivery of Ranibizumab. *Mol. Pharm.,* (2016); *13*(9), 2923-2940.
191. Thakur SS, Chen YS, Houston ZH, Fletcher N, Barnett NL, Thurecht KJ, Rupenthal ID, Parekh HS: Ultrasound-responsive nanobubbles for enhanced intravitreal drug migration: An ex vivo evaluation, *European J of Pharm & Biopharm,* (2019); 136 102–107.
192. Basuki JS, Qie F, Mulet X, Suryadinata R, Vashi AV, Peng YY, Li L, Hao X, Tan T, Hughes TC: Photo-modulated therapeutic protein release from a hydrogel depot using visible light. *Angew Chem Int Ed Engl*, (2017); 56:966–971.
193. Sharma A, Tandon A, Tovey JC, Gupta R, Robertson JD, … Mohan RR: Polyethylenimine-conjugated gold nanoparticles: gene transfer potential and low toxicity in the cornea. *Nanomedicine*, (2011); 7:505–513.
194. Tandon A, Sharma A, Rodier JT, Klibanov AM, Rieger FG, Mohan R: BMP7 gene transfer via gold nanoparticles into stroma inhibits corneal fibrosis in vivo. *PLoS ONE,* (2013); 8:e66434.
195. Mitra M, Kandalam M, Rangasamy J, Shankar B, Mahswari UK, Swaminathan S, Krishnakumar S: Novel epithelial cell adhesion molecule antibody conjugated polyethyleneimine-capped gold nanoparticles for enhanced and targeted small interfering RNA delivery to retinoblastoma cells. *Mol Vis*, (2013); 19:1029–1038.
196. Pereira DV, Petronilho F, Pereira HR, Vuolo F, Mina F, Possato JC, … Dal-Pizzol F: Effects of gold nanoparticles on endotoxin-induced uveitis in rats. *IOVS*, (2012); 53:8036–8041.
197. Kim JH, Kim MH, Jo DH, Yu YS, Lee TG, Kim JH: The inhibition of retinal neovascularization by gold nanoparticles via suppression of VEGFR-2 activation. *Biomaterials*, (2011); 32:1865–1871.
198. Bengani LC, Hsu KH, Gause S, Chauhan A: Contact lenses as a platform for ocular drug delivery, *Expert Opin. Drug Deliv*, (2013); 10 1483–1496.
199. Carvalho IM, Marques CS, Oliveira RS, Coelho PB, Costa PC, Ferreira DC: Sustained drug release by contact lenses for glaucoma treatment – a review, *J. Control. Release*, (2015); 202, 76–82.
200. Jung HJ, Chauhan A: Temperature sensitive contact lenses for triggered ophthalmic drug delivery. *Biomaterials*, (2012); 33, 2289–2300.
201. Kim HJ, Zhang K, Moore L, Ho D: Diamond Nanogel-embedded contact lenses mediate lysozyme-dependent therapeutic release, *ACS Nano*, (2014); 8, 2998–3005.
202. Behl G, Iqbal J, O'Reilly NJ, Mcloughlin P, Fitzhenry L: Synthesis and characterization of poly(2-hydroxyethylmethacrylate) contact lenses containing chitosan nanoparticles as an ocular delivery system for dexamethasone sodium phosphate *Pharm. Res*, (2016); 33, 1638–1648.
203. Helaly FM, El-Sawy SM, Hashem AI, Khattab AA, Mourad RM: Synthesis and characterization of nanosilver-silicone hydrogel composites for inhibition of bacteria growth *Cont. Lens Anterior Eye*, (2017); 40,59–66.
204. Wang MTM, Ganesalingam K, Loh CS, Alberquerque T, Al-Kanani S, Misra SL, Craig JP: Compatibility of phospholipid liposomal spray with silicone hydrogel contact lens wear. *Cont. Lens Anterior Eye*, (2017); 40, 53–58.
