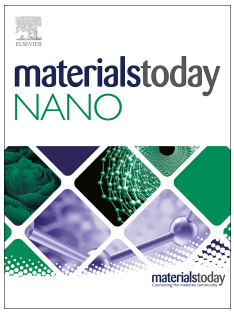


Recent advances in drug delivery systems for glaucoma treatment

Kapil D. Patel, Lady Barrios Silva, Yuli Park, Taleen Shakouri, Zalike Keskin-Erdogan, Prasad Sawadkar, Kyong Jin Cho, Jonathan C. Knowles, David Y.S. Chau, Hae-Won Kim

PII: S2588-8420(22)00006-2

DOI: https://doi.org/10.1016/j.mtnano.2022.100178

Reference: MTNANO 100178

To appear in: *Materials Today Nano*

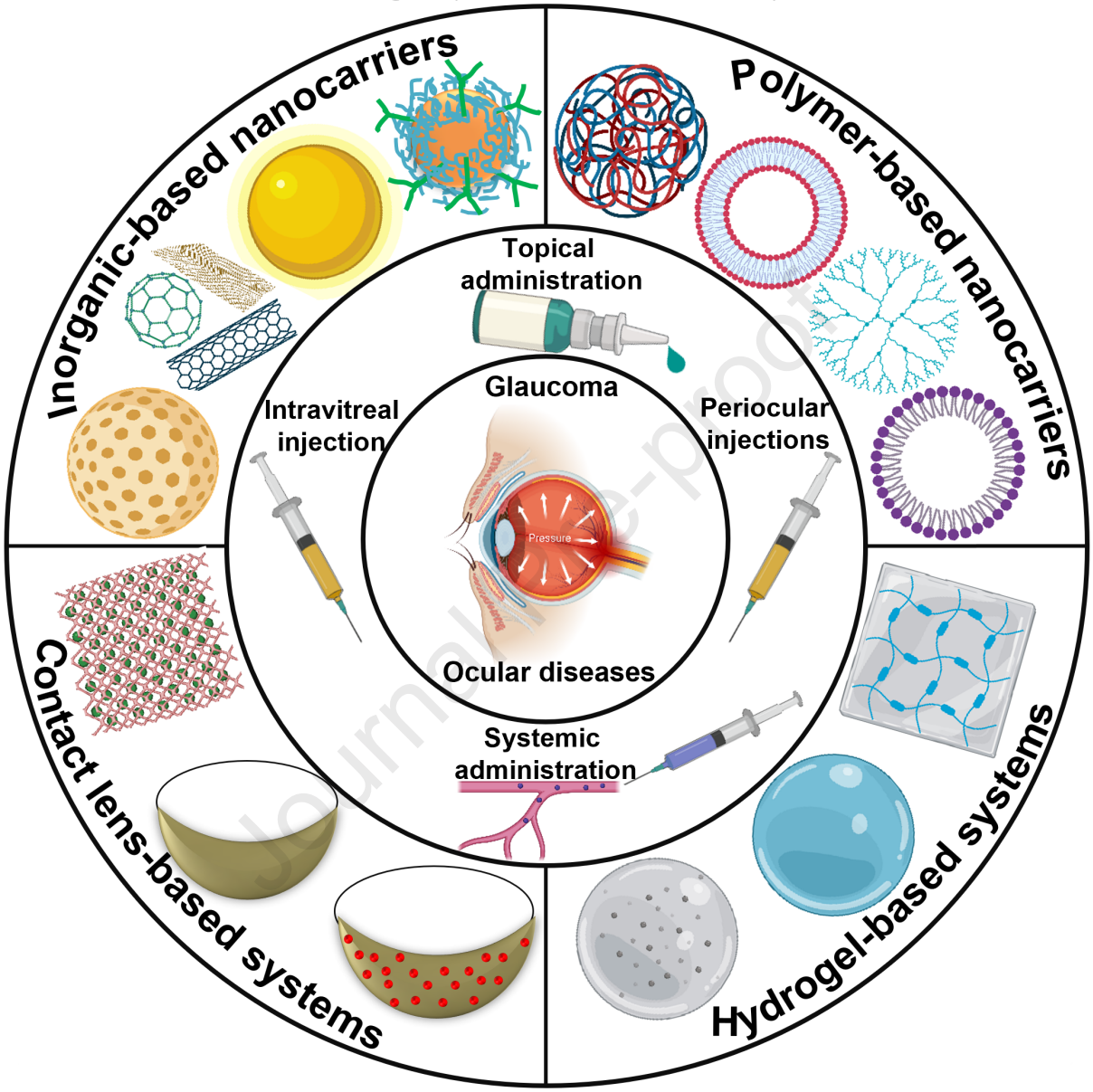
Received Date: 4 November 2021

Revised Date: 11 January 2022

Accepted Date: 30 January 2022

Please cite this article as: Patel K.D., Silva L.B., Park Y., Shakouri T., Keskin-Erdogan Z., Sawadkar P., Cho K.J., Knowles J.C., Chau D.Y.S. & Kim H.-W., Recent advances in drug delivery systems for glaucoma treatment, *Materials Today Nano*, https://doi.org/10.1016/j.mtnano.2022.100178.

1. Formulations (inorganic nanoparticles, polymers nanoparticles, hydrogel-based, contact lens/implants)
2. Administrations (topical, intravitreal, periocular, systemic)
3. Targets (Glaucoma/ocular diseases)



Recent advances in drug delivery systems for glaucoma treatment

Kapil D. Patel[1,2,3,9,*], Lady Barrios Silva[6], Yuli Park[5], Taleen Shakouri[6], Zalike Keskin-Erdogan[6], Prasad Sawadkar[2,8], Kyong Jin Cho[5], Jonathan C. Knowles[1,2,3,4,7], David Y.S. Chau[2,6], Hae-Won Kim[1,2,3,4,*]

[1]Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Cheonan, 31116, South Korea

[2]UCL Eastman-Korea Dental Medicine Innovation Centre, Dankook University, Cheonan, 31116, Republic of Korea

[3]Department of Nanobiomedical Science & BK21 NBM Global Research Center for Regenerative Medicine, Dankook University, Cheonan, 31116, South Korea

[4]Department of Biomaterials Science, College of dentistry, Dankook University, Cheonan, 31116, Republic of Korea

[5]Department of Ophthalmology, College of Medicine, Dankook University Hospital, Cheonan, 31116, South Korea

[6]Division of Biomaterials and Tissue Engineering, Eastman Dental Institute, University College London, Royal Free Hospital, Rowland Hill Street, London, NW32PF, London, UK

[7]Centre for Precision Healthcare, UCL Division of Medicine, University College London, 5 University St, London WC1E 6JF

[8]Division of Surgery and Interventional Science, University College London, London W1W7TY, UK

[9]Department of Materials Science and Engineering, Korea University, Seoul 02841, South Korea

---

*Corresponding author, Kapil D. Patel (kapildpatel20@gmail.com, kimhw@dku.edu)

For: Materials Today Nano

Contents

1. Introduction
2. Glaucoma: treatment, prevention, and necessity of carriers for ocular drug delivery
    - 2.1. Necessity of carriers for ocular drug delivery
    - 2.2. Topical administration
    - 2.3. Ocular and periocular injections
    - 2.4. Systemic administration of ocular nanosystems
    - 2.5. Metabolic clearance of nanocarriers
3. Development of carriers for ocular drug delivery
    - 3.1. Inorganic-based carriers
        - 3.1.1. Magnetic nanoparticles (MNPs)
        - 3.1.2. Gold nanoparticles (AuNPs)
        - 3.1.3. Silver nanoparticles (AgNPs)
        - 3.1.4. Mesoporous silica nanoparticles (MSN)
        - 3.1.5. Carbon-based nanocarriers (CBNs)
        - 3.1.6. Ceria-based nanoparticles (CeNPs)
    - 3.2. Polymer-based nanocarriers
        - 3.2.1. Collagen
        - 3.2.2. Gelatin
        - 3.2.3. Chitosan
        - 3.2.4. Sodium alginate
        - 3.2.5. Hyaluronic acid (HA)
        - 3.2.6. Silk-fibroin (SF)
        - 3.2.7. Synthetic polymer-based carriers
        - 3.2.8. Other carriers
    - 3.3. Hydrogel-based delivery systems
    - 3.4. Contact lens-based delivery systems
4. Concluding remarks
5. References

ABSTRACT

Glaucoma is a chronic eye disease and the second most leading cause of irreversible impaired vision in the world, which is primarily linked with high intraocular pressure (IOP). The treatment of glaucoma is currently challenging due to the innate mechanics of ocular barriers that limit the penetration of ophthalmic drugs. To tackle this, various carriers (inorganic-, polymeric-, hydrogel-, or contact lens-based) with specialized physical and chemical properties have been intensively studied. These carriers-drug formulations have shown significant improvement in various aspects such as ocular barriers penetration, bioavailability, sustained release of drug, tissue targeting, and lowering the IOP. The delivery systems can be administered through various routes (intravitreal or periocular injection, or systemic route), enabling the drugs to reach the damaged sites and help to recover the damaged optical nerves, thus considered an effective treatment strategy for glaucoma. In this review, we thoroughly investigate the recent advances in ocular delivery formulations focusing on glaucoma, including nanocarrier types, delivery route, and the efficacy in vitro and in vivo, clinical availability, and future outlook.

Keywords: Nanocarriers, Ocular Drug Delivery, Glaucoma, Hydrogel, Contact Lens

1. Introduction

Glaucoma is a chronic ocular disorder characterized by dysfunctional of optical nerve and associated visual field loss by progressive impairment or retinal ganglion cells (RGCs) death and second most prominent reason of blindness after age-related macular degeneration globally [1,2]. The World Health Organization (WHO) report entitled "world report on vision" published in 2020 claim that at least 76 million individuals are suffering from glaucoma and is predicted a significant surge of 1.3 times by 2030 [3]. Currently, the most frequently used medication method to management and treatment of glaucoma is topical administration (*i.e.,* eye drop). However, the major challenges are limited period precorneal residence with the desired dose, inadequate corneal access, and low ocular bioavailability [4]. A sum of 74% of glaucoma patients use topical treatment with eye drops to an alternative treatment method such as 3-monthly subconjunctival injections [5]. Injections or implants in the subconjunctival area have been reported to be non-invasive and well-accepted by many patients in the management and treatment of glaucoma. Recent years, a huge surge has been found for the development of controlled and on demand sustained ocular drug delivery systems for glaucoma treatment [6,7]. An excessive intra ocular pressure (IOP) often associates with glaucoma. Nanocarriers are promising, safe and effective approach to address the low bioavailability and subsequent IOP fluctuations via a controlled delivery system with sustained drug concentration over extended periods of time.

In the recent years, different kinds of nanocarriers are synthesized for safe and efficient ocular drug delivery applications. These nanocarriers include inorganic nanoparticles, polymer nanoparticles, hydrogels, contact lenses and others smart nanocarriers. Inorganic nanoparticles-based carriers such as iron oxide, silver, gold, carbon-based nanomaterials (CBNs), and mesoporous silica-based nanoparticles (MSNs) were developed and applied for ocular delivery as these nanocarriers possessed specific characteristics such as controlled sized, shape, large surface area, versatile surface functionality, biocompatibility, increased dissolution rate, penetration ability, distribution, and the ability to glue to the cell surface membrane. Similarly, polymer-based nanocarriers, for example nanocapsules, nanoparticles, micelles, liposomes, nanocapsules, and dendrimers have also been widely explored with different therapeutic molecules including drugs, growth factors, proteins, genes, and peptides [8]. Other biomaterials like hydrogel-based

injections as well as contact lenses, and implants have also been studies widely for direct and controlled delivery approach.

Ocular drug administration through minimally invasive approaches include topical eye drops, eye ointments and gel drops to treat various eye diseases, however, these treatment approaches are not always effective. The effectiveness of the medications available for retinal and optic nerve related disease treatment are limited due to encumbers made by the blood-retinal barrier (BRB), and entering systemic circulation [9]. The eye drops which are extensively used for controlling IOP associated with glaucoma and also for the preservation of corneal endothelial cells [10]. However, physiological barriers of eyes such as the corneal epithelial barrier (CEB) limits the bioavailability and effectiveness of topical eye drops, eye ointments and gel drops. In fact, approximately 4 to 5% of the drug for a short time is retained by ocular layer and very low amount of the drug can cross the CEB and reaches to cornea [11]. To overcome the above physiological barriers, recently, the ocular drug administrative approaches such as ocular/preocular injections and systemic administration of ocular nanosystems have been developed. However, these approaches are still in developmental stages and used in very severe cases. Finally, the penetration and distribution of the nanocarriers are also important for the safety and long-term side-effects.

In this review, we have emphasized the formulations and rationale of various types of nanocarriers and particularly the clinical solution for glaucoma treatment, while addressing their drug loading capacity, physical and biological limitations, strategies of drug delivery and distribution and penetration of nanocarriers. We have also highlighted the three main routes for ocular drug administration and as well as major challenges and opportunity and future prospective for the nanocarriers developments and their effective application in glaucoma drug delivery. The goal of this review is summarized in **Figure1**, the outer concentric circle explains the various biomaterials (inorganic nanoparticles, polymers nanoparticles, hydrogel-based materials, and contact lens/implants) for drug formulations, the middle co-centric circle describes the administrative approaches (topical, intravitreal, periocular, systemic) for drug delivery, and inner co-centric circle represents the target disease (*i.e.*, glaucoma).

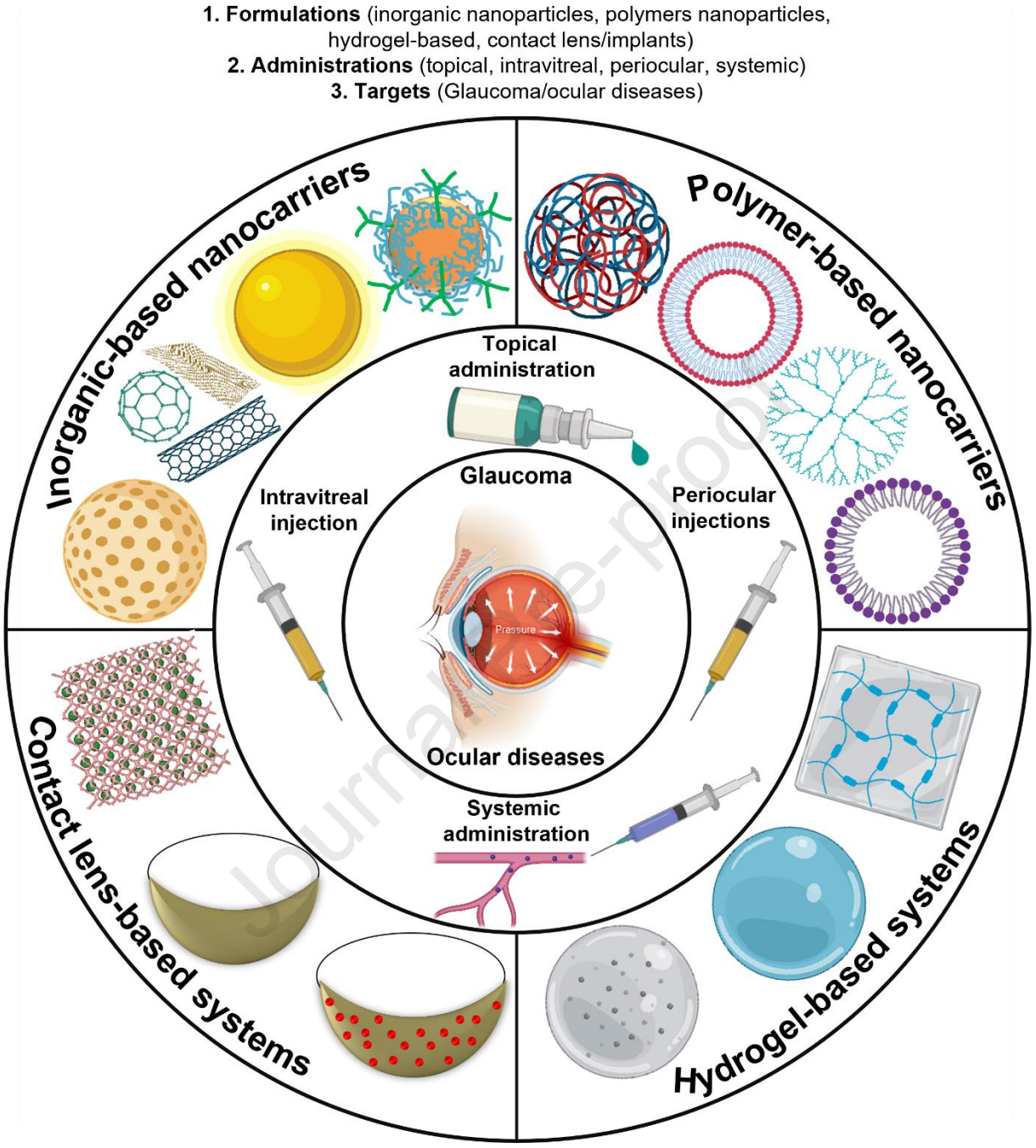


Figure 1. Schematic illustration of different types of nanomaterials (inorganic nanoparticles, polymers nanoparticles, hydrogel-based, contact lens/implants) formulations and their various ocular delivery administration in glaucoma applications discussed in this review. Rationales of nanocarriers and drug formulations, strategies, for drug delivery, distribution, penetration, and limitations in ocular delivery were summarized. Figure created by authors using BioRender.com.

2. Glaucoma: treatment, prevention, and necessity of carriers for ocular drug delivery

Glaucoma is a type of ocular neuropathy that progresses over time and can lead to blindness. The loss of RGCs results a permanent visual field defects and it affects more than 76 million individuals worldwide [12]. Understanding the mechanism and following alterations of glaucoma yields the foundation of therapeutic approach, therefore the sole purpose of this review is to provide a well-organized and comprehensive understanding of basic mechanisms and the treatment of glaucoma. A schematic illustration of glaucoma disease and related issues are summarized in **Figure 2**. The normal and blocked drainage channel are presented in **Figure 2 (i-ii)**. Glaucoma conditioned eyes shows pressure buildup which eventually causes change in optic nerve as is presented in **Figure 2 (iv)**. Fluid flow through the anterior and posterior cambers and corresponding components in **Figure 2 (v)** and finally **Figure 2 (vi)** is a schematic illustration of high IOP effects and oxidative stress on Muller cell and astrocytes activation causing optical nerve damage and retinal ganglion cells damage seen in glaucoma disease.

While the principal change in glaucoma is RGC loss leading to the atrophy of related retinal layers [13], it is the increased intraocular pressure (IOP) that has been understood as the cause of glaucoma. IOP elevation result in mechanical damage which leads to RGCs loss and corresponding axons, which in turn activates astrocytosis that accompanies excessive creation of nitric oxide through activation of nitric oxide synthase [14]. As a result, peroxynitrite is created when reactive oxygen species (ROS) react with nitric oxide, which destructs DNA and proteins within the cells eventually resulting in cell death. What is more increased IOP at lamina cribrosa reduces the neurotrophins retrograde axonal transport. The impairment of axonal transport results in a lack of the crucial elements needed to maintain homeostasis in RGCs. It has been suggested that astrocytes and cells in lamina cribrosa can perceive the environment and react to the stimulus by remodeling the extracellular matrix [15]. IOP elevation also impairs microvascular supply of the optic disc potentially leading to the ischemia of RGCs [16]. Currently, glaucoma is considered as chronic optic neuropathy. Glaucomatous optic neuropathy is a result of degeneration of the optic nerve associated with progressive loss of RGCs, blood supply disorders of the optic disc and activation and corresponding change in glial cells. Preventing the damage of the optic nerve, maintaining the patient's visual field, and quality of life by restricting adverse effects of therapy are the current aims of glaucoma treatment. Among multiple

risk factors of glaucoma, IOP is the most critical and only potentially changeable factor. The progression of glaucoma is impeded by IOP reduction. Therefore, it is IOP that needs to be considered as the first element to be altered at the beginning of the treatment and the prime proven glaucoma management approach focuses on lowering IOP. The treatment involves topical anti-glaucoma eye drops with escalation to oral medications, laser, and glaucoma surgery. The glaucoma treatment can be classified as summarized in **Table 1**.

Topical anti-glaucoma eye drops lower IOP and several medications with different mechanisms are of use. Cholinergic agonists contract ciliary muscle which causes lese to become more spherical and thus increased the focusing power, and tighten the trabecular meshwork (TM) cells thus endowing increased trabecular outflow of the aqueous humor. Alpha-adrenergic agonists decrease creation of aqueous humor, and carbonic anhydrase inhibitors lower aqueous humor secretion through reducing the enzyme activity in ciliary body. Beta-adrenergic receptor antagonists diminish the aqueous humor production by ciliary body; however, it can generate cardiac or respiratory side-effects. Prostaglandin analogues reduce IOP by increasing uveoscleral outflow with adverse-effects such as conjunctival hyperemia and trichiasis. Therefore, conventional topical eye drops for anti-glaucoma treatment may be poorly tolerated and the regular needed reapplication into the eye is usually associated with poor compliance.

Fixed drug combinations have been developed to enhance the patient's compliance and to decrease exposure to preservatives contained in the eye drops. Injectable sustained release medications are another approach to ameliorate the patient's compliance and engineered to release the drug over a prolonged period. Further studies are being carried out about neuroprotection of the optic nerve in the treatment of glaucoma.

Medical therapy may not lower IOP to targeted levels, and even with maximum medical management glaucoma it may progress and result in the deterioration of the optic nerve. In these cases, trabeculoplasty can be considered to reduce IOP in open angle glaucoma. In the case of Argon laser trabeculoplasty (ALT), the laser target are TM cells containing pigment causing coagulative necrosis and thermal damage. Laser burns causes shrinkage of trabecular meshwork thereby enhancing outflow facility of aqueous humor. The amount of injury caused by the argon laser goes beyond the target area that contains melanin. ALT brings about serious side effects such as peripheral anterior synechiae, uveitis, and transient IOP spikes [17]. On

the other hand, a double-frequency Q-switched Nd:YAG laser is used for selective laser trabeculoplasty (SLT). The mechanism by which SLT reduces IOP appears to include the following mechanisms: stimulation of cell and extracellular matrix production and turnover, displace trabecular cells, and mechanical expansion of the Schlemm's canal [18]. Unlike ALT, SLT selectively targets pigmented cells in the trabecular meshwork minimizing structural and thermal destruction to neighbouring cells. The risk of acute angle closure attack is diminished by laser iridotomy. Diode laser cycloablation destroys the ciliary body and reduces IOP by decreasing secretion of aqueous humor.

The reduction of glaucoma surgeries reported can be ascribed to the improvement of pharmacotherapy [19,20]. Conventional glaucoma surgery intends to make an outflow opening in the trabecular meshwork that aids outflow of aqueous humor and consequently IOP reduction. Trabeculectomy is designed to eliminate a part of the trabecular meshwork to facilitate outflow of aqueous humor. However, it can be unsuccessful because of the excessive scarring around the opening, excessive outflow followed by hypotony, and choroidal detachment. Extended utilization of Glaucoma drainage implants (GDIs) have been validated by the tube versus trabeculectomy (TVT) study gaining popularity even in cases of nonrefractory glaucoma [21]. Glaucoma surgery is followed by high rates of complications which include shallow anterior chambers, hypotony, choroidal effusions, and hyphema. Moreover, the possible long-term difficulties are blebitis, wound leakage, and endophthalmitis.

Therefore, it is important and significant to overwhelm the flaws of traditional anti-glaucoma eye drops, which is achievable through changing routes of administration. A key challenge for glaucoma treatment is to create a safer and effective means of long-lasting drug delivery to the optic nerve. The administration of anti-glaucoma therapy must be developed to improve the absorption as well as the retention time in the cornea in a way to decrease side effects, such as ocular surface disorder which is caused by frequent instillations of the eye drop which leads to unfavorable compliance to patient. Moreover, frequent instillation of concentrated drug at cornea and conjunctiva causes toxic effects and cellular damage. Therefore, novel anti-glaucoma drug delivery should easily flow through cornea and have good histocompatibility, no toxicity, and no immunogenicity. Recently, several delivery vehicles in form of inserts, ointments, suspensions, and gel formulations have been formulated to enhance ophthalmic bioavailability and increase the residence

time. However, they have not been commonly applied due to the blurred vision and ocular irritation leading to low patient compliance.

Traditional treatment of ocular conditions includes the use of free drug solutions administered topically, as eye drops or ointments, or more invasively, by intravitreal injections or implants. However, these traditional administration strategies have significant limitations. Current research in this field has focused on developing alternative administration and drug encapsulation strategies such as nanomedicine, with sole objective of improving patient compliance and enhancing therapeutic efficacy by treating conditions at the molecular level [22,23]. The sub-sections will briefly review the administration strategies to deliver drug using nanocarriers to cornea and how these have overcome traditional limitations of each route.

Table 1. Classification of glaucoma treatment

Medical therapies	Cholinergic agonist, beta blocker, prostaglandin agonist, alpha adrenergic agonist, carbonic anhydrase inhibitor
Laser therapies	Trabeculoplasty, iridotomy, iridoplasty, cyclophotocoagulation
Surgical therapies	Trabeculectomy, glaucoma drainage implants, deep sclerectomy, vascocanalostomy, goniotomy, trabeculotomy

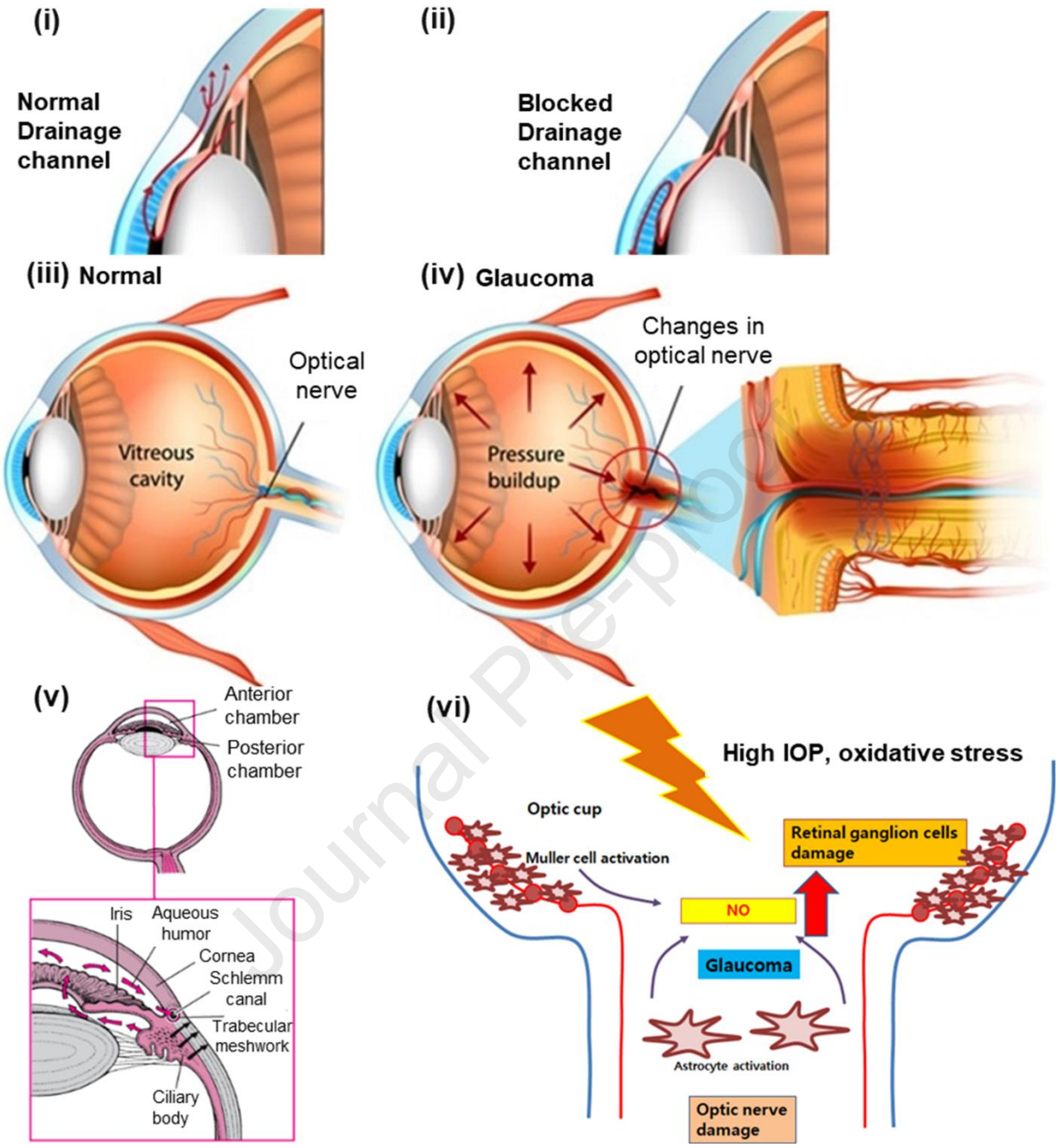


Figure 2. (i) Schematic illustration of normal drainage channel, **(ii)** blocked drainage channel. Normal and glaucoma conditioned eye, **(iii)** vitreous cavity and optical nerves in normal condition eye, **(iv)** IOP build-up in vitreous cavity and changes in optical nerves in glaucoma conditioned eye. **(v)** An open-angle glaucoma vector images shows fluid is generated at ciliary body behind the iris (*i.e.*, posterior chamber), and flowing through front portion of eye (*i.e.*, anterior chamber), and finally out through the drainage canal or uveoscleral root as shown on black arrow. **(vi)** High IOP and oxidative stress leads to retinal ganglion cells damage and causes nerve cells damage resultant glaucoma.

2.1. Necessity of carriers for ocular drug delivery

This section includes basic properties of carriers, strategies, and limitations for ocular drug delivery. Nanoparticles size, shape, composition, surface chemistry, molecular weight of functionalized polymers, and the role of various types of surfactants used in carriers formulation will also be discussed. Furthermore, the targeted delivery approach via the use of specific targeting molecules or intrinsic/extrinsic properties of carriers and other possible strategies and their limitations are also summarized. Delivering therapeutic biomolecules to a targeted tissue/specific cell, is important to, first, extend the period of therapy in the desired area; second, promote uniform distribution of the therapeutics in the desired area; third, minimized the unspecific binding to the surrounding tissues and finally the consecutive reduction of the dose necessary for therapeutic effect [24]. Particularly, in ocular drug delivery, 90% of current formulations are in solution as in from of eye drops [25]. However, the main challenges in ocular delivery are a high and rapid elimination rate due to ocular tear turnover and the nasolacrimal duct drainage system [26]. In addition to this, the nature of ocular pharmaceutics has shown extra challenges which further compromise patient compliance and so treatment outcome. For example, pharmaceutics such as prednisolone Pred Forte eye drops for non-infectious anterior uveitis treatment have been investigated which require repeated application of up to every 3 hours for 14 days to achieve clinically effective bioavailability [27]. Chemical nature of many pharmaceuticals such as moxifloxacin 0.5% w/v ophthalmic solution [25,27] or ciprofloxacin antibiotics [28], have led to core issues such as poor tissue penetration, tissue residence time and /or rapid drug degradation. In addition to the aforementioned challenges, several adverse-effects reports such as blurred vision by drug formulations such as Acyclovir 3% ocular ointment (Zovirax®) [29], eye irritation, redness, burning and/or stinging sensation by drugs such as 0.05% Cyclosporine-A emulsion Restasis® [30], local undesirable reactions like increase in IOP, cataracts and glaucoma by overexposure of ocular tissue to steroid drugs [31,32], and systemic side effects such as kidney related hypertension due to systemic absorption of cyclosporine drugs residues from topical administration [33]. Ideally, ocular formulations should have sustained drug release profiles, higher penetrability, and tissue residence time, as well as being isotonic, biodegradable, biocompatible, non-immunogenic, non-sensitizing, and non-irritating. In this context, research in the recent years has explored and advanced the field of nanotechnologies to develop drug carriers that meet all these requirements. For nanocarriers based drug delivery, it is essential to stablish compatibility of the drug and carrier chemistries, this ensures drug loading and NC-drug linkages that are

stable but also responsive to local tissue composition and pathological events such as increase of pH during ocular inflammation. For example, Soiberman *et. al.* explored subconjunctival injectable gels of hyaluronic acid and G4-PAMAM dendrimers conjugated with dexamethasone# as a potential strategy to enhance drug loading, sustained delivery, and corticosteroids bioavailability via using neutrally charged PAMAM dendrimers [32]. They functionalized dexamethasone (DEX) using succinic anhydride to add a carboxylic acid terminal group and reported that the resulting functionalized DEX had similar TNF-α suppression abilities when compared to free-DEX in vitro. Moreover, the chemistry of the DEX also showed that DEX-to-dendrimer link was composed of two ester bonds which made drug release susceptible to hydrolysis and responsive to the changing aqueous pH conditions of inflammatory eye pathologies.

It is also essential for nanocarriers-based ocular drug delivery to include targeting systems. This can be achieved by passive methods, such as carefully choosing carrier type (*i.e.*, liposomes have intrinsic higher penetration abilities) or use permeabilization enhancer agents. Active methods, on the other hand, include nanocarriers surface coatings and/or functionalization processes such as use of natural cell membranes (i.e. RBC) to increase circulation time, cell anchoring and uptake, coating with hybridized DNA or protein structures such as albumin or antibodies for high affinity and preventing opsonization [24].

2.2. Topical administration

The eye drops is a simple and common approach to deliver nanocarrier systems targeting ocular conditions of the anterior section such as cornea, anterior uveitis, and viral and bacterial infections. With traditional formulations, topical administration has several limitations including: 1) restricted dosage due to cul-de-sac accumulation; 2) limited drug bioavailability due to dilution and washout by tear fluid drainage systems and blinking reflex; 3) need to frequent administration resulting in low compliance and precorneal film damage; 4) poor penetration into aqueous humor and posterior segment; and 5) risk of inadequate application and storage [34,35]. However, due to the versatility of self-administrable therapies, most ocular nanocarriers are being developed to be administered topically as eye drops. To overcome the limitations mentioned above, strategies such as the incorporation of mucoadhesive properties in the nanocomposite eye drop formulation has been introduced. Recently, Terreni *et. al.* have developed a collaborative approach formulated on association of mucoadhesive polymers, nanomicelles and hyaluronic acid, which was favorable for Cyclosporine-A delivery as dry eye syndrome treatment [36]. Mahboobian *et. al.* have developed

thermosensitive incorporated polymer poloxamer to produce an in situ gelling nanoemulsions eye drops that form a non-flowing transparent gel upon contact with ocular surface temperature [29]. As a result, nanocarriers delivered as eye drops have the potential to increase retention time, reduce administration frequency, maximize pharmacological effects and lead to better patient compliance. Ocular drug nanocarrier eye drops targeting the posterior segment have also been developed. Abdel-Rashid *et. al.* reported positive results while administrating acetazolamide loaded nanogel/vesicles composite to treat glaucoma ocular hypertension in vivo rabbit models [37]. Similarly, Yadav *et. al.* reported that mucous permeable lipid nanoparticles loaded with atorvastatin administrated as eye drops had the potential to treat aged-related macular degeneration [38].

In addition to eye drops, contact lenses are also being researched as an administration strategy to deliver drug-loaded nanocarriers. Nasr *et. al.* reported the potential to administer loteprednol etabonate loaded nanoparticles by loading them into poly HEMA hydrogels contact lenses, which have a significantly extended release profile compared to conventional free drug loaded contact lenses [39]. Prakash *et. al.* developed biodegradable polymeric contact lenses to deliver drug loaded nanoparticles for a more sustainable ocular drug delivery, while also minimizing ocular infection risks due to patient mishandling conventional contact lenses [34,40]. Further tuning of cytotoxicity and antimicrobial effects is still required, such as the incorporation of metal nanoparticles (silver or copper) into contact lenses [41].

In case of topical administration, the main disadvantages are limited dosage, and poor bioavailability and compliance related with low penetration ability. Nanocarriers can increase the localization and retention time, resistance to washout, enabling penetration of tissue structure with sustained delivery. This is possible by mucoadhesive or mucus-penetrating property of nanocarriers. For example, crosslinked nanocarriers loaded in contact lenses [39], or nanocarriers made from mucoadhesive [36] or thermo-responsive/gelling polymers [29], have been shown to increase retention time of therapeutics for ocular inflammation, dry eye disease, or other eye-related infections. Also, surfactant-based systems such as nanovesicles made of edge activators (membrane softening agents) or solid lipid nanoparticles have been reported [37,38] to enhance the permeability for the treatment of high intraocular pressure and age-related macular degeneration.

2.3. Ocular and periocular injections

Drug delivery to posterior segment is challenging due to anatomical and physiological barriers of cornea and conjunctival epithelium and blood-aqueous and retinal barriers, all which significantly limit drug diffusion from anterior segment and systematic circulation [22,42,43]. Ocular nanocarriers targeted to treating structures of the posterior segment are usually administered using intravitreal injections using 27 to 30-gauge needle into the vitreous humor. The sustained release and biodegradable nature of nanocarriers significantly reduce the frequency of injections needed thus overcoming limitations of free-drug solution injections such as the need for frequent administration, which in turn increases the risks of ocular hypertension, haemorrhages, infections, inflammation, and retinal detachment [34]. For example, Qiu *et. al.* used intravitreal injections to deliver fenofibrate-loaded biodegradable PGLA nanoparticles in rats to diminish retinal leakage and oedema while also reducing the drug dose and frequency of injections needed. An alternative route often used to deliver ocular nanocarriers are the periocular injections including the subconjunctival, peribulbar, retrobulbar and subtenon routes. These routes allow to deliver the drug near the eyeball without puncturing the sclera [23]. Subconjunctival injection is one of the most used preocular injection for nanocarrier delivery since the permeable features of the membrane allows for continuous release of drug into posterior segment [22]. Soiberman *et. al.* and Lin *et. al.* have reported using subconjunctival injections to deliver dexamethasone-loaded nano dendrimers in a rat and rabbit models respectively, to treat dry eye disease [32,44]. This administration route was also used by Wong *et. al.* to deliver prednisolone phosphate loaded-nano liposomes to treat anterior uveitis in rabbits [31].

Intravitreal and periocular injections are an invasive form of treatment for ocular disease management. Advantages include longer sustained release, lower clearance rate, biodegradability, and enhanced permeability, which reduces the number of injections and waste-extraction procedures, and improves the targeting of ocular posterior structure. PEGylated functionalized liposomes, such as liposomal nanocarrier systems were used to deliver steroids via subconjunctival injections for the treatment of uveitis in rabbits [31]. Also, carboxylic acid terminal functionalization was used to enhance solubility, and thiol-ene click chemistry was used to get cross-linkable nanocarrier systems with sustained drug release property, which improves the attenuation of corneal inflammation via subconjunctival injections [32].

2.4. Systemic administration of ocular nanosystems

Systemic administration is almost never used for ocular therapeutics since only 2% gets through the blood ocular barriers. In this approach, nanocarriers are loaded with specific drugs, antibodies, and growth factors to control IOP, pain, or inflammation and as antiangiogenic therapeutics. This approach, however, has limited success to treat the posterior segments due to physiological barriers. However, metal, and inorganic nanoparticles such as gold nanoparticles which can be as small as 20 nm, inert, non-immunogenic, non-toxic, and also able to penetrate the BRB and have been suggested as potential anti-angiogenic element for diabetic retinopathy and macular degeneration treatment. Guo *et. al.* reported to have successfully delivered cyanine-5 fluorescent dye labelled dendrimers in rodent and non-primate models of non-arteritic anterior ischemic optic neuropathy using systemic intravenous injections [45]. Moreover, they found that the nanocarriers selectively accumulated in the inflammatory cells infiltrated in the ischemic lesions of the anterior optic nerve and laminar region for up to 4 weeks.

The major limitation in the systemic route delivery of nanocarriers is the difficulty in crossing blood brain barriers related with their size, and their selectivity to the ocular compartment to avoid systemic toxic effects. When the 4th generation PAMAM dendrimer nanoparticles were developed as smaller as 4 nm, the selective targeting of circulating inflammatory cells accumulated in initial stages of optic nerve ischemic lesions was possible via systemic administration [45].

2.5. Metabolic clearance of nanocarriers

The materials used in the nanocarrier systems are xenobiotic and undergo breakdown into metabolites. Eye is one of the organs that has an independent metabolic activity. There are a variety of metabolic enzymes, esterases, hydrolysis enzymes and peptidases distributed across the ocular tissues. For example, phase I metabolic oxidase cytochrome P-450, which has a role in the oxidation of PEG at systemic level, is expressed in the cornea, iris, and retina. These also possess esterase enzymatic activity related to prodrug metabolism [46], which is responsible for the breakdown of PLGA in the liver [47].

On the other hand, phase II metabolizing enzyme, such as glutathione S-transferase, is expressed in the corneal epithelium, and play a key role in free-radical scavenging and conjugation to phase I metabolites. The aqueous humor also has a metabolic activity of the eye. Although at low levels, macrophage-like cells exist in the vitreous peripheral region and release enzymes needed for the metabolism of lipids [46]. It is

estimated that the aqueous humor is replaced every 100 min [48], which adds to metabolite clearance from ocular area.

Depending on the material composition, the degradation rate of nanocarriers is largely affected. For example, PLA and PLGA can undergo hydrolysis into lactic acid, and glycolic, and these metabolites can enter the Krebs cycle, ultimately generating water and CO_2. In case of polysaccharides, such as chitosan and hyaluronic acid, degradation is mediated by enzymatic hydrolysis, and the oxygen radical mediated fragmentation is also involved in hyaluronic acid. Metabolites from these reactions can be used in the biosynthesis process, ultimately generating water and CO_2. In case of PEG, breakdown usually occurs via oxidation, mainly to nontoxic metabolites [47].

The inorganic-based nanocarriers, which are normally considered resistant to degradation, can also undergo a degree of enzymatic and non-enzymatic degradation of surface chemical groups. Moreover, clearance sometimes involves the excretion without degradation [47]. In case of AuNPs and silica NPs, clearance is known to be mediated via cellular internalization and posterior dilution during cell proliferation [49]. The clearance mechanism of inorganic-nanoparticles is thus considered to depend on the composition and size, which yet needs more in-depth studies.

The unbroken materials and the corresponding metabolites enter regional lymph nodes and then circulate systematically. Although the systemic clearance is possible via the spleen (materials >200 nm), enzymatic metabolism in the liver, and glomerular filtration in the kidney [47], strategies to improve tissue targeting and reducing systemic distribution are needed, which may be possible in part by the control of size and surface chemistry of nanocarriers.

3. Development of carriers for ocular drug delivery

In medicine, nanoparticles as drug delivery carriers have been extensively studied for various fields such as cancer theranostics, tissue engineering, cell reprogramming, regenerative medicine, and eye related diseases. The recent progress in nanoparticles-based carriers for therapeutic approaches has proved significant benefits to address the problem associated to traditional delivery system and improved the therapeutic efficiency [50-53]. Several attempts have been made to promote the biocompatibility, and sustain drug release from the nanoparticles-based carriers. Particularly, glaucoma application, the physicochemical, and biological properties of nanocarriers are more important compared to other delivery

applications because of the biological barriers. The particles size, shape, structure, degradation, dispersibility, bioavailability, and biocompatibility are the important parameters for the nanoparticles-based drug delivery carriers in the glaucoma. Therefore, to avoid these barriers, nanoparticles-based delivery carriers need favorable biological properties which include chemical surface modifications and other strategies for decreasing toxicity, prolonging the residing time, and lowering the precorneal drug loss by rapid tear fluid turnover thus increasing drug ability of penetration to corneal layers (epithelium, stroma, and Descemet's membrane, and endothelium), and aqueous humor [54,55]. In this part, we will discuss the nanoparticles-based carriers such as inorganic nanoparticles-based, polymers-based, hydrogel-based, and other possible nanocarriers in ocular delivery applications. The advantages and disadvantages of different types of nanocarriers for ocular applications are summarized in **Table 7**.

3.1. Inorganic-based nanoparticles

The eye is a complex organ and most challenging for targeted therapeutic approaches due to its biological structure, functioning, and barriers of the cornea, which poorly permeable to the drugs. The treatment of eye diseases (glaucoma, keratoconus, ocular hypertension, and uveitis) and pathologic condition of ocular neovascularization in retina is a demanding assignment. Therefore, ocular drug delivery systems based on different nanocarriers are needed to improve for safely, bioavailability, and sustain delivery of ocular therapeutics. Recently, inorganic nanoparticles-based carriers in ocular drug delivery with improved penetration, prolonged residence in the anterior eye chamber have been developed and studied [56,57]. However, to target the posterior chamber is still a challenging topic in ocular delivery.

Recently, interests have significantly grown in area of nanomedicine for inorganic nanoparticles-based delivery system. Particularly, inorganic nanoparticles-based carriers such as iron oxide, silver, gold, silica, carbon-based nanomaterials, and bioactive glass have been used for drugs, proteins, and genes delivery applications. Recent examples of inorganic-based nanocarriers for ocular applications are summarized in **Table 2**. Inorganic nanoparticles possess specific characteristics including controlled structure/size, shape, specific surface area, versatile surface functionality, biocompatibility that endow to overcome challenges such as saturation solubility, penetration ability, distribution, increased dissolution rate, and glueyness to the cell surface membrane. A homogenous size of nanoparticles and suspension in marginal liquid medium

are important parameters for effective delivery of drug. The surface modification, generally in the form of chemical modifications, are achieved by surfactant use known as nano-suspension, however recent methods can also modify the nanoparticles surface during preparation without use of toxic chemicals. The dispersing media are generally water or organic solvent like polyethylene glycol and oils [58,59]. There are several nanoparticles such as iron oxide, silver, gold, silica, and carbon-based nanomaterials utilized as therapeutic delivery vehicles. However, most nanoparticles are still in the first stage of clinical trial and only a few of them have been accepted for clinical use.

3.1.1. Magnetic nanoparticles (MNPs)

In the last few decades, iron oxide nanoparticles (Fe_3O_4NPs) have been explored to synthesis, and engineered for diversified biomedical applications including detection, sensing, imaging, cells and exosomes separation, cancer therapy, tissue regeneration, and drug delivery. Many researchers have shown that use of magnetic-based nanomaterial is a promising therapeutic delivery carrier as it can provide controlled and sustained release as well as improve availability of bioactive molecules [60]. Particularly, MNPs have shown promising biodegradable and non-toxic drug delivery carriers in ocular delivery system [61]. It is important to highlight that the cellular uptake, cytotoxicity does not only depend on the nanoparticles size, composition but also these results depend on the cell types, cell lines, culture condition [62,63]. Cornell have demonstrated the MNPs as potentials vehicle for corneal endothelium repair [64]. Although, MNPs have not been utilized for test in human eye diseases, and has also been investigated for preliminary evidence from in vivo studies on rat, which results confirmed the nontoxicity in ocular structures [61,65]. Moreover, MNPs is also potential nanoparticles for in vivo imaging by means of MRI technique [66]. Physicochemical properties such as size shape, surface chemistry, magnetization, have significant role to produce ROS to treat the diseases. The redox activity of MNPs is highly depends oxidation states of iron (Fe) together with on physicochemical parameters. The oxidation states of Fe (2+ and 3+) are important because it play series of reaction in ROS generation [67]. Iron oxide nanoparticles can exist in many forms which two of the most common forms of iron oxide nanoparticles being magnetite (Fe_3O_4) and maghemite (Fe_2O_3). Magnetic Fe_3O_4NPs with size range from 1 – 100 nm with core-shell structure, where Fe_2O_3 as core and polymers or functional chemical groups are generally as shell structure. A number of biomolecules including antibodies, amines, hydroxyl, thiol, biotin, carboxyl, and streptavidin can act as chemical functional groups as well as

other functional groups with disulfide cross-linkers [68,69]. For example, Giannaccini *et. al.* have used MNPs in the form iron oxide as intraocular delivery to target retinal pigmented epithelium (RPE) layer [70]. They used MNPs as nanocarriers for ocular drug delivery via intraocular injection in *Xenopus* embryos which was capable of targeting the retinal RPE layer and being sustainable for weeks. Moreover, localization and distribution of MNPs in the RPE layer was independent of particles physicochemical properties. Raju *et. al.* investigated in vivo distribution of MNPs using magnetic resonance imaging (MRI) after intravenous injection and therapeutic and diagnostics role in the eye [66]. They used two different size MNPs (50 nm and 4 µm) and intravitreal injection into the left eye of adult Sprague-Dawley rats, same volume of PBS into opposite eye as control and in vivo distribution of MNPs was analyzed at designated time points of injection using MRI. At one hour, both 50 nm and 4 µm MNPs were detected in in/ex vivo by high resolution MRI and clear visualization, respectively. However, only 4 µm MNPs were detected by MRI in the eye at 5 weeks of post injection, while 50 nm size MNPs was not noticed at same time point. The finding suggests that the 50 nm sized MNPs cleared quickly compared to 4 µm MNPs, proving that nanoparticles may provide a definite advantage over micron-sized particles when considering long-term persistence risks. Levy *et. al.* demonstrated core-shell iron oxide superparamagnetic nanoparticles as growth factors release in sustained manner and its effect to increase proliferation and differentiation of human mesenchymal stem cells (hMSCs) using bioactive, human serum albumin (HSA) modified near infra-red (NIR) fluorescence [71]. They used HAS-modified Fe_3O_4NPs covalently conjugated with fibroblast growth factor 2 (FGF 2) for enhancement of proliferation, clonal expression of hMSCs and their adipose and osteogenic differentiation. Recently, Tzameret *et. al.* assessed the drug delivery and in vivo MRI potentials of HAS modified Fe_3O_4NPs in retinal degeneration rat animal model [72]. They injected 20 nm HAS modified Fe_3O_4NPs into one eye of the rats and other eye with PBS as control. They reported long-term persistence of nanoparticles in posterior division of eyes treated with nanoparticles and found that MRI could be used for tracking the nanoparticles in future translational and clinical studies. After demonstrating the rapid and persistently localization within the REP of intravitreal-injected MNPs. Giannaccini *et. al.* have investigated the localization pattern of VEGF functionalized-MNPs in eye posterior layer [73]. They found that the covalently functionalized VEGF-MNPs change the localization fate of intravitreal injected MNPs to the choroid, while polypeptide (poly-L-lysine) functionalized-MNPs as control showed same localization pattern of the bare

MNPs particles. Therefore, the functionalized MNPs with specific biomolecules may be a potential candidate in cell specific targeting eye posterior segment.

3.1.2. Gold nanoparticles (AuNPs)

Last few years, gold nanoparticles (AuNPs) have attracted enormous attention within the research community as well as in industry for their advanced application in diversified field. AuNPs is a novel material which is in use for a different area in biomedicine including theranostics, catalyst, therapeutic delivery, biochemical, biosensing, photothermal and photodynamic therapy in cancer and antibacterial activities. The unique optical properties (shape and size dependent), low cytotoxicity, high stability, and easy surface modification make AuNPs highly attractive nanocarriers in therapeutic delivery and nanomedicine. Moreover, surface functional groups, surface charge, polarity, and ligand coupling influence the physicochemical properties and contribute important role in targeted drug delivery [74,75]. The drug release can be controlled as drug molecules bind either covalently bound or bound via supramolecular interaction to the AuNPs surface and endow administered delivery of therapeutic molecules in ocular system. AuNPs-based drug delivery system enables specific targeted delivery and controlled release of drugs with sensitive bioimaging modality for early diagnosis of the disease [76-78]. Kim *et. al.* investigated the AuNPs administered intravenously and cross the BRB and spread in retinal layer without any retinal toxicity [79]. They found that while the larger size (100 nm) AuNPs were not noticed, smaller size (20 nm) AuNPs passes BRB and spread in the retinal area after intravenous administration into C57BL/6 mice. This means that the smaller size AuNPs may be a potential candidate for therapeutic delivery in retinal area. AuNPs did not express any structural abnormality and cellular toxicity to retinal, astrocytes, endothelial, and retinoblastoma cells. Vision loss among all age groups is mostly due to the pathological angiogenesis in the retina. In another study, same research group have demonstrated AuNPs as a retinal neovascular inhibiter through suppression of VEGFR-2 activation in ROP animal model [80]. They found that the AuNPs not only effectively suppresses the angiogenesis but also prevented auto phosphorylation of VEGFR-2 by induction of VEGF to prevent the consequent instigation of ERK 1/2 pathway. Cho *et. al.* have also used topically administered AuNPs to inhibit corneal neovascularization in mice [81]. They found that neovascularized area in AuNPs treated group was greatly reduced by 39%, and also VEGFR-2 expression was reduced which inhibited the inflammation.