205. Kapoor Y, Thomas JC, Tan G, John VT, Chauhan A: Surfactant-laden soft contact lenses for extended delivery of ophthalmic drugs. *Biomaterials*, (2009); 30, 867–878.
206. Hiratani H, Fujiwara A, Tamiya Y, Mizutani Y, Alvarez-Lorenzo C: Ocular release of timolol from molecularly imprinted soft contact lenses. *Biomaterials*, (2005); 26, 1293–1298.
207. Di Trani N, Jain P, Chua C, Ho J, Bruno G, Susnjar A, Pons-Faudoa FP, Sizovs A, Hood RL, Smith JW, Ballerini A, Filgueira CS, Grattoni A: Nanofluidic microsystem for sustained intraocular delivery of therapeutics *Nanomedicine: Nanotechnology, Biology, and Medicine*, (2019); 16, 1–9.
208. Fulgêncio GdeO, Viana FAB, Ribeiro RR, Yoshida MI, Faraco AG, Cunha AS: New mucoadhesive chitosan film for ophthalmic drug Adv Ther delivery of timolol maleate: *in vivo* evaluation. *J Ocul Pharm Ther*, (2012); 28(4): 350-8.
209. Jung HJ, Chauhan A: Extended release of timolol from nanoparticle-loaded fornix insert for glaucoma therapy. *J Ocul Pharmacol Ther* (2013); 29:229–35.
210. Rathod LV, Kapadia R, Sawant KK: A novel nanoparticle impregnated ocular insert for enhanced bioavailability to posterior segment of eye: *in vitro, in vivo* and stability studies. *Mater Sci Eng C Mater Biol Appl,* (2017); 1(71):529–40.
211. Franca JR, Foureaux G, Fuscaldi LL, Bravo R, … Faraco AAG: Bimatoprost-loaded ocular inserts as sustained release drug delivery systems for glaucoma treatment: *in vitro and in vivo* evaluation. *PLoS One,* (2014); 9(4): e95461.
212. De Souza F, Júlio NM, Kamila PSDO, Patrício GM, Fernandes-Cunha MG, Da Silva CEDM, Da Silva Jensen GR: Ocular inserts based on chitosan and brimonidine tartrate: development, characterization and biocompatibility, *J. Drug Delivery Sci. Technol*, (2016); 32, 21–30.
213. Navratil T, Maynor B, Yerxa B: Improving outcomes In ophthalmology via Sustained drug delivery. *ON Drug Delivery*, (2014); 48.
214. Canelas DA, Herlihy KP, DeSimone JM: Top-Down Particle Fabrication: Control of Size and Shape for Diagnostic Imaging and Drug Delivery. *Wiley Interdiscip Rev Nanomed Nanobiotechnol,* (2009); 1(4): 391–404.
215. Garcia A, Tully J, Das S, Pegoraro T, Trevino L, Maynor B, Navratil T, Yerxa BR: Precisely Engineered Biodegradable Drug Delivery Systems for the Extended Release of Prostaglandin Analogues in the Anterior Chamber. *IOVS*, (2014); 55, 5268
216. Birch DG, Bennett LD, Duncan JL, Weleber RG, Pennesi ME: Long-term follow-up of patients with retinitis pigmentosa receiving intraocular ciliary neurotrophic factor implants. *Am. J. Ophthalmol,* (2016); 170, 10-14.

217. Wong FSY, Tsang KK, Lo ACY: Delivery of therapeutics to posterior eye segment: cell-encapsulating Systems. *Neural Regeneration Research*, (2017); 12: 4.
218. Lee JW, Park JH, Prausnitz MR: Dissolving microneedles for transdermal drug delivery. *Biomaterials*, (2008); 29:2113–2124.
219. Song HB, Lee KJ, Seo IH, Lee JY, Lee SM, Kim JH, Kim JH, Ryu W: Impact insertion of transfer-molded microneedle for localized and minimally invasive ocular drug delivery. *J Control Release*, (2015); 209:272–279.
220. Jiang J, Gill HS, Ghate D, McCarey BE, Patel SR, Edelhauser HF, Prausnitz MR: Coated microneedles for drug delivery to the eye. *IOVS*, (2007); 48:4038–4043.
221. Than A, Liu C, Chang H, Duong PK, Cheung CMG, Xu C, Wang X, Chen P: Self-implantable double-layered micro-drug-reservoirs for efficient and controlled ocular drug delivery. *Nat Commun,* (2018); 9, 4433.
222. Thakur RRS, Tekko IA, Al-Shammari F, Ali AA, McCarthy H, Donnelly RF: Rapidly dissolving polymericmicroneedles forminimally invasive intraocular drug delivery. *Drug Deliv. Transl. Res*, (2016); 6 800–815.
223. Chiang B, Venugopal N, Edelhauser HF, Prausnitz MR: Distribution of particles, small molecules and polymeric formulation excipients in the suprachoroidal space after microneedle injection, *Exp. Eye Res*, (2016); 153, 101–109.
224. Hartman RR, Kompella UB: Intravitreal, subretinal, and suprachoroidal injections: evolution of microneedles for drug delivery. *J. Ocul. Pharmacol*, (2018); 34, 1–13.
225. Rowe-Rendleman CL, Durazo S, Kompella UB, Rittenhouse KD, Di Polo A, Weiner AL, Grossniklaus HE, Naash MI, Lewin AS, Horsager A, Edelhauser HF: Drug and gene delivery to the back of the eye: from bench to bedside. *IOVS*, (2014); 55, 2714–2730.
226. Coursey TG, Henriksson JT, Marcano DC, Shin CS, Isenhart LC, Ahmed F, De Paiva CS, Pflugfelder SC, Acharya G: Dexamethasone nanowafer as an effective therapy for dry eye disease. *J Control Release*, (2015); 213:168–174.
227. Bian F, Shin CS, Wang C, Pflugfelder SC, Acharya G, De Paiva CS: Dexamethasone drug eluting nanowafers control inflammation in alkali-burned corneas associated with dry eye. *IOVS*, (2016); 57:3222–3230.
228. Yuan X, Marcano DC, Shin CS, Hua X, Isenhart LC, Pflugfelder SC, Acharya G: Ocular drug delivery nanowafer with enhanced therapeutic efficacy. *ACS Nano*, (2015); 9:1749–1758.
229. Gutierrez-Hernandez JC, Caffey S, Abdallah W, Calvillo P, Gonzalez R, Shih J, Brennan J, Zimmerman J, Martinez-Camarillo JC, Rodriguez, Varma R, Santos A, Sanchez G, Humayun M: One-year feasibility study of replenish MicroPump for intravitreal drug delivery: a pilot study, *Transl. Vis. Sci. Technol*, (2014); 3:1–8.
230. Saati S, Lo R, Li PY, Meng E, Varma R, Humayun MS: Mini drug pump for ophthalmic use. *Curr. Eye Res*, (2010); 35: 192–201.
231. Song P, Kuang S, Panwar N, Yang G, Tng DJH, Tjin SC, Ng WJ, Majid MBA, Zhu G, Yong KT: A self-powered implantable drug-delivery system using biokinetic energy. *Adv. Mater*, (2017); 29;11.
232. Pirmoradi FN, Jackson JK, Burt HM, Chiao M: On-demand controlled release of docetaxel from a battery-less MEMS drug delivery device. *Lab Chip*, (2011); 11: 2744–2752.
233. Wilson DF, Vinogradov SA, Grosul P, Vaccarezza MN, Kuroki A, Bennett J: Oxygen distribution and vascular injury in the mouse eye measured by phosphorescence-lifetime imaging. *Appl. Opt,* (2005); 44: 5239-5248.
234. Tari SR, Barile GR, Kompella UB: Polychromatic, diversely-sized particles for angiography. In: Office, U.S.P. (Ed.), A61K 51/00; A61K 49/00; A61K 9/127; A61K 9/14; A61K 9/16 Ed. (2009); p. 19.