Endotoxin-induced uveitis (EIU) is an animal model of ocular inflammation. In EIU condition, an acute inflammation occurs in bilateral anterior region. The EIU is primarily characterized by formation of blood-ocular barrier (BOB) and inflammatory cells accumulation [82]. It has been also noted that both the oxidative stress and inflammation help in this process of pathogenesis and oxidative markers elevated in the EIU [83]. Pereira *et. al.* have assessed AuNPs role as an anti-inflammatory in EIU rat model. They found that the AuNPs topical administration leads to suppression oxidative stress and inflammatory factor related damage in retina [84]. This reduction in inflammatory factors and oxidative stress related damage are interfering by TLR4-NF-κB pathway. Gene delivery approach is another platform for eye related disease treatment using inorganic nanoparticles. AuNPs have been utilized as gene delivery carriers and proven to cause significant transfection of genes into mammalian cells and lead to ensuing gene expression [85-87]. Sharma *et. al.* have developed polyethylenimine-conjugated AuNPs for gene delivery in the human corneal fibroblast cells, and performed detailed studies of endocytosis and clearance of AuNPs in rabbit model and toxicity, bio-safety of modified AuNPs in the rabbit cornea [88]. After topical administration of developed polyethylenimine-conjugated AuNPs, the rabbit cornea tissues were collected for 12 h, 3 day and 7 days exhibited AuNPs stromal uptake with gradual clearance over time. The effective therapeutic dose of AuNPs did not exhibit any cell morphology change, or inflammation response. Furthermore, lack of changes in the rabbits behavior show that the topical administration of AuNPs on rabbit corneas did not appear to be myopia or hyperopia. Tandon *et. al.* have demonstrated that delivery of bone morphogenetic protein 7 (BMP7) gene via polyethylenimine-conjugated AuNPs modulate corneal wound healing and also inhibits fibrosis by counter balancing of TGFβ1-mediated profibrotic Smad [89]. Salem *et. al.* have investigated a formulation of liposomal flucytosine capped with AuNPs for improving intraocular penetration and therapeutic efficacy [90]. They found that the topical administration of flucytosine capped with AuNPs can effectively treat the experimental *C. albicans* infection of cornea.

3.1.3. Silver nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) have long history of biomedical use in antibacterial, anti-inflammatory, antioxidant activities, and drug delivery. AgNPs have antiangiogenic properties and effectively suppress retinal endothelial cell survival. AgNPs have also been used to reduce inflammation and accelerate wound healing and have been used after burn injuries as adjunctive agents [91]. AgNPs inhibit both fibroblast

proliferation and cytokine production leading to anti-inflammatory effects allowing for its use in wound healing with diminished scar tissue formation [92-94]. The antibacterial activity of AgNPs may be because they damage the bacterial cell membrane. The mechanism of antibacterial activities of AgNPs is still partially understood [95,96]. Sondi *et. al.* demonstrated that bacterial activity of AgNPs and constituent of the bacterial cell membrane caused structural change and membrane damage and leading to apoptosis [97]. McQuillan *et. al.* have suggested that AgNPs interact with the outer and inner bacterial cell wall, silver ions inter into cell and causes the transcriptional regulation [98]. Moreover, electrostatic interactions between positively charged ions and negatively charged bacteria surface leads to subsequent blocking of electron transfer and translocation of ATPase is not up regulated [99]. Several studies have demonstrated that the silver nanoparticles are safe, biocompatible, however large particles size and extreme concentrations can cause cytotoxicity as well as genotoxicity. Topical administration of AgNPs in mice and rabbits ocular are compatible and well tolerated [100,101]. Moreover, AgNPs and silver nitrate have also been used in ophthalmology as agents to prevent ophthalmia neonatorum [102]. Considering all these favorable properties of the silver nanoparticles, Luo *et. al.* have developed gelatin functionalized-silver nanoparticles (denoted as G-AgNPs) as nanocarriers for antibacterial, and antiangiogenic treatment in bacterial keratitis (BK) [103]. AgNPs was synthesized through chemical reduction method using silver nitrate in presence of maltose and further formulation with functionalization of gelation **(Figure 3 (i))**. Bare AgNPs were aggregated, after gelatin modification particles were dispersed (Figure 3B (ii)) and exhibited the antibacterial properties against *S. aureus* after in Luria broth (LB) agar plates and treatment for 1 day **(Figure 3 (iii))**. Postoperative evaluation results showed significant reduction in transparency of cornea and due to bacterial infection in control group rabbit **(Figure 3 (iv))**. The intrastromal injection of silver nanoparticles exhibited poor tissue clarity, while G-AgNPs injection reduced the cloudiness and showed clear transparency. Moreover, the angiogenic activity of the AgNPs and G-AgNPs in CAM assay demonstrated that the gelatin functionalization reduced the vascular network **(Figure 3 (v))**. The *in vivo* angiogenic potential of AgNPs and G-AgNPs in rabbit model of experimental CNTV confirmed the effect of Ag-NPs as antiangiogenic activity that is ability to inhibit the blood vessel formation **(Figure 3 (vi))**. Furthermore, angiogenesis is an important and very complex process and has central role in capillary network formation of endothelial cell [104]. In diabetic patient, vision loss is fundamentally due to pathological neovascularization in ROP, and age-

related macular neovascularization [105]. Gurunathan *et. al.* have demonstrated AgNPs as antiangiogenic molecule through targeting PI3K/Akt pathways activation [106]. In addition, functionalized Ag nanoparticles exhibited greater antiangiogenic effect mainly because of presence of gelatin molecules. In this regard, it has been reported that gelatin possess RGD sequence, so the presence of RGD sequence enhance the anti-nonvascular ability of gelatin functionalized AgNPs [107]. The RGD sequence is favorable to bind the specific integrins on $\alpha_v\beta_3$ receptors, and inhibits the activation of PI3F/Akt signaling pathway [108].

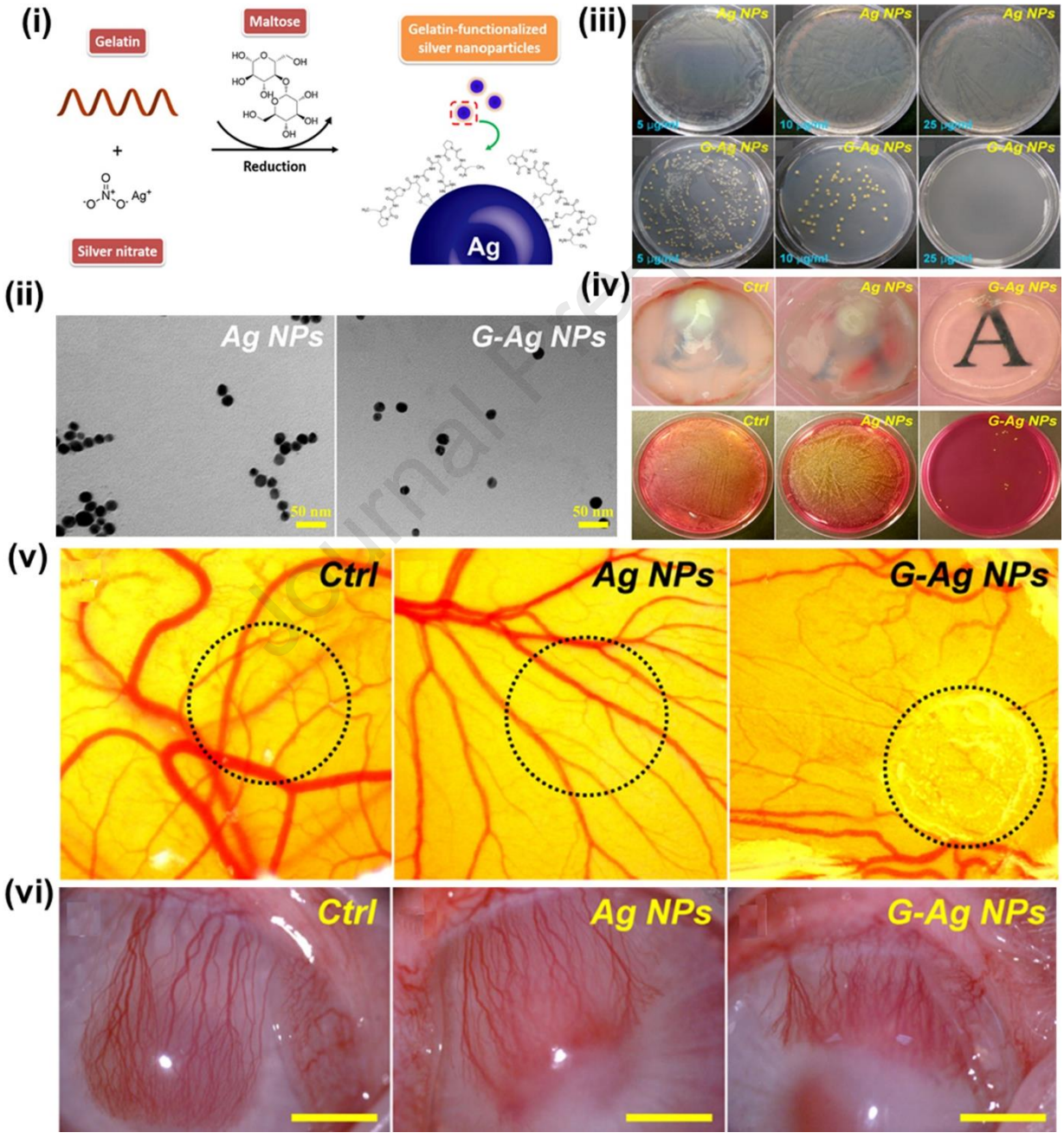
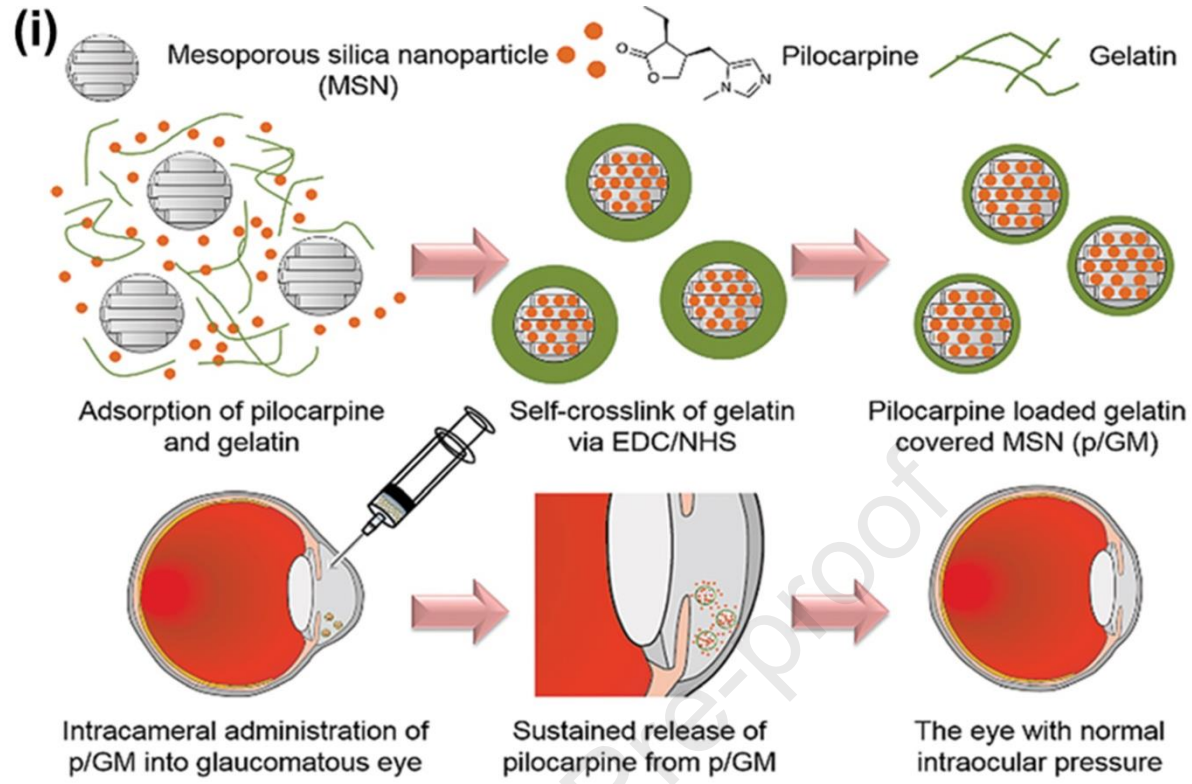
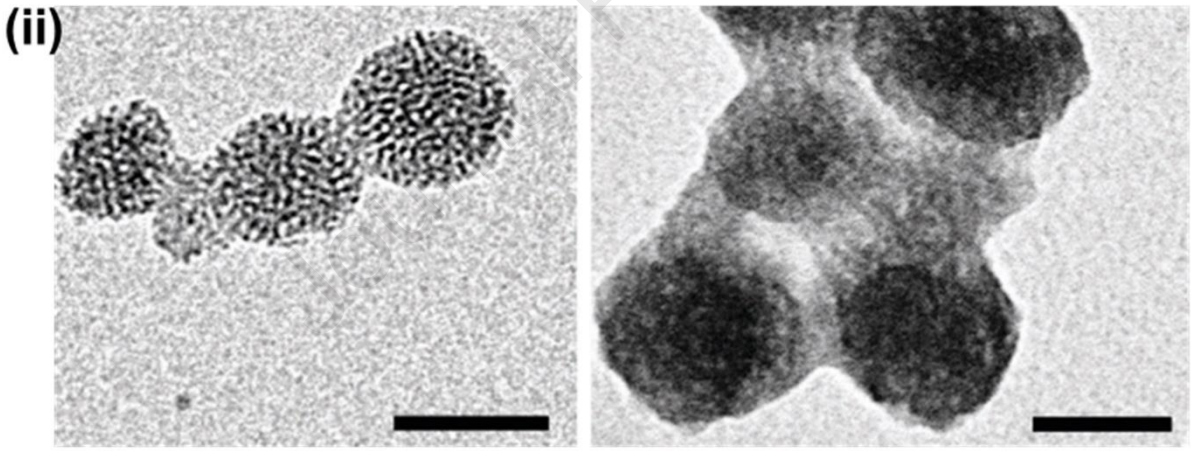


Figure 3. Inorganic nanoparticles nanomaterials in ocular applications; silver nanoparticles (AgNPs) and gelatin modified G-Ag NPs for BK treatment; **(i)** Schematic diagram of environment friendly synthesis of G-AgNPs, **(ii)** TEM images of AgNPs and G-AgNPs, **(iii)** Optical images of bacteria exposed with Ag NPs or G-Ag NPs with varying concentration 5, 10, and 25 µg/mL, **(iv)** Optical images of corneal (typescript beneath) samples of rabbit eyes at day 3 after surgery and through an experiment caused BK and then injection of AgNPs or G-Ag NPs via intrastromally, **(v)** Optical images of CAM vasculature after exposure with AgNPs or G-Ag NPs at day 1, **(vi)** Representative optical images of slit-lamp of VEGF-A165-induced rabbit CNV treated with AgNPs or G-Ag NPs, Ctrl samples were treated with PBS injections only and these images were taken at day 3 (scale bars: 2 mm). Reproduced with permission from Elsevier from ref. [103].

3.1.4. Mesoporous silica nanoparticles (MSN)

MSN is a well know inorganic nanoparticle with various therapeutic delivery applications. MSN have been extensively explored in anticancer drug delivery for excellent biocompatibility, control biodegradation, which is important aspect of their future role in clinical application. MSN have large surface area, pore size, and versatile physicochemical properties which endow high drug loading capacity. Moreover, tunable surface chemistry of MSN improve the biocompatibility, and increase permeability and retention (EPR) effect in cancer cells [109]. The large number of silanol groups presence on MSN surface endow unique feature for controlling the drug loading and release. However, traditional design concept, synthesis approach, and surface modification with active groups is not enough to improve the control drug release. Liao *et. al.* have demonstrated sustained release properties of gelatin functionalized-mesoporous silica nanoparticles (G-MSN) for intracameral pharmacotherapy of glaucoma [110]. They found that the up-regulated expression of matrix metalloproteinase-2 (MMP-2) in anterior chamber causes degradation of gelatin which further create a moderately acidic environment that allowed release of pilocarpine from G-MSN and reduced IOP. Cellular and animal studies results confirmed the controlled pilocarpine release and its consequence in IOP reduction and thus developed nanocarriers can be potentially useful in glaucoma treatment. Schematic illustration of drug loaded MSN and covered with G-MSN are presented in **Figure 4 (i)**, further the deliver the drug when administered through intracameral into anterior chamber to reduce the IOP, **Figure 4 (ii)** present the TEM images of MSN and G-MSN, and cumulative release profile exhibited long-tern sustained release of pilocarpine from G-MSN compared to only MSN in presence of MMP-2 solution are shown in **Figure 4 (iii)**. This study confirmed that MSN-based inorganic nanoparticles are suitable to use as ocular delivery carriers for glaucoma, and other eye related disease treatments.


(i)
Mesoporous silica nanoparticle (MSN)
Pilocarpine
Gelatin
Adsorption of pilocarpine and gelatin
Self-crosslink of gelatin via EDC/NHS
Pilocarpine loaded gelatin covered MSN (p/GM)
Intracameral administration of p/GM into glaucomatous eye
Sustained release of pilocarpine from p/GM
The eye with normal intraocular pressure


(ii)

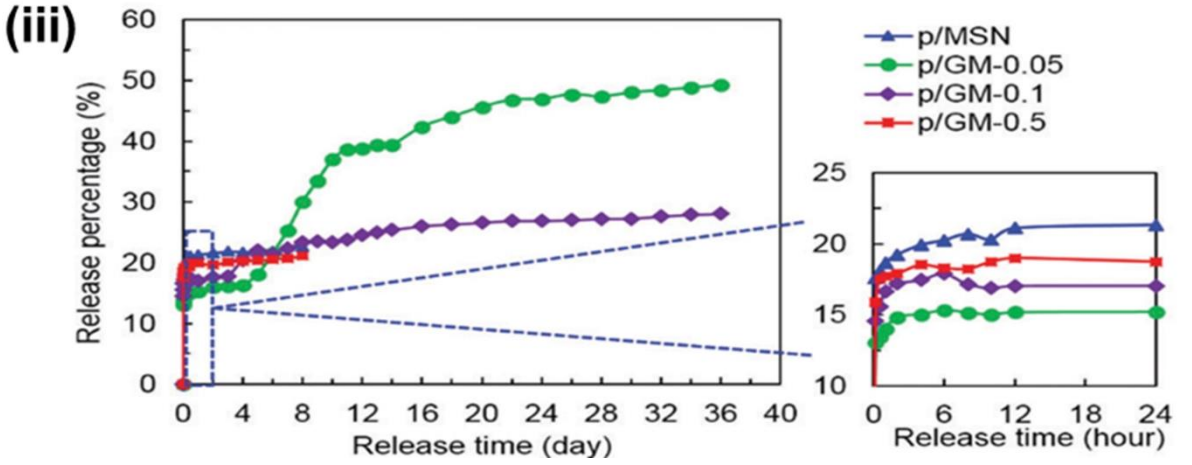

(iii)
Release percentage (%)
Release time (day)
Release time (hour)
p/MSN
p/GM-0.05
p/GM-0.1
p/GM-0.5

Figure 4. Mesoporous silica nanoparticles (MSN)-based inorganic nanocarriers for ocular drug delivery; **(i)** Schematic illustration of pilocarpine loaded-MSN covered by gelatin to deliver the drug when administered through intracameral into anterior chamber to reduce the IOP, **(ii)** TEM images of MSN and G-MSN (scale bar is 50 nm), **(iii)** Release of drug (pilocarpine) from MSN and G-MSN in enzymatic solution. Reproduced with permission from the Royal Society of Chemistry from ref. [110].

3.1.5. Carbon-based nanocarriers (CBNs)

In carbon-based nanomaterials (CBNs), graphene is an important material in several forms including carbona nanotubes (CNTS), graphene (G), graphene oxide (GO), reduced graphene oxide (rGO), and few layer graphene (FLG) with sp^2 and sp^3 hybridized carbon atoms [111]. GO properties like large surface, good chemical stability, good mechanical strength, and electrical conductivity, easy biofunctionality, and biocompatibility endow great promise for biomedical applications. Therefore, GO and other derivatives can be potential candidate for creating barrier layers in multilayers films by trapping molecules of interest for controlled drug delivery applications [112,113]. Moreover, the molecular structure of therapeutics endows to be bound with the basal plane of GO surface via π-π stacking between residues of aromatic amino acid and sp^2 hybridized carbon atoms, negative functional groups of GO, electrostatic interactions between residues of basic amino acid in proteins and delivered in a hydrophilic carrier [114]. The other aspect such as of large surface area, surface volume and its ratio of the GO is high drug loading compared to traditional drug delivery systems. The IOP is an important and prime parameter for the diagnosis as well as the treatment of glaucoma. The increase in IOP is often due to the blockage of outflow system which leads to the progressive apoptosis of RGCs. Currently, lowering of IOP is in use and trusted technique for treating glaucoma. Moreover, single measurement of the IOP in clinic can be misleading for diagnosis given the circadian rhythm changes depending on the body gesture. Xu *et. al.* have developed highly transparent, sensitive, linear, and biocompatible, non-invasive graphene sensors for concurrent and continuous monitoring of IOP [115]. They designed Wheatstone bridge using few layers graphene as sensing material, which was consisting of two strain gauges towards radial direction, and two compensating resistors at the edge of the sensor for fluctuation of IOP measurement. Lai *et. al.* have developed ultrastrong trapping of VEGF on bovine serum albumin (BSA)-functionalized GO (designated as BSA-GO) for antiangiogenic application [116]. They found that the binding affinity of VEGF on BSA-GO was very high (*i.e.*, five orders of magnitude stronger) compared to any other abundant plasma contains proteins including fibronectin,

transferrin, human serum albumin, and immunoglobin G. Moreover, BSA-GO samples not only strongly blocks neovascularization in cornea induced by VEGF-A_{165} and disturbs angiogenesis in chick chorioallantoic membrane but also inhibit proliferation, migration, and HUVECs tube formation. Thus, GO can be suitable candidate for therapeutic antiangiogenic through ultrastrong VEGF absorption. Recently, Cibecchini *et. al.* have shown the antiangiogenic effects of GO in primary human endothelial cells due to induction of oxidative stress [117]. An *et. al.* have evaluated the toxicity of GO and rGO using morphological change and molecular biological techniques in vivo and in vitro [118]. They found that GO exhibited clear intraocular inflammation while rGO showed no significant toxicity in the eyeballs of the mice. Moreover, frequent exposure of GO can cause an increase in corneal stromal layer, cytotoxicity to corneal epithelial cells of rat, intraocular inflammation, and iris neovascularization.

3.1.6. Ceria-based nanoparticles (CeNPs)

Pilocarpine, brimonidine, timolol and carteolol, latanoprost and brinzolamide are anti-glaucoma medicine that have been explored in glaucoma therapies [119]. Luo *et. al.* have explored an anti-glaucoma formulation which is based on dual-functional nanocarrier platform made of hollow ceria nanoparticles (hCeNPs) which was further modified with chitosan and ZM241385 [120]. They found that the functionalized hCeNPs can inhibit the H_2O_2-induced ROS production and suppress the inflammatory cytokines, such as IL-6 and MCP-1 in IL-1β stimulated SIRC cells. Dual-functionalized hCeNPs and the targeting strategy to ciliary body are schematically illustrated in **Figure 5**. The nanoparticles significantly attenuated the progression of experimental glaucoma compared to commercially available eye drops. The nanoparticle treatment showed 42-times longer time with lower IOP, highlighting the improved treatment efficacy in the ciliary body.

Hollow ceria nanoparticles (hCeNPs)-based inorganic carriers have also been used to load and deliver antiglaucoma drug pilocarpine via topical administration [2]. The pilocarpine was loaded into hollow cavity of the mesoporous CeNPs which was then capped by chitosan and ZM241385 to achieve penetration of epithelial tight junctions and controlled drug delivery to the intraocular ciliary body tissue. In this study, the *in vitro* drug release from hCeNPs was controlled by pH-stimuli responsive mechanism of chitosan. The pilocarpine loading was enabled by protonation of amino groups (at pH 5.5) of chitosan and thus swelling due to increased positive surface charge on the chitosan backbone. The stability of loaded drug molecules was then achieved via deprotonation (at pH 7.4) of chitosan which led to weakening the electrostatic

repulsion, shrinkage of chitosan and closure of the pore channels of the hCeNPs preventing drug molecules leakage. The pH-responsive drug release at pH 6.5 showed controlled release over 7 days. The initial burst release was due to the loosely bound drug molecules on the surface whereas later sustained release was due to diffusion of drug molecules through the chitosan layer. In the case of MSN, drug molecules were loaded in mesopore channels followed by functionalization with stimuli-responsive molecules to control the drug release under specific stimulus conditions (pH, light, temperature, redox, enzyme, etc.) [3].