235. Doolittle ND, Christoforidis GA, Frank JA, Lindau M, Lockman PR, Manninger SP, Qiang Y, Spence AM, Stupp SI, Zhang M, Neuwelt EA: Imaging and nanomedicine for diagnosis and therapy in the central nervous system: report of the Eleventh Annual Blood-Brain Barrier Disruption Consortium Meeting. *Am J Neuroradiol,* (2006); 27: 715–721.
236. Tam AL, Gupta N, Zhang Z, Yücel YH: Quantum dots trace lymphatic drainage from the mouse eye. *Nanotechnology*, (2011); 22:425101.
237. Raveendran S, Lim H, Maekawa T, Matham MV, Kumar S: Gold nanocages entering into the realm of highcontrast photoacoustic ocular imaging. *Nanoscale*, (2018); 10, 13959.
238. Nguyen-Vu VP, Li Y, Qian W, Liu B, Tian C, Zhang W, Huang Z, Ponduri A, Tarnowski M, Wang X, Paulus YM: Contrast Agent Enhanced Multimodal Photoacoustic Microscopy and Optical Coherence Tomography for Imaging of Rabbit Choroidal and Retinal Vessels *in vivo, Scientific Reports,* (2019); 9: 5945.
239. Cohen-Karni T, Timko BP, Weiss LE, Lieber CM: Flexible electrical recording from cells using nanowire transistor arrays. *Proc Natl Acad Sci*, (2009); 106:7309–7313.
240. Kim W, Lee JC, Shin JH, Jin KH, Park HK, Choi S: Instrument-free synthesizable fabrication of label-free optical biosensing paper strips for the early detection of infectious keratoconjunctivitides. *Anal Chem,* (2016); 88:5531–5537.
241. Cialla D, Marz A, Bohme R, Theil F, Weber K, Schmitt M, Popp J: Surface-enhanced Raman spectroscopy (SERS): progress and trends. *Anal Bioanal Chem*, (2012); 403:27–54.
242. Butler HJ, Fogarty SW, Kerns JG, Martin-Hirsch PL, Fullwood NJ, Martin FL: Gold nanoparticles as a substrate in bioanalytical near-infrared surface-enhanced Raman spectroscopy. *Analyst,* (2015); 140:3090–3097.
243. Verma MS, Chen PZ, Jones L, Gu FX: "Chemical nose" for the visual identification of emerging ocular pathogens using gold nanostars. *Biosens Bioelectron*, (2014); 61:386–390.
244. Wang S, Riahi R, Li N, Zhang DD,Wong PK: Single cell nanobiosensors for dynamic gene expression profiling in native tissue microenvironments. *Adv Mater,* (2015); 27:6034–6038.
245. Andoralov V, Shleev S, Arnebrant T, Ruzgas T: Flexible micro(bio)sensors for quantitative analysis of bioanalytes in a nanovolume of human lachrymal liquid. *Anal Bioanal Chem,* (2013); 405:3871–3879.
246. Kim W, Lee SH, Kim SH, Lee JC, Moon SW, Yu JS, Choi S: Highly reproducible Au-decorated ZnO nanorod array on a graphite sensor for classification of human aqueous humors. ACS ApplMater Interfaces. 2017;9:5891–5899.
247. Goto K, Kato D, Sekioka N, et al. Direct electrochemical detection of DNA methylation for retinoblastoma and CpG fragments using a nanocarbon film. *Anal Biochem*, (2010); 405:59Y66.
248. Kato D, Sekioka N, Ueda A, Kurita R, Hirono S, Suzuki K, Niwa O: A nanocarbon film electrode as a platform for exploring DNA methylation. *J Am Chem Soc* (2008); 130:3716.
249. Kato D, Sekioka N, Ueda A, Kurita R, Hirono S, Suzuki K, Niwa O: Nanohybrid carbon film for electrochemical detection of SNPs without hybridization or labeling. *Angew Chemie Int Ed Engl,* (2008); 47:6681Y6684.
250. Takeda A, Baffi JZ, Kleinman ME, Cho WG, Nozaki M, Yamada K, … Ambati J: CCR3 is a target for age-related macular degeneration diagnosis and therapy. *Nature*, (2009); 460:225–230.
251. Prow T, Grebe R, Merges C, Smith JN, McLeod DS, Leary JF, Lutty GA: Nanoparticle tethered antioxidant response element as a biosensor for oxygen induced toxicity in retinal endothelial cells. *Mol Vis*, (2006); 12:616–25.

252. Tang M, Ji X, Xu H, Zhang L, Jiang A, Song B, Su Y, He Y: Photostable and Biocompatible Fluorescent Silicon Nanoparticles-Based Theranostic Probes for Simultaneous Imaging and Treatment of Ocular Neovascularization, *Anal. Chem*, (2018); 90, 8188−8195.
253. Chen J, Patil S, Seal S, McGinnis JF: Rare earth nanoparticles prevent retinal degeneration induced by intracellular peroxides. *Nat Nanotechnol*, (2006); 1:142–50.
254. Zhou Y, Li L, Li S, Li S, Zhao M, Zhou Q, Gong X, Yang J, Chang J: Autoregenerative redox nanoparticles as an antioxidant and glycation inhibitor for palliation of diabetic cataracts† *Nanoscale*, (2019); 11: 13126.
255. Makky A, Michel JP, Maillard P, Rosilio V: Biomimetic liposomes and planar supported bilayers for the assessment of glycodendrimeric porphyrins interaction with an immobilized lectin. *Biochim Biophys Acta,* (2011); 1808(3):656-666.
256. Wang ZJ, Chauvin B, Maillard P, Hammerer F, Carez D, Croisy A, Sandrè C, Chollet-Martin S, Prognon P, Paul JL, Blais J, Kasselouri A: Glycodendrimeric phenylporphyrins as new candidates for retinoblastoma PDT: blood carriers and photodynamic activity in cells. *J Photoch Photobio B*, (2012); 115:16-24.
257. Butterworth KT, McMahon SJ, Currell FJ, Prise KM: Physical basis and biological mechanisms of gold nanoparticle radiosensitization. *Nanoscale*, (2012); 4(16):4830-4838.
258. Chang MY, Shiau AL, Chen YH, Chang CJ, Chen HH, Wu CL: Increased apoptotic potential and dose-enhancing effect of gold nanoparticles in combination with single-dose clinical electron beams on tumor-bearing mice. *Cancer Sci*, (2008); 99(7):1479-1484.
259. Berbeco RI, Ngwa W, Makrigiorgos GM: Localized dose enhancement to tumor blood vessel endothelial cells via megavoltage X-rays and targeted gold nanoparticles: new potential for external beam radiotherapy. *Int J Radiat Oncol Biol Phys*, (2011); 81(1):270-276.
260. Leonardi M, Pitchon EM, Bertsch A, Renaud P, Mermoud A: Wireless contact lens sensor for intraocular pressure monitoring: Assessment on enucleated pig eyes. *Acta Ophthalmol*, (2009); 87, 433–437.
261. Rizq RN, Choi WH, Eilers D, Wright MM, Ziaie B: Intraocular pressure measurement at the choroid surface: A feasibility study with implications for implantable microsystems. *British J. Ophthalmol*, (2001); 85, 868–871.
262. Dresher RP, Irazoqui PP: A compact nanopower low output impedance CMOS operational amplifier for wireless intraocular pressure recordings. In Proceedings of the 2007 29th Annual *International Conference of the IEEE Engineering in Medicine and Biology Society*, (2007); 6055–6058.
263. Chen PJ, Rodger DC, Saati S, Humayun MS, Tai YC: Microfabricated implantable parylene-based wireless passive intraocular pressure sensors. *J. Microelectromechanical Syst,* (2008); 17, 1342–1351.