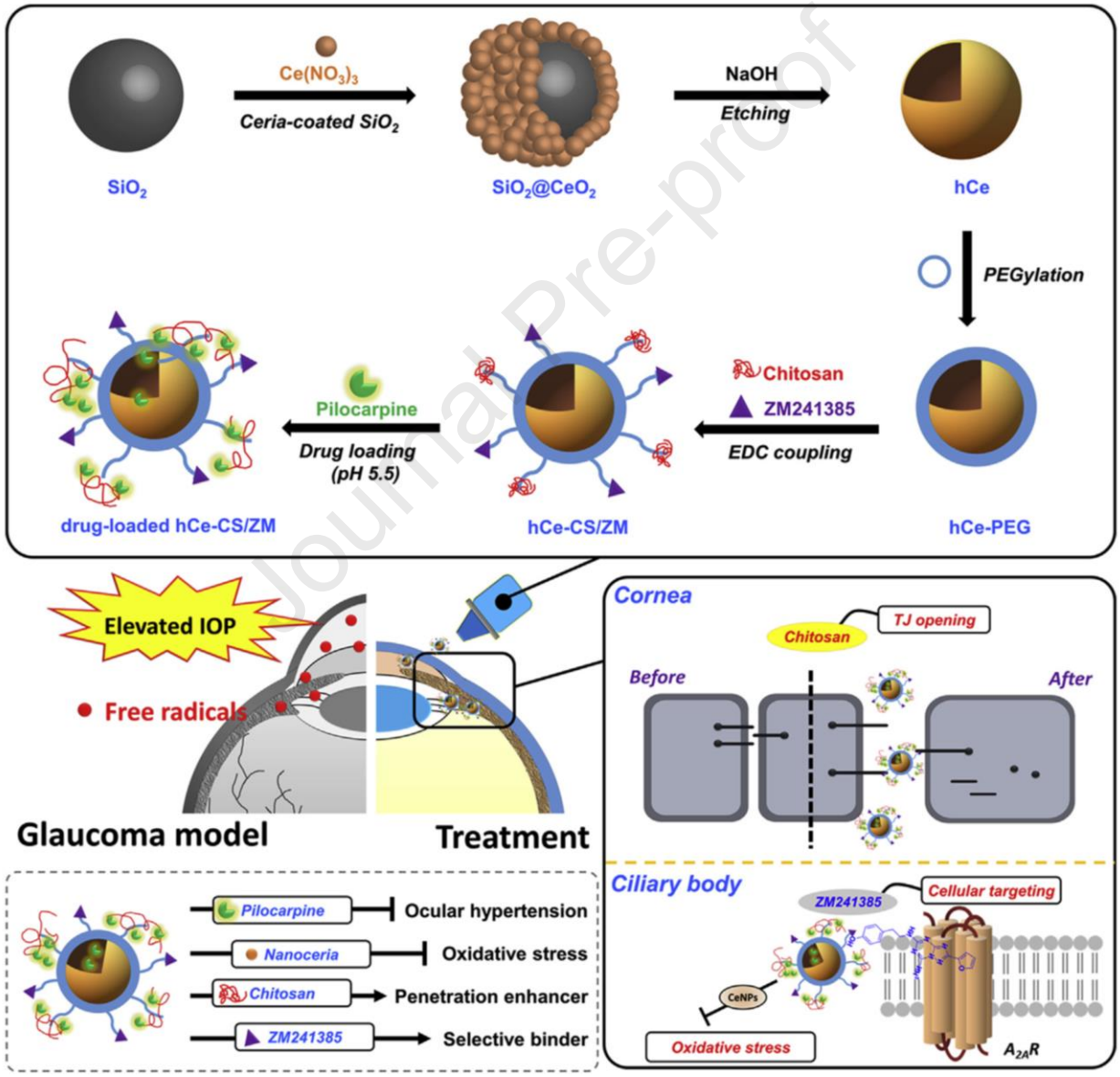


Figure 5. Schematic diagrams describe the design of nanoparticles-based eye drops to treat glaucoma. Preparation of hollow ceria nanoparticles (hCeNPs) and double modification with chitosan/ZM241385 and

filling with pilocarpine in drop form. Underside schemes diagrams explains the topical administration of nanoparticles-based eye drop formulation and corresponding pharmacological functions in corneal epithelial tight junctions opening, and allowing drug to ciliary body which reduces the oxidative stress, and inflammation in glaucoma. Reproduced with permission from Elsevier from ref. [121].

Table 2: Summary of inorganic-based nanocarriers for ocular applications.

Materials	Drug/biomolecules	Targets	Refs.
MNP	Vascular endothelial growth factor (VEGF)	Choroidal layer in the posterior segment	122
MNP	MNP-label MSCs for stem cell delivery	Trabecular meshwork tissue to reduce IOP to treat glaucoma	123
SPIONs	Rat mesenchymal stem cells	Retina: age-related macular degeneration and retinitis pigmentosa	124
MNPs	Intraocular drug delivery system	Specific target to the retinal pigmented epithelium (REP) layer after intraocular injection in *Xenopus laevis*	70
Iron oxide NPs (IONPs)	Fibroblast growth factor 2 (FGF2)	Augmentation of human mesenchymal stem cells (hMSCs)	125
IONPs	Human serum albumin (HAS)	Posterior segment of the eye in rat model of retinal degeneration	72
MNPs-PDMS-based contact lens	On-demand one-directional drug delivery	Periocular eye disease therapy	126
AuNPs	20 nm AuNPs can cross retinal barrier and deliver the drug	Cross the blood-retinal barrier (BRB)and distributed in retinal layers	127
AuNPs	Suppression of VEGFR-2	To target the inhibition of retinal neovascularization	128
AuNPs	Topical administered of AuNPs	Inhibition of corneal neovascularization in mice	81
AuNPs	Topical administered in rat eyes	Endotoxin-induced uveitis (EIU) rat model and to supress oxidative stress and inflammation	84
Polyethylenimine-conjugated AuNPs	Corneal gene therapy	*In vitro* in human cornea and *in-vivo* in rabbit cornea	129
Hyaluronic acid coated AuNPs	Topically applied drug delivery vehicles	Therapeutic delivery to retina and the retinal pigment epithelium	130
Hyaluronic acid coated AuNPs	Intraocular drug delivery	AuNPs inhibits the advanced glycated end products, cells death and neovascularization	131
AuNPs	AuNPs in ophthalmic imaging such as optical coherence tomography and photoacoustic imaging	Ophthalmic molecular imaging for the early detection of eye disease before morphological change	132
AgNPs	Anti-fungal, anti-bacterial, and anti-inflammatory	Ani-vasopermeability to vascular endothelial pathological component for retinal diseases	133
Gelatin functionalized AgNPs	Anti-bacterial and anti-angiogenic	Bacterial keratitis	103
MSN	Sustained release of nitric oxide	Efficient treatment of primary open-angle glaucoma	134

Gelatin-functionalized MSN	Pilocarpine: an anti-glaucoma drug	Glaucoma in rabbit animal model	110
MSN	Bevacizumab: inhibition of corneal neovascularization and retinal neovascularization *in vivo*	Anti-angiogenic therapy	135
Sol-gel MSN	Different pore size microparticles	15 µm silica with 10 nm pore was safe in both rabbit and guinea pig eyes and particles lasted in the vitreous for longer than two months	136
Light-sensitive MSN and contact lens	Ocular drug delivery	Nanoparticles in contact lenses for targeted and stimuli-responsive ocular drug delivery application	137
MSN	Versatile drug delivery carriers	Various routes of administration for ocular drug delivery	138
SiNPs	50, 100, and 150 nm size of SiNPs show no cytotoxicity	Human corneal epithelial cells (hCECs)	139
BSA-functionalized GO	VEGF trapped	Chick chorioallantois membrane (CAM) and rabbit corneal neovascularization (CNV)	116
GO and rGO	Mechanism of ocular toxicity of GO and rGO	Eyeballs of the mice	118
CeO_2 NPs	Therapeutic and cytotoxic effect of nanoceria	Non-toxic retention of nanoceria in urine eyes	140
CeO_2NPs	Intraocular pilocarpine drug delivery	Glaucoma therapy by pushing limits of static and dynamic ocular barriers	121
Nanoceria	Y-27632	Y-27632-mediated alleviation of ocular hypertension	141
Ceria-glycol chitosan nanosystem	Topical drug delivery as eye drop	To target dry eye disease treatment	142

3.2. Polymer-based nanocarriers

Over the last few decades, polymer (natural/synthetic)-based nanocarriers for drug delivery applications have gained enormous interests among the researcher, clinician, and pharmaceutical industries. The polymer properties such as biocompatibility, biomimetic, biodegradability, and easily control chemical functionality makes them an important candidate for drug delivery applications. By using the controlled biodegradable polymers carriers, the drug delivery time spam can be tuned from a few days to weeks or even months. The biodegradability of polymers has a vital role in drug release mechanism which interplay between diffusion process and polymers erosion and drug release in controlled manner. Polymer-based nanocarriers can be prepared by use of synthetic, semi-synthetic, and natural polymers depending on the requirements. Moreover, polymer-based nanocarriers can be effectively useful for the delivery of biomolecules (proteins, drugs, genes, and growth factors) to any parts of the body such as the cornea,

inflammatory regions, and central nerve system, where they have to work against BBB [143]. Selection of polymers and preparation methods for polymer-based nanocarriers depend on the type of drug, the delivery patterns required (controlled/burst) and the targeted tissue.

The natural polymer-based nanocarriers are highly biocompatible and biodegradable and can be used for soft tissues such as nerve, cartilage, muscle, cornea targets, wound dressing, and drug delivery applications. Synthetic and semi-synthetic polymer-based nanocarriers were utilized for sustaining and long-term controlled drug release [144]. Natural polymers are macromolecules that are originated / extracted from animal or natural products, while synthetic and semi-synthetic polymers are macromolecules synthesized in the laboratories and industrial factories through complex chemical reactions. Natural polymers such as alginate, collagen, chitosan, elastin, gelatin, hyaluronic acid, silk; and synthetic polymers like polycaprolactone (PCL), polyvinyl, polyamides, glycols, poly vinyl ethers, poly(lactic acid) (PLA), polyurethane (PU), and poly(L-lactide) (PLLA); and semi-synthetic polymers such as gelatin methacrylate (GelMA), methacrylate-silk (MA-silk), methacrylate-hyaluronic acid (Me-HA) have been utilized as drug delivery carrier. The polymers-based nanocarriers allowed for a controlled release mechanism which maintains therapeutically effective concentrations within the area of target over a period of few months [6, 7]. Recent examples of polymers-based nanocarriers for ocular applications are summarized in **Table 4**.

3.2.1. Collagen

Delivery of therapeutic molecules with effective dose, minimum loss, minimal invasive, maintaining patient compliance, and less systemic absorption are important factors in the development of the ocular drug delivery carrier. Major portion of cornea is made out of collagen, a natural protein which were used for different ocular applications including shields and drug delivery carriers [145]. Collagen shields have been utilized as corneal bandages and pharmacological delivery devices [146]. In eye, collagen shield encapsulated or physically absorbed with drug molecules can sustain for maximum 3 days prior to complete degradation [147]. Collagen shields has been used for various ocular applications including antibacterial [148] [149], anti-inflammatory [150], anti-coagulant [151], anti-fungal [152], immunosuppressive [153], steroidal [154], and antiviral [155] delivery. Agban *et. al.* have demonstrated metal oxide nanoparticles (ZnO, TiO_2, and ZnO/PVP) cross-linked collagen shields with pilocarpine hydrochloride drug release in sustainable manner in ocular delivery

of for glaucoma treatment [145]. The ZnO/PVP nanoparticles were used for the crosslinking agent with 1:1 of collagen, and fabricated collagen shield exhibited sustained release for 14 days.

3.2.2. Gelatin

Gelatin is also a natural polymer and denatured from of collagen, usually obtained by acid or alkaline hydrolysis of collagen. Gelatin has been extensively used as biomaterials in different form such as microparticles, nanoparticles, 3D scaffolds, membrane, electrospun nanofibers, and hydrogel for drug delivery, and tissue engineering biomedical applications [156-160]. Gelatin has been considered as a polyampholyte and has both anionic and cationic groups together with hydrophobic which leads to tailored degradation [158]. The presence of lysine and arginine amino acids groups endow 13% positive charge, glutamic and aspartic acid groups contributes 12% negative charge, and amino acids such as leucine, isoleucine, methionine and valine groups contributes 11% hydrophobic chain in gelatin [159]. Gelatin-based nanocarriers have been developed and well-established over the past three decades as one of the best candidates for drug and gene delivery applications [159,161]. The first ocular application of gelatin-based particulates was utilized in 1989, although no use of this was recorded until 2004 [162]. Shokry *et. al.* have fabricated the Timolol Maleate, an anti-glaucoma drug, loaded gelatin nanoparticles using double desolvation method by glutaraldehyde (GA) crosslinking [163]. They optimized the nanoparticles size 205 nm with drug entrapment efficiency of 74.72 % **(Figure 6 (i))**. The in vitro release profile of timolol maleate showed early burst effect and later followed by a control release profile. Two different formulations of drug loaded gelatin nanoparticles exhibit two step drug release profile where first stage (early time point) rapid release and second stage controlled release **(Figure 6 (ii))**. The selected formulation of drug loaded gelatin nanoparticles was studies in vivo and equated with commercially available timolol formulation on albino rabbit and they found that the developed nanocarriers exhibits better IOP lowering and sustainable efficiency **(Figure 6 (iii))**. The Hematoxylin and Eosin (H&E) results of 1 week treated tissue indicating moderated oedema of grade II, while 2 week treated exhibited mild oedema of grade I **(Figure 6 (iv))**. The H&E for control, retinal layers, and the ciliary body, has showed normal structure for 2 weeks of the drug loaded particles treatment **(Figure 6 (v))**. Pérez *et. al.* have developed hybrid system made of gelatin nanoparticles-HPMC as ocular topical administration for effective antihypertensive agent [164]. They used gelatin to load timolol maleate in nanoparticle structure and a hybrid system was added hydroxypropyl

methylcellulose (HPMC) solution. Gelatin nanoparticles exhibited similar hypertension effect and commercially available o.5% timolol maleate 5-fold lower drug concentration in in vivo.

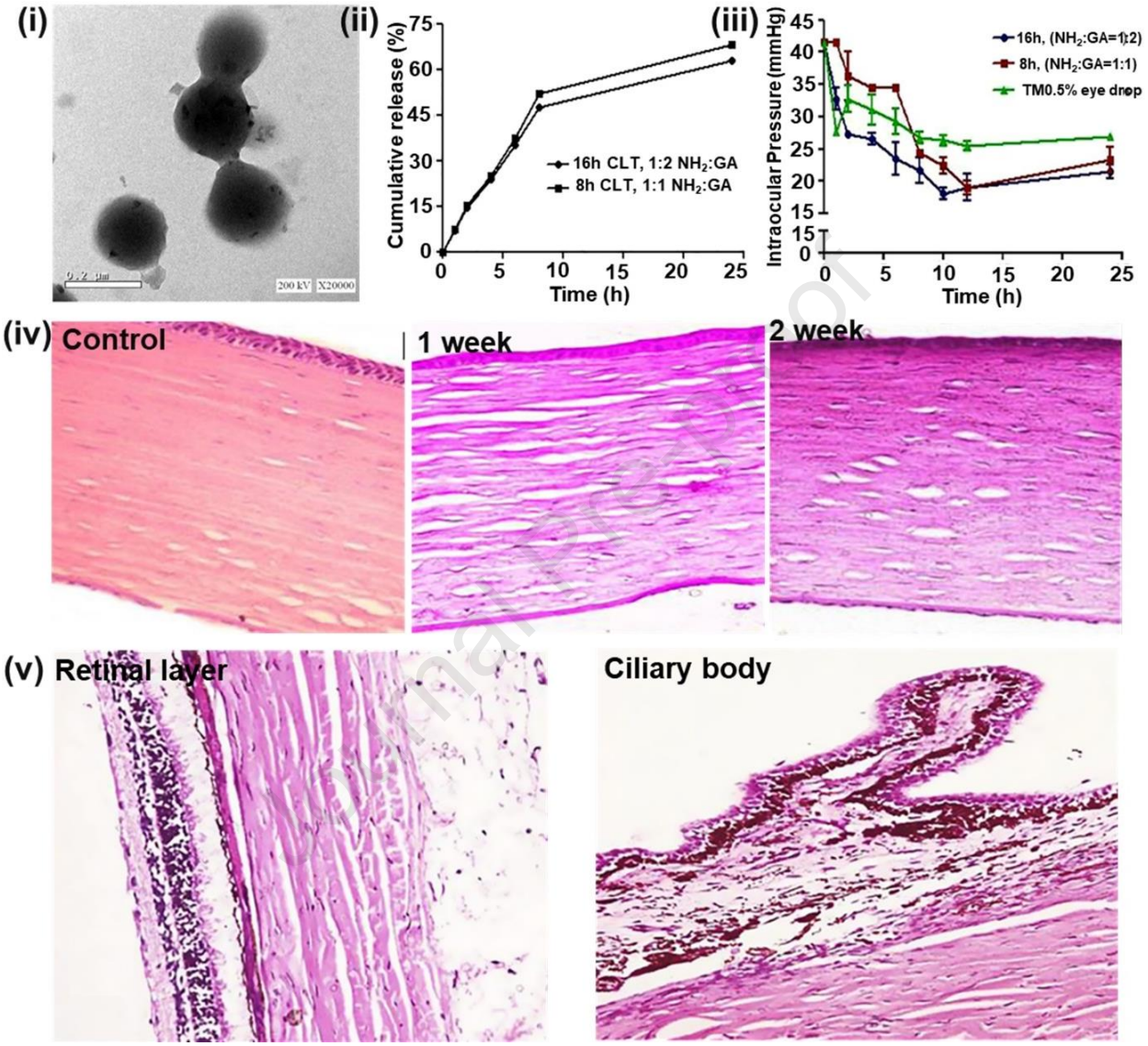


Figure 6. Natural polymers-based nanocarriers in drug delivery and safe *in vivo* efficacy; Transmission electron microscope image of Timolol Maleate loaded gelatin nanoparticles; **(i)** gelatin nanoparticles, **(ii)** Timolol Maleate release profile from the gelatin nanoparticles, **(iii)** IOP profile for 5.5 g of plunger drug loaded gelatin nanoparticles formulation *vs* 0.5% commercial product, **(iv)** H&E staining of corneal tissue of control group, 1 and 2 weeks of treatment, **(v)** H&E staining of the retinal layer, and the ciliary body after treatment with drug loaded gelatin nanoparticles at 14 days. Reproduced with permission from Elsevier from ref. [165].

3.2.3. Chitosan

Chitosan, another naturally occur polymer, which is usually obtained from the sea animal such as crab, shrimp, crustaceans, fungi, and exoskeleton of insects [166,167]. The main process for the extraction of chitosan from its parent polymer chitin is deacetylation, widely available in the nature. Chitin is parental form of chitosan, which is insoluble in most of the organic solvents, while chitosan is soluble in acidic aqueous of pH 6.0 of bellow mainly due to quaternization of amine groups (p*Ka* 6.3) [168,169]. Chitosan is a biodegradable, bioactive, cytocompatible, and mucus adhesive polymer which has been used greatly in biomedical application [170]. Chitosan can be used to produce microparticles, nanoparticles, film membrane, electrospinning nanofibers, 3 D scaffolds, and hydrogels for wound healing, drug delivery, antibacterial and tissue regeneration applications. Chitosan exhibits positive surface charge (*i.e.,* cation) due to large amount of amin groups and forms various salts [171]. Therefore, positive charge chitosan and negatively charge corneal mucin can bind through charge-charge electrostatic interactions and hence leads to long-term bioavailability [172]. Kao *et. al.* have synthesized chitosan-carbopol nanoparticles loaded with antiglaucoma drug pilocarpine and evaluated the sustain release effect of pilocarpine in vitro and in vivo. The drug release was analyzed using miotic tests, and compared with free pilocarpine, encapsulated in gel, and also loaded in liposome [173]. Chitosan- carbopol nanoparticles showed slow release of pilocarpine compared to the other formulation (free, gel and liposome), and exhibited long-lasting pupil diameter reduction of rabbit in vivo miotic tests. Katiyar *et. al.* have formulated chitosan nanoparticles encapsulated with dorzolamide and dispersed in alginate gel to achieve long-term sustain release for glaucoma treatment [174]. The *in-situ* gel nanoparticles formulation exhibited long-term controlled release of drug, and ex vivo permeation results showed *in-situ* gel nanoparticles formulation is significantly high than the marketed solution available for glaucoma management. Papadimitriou *et. al.* have formulated chitosan nanoparticles with dorzolamide hydrochloride for efficient ocular and pramipexole hydrochloride for oral formulations using gelation technique [175]. The prepared formulations possess mucoadhesive property which decreases with increasing contents of the drug, and exhibited sustained release. Cho *et. al.* have formulated hexanoyl glycol chitosan for preocular drug delivery to the treatment of the glaucoma [176]. They found that the hexanoyl glycol chitosan-based formulation can be used long-term retention at preocular surface and also enhanced the bioavailability of the drug. Lin *et. al.* have synthesized pilocarpine drug loaded chitosan-poly(acrylic acid) nanoparticles and tested as ophthalmic drug delivery carrier [177]. They found that the size carriers drastically

enlarged with change in pH of solution and in both in vitro and in vivo results exhibited controlled pilocarpine release. Recently, Sun *et. al.* developed topical eye drops using mucoadhesive phenylboronic acid conjugated chitosan oligosaccharide-vitamin E voriconazole-loaded nanomicelles for fungal keratitis application [178]. These examples of chitosan with different drug formulations confirmed the promising role of chitosan as biomaterials for glaucoma drug delivery.

3.2.4. Sodium alginate

Sodium alginate is commonly known as alginate, a naturally occur polymer and has been used for various ocular delivery systems, either alone or collaboration with other materials [179].The alginate is an anionic polysaccharide which is abundantly spread in cell walls of the brown algae in viscous gum in presence of water. Alginate has been used as promising biomaterials for cell and drug delivery systems due to adaptable mechanical, physicochemical, selective chemical modification, biodegradation, gelation, biocompatibility, and mucoadhesiveness [180-182]. Moreover, the mechanical, biodegradation, gelation, biocompatibility, and cell affinity of the alginate-based nanoparticles can be modulated by proper physical or chemicals crosslinking or surface tailoring using specific targeting moieties [183]. Liu *et. al.* have demonstrated an antibacterial formulation of alginate/HPMS-based an ophthalmic drug delivery system of gatifloxacin using ion-activation *in situ* gelation method [184]. The in vitro drug release and in vivo precorneal retention of the formulation exhibited long-term retention which was better than alginate or HPMC-drug formulation alone. Thus, gelatin with HPMC formulation in in-situ gel carrier can enhanced the bioavailability. Similarly, Lin *et. al.* have developed alginate/pluronic formulations using in-situ gel technique for pilocarpine delivery in ophthalmic application [185]. Taghe *et. al.* have prepared different combed formulated of chitosan-alginate-tripolyphosphatesodium and chitosan-tripolyphosphatesodium nanoparticles for prolonged topical ophthalmic delivery ofloxacin [186]. Motwani *et. al.* have synthesized two natural polymer chitosan-alginate-based nanoparticles formulation using ion gelation technique for drug delivery [187]. The nanoparticles formulation exhibit burst release for initial 4 h and followed by slow drug release for 24 h following non-Fickian diffusion mechanism of drug release. Overall, these examples suggest that the alginate-based drug-nanoparticles formulation can be effective and potential candidate for long-term controlled drug delivery application in glaucoma.

3.2.5. Hyaluronic acid (HA)

Hyaluronic acid (HA) is also a natural polymer with exceptional biocompatibility, biodegradability, amenability to chemical modification, and low immunogenicity and has been long used as biomaterials for therapeutic and cell delivery, wound healing, and soft tissue regeneration [188-190]. Moreover, hyaluronic acid has normally been valued to control tissue mechanics and remodeling of extracellular matrix (ECM) of connective tissues through distinctive biophysical properties as well as organize the ECM proteins [191,192]. HA receptors are well known in cell singling which can trigger cell signals and modulate the cell activities such as survival, adhesion, proliferation, migration, and specific linage differentiation [193]. Aragona *et. al.* have investigated the effect of sodium hyaluronate containing artificial tears in topical form on 86 patients with moderate to severe dry eye [194]. They found that the sodium hyaluronate formulation with artificial tear may improve the damaged ocular surface in dry eye syndrome. Fuente *et. al.* have synthesized hyaluronic acid-chitosan nanoparticles formulation and investigated the mechanism of effectiveness for the gene delivery in cornea and conjunctiva [195]. They found that the developed nanoparticles formulation was effective to offer high level of transfection without affecting cell viability. Moreover, the hyaluronic acid-chitosan nanoparticles formulation were internalized through endocytosis which was mediated by the hyaluronic receptor CD44 for targeting and transfection of gene to ocular surface. Apaolaza *et. al.* have developed solid lipid of protamine and hyaluronic acid nanoparticles formulation for gene delivery [196]. The particles-gene complexes had positive charge and particles size 240 – 340 nm which was transfected into two different cells namely ARPE-19 retinal and HEK-293 kidney cells in an effective manner. They found that the hyaluronic acid can effectively modulate the condensation of DNA mainly because of protamine inside the cell, if the nanocarriers were internalized via nondegradative endocytosis.