264. Swain MV, Nohava J, Eberwein P: A simple basis for determination of the modulus and hydraulic conductivity of human ocular surface using nano-indentation. *Acta Biomater*, (2016); 50: 312-321.
265. Sretavan DW, Chang W, Hawkes E, Keller C, Kliot M: Microscale surgery on single axons. *Neurosurgery*, (2005); 57: 635–646.
266. Kim P, Lieber CM: Nanotube nanotweezers. *Science,* (1999); 286:2148 –2150.
267. Bhisitkul RB, Keller CG. Development of microelectromechanical systems (MEMS) forceps for intraocular surgery. *Br J Ophthalmol,* (2005); 89:1586-1588.
268. Ponnusamy T, Yu H, John VT, Ayyala RS, Blake DA: A novel antiproliferative drug coating for glaucoma drainage devices. *J. of Glaucoma*, (2014); 23: 526–534.
269. Shaunak S, Thomas S, Gianasi E, Godwin A, Jones E, Teo I, Mireskandari K, Luthert P, Duncan R, Patterson S, Khaw P, Brocchini S: Polyvalent dendrimer glucosamine conjugates prevent scar tissue formation. *Nat Biotechnol*, (2004); 22:977–84.
270. Ye H, Qian Y, Lin M, Duan Y, Sun X, Zhuo Y, Ge J: Cationic nano-copolymers mediated IKKβ targeting siRNA to modulate wound healing in a monkey model of glaucoma filtration surgery. *Mol Vis,* (2010); 16: 2502–10.
271. Pan T, Brown JD, Ziaie B: An artificial nano-drainage implant (ANDI) for glaucoma treatment. In *Proceedings of the 2006 International Conference of the IEEE Engineering in Medicine and Biology Society*, New York, NY, USA, 30 August–3 September (2006); 3174–3177.
272. Paschalis EI, Chodosh J, Sperling RA, Salvador-Culla B, Dohlman C: A novel implantable glaucoma valve using ferrofluid. *Plos One*, (2013); 8, e67404.
273. Maleki T, Chitnis G, Park JH, Cantor LB, Ziaie B: Biodegradable microfabricated plug-filters for glaucoma drainage devices. *IEEE Trans Biomed Eng*, (2012); 59: 1507–1513.
274. Harake RS, Ding Y, Brown JD, Pan T: Design, fabrication, and in vitro testing of an anti-biofouling glaucoma micro-shunt. *Ann Biomed Eng,* (2015); 43: 2394–2405.
275. Lin YX, Hu XF, Zhao Y, et al., Photothermal ring integrated intraocular lens for high-efficient eye disease treatment. *Adv Mater*., (2017).
276. Yamamoto S, Manabe N, Fujioka K, Hoshino A, Yamamoto K: Visualizing vitreous using quantum dots as imaging agents. *IEEE Trans Nanobioscience,* (2007); 6(1):94-98.
277. Nguyen-Vu TDB, Chen H, Cassell AM, Andrews RJ, Meyyappan M, Li J: Vertically Aligned Carbon Nanofiber Architecture as a Multifunctional 3-D Neural Electrical Interface. *IEEE Transactions on Biomedical Engineering*, (2007); 54: 6
278. Hecht DS, Ramirez RJA, Briman M, Artukivic E, Chichak KS, Stoddart JF, Gruner G: Bioinspired detection of light using a porphyrin-sensitized single-wall nanotube field effect transistor. *Nano Lett*, (2006); 6: 2031–2036.
279. Frydrych M, Silfsten P, Parkkinen S, Parkkinen J, Jaaskelainen T: Color sensitive retina based on bacteriorhodopsin. *Biosystems,* (2000); 54: 131–140.
280. Noorani B, Tabandeh F, Yazdian F, Soheili ZS, Shakibaie M, Rahmani S: Thin natural gelatin/chitosan nanofibrous scaffolds for retinal pigment epithelium cells. *Int. J. Polym. Mater. Polym. Biomater*, (2018); 67: 754–763.