3.2.6. Silk-fibroin (SF)

Silk-fibroin (SF) is a natural protein and usually obtained from cocoons of *B. mori*. Cocoon is consisting of two main components, first a water-soluble protein (silk-sericin), and second, fibrous hydrophobic protein (silk-fibroin). SF is important component of silk obtained from *B. mori* and consists of two different molecular weight of 390 kDa (heavy chain) and 26 kDa (light chain) via disulfide bond [197]. The chemical modification of SF with specific molecule at defined sites for better interaction and control of biological activity and varied mechanical properties have been reported for tissue engineering [198]. For the same purpose, number of approaches have also been applied for the improvement of the bioactivity of silk. Recently, chemical

modification and blending of silk with other materials have also performed [199,200]. The excellent biological, physical, and chemical properties of silk endow as a robust biomaterial for regenerative medicine application. The versatile surface chemistry and controllable degradation by manipulating the silk structure and interactions between silk molecules and drug molecules allow as delivery candidate. Huang *et. al.* have investigated the feasibility of SFNPs as sustain drug delivery carrier in transscleral ultrasound [201]. They used FITC-conjugated BSA as a model protein to encapsulate in silk fibroin nanoparticles and performed the in vitro study of transscleral under ultrasound of frequency 1MHz, power 0.5 W/cm^2 for 300 second in isolated sclera of rabbit using continuous wave. They found that FITC-BSA-silk fibroin nanoparticles exhibited controlled release, better bioadhesive, co-permeation, and penetration efficiency of nanoparticles formulation significantly improved under ultrasound related to normal delivery. The ultrasound application of delivery does not cause any adverse effect to corneal tissue and nanoparticles biodistribution results also revealed fast and long-term adhesion to out-sclera tissue, and later followed by migration into interior for a week. Dong *et. al.* have investigated SF functionalized liposomes nanoparticle formulation for controlled ocular delivery of ibuprofen [202]. The in vitro release of ibuprofen and its corneal layers infusion from SF functionalized liposomes nanoparticle formulation was relatively better than drug solution and conventional liposome alone.

3.2.7. Synthetic polymer-based carriers

Recently, several synthetic polymer-based formulations have been synthesized and developed as a ocular delivery carries, despite the availability of various natural polymers-based nanocarriers. The arise of synthetic polymers-based nanocarriers formulation are primarily because of their cost-effective manufacturing, biocompatibility, controllable degradability, and long-term drug release. The main synthetic polymer-based nanoparticles are PCL, PLA, and PLGA. PCL is approved by the US food and drug administration (FDA) for development of various biomedical devices and implants. Moreover, PCL can be used to produced nanoparticles, microparticles, nano-, microfibers, scaffolds, and as well as injectable materials. Lee *et. al.* have fabricated PCL-base polymer nanoparticles and nanocapsules formulated with anti-glaucoma drug pilocarpine (PILO) using oil/water/oil double emulsion method [144]. Solid nanoparticles and hollow PCL nanocarriers formulation with PILO loaded were of similar size around 227, and 235 nm, respectively **(Figure 7 (i-ii))**. The drug release profile for solid PCL nanocarriers was burst release while

hallow PCL nanocarriers showed sustained cumulative leased of PILO for 6 weeks, indicating the significance of hollow structure **(Figure 7 (iii))**. The IOP value for glaucomatous eyes after treatment with drug formulated nanocarriers (solid and hallow) both showed an effectiveness on day 5, however only hollow PCL nanocarriers sustained the effectiveness up to 42 days **(Figure 7 (iv))**. Finally, the effect of drug release in eye was evaluated qualitatively and quantitatively by anterior chamber depth in glaucomatous rabbit eyes **(Figure 7 (v-vi))**. They found that the hallow PCL nanocarriers showed three times higher PILO loading capacity compared to solid PCL nanoparticles and thereby better therapeutic effect in the treatment of glaucoma. PLGA is another popular synthetic polymer for drug delivery because it is a co-polymer and biocompatible and biodegradable and moreover it is approved by US FDA for therapeutic delivery and other biomedical applications. PLGA is made of varying composition two different acids of lactic acid (LA), glycolic acid (GA), and depending on the block structure and molar ratio of composition the crystallinity changes and thus the biodegradation. Recently, Pan *et. al.* have synthesized PLGA nanoparticles via co-axial electrospray techniques and formulated with co-delivery of dexamethasone and melatonin for the treatment of glaucoma [203]. They found that the drug encapsulation efficacy was around 85% and exhibited sustained resales over two weeks. The cell viability against R28 cells was above 90% for dexamethasone and melatonin concentration of 15 µg/mL and showed enhanced retinal penetration efficacy, which subsequently help to reduce the IOP significantly and improve the management of glaucoma condition. Nguyen *et. al.* have developed hallow PLA with varying shell width of 10 to 100 nm and utilized for long-term controlled release for pharmacological management of glaucoma. They found that the nanoparticles with shell thickness of 70 to 100 nm had low capacity of drug encapsulation and ultrathin thickness showed faster drug release compared to thick shell nanoparticles. The optimum shell thickness was 40 nm which endow long-term drug release (56 days) and effective in glaucomatous condition in rabbit eyes.

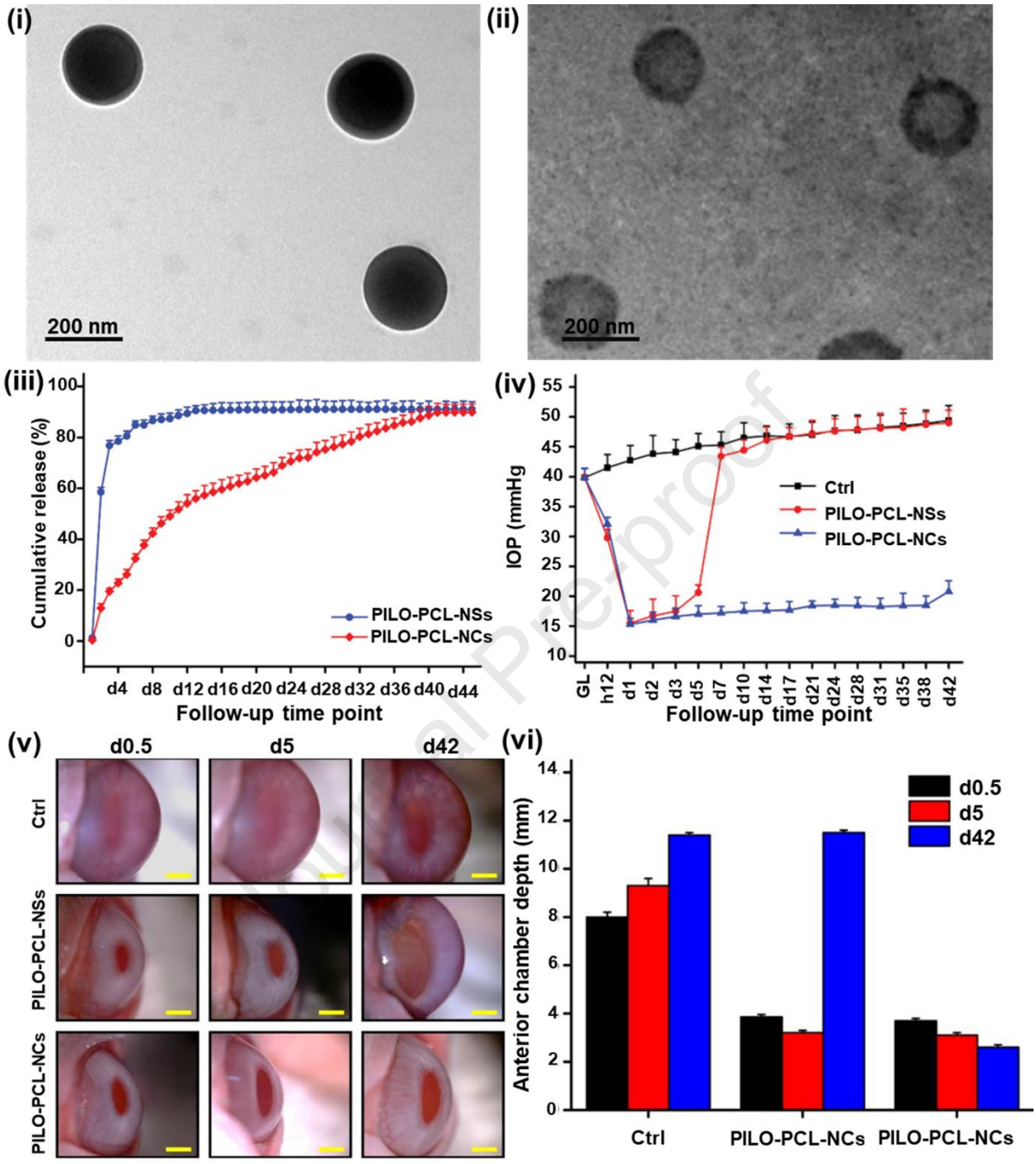


Figure 7. Synthetic polymer-based nanocarriers for anti-glaucoma drug delivery; TEM image of pilocarpine drug loaded PCL nanospheres and nanocapsules; **(i)** PILO-PCL NSs, **(ii)** PILO-PCL NCs, **(iii)** Pilocarpine release profile from PCL nanosphere and nanocapsules, **(iv)** The IOP values of after treatment with 20 µL of both nanocarriers separately. **(v)** The slit-lamp biomicroscope images (side view) of eyes treated with drug loaded nanocarriers, and **(vi)** corresponding glaucomatous rabbit eyes anterior chamber depth at different time points. Reproduced with permission from the Royal Society of Chemistry from ref. [144].

3.2.8. Other nanocarriers

In this subsection, dendrimers, nanomicelles, nanoliposomes and niosomes, and nanoemulsions were discussed. Many studies regarding these nanocarriers share three common limitations; first, many research groups limit their observations to short or mid-term studies to assess the effect of the nanocarriers that will be used for chronic conditions such as dry eye disease. Second, most of the research groups do not investigate the conjunctive effect of nanocarriers type, composition and the administration at different pathological timelines which could be used to further improve therapeutics. The third limitation is that few research groups have investigated the potential development of drug resistance over time or with repeated treatments when using the nanocarriers based delivery systems.

The word dendrimer comes from a Greek word *Dendron* which literal meaning is 'tree'. Dendrimers are polymeric structures than can be found in star-shaped, highly branched, star-shaped polymeric structures with nanometer-scale dimensions and a well-defined architecture [204]. Their structure includes three regions: a core molecule or atom, the branches, which are formed by repeated units that define the geometrical arrangement and the surface terminal groups, which can be finely tuned to match the drug cargo [26]. Dendrimer-based nanocarriers have been studied for ophthalmic drug delivery applications by several research groups [205-207]. The safe use of dendrimers as nanocarriers has been possible thanks to their suitable physicochemical properties. Dendrimers-based nanocarriers of higher generation (G2, G3, G4 and G7) have a spherical configuration which facilitates drug loading, while lower generation dendrimers are more open and amorphous. Their size normally ranges from 1 to 100 nm which enhances site targeting and retention of the loaded drug [26]. Moreover, the degree of uniformity in the molecular structure (monodispersity) of dendrimers is determined by steps such as reagent removal or dimer bridging during production and is a tool for research reproducibility and consistency [26]. The biocompatibility and drug loading capacity of dendrimers nanocarriers are determined by the nature, surface charge, and complexity of dendrimer's terminal groups. While cationic dendrimers (*i.e.*, PMAM, PPI and Poly-l-lysine) are capable of interacting with negatively charged cellular membrane allowing higher cell internalization, load compacting and transfection potential, these same interactions can promote the formation of cell membrane nanoscale holes and cell lysis thus having a higher and dose dependent cytotoxicity profile and a greater tendency to aggregate [208,209]. On the other hand, neutrally charged hydroxyl dendrimers are effective to deliver drugs with a good safety profile, but the lack of cell interaction limits their use as gene vector carriers.

Dendrimer engineering allows to achieve therapeutic requirements while also reducing cytotoxicity. For example, dendrimer functionalization by glycosylation, acetylation and PEGylation allows for surface charge neutralization (as show in Table 8) [210,211]. The degree of surface acetylation of dendrimers such as polypropylene imine (PPI) have been shown to be proportionally related to the drug-loading capacity [212]. Mastorakos *et. al.* have observed that a potent ocular corticosteroid called triamcinolone acetonide (TA) also acted as a nuclear localization signaling agent when added to drug-dendrimers systems [208]. Polypropylene imine is primary amine terminal hyperbranched dendrimers produced by the divergent method. Topical instillation of acetazolamide (ACZ) loaded PPI dendrimers on albino rabbit models was reported by Mishra et. al., to have a delayed (at 6h) but longer sustained (4h) release of the drug achieving significantly lower IOP with less ocular irritation compared to conventional ACZ solution [213].

Functionalization of polyamidoamine (PAMAM) dendrimers has been possible in five different ways to achieve gene therapeutics: 1) adding hydroxyl to PAMAM to neutralized this cationic dendrimer; 2) functionalize the dendrimer with TA to enhance nuclear location of the gene vectors in the dendrimer structure; 3) minimal addition of primary amines to enhance cell-nanocarriers interactions and consequently cellular uptake, 4) protect the amine groups with FMCO groups; and finally 5) coat the dendrimer with hydrophilic and nearly neutral polyethylene glycol (PEG) for colloidal stability of the system. Using these highly functionalized dendrimer nanocarriers, Mastorakos *et. al*. achieved the delivery and transfection of luciferase gene into human RPE cells, therefore introducing the potential of nanocarriers to be used for geneotherapy in retinal diseases including Sargardt's disease and retinitis pigmentosa [208].

PAMAM are silicon containing positively charged dendrimer which are produced by the divergent method from ammonia [26,209]. Recently, Lin *et. al.* reported that comparing to free-Dex treatment, subconjunctival injection of dexamethasone loaded PAMAM-dendrimers (D-Dex) in a rabbit model of dry eye disease showed significantly higher percentage recovery of tear production, increased tear break up time, preocular film protection and upregulation of aquaporin (AQP) proteins for tear secretion in 2 weeks, which led to 56% higher number of rabbits recovery [44]. It was observed that compared to saline group, D-DEX applied rabbits revealed significantly lower amount of proinflammatory factors MMP9, IL6, IL8 and TNF- α. Moreover, T lymphocyte staining marker was attenuated in the D-DEX group and 6 out of 9 rabbits treated with DEX loaded dendrimers also showed expression of anti-inflammatory factor IL10. Other less common dendrimer

types include liquid crystalline dendrimers with rod like or disc like structure, core-shell dendrimers, which are highly organized structures used as building blocks; chiral dendrimers, with varying branches of similar chemistry which allow further functionalization; peptide dendrimers, with amino acids in the peptide core, branches and/or terminal groups; glycodendrimers, made of sugar molecules like mannose or galactose; hybrid dendrimers, composed of branched and linear polymers; and layered or micelle dendrimers i.e. PAMAM core and an organosilicon exterior [214,215]. In addition to the properties mentioned above, research in the recent years has demonstrated that aspects such as final formulation, administration route, pathological progression timeline and local environment (*i.e*., pH changes during inflammation) also determine the efficacy and drug release profiles of dendrimers nanocarriers in ocular delivery. For example, Soiberman *et. al.* observed that compared to free-dexamethasone gels, DEX/G4OH-PAMAM dendrimers delivered within a thiolate hyaluronic acid hydrogel (nanocomposite) had a longer drug retention profile in vitro of 5 to 7 days depending on local pH environment; lasting for longer at pH 7.49, which is like that of ocular inflammation [32]. Furthermore, they have also reported that DEX/G4OH-PAMAM gels showed a 15.3% lower macrophage cytotoxicity and superior clinical outcomes in vivo reducing postoperative intraocular pressure, central corneal thickness, corneal neovascularization, corneal opacity, and inflammatory cytokine production for 7-14 days in corneal alkali burn rat models. Sudden optic nerve related vision loss can be caused by the optic nerve inflammation and retinal ganglion cell death following non-arteritic anterior ischemic optic neuropathy (NAION) lesions. Guo *et. al.* have reported that the time and route of administration was vital for targeted delivery and functionality of dendrimers in rodent and non-human primate models [45]. Their finding suggest that the Cy5 dye loaded hydroxyl-PAMAM dendrimers were only able to reach and aggregate into infiltrated macrophage and activated astrocytes when delivered via systemic intravenous administration at the early stages of the lesion, which suggested that dendrimer entrance in the area is dependent on the disruptions of the blood brain barrier occurring during disease progression. This pathology dependent biodistribution of drug-loaded dendrimers has also been reported 2 weeks after subconjunctival application, when dexamethasone-dendrimers were only located within the inflamed areas of the lacrimal gland [44].

To treat blindness related diseases using virus-based nanocarriers have also been utilized as drug, and gene delivery carriers, though it is not fully safe. For example, Rajala *et. al.* have design and fabricated the

artificial virus liposome-protamine-DNA (LPD) complex-based nanoparticles formulation and improved with cell membrane penetrating as well as nuclear localization signaling peptides to deliver drug and genes for treatment of blinding eyes [216]. The LPD and liposome-based targeted delivery nanocarriers loaded with plasmid DNA and other biomolecules were synthesized **(Figure 8 (i))**. The peptide conjugated nanocarriers can penetrate both cell as well as nuclei and thus successfully delivered the gene in cells **(Figure 8 (ii))**. The expressed of complexed GFP to HEK-293T mammalian cells can be observed by fluorescence microscopy **(Figure 8 (iii))**. Further, the subretinal injection of these nanocarriers into eye can targets the gene and subsequently promote cell-specific gene expression **(Figure 8 (iv))**. Finally, transfected green fluorescent protein in mammalian cells as well as mouse eye were observed by green fluorescence using fluorescence microscope **(Figure 8 (v))**.

In recent years, micelles-based nanocarriers have been utilized for the potential in lipophilic drugs delivery. Nanomicelles are structures of hydrophobic cores and polar shells produced by aggregation of amphiphilic polymeric surfactant block molecules in aqueous conditions [217]. In these colloidal structures, the polar head of the surfactants forms an outer shell, making the system soluble, while the polymer hydrophobic tails remain in the core where lipophilic drugs can be encapsulated with high chemical stability [218]. During design and production of nano micellar formulations, there are many aspects which will influence the final micelle size, surface charge, transport efficiency, drug releasing profile and chemical stability (over time, transport, storage, and specific physiological conditions), these includes surfactant type, critical micellar concentration (CMC), other additives, drug to surfactant ration, and administration routes and storage. Other types of nanocarrier, such as niosomes, which is vesicular nanocarriers exploited for enhanced therapeutic drug delivery in clinical application. Niosomes are composed of bilayer hydrophobic membrane enclosing a core field with aqueous phase, and thus the carrier can encapsulate and deliver both hydrophobic and hydrophilic drugs. Recently, Momekova *et. al.* summarized the multifunctional niosomes-based nanocarriers for advanced drug delivery applications [219].

Surfactant plays very important role in stabilizing the nanocarriers, loading and delivering of drug. Zhang *et. al.* have investigated that the combining block polymer monomethoxy PEG (MPEG) with supramolecular cross-linkable polymers such as alpha-cyclodextrin (α-CD), meant that micellar formulations could be produced as micellar hydrogels for ocular delivery of diclofenac (as illustrated in Table 8) [220]. They found

that the micellar hydrogels of higher α-CD concentrations had a significantly slower release profiles releasing 91-103 % of the diclofenac in 216 h compared to only 12 h from normal micelles. CMC is another important parameter defined by concentration at which the surfactants start forming micelles. Ideally polymers with lower CMC are preferred to allow higher stability of nanomicelles when these are further diluted in the aqueous eye environment [221]. Other additional parameters such as additives like self-assembling surfactants, drug to surfactant ratio, administration routes, and storage have also crucial role in nanocarriers development. The strong interlock hydrogen bonds between the surfactants of the nanomicelles determine the thermodynamic stability of the whole system [222]. Similarly, the compatibility of a drug with the surfactants forming the micelle is essential in tuning amount of drug loading and release profiles. The higher the affinity the slower is the release rate, which allow for sustained drug release [223]. The high lipophilicity of the corneal epithelium can limit the trans-corneal penetration of topically administrated nanomicellar formulations, therefore limiting these therapeutics to treating anterior segment conditions. However, interest is growing in studying conjunctival/scleral pathways potential to deliver nanomicellar formulations to the posterior segment of the eye [33]. In terms of storage and transport, it is predictable that changes in temperature could affect nanomicelles, increasing the tendency for drug leakage. By dehydrating the nanomicellar formulations using freeze drying, these could be stored for three months at 4 °C, with only an increase in CyA micelle size but no aggregation or changes in height ratio, spherical shape and monodispersity of the formulation [30]. Of note the nanomicelles are translational with clinically relevant/measurable properties, including cell viability, ocular irritation, penetration, and physiological changes, as listed in **Table 3**.

Table 3. Summary of nanomicellar ocular delivery products and the corresponding clinically relevant results.

Nanomicellar product	Clinically relevant results	Refs.
Diclofenac loaded into block polymer monomethoxy PEG and α-CD-based micellar supramolecular hydrogel	Hydrogel concentration dependant cytotoxicity of L929 and hCEC. Significantly higher corneal tissue retention. No morphological damages in vivo instillation of albino rabbits.	220
Myricetin/PVCL-PVA-PEG micelles vs Myricetin solutions and Diclofenac sodium eye drops	Significantly higher (>99%), time and concentration dependant hCEC viability. No tear shedding, no inflammatory cell infiltration, no damages in the conjunctiva, cornea, or iris. Dose dependant considerably higher anti-inflammatory effects, with the most efficient concentration being 4 mg/ml. Penetration and	224

	distribution was 2-4-fold stronger in the whole cornea depending on time but specifically in the epithelium.	
Cyclosporine-A loaded into vit-E-TPGS and OPEE micelles with hyaluronic acid VS cyclosporine emulsion Ikervis®	Concentration dependant significant reduction of RCE cytotoxicity. Increased precorneal drug retainment 4-fold lower elimination rate. No damage to corneal tissue integrity.	218
Curcumin loaded into PEG-DSPE/solutol HS15/Gellan-gum	Higher ocular retention time (50 vs 10 min). No ocular irritation in cornea, iris and conjunctiva of rabbit eyes.	225
Cyclosporin-A loaded into mPEG-PLA micelles vs CyA emulsion	Higher HCEC viability and internalization. 4.8-fold higher corneal tissue concentrations and slower elimination rates.	30
Cyclosporin loaded into aqueous nanomicellar formulation Cequa ® (OTX-101)	Enhanced transport by the conjunctiva/scleral path. Significantly higher tear production and lower ocular inflammation (OTX-101 0.09%) mild – moderate (but not new) side effects.	33

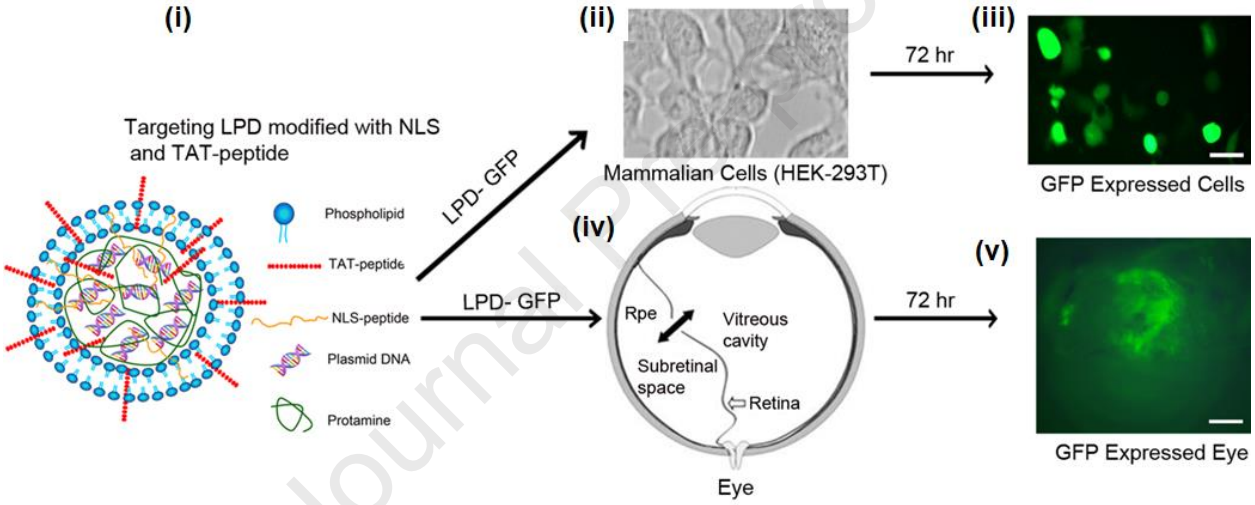


Figure 8. Schematic demonstration of multifunctional nanoparticles functionalized with nuclear localization signaling (NLS) as well as cell penetrating transactivator of transcription (TAT) peptides to deliver into posterior segment of eye, **(i)** A biocompatible lipid molecule was packed with biomolecules such as plasmid DNA, NLS and TAT peptide and protamine as a nonviral drug delivery carrier, **(ii)** Optical image of eye cells penetrating and nuclei targeting functions by functionalized nanoparticles, **(iii)** Expressed complexed GFP in HEK-293T cells; **(iv)** Subretinal injection into eye targets gene and stimulate the expression of gene to cell-specific, **(v)** GFP expression in mammalian cells and mouse eye analyzed by fluorescence after transfection. Reproduced with permission from the American Chemical Society from ref. [216].

As previously highlighted, conventional treatments for ocular disease such as glaucoma limited by considerable drawbacks such as their limited bioavailability to target tissues or adverse ocular effects as well as the poor and inadequate patients compliance. Therefore, there has been an emerging interest in nanotechnology and development of nanocarriers to short out ocular barriers, endowed interactions with specific ocular tissues and provide optimized targeted drug delivery. Nanocarriers has opened up new

possibilities and perspectives in the treatment of glaucoma in various ways such as safeguarding low eye irritation, high bioavailability of drug, and improving tolerance of ocular tissue [226]. For example, Li *et. al.* looked at designing a nanoparticle drug-loading system in which drug molecules were packed in PEG-PSA materials that improves bioavailability and shorten half-life of the drug (as shown in Table 8). They tested a combinational treatment of brinzolamide, which is an effective aqueous humor inhibitor, and mi-RNA-124 transported through nanoparticle in rabbits and mice eyes, and found that the miRNA/BRZ formulation was safe and efficient in IOP reduction [227].

Table 4. Summary of polymer-based nanocarriers for ocular applications.

Materials	Drug/molecules	Targets	Refs.
Collagen shields	Tobramycin cyclosporin, dexamethasone	Drug delivery device to the rabbit eyes	146
PVA/anionic collagen membranes	Ciprofloxacin hydrochloride	Antibacterial and potentials use in treatment of ulcerative keratitis	228
Alginate microsphere-collagen composite hydrogel	Bovine serum albumin (BSA)	Ocular drug delivery and corneal substitute for transplantation	179
Collagen-based corneal scaffold	Vancomycin	Prevention of anti-infective and perioperative bacterial infections	149
Cationic lignin-based hyperbranched polymers	Polymyxin B, colistin	To target *Pseudomonas* keratitis in rabbit eyes	229
Collagen suprafamily	Drug delivery and tissue engineering	Biomaterials for tissue engineering	230
Polydopamine-crosslinked gelatin	Vancomycin	Anti-bacterial, anti-fungal and wound healing	231
Collagen membrane	B-cyclodextrin dialdehyde and ofloxacin	Bacterial keratitis	232
Natural biomaterials	Drug delivery and tissue engineering	Corneal tissue engineering, repair and regeneration	233
Cornea-derived decellularized extracellular matrix (Co-dECM)	3D bioprinting and high transparency	Corneal diseases based on corneal-specific ability	234
Chitosan nanoparticles	Daptomycin	Ocular treatment of bacterial endophthalmitis	235
Glycol chitosan DNA nanoparticles	Plasmid DNA	Retinal gene delivery application	236
Chitosan nanoparticles	5-fluorouracil	Ophthalmic delivery application	237
Mucoadhesive polymer formulations	Ketoconazole	Rabbit fungal keratitis and associated inflammation	152
Chitosan nanoparticles	Dorzolamide hydrochloride	Glaucoma treatment	174
Chitosan nanoparticles	Dorzolamide hydrochloride and pramipexole	Ocular drug delivery for idiopathic Parkinson's disease	175

Thermoresponsive hexanoyl glycol chitosan	Brimonidine	Topical drug delivery for glaucoma treatment	176
Chitosan-PAA nanosuspension	Pilocarpine	Ophthalmic drug delivery for glaucoma treatment	177
Gelatin nanoparticles	Timolol Maleate	Albino rabbit, and normotensive rabbit	165
Alginate-pluronic solution	Pilocarpine	New Zealand albino rabbits	238
Core-shell nanoplatform composed of amino acid-functionalized dendrimers, hyaluronic acid-1,2-dioleoylphosphatidylethanonlamine (DOPE)	Gene delivery	Retinal disease such as age-related muscular degeneration (AMD)	239
Cyclodextrin-based nanohybrid hydrogels with polydopamine	Dexamethasone	*In vitro* ocular drug delivery for inflammatory diseases	240
Hyaluronan with ε-polylysine micelle nanogel	Ferulic acid	Rabbit corneal alkali burn model and corneal wound healing	241
E-tocopheryl polyethylene glycol succinate-PCL nanoparticles	Brimonidine tartrate	*In-situ* gel for intraocular pressure reduction in glaucoma patients	242
Silk-fibroin coated liposomes	Ibuprofen	Ocular drug delivery in albino New Zealand rabbit	243
PLGA nanoparticles	Dexamethasone and melatonin	Glaucoma treatment	203
PCL nanocarriers	Pilocarpine	Glaucoma treatment in rabbit animal model	144
Liposome	Topical delivery of Avastin	Posterior segment of the eye using annexin A5-mediated liposomes	244
Cubosomes	LM22A-4, a small neurotrophic factor	To prevent the progression of RGCs loss in glaucoma	245
G5-PAMAM dendrimer	Polymer-mediated intracellular superoxide dismutase delivery	Treatment of retinal ischemic disorder by direct delivery of antioxidant proteins	246

3.3. Hydrogel-based delivery systems

Hydrogels are generally soft materials made of water and polymers (natural/synthetic/semi-synthetic) and can be engineered through physical or chemical crosslinking. The physically crosslinked hydrogels involves hydrogen bonding, ionic bonds, van der Waals, hydrophobic, electrostatic interactions and chemically crosslinked hydrogels involves only covalent bonds. The mechanical strength of hydrogel mostly depends on three different parameters namely molecular weight, concentration, and types of crosslinking (physical, ionic, and chemical). Hydrogel-based systems can provide controlled and therapeutically beneficial outcomes of drug delivery[247,248]. The controlled drug delivery from hydrogel system is mainly due to tunable physical and degradation, and ability to protect encapsulated drug/therapeutic molecules from being ineffective /denatured. Hydrogels have a controlled mechanism for drug release which involves a regulation

through the network degradation. Biodegradation of a polymer networks depends on polymers backbone, type of crosslinking (physical, chemical, ionic, enzymatic), and it is molecular weight as well as typically mediated by hydrolysis, enzymatic degradation [249,250]. The hydrogels are formed by various crosslinking mechanisms, which include physical, chemical and ionic cross-linking. For example, collagen is physically crosslinked by the gelation mechanism of self-assembling fibrils under appropriate pH, temperature and ionic strength conditions. Alginate is a unique polymer that can be crosslinked by ions, such as the use of calcium salts. On the other hand, many polymers were modified to be crosslinked chemically [251]; for instance, the gelatin is methacrylated (GelMA) to allow chemical crosslinking via UV irradiation (as shown in Table 8) [252].

Recently, hydrogel-based formulations with sustained ocular drug delivery carriers have attracted significant interest due to efficient delivery at the target site without causing systemic adverse effects and drug loss. This could be linked with the fact that hydrogels are a combination of water molecules in cross-linked polymeric matrix, which endow quite enough space for drug/therapeutic molecules encapsulation and their slow release. Various types of hydrogels have been developed with enhanced contact time of formulation in ocular delivery applications [253]. Stimuli-responsive hydrogels such as pH, temperature, enzyme and ion-activated have also been formulated for ocular delivery as well as glaucoma treatment [254]. In this regard, Fedorchak *et. al*. have developed a non-invasive combined approach of gel-microsphere (GMS)-based eye drop which contained drug loaded polymer microspheres in thermoresponsive hydrogel [255]. The hydrogel/microspheres-based eye drop formulation can sustain for long-term and topically retained for glaucoma treatment. The scanning electron microscope observation of the gel matrix confirmed the homogeneous distribution of microspheres **(Figure 9 (i))**. After administration of GMS drop in eyes, the location of gel/microspheres can be easily found **(Figure 9 (ii))**. The *in vitro* drug release profile from gel/microsphere-based GMS drops was determined and the maximum amount of released brimonidine drug was around 60 µg for 32 days **(Figure 9 (iii))**. The release profile confirmed the sustained, long-term linear release kinetics which is mainly due to the spherical microcarriers in hydrogel matrix. After drug admiration, the percentage baseline **(Figure 9 (iv))** IOP results confirmed the significantly lower compared to the control, while the actual **(Figure 9 (v))** IOP results do not exhibited statistically significant value.

Nanohydrogels or nanogels have been described as hydrogel particles in the size scale of 1 to 100 nm [256] More specifically they are 3D crosslinked networks with a high swelling ratio and tuneable mechanical properties. These materials are very similar to biological tissues due to the high-water content and carbon-based chemical composition, which makes them highly biodegradable, biocompatible, and responsive to environmental conditions including pH and temperature. Their high-water content and good swelling behaviour favors drug loading, diffusion properties and permeability features, all of which are important for their use as pharmacological vehicles [204,257,258]. Depending on the production methods of the nanogels, it is divided in two categories, namely physical and chemical nanogels. Chemical nanogels are produced by the crosslinking of polymer precursors or heterogeneous polymerization of monomers in which non-reversible covalent bonds are formed. Alternatively, in physical nanogels are crosslinked by non-covalent forces such as electrostatic attraction [24,257]. Other production methods include molecular imprinting, top-down high-pressure homogenization, and particle replication in non-wetting templates [258,259].

The type of polymer which is used and their properties such as hydrophilicity are important to determine efficacy of the final nanogels as drug carriers. For example, hyaluronic acid (HA), a polysaccharide natural biopolymer is often introduced as functionalization agent in hybrid nanogels with the aim of decreasing toxicity, enhancing antibacterial and bio-adhesiveness [260]. For example, Grimaudo *et. al.* have produced a physical hyaluronic acid nanogel as a vehicle of ferulic acid-loaded micelles as a treatment option for chronic cornea injuries [261]. They found that the use of the HA nanogels exhibited acceptable in vitro cytocompatibility and higher target tissue retention ex vivo, which could be explained by the ability of HA to form non-covalent bonds with mucosal layers. On the other hand, polymer hydrophilicity determines a good swelling behaviour and therefore good biocompatibility. For example, Khan *et. al.* have developed nanogel using a hydrophilic copolymer poloxamer 407 and monomer 2-acrylamido-2-methylpropane sulfonic acid (AMPS) crosslinked using methylene bisacrylamide (MBA) as a carrier of olanzapine, an antipsychotic drug [262]. They were able to produce improved swelling behaviour nanogels in a pH and component concentration dependent manner. The resultant nanocarriers were 161.45 nm in size and had a small polydispersity index with poor tendency for aggregation. Additional characteristics reported for this carrier such as a 38-fold increase in drug solubility in both acidic and alkaline conditions, tuneable entrapment efficiency by

controlling polymer concentrations and no toxicity in vital organs upon oral administrations in vivo, could make this nanocarrier a potential candidate for research in ocular delivery.

Other polymers such as the supramolecular cyclodextrins have also been used as functionalization agents of nanogels. Recently, Argenziano *et. al.* have produced nanogel particles of PAA and cyclodextrins as carriers of dexamethasone [258]. They found that compared to free-dexamethasone, nanogel carriers containing β- cyclodextrins showed slower and prolonged drug release profile (releasing only 14% over 6 h compared to 50%), a significantly different concentration-response curve, significantly better drug effectiveness in inhibition of COX-2 expression and Jurkat cell adhesion (-35% and -40%, respectively) at lower concentrations formulations. Abdel-Rashid *et. al.* have developed nanocomposite of acetazolamide-loaded nanovesicles embedded in chitosan-sodium tripolyphosphate nanogels for glaucoma-related IOP reduction in albino rabbits [37]. In their comparative study, they found that compared to nanovesicles only, the nanocomposite showed a higher entrapment efficiency, longer mucoadhesion time and a gradual and prolonged drug release profile decreasing IOP to normal over 45 min sustaining the effect over 24 h, without irritation, inflammation or less tear production.

The importance of hydrogel-based drug delivery systems particularly for hydrophobic drugs was also highlighted [263]. For example, Sharifi *et. al.* fabricated gelatin-based adhesive hydrogel for ocular tissue engineering [264], whereby they grafted the glycidyl methacrylate into the backbone of gelatin via epoxide ring-opening reaction (as illustrated in Table 8), and obtained elastic protein-based hydrogel named GELGYM which is stretchable ~4-times higher than GelMA control hydrogel, and resists tensile stress up to 1.95 MPa. The same group further developed photo-crosslinkable glycidyl methacrylate (GM) and N-vinylpyrrolidone (VP)-based hydrogel with high mechanical strength and cellular adhesion and proliferation [265]. In addition, Jumelle *et. al.* fabricated photo-crosslinkable hydrogel-based adhesive patch for corneal incisions and open globe injuries; the hydrogel system was composed of hyaluronic acid glycidyl methacrylate (HAGM), gelatin methacryloyl (GelMA), and polyethylene glycol diacrylate (PEGDA) to achieve bioadhesive, transparent, degradable, and mechanically suitable patch (as shown in Table 8) [266]. Kong *et. al.* also fabricated fiber-reinforced GelMA-based hydrogel for corneal stroma tissue regeneration. They used orthogonally aligned poly (ε-caprolactone)-poly (ethylene glycol) (PECL) sub-microfibers, which was further perfused by GelMA hydrogel to achieve 3D fiber-reinforced hydrogel [267]. These GelMA-based

hydrogel platforms were thus highlighted for therapeutic and tissue regeneration applications including ocular drug delivery [268]. In addition, Andreadis *et. al.* fabricated *in-situ* gelling nanofiber films composed of poly(vinyl alcohol) and poloxamer 07 for ocular drug delivery of TM to lower the IOP in a conventional eye drops [269]. An acellular porcine corneal stroma (APCS) has also been employed for corneal wound healing application [270]. Eggshell membrane (ESM), which is an abundant resource of innate complex structure and protein compositions provided by nature, has shown potential biomaterials for corneal wound healing application [271]. Liu *et. al.* fabricated cationic self-assembled peptide-based supramolecular hydrogels at pH 5-7 which exhibited a promising ocular delivery vehicle [272]. Ocular infection is very common and sometimes challenging to manage due to low bioavailability of drugs and delivery vehicles-associated adverse effects. Xinxin *et. al.* demonstrated spontaneous self-assembly of ibuprofen-peptide for the ocular inflammation treatment [273]. Agarwal *et. al.* developed topical semifluorinated alkane-based azithromycin eye drops for ocular infections management [274]. Recent examples of the hydrogel-based delivery systems for applications are summarized in **Table 5**.

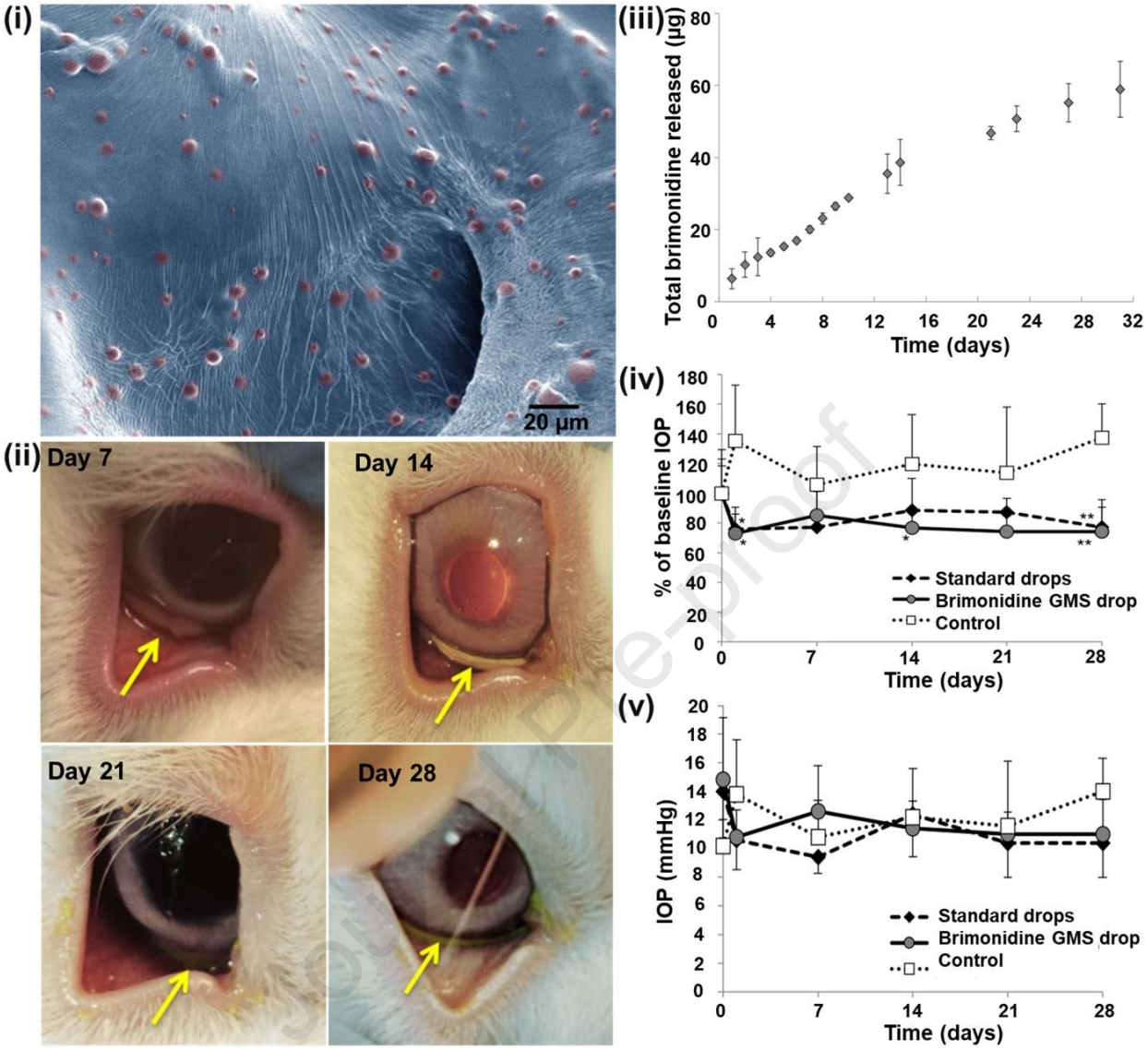


Figure 9. Hydrogel-based ocular drug delivery; **(i)** SEM image of drug loaded PLGA microsphere (red colored) containing pNIPAAm hydrogel in blue color, **(ii)** Optical mages of fluorescein stained hydrogels in inferior fornix at day 7, 14,21, and 28. Yellow arrows indicating the hydrogels location in the treated eye. **(iii)** *In vitro* release profile of brimonidine from microsphere-polymer hydrogel formulation. Hypotensive effect of aqueous BT and microsphere-polymer-BT hydrogel formulation drops; **(iv)** actual IOP, **(v)** corresponding IOP in percentage with respect to average baseline. Reproduced with permission from the Nature Publishing Group from ref. [255].

Table 5. Summary of hydrogel-based systems for ocular applications.

Materials	Drug/molecules	Targets	Refs.
Acellular porcine corneal stroma-derived hydrogel	Corneal ECM of collagens and growth factors	Better corneal epithelial and stroma cell biocompatibility and corneal wound healing	[270]
Hydrogel-based materials	Drug delivery	Corneal wound healing	[275]

Hydrogel/PLGA nanoparticles	Brimonidine and timolol maleate	Long-term sustained delivery for glaucoma treatment	[276]
Collagen-based corneal graft using PCL	Mechanically robust corneal graft	Corneal tissue engineering and wound healing	[277]
Human collagen type I-plant recombinant hydrogel	Aligned and random collagen hydrogel	Subcutaneous implantation in rats and corneal implantation in minipigs for corneal regeneration	[278]
Polymers-based scaffolds	Scaffolds	Corneal tissue regeneration	[279]
Collagen hydrogel crosslinked with PEG	*In-situ* hydrogelation	Therapy for corneal defects	[280]
Porous collagen-based hydrogel	Nerve growth factor beta (NGF-β)	Corneal stromal regeneration and sustained local drug delivery	[281]
Collagen I and IV	Collagen-based biomaterials	Transparent ocular tissues and variety of ocular diseases	[282]
Collagen-hyaluronate hydrogel	*In-situ* forming copolymer	To treat deep corneal stromal defects	[283]
Hyaluronic-collagen hydrogel	Bio-orthogonally crosslinked	Suture-free corneal defect repair and regeneration	[284]
Thermo-gelling dendronized chitosan	Transparent hydrogel	Corneal tissue substitutes and corneal defects engineering	[285]
Hyaluronic acid hydrogel	Stem cell delivery	Sutureless corneal epithelium and stroma regeneration	[286]
Collagen-hyaluronic acid hydrogel	Interpenetrating polymer network	*In-situ* forming corneal defect filler	[287]
Supramolecular host-guest hyaluronic acid hydrogels	Hydrogel for wound healing	Corneal wound healing via dynamic spatiotemporal effects	[288]
Collagen-hyaluronic acid-based hydrogels	Tissue regeneration	Biomedical application of composite hydrogels	[289]
Hyaluronic acid hydrogel	Tissue regeneration	Healing of corneal epithelial defects	[290]
Collagen mimetics patches	LiQD cornea	Corneal transplantation	[291]
pHEMA-hydrogel	Cyclosporine A and Brij98	Ocular drug delivery and surfactant transport	[292]
Chitosan-gelatin hydrogel	Timolol maleate	Ocular drug delivery with enhanced therapeutic efficacy and reduced side effects	[293]

3.4. Contact lens-based delivery systems

Contact lenses are suitable platform for targeted therapeutic delivery to cornea with extended time; however, the commercial contact lenses release ocular drugs limited to 1-2 hours [294]. The commercial contact lenses are effectively applied for vision improvement, management and treatment of microbial keratitis and ocular delivery for glaucoma treatments [295-298]. The use of contact lenses is easy and comfortable compared to glass and also provides high capacity of drug loading and long-term sustained release which makes contact lenses-based devices fitting candidate for drug delivery in eye [299]. Nowadays,

new developments in hydrogel-based delivery research have enabled soft contact lenses to load and timely drug release at required rate to achieve better therapeutic effects with less possible adverse effects.

Contact lenses are divided in three different classes, namely rigid gas-permeable, soft, and hybrid contact lenses. Currently, approximately 9% of all contact lens are rigid gas-permeable contact lens, which are mainly made of poly(methyl methacrylate) (PMMA) and fluorosilicone acrylate. Hydrogel and silicone-based soft contact lenses cover 89% of total contact lens market. Most of the hydrogel-based contact lenses are made of polymerization of HEMA to PHEMA [8]. The hydrophilic monomers contents in the soft lens increases the drug loading capacity, while silicone content control the sustained drug release. Because of difficulty in applying and removing from the eye, only 2% of total contact lens are made of hybrid contact lens.

The contact lens-based ocular delivery systems for glaucoma are summarized in **Table 6**. Huang *et. al.* have fabricated a hybrid hydrogel-based contact lens containing quaternized chitosan (HTCC), GO, and AgNPs with a combination of antibacterial as well as antifungal compound and investigated its functions [295]. Hydrogel formations involve electrostatic crosslinking mechanism between GO and HTCC which endowed strong mechanical strength. GO acts as a drug delivery carriers and presence of anionic groups interact with cationic groups of HTCC to make electrostatic interaction and endow sustained drug delivery platform. Nasr *et. al.* have developed the PCL-based nanoparticles laden contact lenses and evaluated the drug release and corresponding effects in ocular system [39]. The incorporation of polymers nanoparticles in hydrogel-based contact lens does not affect primary features such as transparency, hydrophilicity, mechanical strength, ions, oxygen and other nutrient permeabilities. Hydrogel and nanoparticles involved in the contact lens fabrication showed high cell viability and stimulatory effects. The in vitro drug release from polymer nanoparticles laden hydrogel-based formulation showed drug release for approximately 2 weeks. Kim *et. al.* have developed nanodiamond (ND)-incorporated contact lenses that can provide long-term therapy with enzymatically initiated release of TM [299]. Polyethylenimine functionalized NDs were cross-linked with chitosan **(Figure 10 (i))**, and formulated with TM in nanogel, which was further incorporated within a polyHEMA matrix and created as contact lenses **(Figure 10 (ii))**. The drug loaded NDs-nanogel was encapsulated in polyHEMA matrix and finally made into contact lens **(Figure 10 (iii))**. Next, developed drug-eluting hydrogel-based contact lens formulation exhibited the enzyme-triggered (lysozyme)

cumulative drug release of approximately 9.14 µg in one day **(Figure 10 (iv))**. Chauhan *et. al.* have developed thermo-sensitive hydrogel-based contact lens for drug delivery in cornea [294]. They used hydroxyl methyl methacrylate (HEMA) gels as base materials and incorporated TM loaded nanoparticles in the gel matrix to construct contact lens. The in vitro release of TM showed 2-4 weeks controlled release and was further checked in vivo. Lee *et. al.* have developed ocular delivery system using poly(2-hydroxyethyl methacrylate) (pHEMA)-hydrogel-based contact lenses [300]. They incorporated vitamin E in pHEMA-gel and formulated as contact lenses which showed significantly improved loading capacity of two hydrophobic glaucoma drugs (timolol, and brimonidine) and hydrophilic drug surrogate in lenses by 19.1%, 18.7%, and 37.5%, respectively. Kapoor *et. al.* synthesized Brij 98 surfactant-loaded pHEMA hydrogel formulations and showed release of cyclosporine A (CyA) for extended time periods in controlled fashion, and thus contact lens made of pHEMA hydrogel formulations will be suitable for delivery of CyA [292]. Recently, Zhu *et. al.* have developed the silicone hydrogel-based lenses (balafilcon A, and senofilcon A) and demonstrated the micromechanical adhesion of dehydrating hydrogels to corneal tissues [301]. One recent work systematically evaluated the advantage, reproducibility, safety profile, and drug delivery potential of the commercially available soft contact lenses [302]. Of note, Ma *et. al.* engineered commercially available soft contact lens with AgNPs-modified zwitterionic poly (carboxybetaine-co-dopamine methacrylamide) copolymer for keratitis infection treatment [303], and also, the advantages and challenges of nanoparticles-laden contact lenses as ocular drug delivery applications were demonstrated well [304].

The drug encapsulation/loading in contact lenses-based systems were achieved by various methods, including soaking/absorption, ionic interactions, molecular imprinting, cyclodextrins complexing, and incorporation of drug loaded nanoparticles. Moreover, drug loaded nanoparticles can be incorporated in several ways; first, during the polymerization process via dispersion in pre-monomer mixture, second, soaking of contact lenses in drug loaded nanoparticles suspension, and third, immobilization of drug loaded nanoparticles on the surface of the contact lenses. Drug release mechanisms from contact lenses are mainly dependent on loading localization. In case of drug loading via nanoparticles within contact lens the drug molecules diffuse from the nanoparticles to the contact lens matrix, and next from contact lens matrix to precorneal region. In case of contact lens with surface-immobilized drug-loaded nanoparticles, drug molecules directly release to the precorneal region.