281. Yang TC, Chuang JH, Buddhakosai W, Wu WJ, Lee CJ, Chen WS, Yang YP, Li MC, Peng CH, Chen SJ: Elongation of axon extension for human iPSCderived retinal ganglion cells by a nano-imprinted scaffold. *Int J Mol Sci,* (2017); 18: 2013.
282. Arabpour Z, Baradaran-Rafil A, Bakhshaiesh N, Ai J, Ebrahimi-Braough S, Malekabadi H, Nazeri N, Vaez A, Salehi M, Sefat F, Ostad S: Design and Characterization of Biodegradable Multi Layered Electrospun Nanofibers for Corneal Tissue Engineering Applications. *J Biomed Mater Res A,* (2019); 107 (10), 2340-2349.
283. Wu Z, Kong B, Liu R, Sun W, Mi S: Engineering of corneal tissue through an aligned PVA/collagen composite nanofibrous electrospun scaffold. *Nanomaterials*, (2018); 8: 124.
284. Nibourg LM, Gelens E, De Jong MR, Kuijer R, Van Kooten TG, Koopmans SA: Nanofiber-based hydrogels with extracellular matrix-based synthetic peptides for the prevention of capsular opacification. *Exp. Eye Res*. (2016); 143: 60–67.
285. Xi Y, Dong H, Sun K, Liu H, Liu R, Qin Y, Hu Z, Zhao Y, Nie F, Wang S: Scabinspired cytophilic membrane of anisotropic nanofibers for rapid wound healing. *ACS Appl. Mater. Interfaces*, (2013); 5: 4821–4826.

286. Borras T: Advances in glaucoma treatment and management: gene therapy. *IOVS*, (2012); 53:2506–2510.
287. Du Y, Yun H, Yang E, Schuman JS: Stem cells from trabecular meshwork home to TM tissue in vivo. *IOVS*, (2013); 54:1450–1459.
288. Torrejon KY, Pu D, Bergkvist M, Danias J, Sharfstein ST, Xie Y: Recreating a human trabecular meshwork outflow system on microfabricated porous structures. *Biotechnol Bioeng,* (2013); 110: 3205–3218.
289. Neelesh K. Mehra, Defu Cai, Lih Kuo, Travis Hein & Srinath Palakurthi: Safety and toxicity of nanomaterials for ocular drug delivery Applications, *Nanotoxicology*, (2016); 1-25.
290. Srivastava V, Gusain D, Sharma YC: Critical review on the toxicity of some widely used engineered nanoparticles. *Ind Eng Chem Res,* (2015); 4:6209–33.
291. U. FDA, Guidance for Industry: Considering Whether An FDA-Regulated Product Involves the Application of Nanotechnology, June 2014.
292. Sadrieh N: FDA Considerations For Regulation of Nanomaterial Containing Products, CDER, FDA, 2006.
293. Ariano P, Zamburlin P, Gilardino A, Mortera R, Onida B, Tomatis M, Ghiazza M, Fubini B, Lovisolo D: Interaction of spherical silica nanoparticles with neuronal cells: size-dependent toxicity and perturbation of calcium homeostasis. *Small*, (2011); 7(6): 766-74.
294. Cohen D, Soroka Y, Ma'or Z, Oron M, Portugal-Cohen M, Bregegere FM, Berhanu D, Valsami-Jones E, Hai N, Milner Y: Evaluation of topically applied copper(II) oxide nanoparticle cytotoxicity in human skin organ culture. *Toxicol In Vitro*, (2013); 27(1): 292-8.
295. Wilson SL, Ahearne M, Hopkinson A: An overview of current techniques for ocular toxicity testing. *Toxicology,* (2015); 327: 32–46.
296. Guidolin K, Zheng G: Nanomedicines Lost in Translation. *ACS Nano,* (2019); 13 (12): 13620-13626.