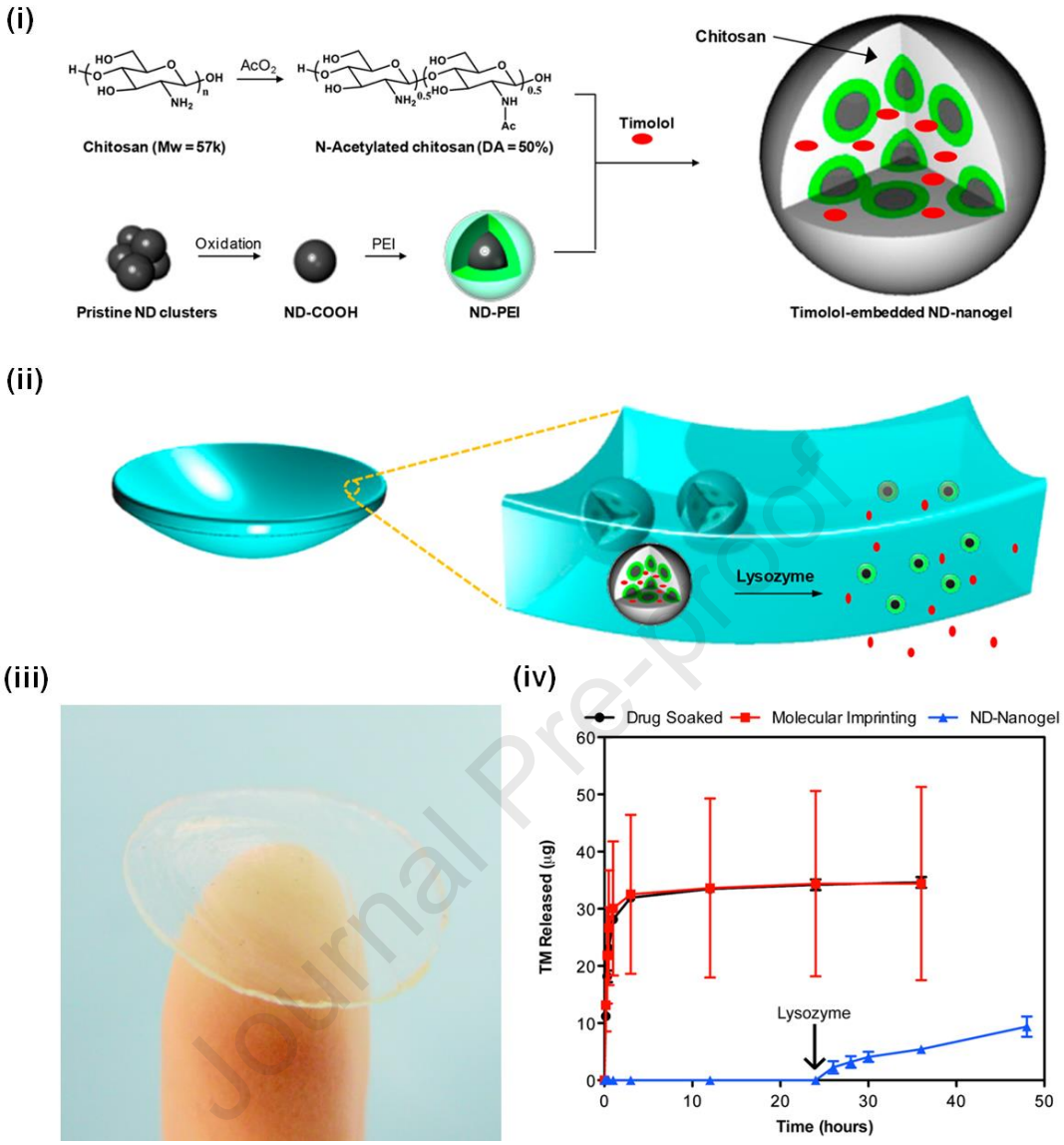


Figure 10. Lysozyme-activated drug-releasing contact lenses for therapeutic delivery; **(i)** Schematic representation of a lysozyme-activated active substance-releasing contact lens with ND nanogel and PEI, chitosan and TM, **(ii)** Action of the tear fluid lysozyme breaks the chitosan, decomposes the ND from nanogels and TM release starts without affecting the lens structure, **(iii)** Incorporation of ND nanogels in PolyHEMA to formulate contact lens, **(iv)** Enzyme-activated TM release profiles from drug soaked lens, with molecular imprinting, and ND embedded in nanogel-based lenses. Reproduced with permission from the American Chemical Society from ref. [299].

Table 6. Summary of contact lens-based delivery systems for ocular applications.

Materials	Drug/molecules	Targets	Refs.
Drug loaded polymeric coating on lens	Cyclosporin A	Efficient prevention of posterior capsular opacification	305
Aligned poly (glycerolsebacate)/	Stem cell therapy	Stem cell-based retinal ganglion cells (RGCs) differentiation and	306

Poly(ε-caprolactone) scaffold		growth of RGCs neurites for glaucoma therapy	
Silicone hydrogel contract lenses	Micromechanical measurement of adhesion	Long-lasting contact lens and dehydration state	301
Contact lens with multifunctional ternary nanocoating	Phytomolecule-coated ZnO nanoparticles: Gallic Acid: Tobramycin	The treatment of bacterial and fungal keratitis	307
Silicone hydrogel contact lenses	Dexamethasone and vitamin E	Protection of corneal damage from UV light	308
Poly-hydroxyethyl methacrylate (p-HEMA) contact lenses	Dexamethasone	Ocular drug delivery	309
Polysiloxane intraocular lens	Injectable and *in-situ* curable	Loss of the eye's ability to change focus and provide clear vision for near objects	310
PLGA-based contact lens	Latanoprost	Ocular drug delivery and treatment of glaucoma	311
PEO-coated silicone hydrogel contact lens	Lotrafilcon A	Elimination of biofouling	312
Silicone hydrogel contact lens	Hydroxypropyl methylcellulose (HPMC)	Molecularly imprinted lens and extended release	313
Commercially available contact lens	Ciprofloxacin	Ocular drug delivery and antimicrobial activity	314
PLGA-HFIP-based contact lens	Dexamethasone	Drug-eluting contact lens and inhibition of VEGF-induced retinal leakage	315
Nanodiamond-embedded lens	Timolol maleate	In vitro model with primary HTM cells and ocular drug delivery	299
pHEMA-hydrogel-based contact lens	Lipophilic vitamins, Timolol maleate, and brimonidine	Ocular drug delivery	300

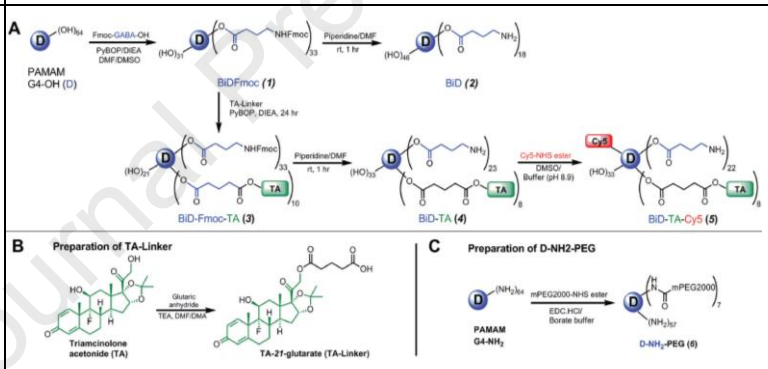
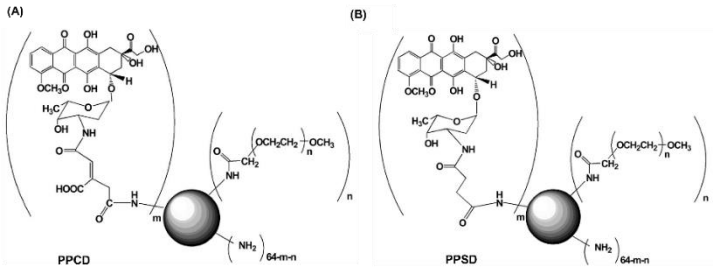
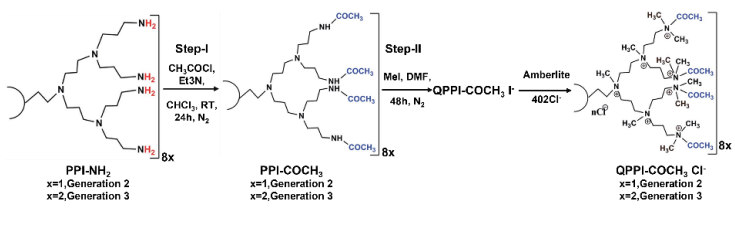
Table 7. Summary of advantages and disadvantages of nanocarriers for glaucoma applications.

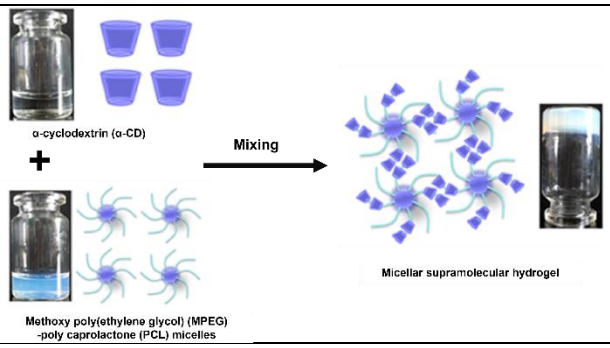
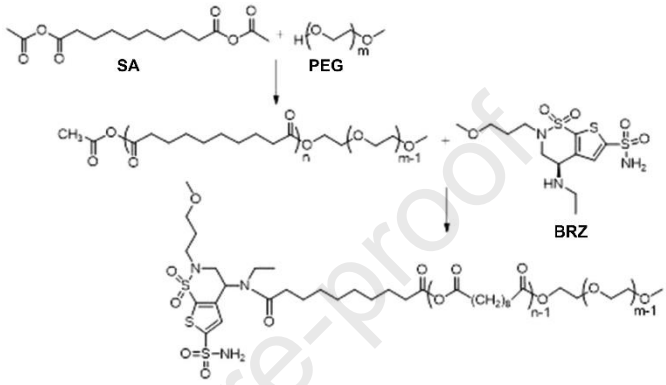
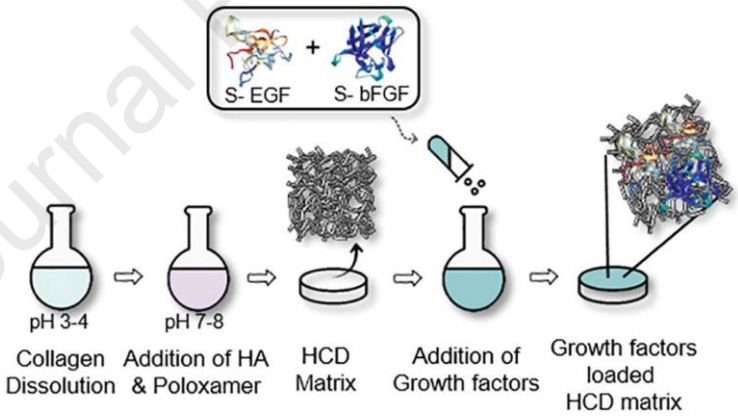
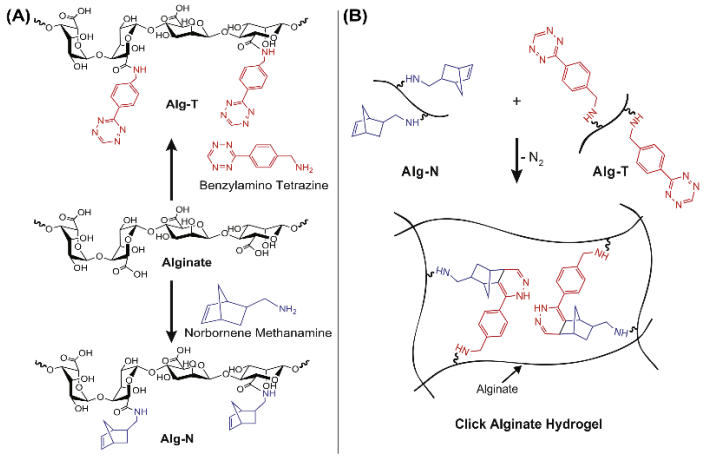
Drug Delivery carriers	Advantages	Disadvantages	Refs.
Inorganic nanoparticles	Inorganic-based nanocarriers are easy to synthesize and control the shape and size, and surface chemistry.	Chemicals are expensive which financially reflects in the production of nanocarriers.	316-318
	Small size, long shelf-life, stable, biocompatible, low cytotoxic, multifunctional, easily combined with functional molecules.	Many steps (chemical /biochemicals) involved to achieving biocompatible and multifunctional nanocarriers.	
	High drug loading capacity, and sustained release.	Initial burst release and poor bioavailability in topical drug delivery application.	
	Increases residence time /bioavailability, precision targeting and combined therapeutic approaches	Physical and physicochemical obstacles of injecting nanocarriers in the eyes and difficult to penetrate RBB.	
Polymers	Polymer-based nanocarriers can be developed as solid	Synthesis involves toxic organic chemicals and is a tedious process. Difficult to scale-up production.	319-323

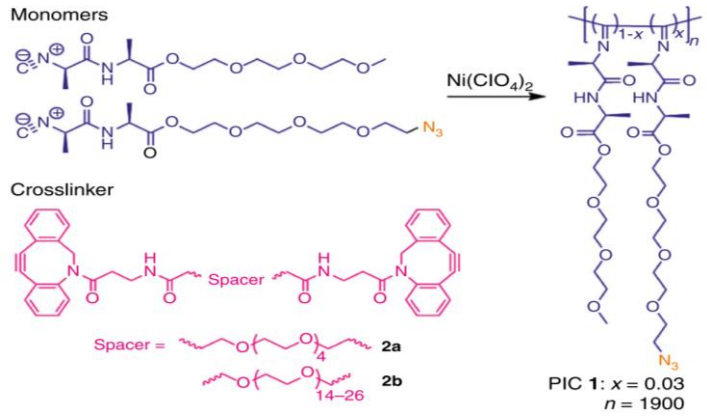
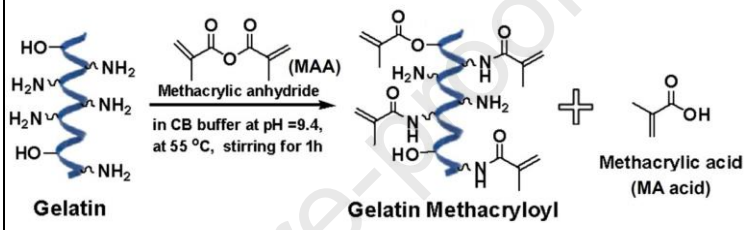
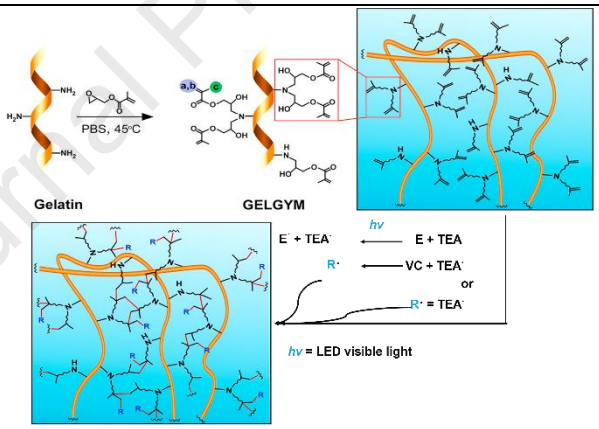
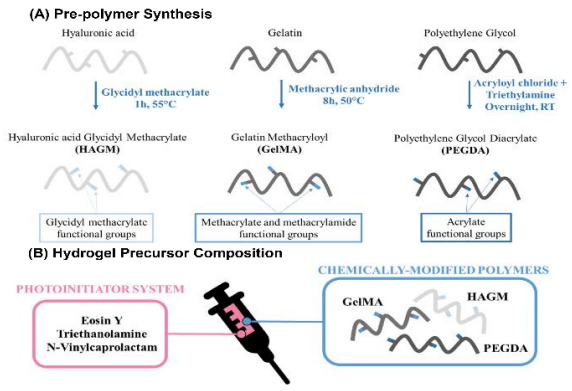
	nanoparticles as well as hollow nanocapsules.		
	Size controlled, stable in aqueous medium, biocompatible, multifunctional, and easily linked with functional molecules.	Synthetic polymers-based nanocarriers are slow degradable.	
	High capacity of drug encapsulation, stable, increased therapeutic efficacy, and reduced toxic exposure to sensitive tissues.	Low solubility, possible leakage and fusion of encapsulated drug molecules, short half-life and less stable	
	Loading of both hydrophilic and hydrophobic drugs, and combined with penetration enhancer, used in all delivery routes.	Complex process to control drug loading/ encapsulation capacity during reaction process (hydrophobic drugs).	
Hydrogels	Hydrogels are easy to fabricate and maintain the shapes for specific tissue graft/scaffolds as an implantation.	Hydrogels are difficult to control at very low scale (micro/nano) and challenging to achieve nanostructured artificial tissue graft.	263,324-326
	The physical and ionic cross-linked hydrogel are easy to degrade but covalently crosslinked hydrogel are difficult to control the degradation.	It is very challenging to control the mechanical stability (stiffness) and degradation of hydrogel at the same time.	
	Hydrogels have a high loading capacity for drug/proteins and other biomolecules.	Initial burst release, and difficult to control the swelling and fast drug release. Possibility of drug denaturation during hydrogel preparation.	
	Hydrogel-based delivery carriers can be suitable for accelerated wound healing, antibacterial, and can be used for soft to hard tissue targets.	Less tissue penetration, poor bioavailability, and very limited potentials as nanocarriers.	
Contact lens	Contact lens fabrication required sophisticated technology and is controllable and reproducible.	The contact-lens fabrication technology is expensive.	300,327-331
	Contact lens-based delivery systems provide increased residence time and high bioavailability with minimal systemic absorption.	Movement around the eye could be annoying to the patient, interfere with vision and fall out	
	Administration frequency reduced, conjunctive/scleral route to internal target, better shelf-life, and no preservative requires.	Sometimes difficult to insert or remove the lens, and potential initial drug burst release upon insertion before start a controlled delivery.	
	Contact lens-based on soft hydrogel are tissue adhesive, biocompatible, mechanically stable and highly transparent.	Contact lens-based delivery systems requires blotting of lens surface and sometimes lenses or probe liquid can dry out	
Dendrimers and nanoliposomes	Easy to synthesize and control branches and functionalization which determines the	Some dendrimers have been reported as cytotoxic, particularly cationic dendrimers.	

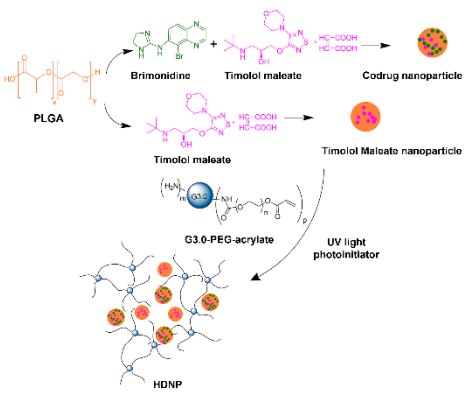
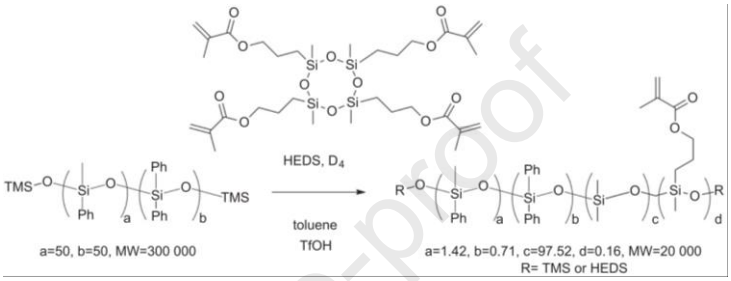
	solubilization and enables for targeted drug delivery.		215,319,331-333
	Dendrimers are capable of carrying hydrophobic as well as hydrophilic drugs and can deliver long-term sustained release. It is possible to load small and large molecules, targeting, and imaging agents, and tissue penetrating enhancers.	Reported correlation between terminal branch amine groups toxicity. There are concerns on biodistribution, biodegradation, chronic toxicity, and pharmacokinetics of PAMAM-based dendrimers.	
	PAMAM-based dendrimers are stable and relatively resistant to hydrolysis.	PAMAM-based dendrimers are expensive.	
	Silica-based dendrimers can be used for gene therapy and polymer-based dendrimers can be used for all kind of delivery applications.	Dendrimers-based delivery carriers are rapidly cleared, and hence have poor bioavailability	

Table 8. Summary of biomaterials synthesis routes and the corresponding chemical reactions.

Synthesis routes	Chemical reactions	Refs.
Preparations of hydroxyl PAMAM dendrimer-based gene vectors; (A) Amine functionalized bifunctional dendrimer (BiD), bifunctional triamcinolone acetonide (BiD-TA), and Cy5-labeled BiD-TA (BiD-TA-Cy5), (B) Triamcinolone acetonide-21-glutarate, and (C) PEGylated amine functionalized PAMAM dendrimer (D-NH_2-PEG).		208
Partially PEGylated polyamidoamine-DOX conjugates (PPCD) and PEG-PAMAM-succinic-DOX conjugated (PPSD) dendrimers		210
Synthesis route of QPPI-NHAc (G2)/(G3) dendrimer from PPI (G2)/(G3) dendrimers.		212

Synthesis route of micellar supramolecular hydrogel composed of α-CD and MPEG-PCL micelles for ocular drug delivery		220
Brinzolamide and miRNA-124 loaded deblock polyethylene glycol-co-polysebacic acid (PSA-PEG) NPs for glaucoma treatment.		227
Synthesis dual growth-factor loaded hyaluronate collagen dressing (dual-HCD) matrix.		334
Fabrication of alginate-based hydrogel via tetrazine-norbornene click chemistry. (A) Modify alginate (Alg) backbone carboxylic acids with tetrazine (Alg-T) or norbornene (Alg-N), (B) Alg hydrogel network mechanism via mixing of Alg-T and Alg-N with the loss of N_2.		335

Synthesis reaction of fibrous hydrogel using semi-flexible (ethylene glycol)-decorated polyisocyanide (PIC) polymers.		251
Fabrication of GelMA from gelatin using MAA via one pot method.		252
Chemical reaction for synthesis of GELGYM using glycidyl methacrylate with gelatin and visible LED light.		264
Synthesis of photo-crosslinkable hydrogel-based adhesive patch; (A) different steps of pre-polymer synthesis, (B) Hydrogel precursor composition polymers.		266

Chemical reaction for synthesis of hybrid dendrimer hydrogel/PLGA nanoparticles loaded with brimonidine and timolol maleate were loaded.		276
Synthesis route of high refractive index polysiloxane as injectable gel.		310

4. Concluding remarks

Glaucoma is a devastating worldwide disease with limited practical treatment options. In this Review, we summarized the limitations of the currently available ocular drug delivery approaches by emphasizing the glaucoma pathophysiology and the available administration techniques, and highlighted the potential of various nanotechnology-enabled carrier platforms.

Polymers or inorganic nanoparticulates are the available choices of carrier composition to load drugs and delivery them to the targeted side at controlled release rates. The nanocarriers were tailored to have optimized compositions and controlled degradability, furthermore, the combination routes to load and deliver drug molecules were developed (as summarized in Table 9); these can determine the loading capacity and release rate of the cargo therapeutic molecules. When using the nanocarriers, one of the important considerations is the physiological barrier that significantly limits the access of drugs, thus effective delivery systems with penetration and targeting ability need to be developed. The surface

functionalization of the nanocarriers such as with natural cell membranes (i.e., RBC) is an example to increase the penetration ability and cell anchoring and uptake with good biocompatibility.

Table 9. Summary of biomaterials, drug molecules, and their combination methods.

Types of Biomaterials	Drug/molecules	Method of combining drug vectors	Refs.
Polymeric NPs	Timolol Maleate	Entrapment loading under mild heating	165
Liposomes (polymer coated)	Ibuprofen	Liposomes containing ibuprofen was prepared by the ethanol injection method.	243
Synthetic polymers (PLGA NPs)	Dexamethasone and melatonin	Coaxial electrospray (CES) method	203
Hydrogels (hexanoyl glycol chitosan)	Brimonidine tartrate (BRT)	Loading by preparation of hydrogels in the buffer that contains pre-dissolved BRT and lyophilization.	176
Contact Lenses (Hydrogel based)	Timolol, Brimonidine and Vitamin E	Soaking, immersing lenses into drug and vitamin containing ethanol solutions for 24 hours.	300
Micelles/Nanogels (pluronic/hyaluronan)	Ferulic acid	Ferulic acid solution in ethanol were added micelle dispersions in water.	261
Hybrid natural/synthetic polymers NPs	Pilocarpine	Material dispersed in simulated tear fluid and the drug powder dissolved in it for 48h continuously stirring.	177
Synthetic polymers-based contact lens (PLGA)	Latanoprost	PLGA and latanoprost solution mixed in ethyl acetate and then solvent evaporated to obtain drug-polymer films.	311
PLGA-HFIP-based contact lens	Dexamethasone	Drug-polymer film fabrication by dissolving dexamethasone and PLGA in hexafluoro isopropanol (HFIP) the apply rotary evaporation.	315
PEO-coated silicone hydrogel contact lens	Lotrafilcon A	Coating on lotrafilcon a contact lenses by thin film deposition of allylamine plasma polymer (ALAPP) grafted of poly(ethylene oxide) dialdehyde.	312
Inorganic NPs (MSN)	Bevacizumab (Avastin®)	Encapsulating bevacizumab into the surface-functionalized NPs via the nanocasting strategy.	135
Nano diamond embedded lenses	Timolol maleate	Drug loading was done by soaking in 0.02 mM of timolol solution for 3 days.	299
Hydrogels (collagen based)	Nerve growth factor beta (NGF-β)	NGF-β powder dissolved in sterile in a sterile MES buffer solution (2-(N-morpholino) ethanesulfonic acid). Then certain amount from this solution incorporated into collagen stock solution during manufacture of the implants	281
Mucoadhesive polymers	Ketoconazole	Gel solutions prepared directly with certain amount of ketoconazole including solutions	152
pHEMA hydrogels	Cyclosporine A (CyA)	CyA powder was dissolved HEMA monomer and stirred at 600 rpm for a period of 5 h then the crosslinker (EGDMA) added to the drug–HEMA mixture degassed by bubbling nitrogen for 10 min.	292

Collagen shields	Tobramycin cyclosporin, dexamethasone	Pre-soaked, *in vitro* adsorption of drug	146
PVA/anionic collagen membranes	Ciprofloxacin hydrochloride	Antibiotic incorporation was achieved by two methods namely soaking and mixing.	228
Alginate microsphere-collagen composite hydrogel	BSA	Loading of BSA in alginate microsphere and wand dispersed in collagen hydrogel.	179
Collagen-based corneal scaffold	Vancomycin	Vancomycin was mixed in collagen solution and chemically crosslinked via EDC/NHS chemical reaction.	149
Cationic lignin-based hyperbranched polymers	Polymyxin B, Colistin	Copolymer and polymyxin B were mixed and kept for absorption.	229
Chitosan nanoparticles	5-fluorouracil	Chitosan NPs was prepared by the ionotropic gelation method and mixed with 5-fluorouracil during the preparation of NPs.	237
Collagen membrane	β-cyclodextrin dialdehyde and ofloxacin	Collagen membrane was chemically crosslinked with incubation β-cyclodextrin dialdehyde and ofloxacin through coprecipitation lyophilization, and kneading.	232
Mucoadhesive polymer formulations	Ketoconazole	Mixing of ketoconazole and mucoadhesive hydrogel.	152
Chitosan nanoparticles	Dorzolamide hydrochloride (DZH)	DZH loaded in chitosan NPs via ionotropic gelation method.	174
Chitosan nanoparticles	DZH and pramipexole hydrochloride	DZH and pramipexole hydrochloride loaded in chitosan NPs via ionotropic gelation method.	175
Core-shell nanoplatform	Gene delivery	Nanocore-DNA condensation using PRHF and NLS.	239
Cyclodextrin-based nanohybrid hydrogels with polydopamine	Dexamethasone	Hydroxy groups of cyclodextrins react with bisacrylamides in the same way and cyclodextrins crosslinked with polymers.	240

As noted, the administration routes should also be considered importantly before the choice of nanocarriers and the target drugs. While the topical administration is the most common approach to deliver nanocarrier systems, the drug delivery to the posterior segment is challenging due to anatomical barriers of ocular system that significantly limit the drug diffusion from the anterior to the posterior region. For this, the periocular injections are often favored; for instance, subconjunctival injection with surface-functionalized polymeric nanocarrier can penetrate the barrier membrane and allow for continuous release of the drug into the posterior segment.

Recently, a therapeutic concept of intracameral targeted delivery has also emerged to treat glaucoma where the nanocarriers with siRNA were delivered to the cells in the aqueous humor outflow pathway in anterior chamber to alter the IOP in the glaucoma patients [336]. Among the particulate forms, the nanogels

in the form of hydrogel particles with sizes of 1-100 nm are considered a promising nanocarrier system that can be developed even to be responsive to environmental conditions including pH and temperature which enables smart drug delivery approaches. Apart from the nanoparticulate forms, the hydrogel-based soft contact lenses designed to have drug loading and release capacity are also believed to be a versatile delivery system where the nanoparticles can be incorporated to partition drugs and release them more sustainably.

Eye is a developmentally protective organ, thus hampers ocular drug delivery. Obstacles to effective drug administration are: drug loss from ocular surface (anterior segment of the eye, cornea, conjunctiva, sclera, anterior uvea), lacrimal fluid-eye barriers, and blood-ocular barriers. Even with prolonged action of drug administration, the corneal and epithelial barriers of the systemic absorption from the conjunctival sac via local blood capillaries and absorption during systemic circulation reduce the ocular bioavailability of drugs. Therefore, it is important to consider these obstacles when formulating the topical drug delivery systems [337].

The topical drug delivery cannot reach posterior parts of the eye (retina, vitreous, and choroid) due to the REP and tight walls of the retinal capillaries from the posterior barrier between the bloodstream and the eye, therefore, when these segments are targeted, higher dosage of drug should be administered either via intravenously or intravitreal way. With the lack of appropriate targeting device, only a small fraction of drugs administered intravenously or orally can reach the retina and choroid. The blood-ocular barriers should also be studied in detail like the blood-brain barriers, to allow us to design delivery systems for effective drug transportation and metabolic expression [337,338]. Moreover, advances in understanding the mechanism of ocular barriers and challenges associated with various drug delivery routes in glaucoma treatment are essential to improve the bioavailability of the administered drug.

As discussed, the advances in biomaterials and nanotechnology have significantly increased the possibility of therapeutic treatment of glaucoma. Even with the potential of the nanocarrier-based delivery systems, most data are still limited with the in vitro experiments or with relatively small animal models, which thus needs stronger evidence with large animal studies to refine the safety and effectiveness for future medical translations.

Acknowledgments

The authors would like to thank the National Research Foundation Korea (NRF) for providing financial support for program for UCL Eastman-Korea Dental Medicine Innovation Centre; 2018K1A4A3A0106425), and other NRF (2021R1I1A1A01050661, 2020R11A1A01071828, 2021R1A5A2022318) fundings.

Conflicts of interest

The authors declare no competing financial interest.

5. References

1. Falkenberg, H. K., and Bex, P. J., *Investigative ophthalmology & visual science* (2007) **48 6**, 2913
2. Wong, W. L.*, et al.*, *The Lancet Global Health* (2014) **2** (2), e106
3. World Health, O., *World report on vision*. World Health Organization: Geneva, 2019
4. de la Fuente, M.*, et al.*, *Advanced drug delivery reviews* (2010) **62** (1), 100
5. Chong, R. S.*, et al.*, *Journal of glaucoma* (2013) **22** (3), 190
6. Voss, K.*, et al.*, *Journal of controlled release : official journal of the Controlled Release Society* (2015) **214**, 1
7. Lai, J.-Y., and Luo, L.-J., *Biomacromolecules* (2015) **16** (9), 2950
8. Tsai, C.-H.*, et al.*, *International Journal of Molecular Sciences* (2018) **19** (9), 2830
9. Urtti, A., *Advanced drug delivery reviews* (2006) **58** (11), 1131
10. Han, S. B.*, et al.*, *Journal of Ophthalmology* (2018) **2018**, 1215868
11. Chang, C.-Y.*, et al.*, *Int J Nanomedicine* (2016) **12**, 279
12. Quigley, H. A., *N Engl J Med* (1993) **328** (15), 1097
13. Weinreb, R. N.*, et al.*, *Jama* (2014) **311** (18), 1901
14. Neufeld, A. H., and Liu, B., *The Neuroscientist* (2003) **9** (6), 485
15. Crawford Downs, J.*, et al.*, *Exp Eye Res* (2011) **93** (2), 133
16. He, Z.*, et al.*, *Clin Exp Optom* (2011) **94** (2), 133
17. Shi, J.-M., and Jia, S.-B., *Int J Ophthalmol* (2012) **5** (6), 742
18. Wang, W.*, et al.*, *PLoS One* (2013) **8** (12), e84270
19. Ramulu, P. Y.*, et al.*, *Ophthalmology* (2007) **114** (12), 2265
20. Campbell, R. J.*, et al.*, *Can J Ophthalmol* (2008) **43** (4), 449
21. Gedde, S. J.*, et al.*, *Am J Ophthalmol* (2012) **153** (5), 789
22. Abd, A. J.*, et al.*, Nanomedicine-Based Delivery to the Posterior Segment of the Eye: Brighter Tomorrow. In *Drug Delivery for the Retina and Posterior Segment Disease*, Patel, J. K.*, et al.*, (eds.) Springer International Publishing, Cham, (2018), pp 195
23. Jiang, S.*, et al.*, *Int J Ophthalmol* (2018) **11** (6), 1038
24. Pinelli, F.*, et al.*, *Gels* (2020) **6** (1), 6
25. Shah, J.*, et al.*, *Pharmaceutics* (2019) **11** (5)
26. Kesharwani, P.*, et al.*, *Progress in Polymer Science* (2014) **39** (2), 268
27. Wang, F.*, et al.*, *Front Pharmacol* (2018) **9**, 91
28. Balguri, S. P.*, et al.*, *International journal of pharmaceutics* (2017) **529** (1-2), 32
29. Mahboobian, M. M.*, et al.*, *Journal of Drug Delivery Science and Technology* (2020) **55**, 101400
30. Yu, Y.*, et al.*, *Drug Deliv* (2018) **25** (1), 888
31. Wong, C. W.*, et al.*, *Scientific Reports* (2018) **8** (1), 6604
32. Soiberman, U.*, et al.*, *Biomaterials* (2017) **125**, 38
33. Mandal, A.*, et al.*, *Pharmaceutical research* (2019) **36** (2), 36
34. Lynch, C.*, et al.*, (2019) **11** (8)
35. Varela-Fernández, R.*, et al.*, *Pharmaceutics* (2020) **12** (3), 269
36. Terreni, E.*, et al.*, *Pharmaceutics* (2020) **12** (3), 253
37. Abdel-Rashid, R. S.*, et al.*, *Int J Nanomedicine* (2019) **14**, 2973
38. Yadav, M.*, et al.*, *Drug Delivery and Translational Research* (2020) **10** (4), 919
39. Nasr, F. H.*, et al.*, *Biomacromolecules* (2016) **17** (2), 485
40. Prakash, M., and Dhesingh, R. S., *Current drug delivery* (2017) **14** (4), 555
41. Kharaghani, D.*, et al.*, *Polymer Testing* (2019) **79**, 106034
42. Levine, D.*, et al.*, *Ophthalmic surgery, lasers & imaging retina* (2020) **51** (3), 132
43. Nayak, K., and Misra, M., *Biomedicine & Pharmacotherapy* (2018) **107**, 1564
44. Lin, H.*, et al.*, *The Ocular Surface* (2018) **16** (4), 415
45. Guo, Y.*, et al.*, *PLOS ONE* (2016) **11** (4), e0154437
46. Duvvuri, S.*, et al.*, *Current Drug Metabolism* (2004) **5** (6), 507
47. Su, C.*, et al.*, *Advanced drug delivery reviews* (2019) **143**, 97
48. Cholkar, K.*, et al.*, 1 - Eye: anatomy, physiology and barriers to drug delivery. In *Ocular Transporters and Receptors*, Mitra, A. K., (ed.) Woodhead Publishing(2013), pp 1
49. Shen, H.-H.*, et al.*, *Nanomedicine* (2015) **10** (13), 2093
50. Singh, R. K.*, et al.*, *Journal of tissue engineering* (2019) **10**, 2041731419877528

51. Mitchell, M. J.*, et al.*, *Nature reviews. Drug discovery* (2021) **20** (2), 101
52. Liu, Y.*, et al.*, *Biomaterials* (2010) **31** (35), 9145
53. Parekh, M.*, et al.*, *Journal of tissue engineering* (2021) **12**, 2041731421990536
54. Kesavan, K.*, et al.*, *Current drug delivery* (2011) **8** (2), 172
55. Ammar, H. O.*, et al.*, *AAPS PharmSciTech* (2009) **10** (3), 808
56. Janagam, D. R.*, et al.*, *Advanced drug delivery reviews* (2017) **122**, 31
57. Omerović, N., and Vranić, E., *Health and Technology* (2020) **10** (1), 61
58. Junyaprasert, V. B., and Morakul, B., *Asian Journal of Pharmaceutical Sciences* (2015) **10** (1), 13
59. Du, J.*, et al.*, *International journal of pharmaceutics* (2015) **495** (2), 738
60. Corem-Salkmon, E.*, et al.*, *Int J Nanomedicine* (2011) **6**, 1595
61. Raju, H. B.*, et al.*, *PLOS ONE* (2011) **6** (5), e17452
62. Shang, L.*, et al.*, *Journal of Nanobiotechnology* (2014) **12** (1), 5
63. Park, M. V.*, et al.*, *Biomaterials* (2011) **32** (36), 9810
64. Cornell, L. E.*, et al.*, *Mil Med* (2016) **181** (5 Suppl), 232
65. Roberto Zysler, A. B., Pablo Gurman, Orlando Hector Auciello, Mario Joaquin Saravia, *20130225906. U.S. Patent.* (2013 Aug 29;)
66. Raju, H. B.*, et al.*, *Clin Exp Ophthalmol* (2012) **40** (1), 100
67. Sims, C. M.*, et al.*, *Nanoscale* (2017) **9** (40), 15226
68. Yanai, A.*, et al.*, *Cell Transplant* (2012) **21** (6), 1137
69. Häfeli, U. O., *International journal of pharmaceutics* (2004) **277** (1-2), 19
70. Giannaccini, M.*, et al.*, *International journal of molecular sciences* (2014) **15** (1), 1590
71. Levy, I.*, et al.*, *Journal of Nanobiotechnology* (2015) **13** (1), 34
72. Tzameret, A.*, et al.*, *Journal of Nanobiotechnology* (2019) **17** (1), 3
73. Giannaccini, M.*, et al.*, *Scientific reports* (2017) **7**, 43092
74. Pissuwan, D.*, et al.*, *Journal of Controlled Release* (2011) **149** (1), 65
75. Rai, M.*, et al.*, *International journal of pharmaceutics* (2015) **496** (2), 159
76. Farooq, M. U.*, et al.*, *Scientific Reports* (2018) **8** (1), 2907
77. Patra, J. K.*, et al.*, *Journal of Nanobiotechnology* (2018) **16** (1), 71
78. Ajnai, G.*, et al.*, *Journal of Experimental & Clinical Medicine* (2014) **6** (6), 172
79. Kim, J. H.*, et al.*, *Nanotechnology* (2009) **20** (50), 505101
80. Kim, J. H.*, et al.*, *Biomaterials* (2011) **32** (7), 1865
81. Cho, W. K.*, et al.*, *Cornea* (2015) **34** (4), 456
82. Rosenbaum, J. T.*, et al.*, *Nature* (1980) **286** (5773), 611
83. Satici, A.*, et al.*, *Eur J Ophthalmol* (2003) **13** (9-10), 779
84. Pereira, D. V.*, et al.*, *Investigative Ophthalmology & Visual Science* (2012) **53** (13), 8036
85. Ghosh, P. S.*, et al.*, *ACS Nano* (2008) **2** (11), 2213
86. Li, D.*, et al.*, *Biomaterials* (2009) **30** (7), 1382
87. Zhou, X.*, et al.*, *Biomaterials* (2008) **29** (1), 111
88. Sharma, A.*, et al.*, *Nanomedicine: Nanotechnology, Biology and Medicine* (2011) **7** (4), 505
89. Tandon, A.*, et al.*, *PLoS One* (2013) **8** (6), e66434
90. Salem, H. F.*, et al.*, *Drug Des Devel Ther* (2016) **10**, 277
91. Fong, J., and Wood, F., *Int J Nanomedicine* (2006) **1** (4), 441
92. Tian, J.*, et al.*, *ChemMedChem* (2007) **2** (1), 129
93. Liu, X.*, et al.*, *ChemMedChem* (2010) **5** (3), 468
94. Wong, K. K.*, et al.*, *ChemMedChem* (2009) **4** (7), 1129
95. Sintubin, L.*, et al.*, *Appl Microbiol Biotechnol* (2011) **91** (1), 153
96. Tang, J.*, et al.*, *ACS Appl Mater Interfaces* (2013) **5** (9), 3867
97. Sondi, I., and Salopek-Sondi, B., *J Colloid Interface Sci* (2004) **275** (1), 177
98. McQuillan, J. S.*, et al.*, *Nanotoxicology* (2012) **6**, 857
99. O'Reilly, J. P.*, et al.*, *Journal of the American Chemical Society* (2005) **127** (6), 1632
100. Kim, J. S.*, et al.*, *Nanotoxicology* (2013) **7** (5), 953
101. Maneewattanapinyo, P.*, et al.*, *J Vet Med Sci* (2011) **73** (11), 1417
102. Murphy, M.*, et al.*, *Journal of Nanomaterials* (2015) **2015**, 696918
103. Luo, L.-J.*, et al.*, *Journal of Colloid and Interface Science* (2019) **536**, 112
104. Wang, Z.*, et al.*, *Semin Cancer Biol* (2015) **35 Suppl** (Suppl), S224
105. Al-Shabrawey, M.*, et al.*, *Expert Rev Ophthalmol* (2013) **8** (3), 267

106. Gurunathan, S.*, et al.*, *Biomaterials* (2009) **30** (31), 6341
107. Sarker, B.*, et al.*, *PLoS One* (2014) **9** (9), e107952
108. Garrigues, H. J.*, et al.*, *J Virol* (2008) **82** (3), 1570
109. Singh, R. K.*, et al.*, *ACS Applied Materials & Interfaces* (2017) **9** (12), 10309
110. Liao, Y.-T.*, et al.*, *Journal of Materials Chemistry B* (2017) **5** (34), 7008
111. Patel, K. D.*, et al.*, *Materials Horizons* (2019) **6** (3), 434
112. Shanshan WANG, Y. L., Xiaobin FAN, Fengbao ZHANG, Guoliang ZHANG, *Front. Chem. Sci. Eng.* (2015) **9** (1), 77
113. Macdonald, M. L.*, et al.*, *Biomacromolecules* (2010) **11** (8), 2053
114. Li, S.*, et al.*, *ACS Applied Materials & Interfaces* (2014) **6** (8), 5704
115. Xu, J.*, et al.*, *ACS Applied Materials & Interfaces* (2020) **12** (16), 18375
116. Lai, P.-X.*, et al.*, *Biomaterials* (2016) **109**, 12
117. Cibecchini, G.*, et al.*, *ACS Applied Materials & Interfaces* (2020)
118. An, W.*, et al.*, *Experimental Eye Research* (2018) **174**, 59
119. Occhiutto, M. L.*, et al.*, *Adv Ther* (2020) **37** (1), 155
120. Luo, L. J.*, et al.*, *Biomaterials* (2020) **243**, 119961
121. Luo, L.-J.*, et al.*, *Biomaterials* (2020) **243**, 119961
122. Giannaccini, M.*, et al.*, *Scientific Reports* (2017) **7** (1), 43092
123. Snider, E. J.*, et al.*, *Scientific Reports* (2018) **8** (1), 12251
124. Yanai, A.*, et al.*, *Cell Transplantation* (2012) **21** (6), 1137
125. Levy, I.*, et al.*, *Journal of nanobiotechnology* (2015) **13**, 34
126. Wang, C., and Park, J., *Micro and Nano Systems Letters* (2020) **8** (1), 1
127. Kim, J. H.*, et al.*, *Nanotechnology* (2009) **20** (50), 505101
128. Kim, J. H.*, et al.*, *Biomaterials* (2011) **32** (7), 1865
129. Sharma, A.*, et al.*, *Nanomedicine* (2011) **7** (4), 505
130. Laradji, A.*, et al.*, *Polymers* (2021) **13** (19)
131. Apaolaza, P. S.*, et al.*, *Experimental Eye Research* (2020) **198**, 108151
132. Chen, F.*, et al.*, *Biomaterials Science* (2021) **9** (2), 367
133. Kalishwaralal, K.*, et al.*, *Journal of controlled release : official journal of the Controlled Release Society* (2010) **145** (2), 76
134. Hu, C.*, et al.*, *Advanced Healthcare Materials* (2018) **7** (23), 1801047
135. Sun, J. G.*, et al.*, *Int J Nanomedicine* (2019) **14**, 1489
136. Sun, Y.*, et al.*, *Drug Deliv* (2020) **27** (1), 703
137. Rodrigues, F. S. C.*, et al.*, *ACS Biomater Sci Eng* (2020) **6** (12), 6587
138. Sábio, R. M.*, et al.*, *Microporous and Mesoporous Materials* (2021) **312**, 110774
139. Park, J.-H.*, et al.*, *Scientific reports* (2016) **6**, 37762
140. Cai, X.*, et al.*, *Mol Vis* (2016) **22**, 1176
141. Luo, L.-J.*, et al.*, *Theranostics* (2021) **11** (11), 5447
142. Yu, F.*, et al.*, *Journal of Controlled Release* (2019) **315**, 40
143. Yadav, H. K. S.*, et al.*, Chapter 17 - Polymer-Based Nanomaterials for Drug-Delivery Carriers. In *Nanocarriers for Drug Delivery*, Mohapatra, S. S.*, et al.*, (eds.) Elsevier(2019), pp 531
144. Lee, C.-H.*, et al.*, *Nanoscale* (2017) **9** (32), 11754
145. Agban, Y.*, et al.*, *International journal of pharmaceutics* (2016) **501** (1), 96
146. Friedberg, M. L.*, et al.*, *Ophthalmology* (1991) **98** (5), 725
147. Liu, W.*, et al.*, *Journal of Materials Science: Materials in Medicine* (2008) **19** (11), 3365
148. Silbiger, J., and Stern, G. A., *Ophthalmology* (1992) **99** (6), 889
149. Riau, A. K.*, et al.*, *ACS Biomaterials Science & Engineering* (2015) **1** (12), 1324
150. Tihan, G. T.*, et al.*, *Comptes Rendus Chimie* (2016) **19** (3), 390
151. Greenwald, Y., and Kleinmann, G., *Expert Rev Ophthalmol* (2008) **3** (6), 627
152. El-Mofty, H. M.*, et al.*, *Journal of Ophthalmology* (2014) **2014**, 173298
153. Reidy, J. J.*, et al.*, *Cornea* (1990) **9** (3), 196
154. Hwang, D. G.*, et al.*, *Archives of Ophthalmology* (1989) **107** (9), 1375
155. Lynch, C. R.*, et al.*, *Frontiers in Bioengineering and Biotechnology* (2020) **8** (228)
156. Echave, M. C.*, et al.*, *Curr Pharm Des* (2017) **23** (24), 3567
157. Fassina, L.*, et al.*, *Conf Proc IEEE Eng Med Biol Soc* (2010) **2010**, 247
158. Patel, K. D.*, et al.*, *Surface and Coatings Technology* (2014) **242**, 232

159. Elzoghby, A. O., *Journal of controlled release : official journal of the Controlled Release Society* (2013) **172** (3), 1075
160. Re, F.*, et al.*, *Journal of tissue engineering* (2019) **10**, 2041731419845852
161. Sahoo, N.*, et al.*, *Int J Biol Macromol* (2015) **81**, 317
162. Hathout, R. M., and Omran, M. K., *Pharm Dev Technol* (2016) **21** (3), 379
163. Shokry, M.*, et al.*, *International journal of pharmaceutics* (2018) **545** (1-2), 229
164. Esteban-Pérez, S.*, et al.*, *Pharmaceutics* (2020) **12** (4)
165. Shokry, M.*, et al.*, *International journal of pharmaceutics* (2018) **545** (1-2), 229
166. Sarbon, N. M.*, et al.*, *J Food Sci Technol* (2015) **52** (7), 4266
167. de Queiroz Antonino, R. S. C. M.*, et al.*, *Mar Drugs* (2017) **15** (5), 141
168. Dash, M.*, et al.*, *Progress in Polymer Science* (2011) **36** (8), 981
169. Patel, K. D.*, et al.*, *Journal of Materials Chemistry* (2012) **22** (47), 24945
170. Busilacchi, A.*, et al.*, *Carbohydrate Polymers* (2013) **98** (1), 665
171. Muzzarelli, R. A. A.*, et al.*, *Carbohydrate Polymers* (2012) **87** (2), 995
172. Goyal, R.*, et al.*, *Journal of controlled release : official journal of the Controlled Release Society* (2016) **240**, 77
173. Kao, H.-J.*, et al.*, *Journal of Pharmacy and Pharmacology* (2006) **58** (2), 179
174. Katiyar, S.*, et al.*, *Carbohydrate Polymers* (2014) **102**, 117
175. Papadimitriou, S.*, et al.*, *Carbohydrate Polymers* (2008) **73** (1), 44
176. Cho, I. S.*, et al.*, *Acta Biomater* (2016) **39**, 124
177. Lin, H. R.*, et al.*, *J Biomater Sci Polym Ed* (2007) **18** (2), 205
178. Sun, X.*, et al.*, *Acta Biomaterialia* (2021)
179. Liu, W.*, et al.*, *Journal of materials science. Materials in medicine* (2008) **19** (11), 3365
180. Schwieger, J.*, et al.*, *Journal of tissue engineering* (2020) **11**, 2041731420911313
181. Kolambkar, Y. M.*, et al.*, *Biomaterials* (2011) **32** (1), 65
182. Kohli, N.*, et al.*, *Journal of tissue engineering* (2021) **12**, 20417314211005610
183. Severino, P.*, et al.*, *Curr Pharm Des* (2019) **25** (11), 1312
184. Liu, Z.*, et al.*, *International journal of pharmaceutics* (2006) **315** (1-2), 12
185. Lin, H.-R.*, et al.*, *Biomacromolecules* (2004) **5** (6), 2358
186. Taghe, S., and Mirzaeei, S., *Brazilian Journal of Pharmaceutical Sciences* (2019) **55**
187. Motwani, S. K.*, et al.*, *European Journal of Pharmaceutics and Biopharmaceutics* (2008) **68** (3), 513
188. Hussain, Z.*, et al.*, *Polymer Reviews* (2017) **57** (4), 594
189. Zhai, P.*, et al.*, *International Journal of Biological Macromolecules* (2020) **151**, 1224
190. Zhu, Y., and Pang, Z., Chapter 13 - Hyaluronic acid in drug delivery applications. In *Natural Polysaccharides in Drug Delivery and Biomedical Applications*, Hasnain, M. S., and Nayak, A. K., (eds.) Academic Press(2019), pp 307
191. Browne, S.*, et al.*, *ACS Biomaterials Science & Engineering* (2020) **6** (2), 1135
192. Loebel, C.*, et al.*, *Journal of Materials Chemistry B* (2017) **5** (12), 2355
193. Wolf, K. J., and Kumar, S., *ACS Biomaterials Science & Engineering* (2019) **5** (8), 3753
194. Aragona, P.*, et al.*, *Br J Ophthalmol* (2002) **86** (2), 181
195. de la Fuente, M.*, et al.*, *Investigative Ophthalmology & Visual Science* (2008) **49** (5), 2016
196. Apaolaza, P. S.*, et al.*, *International journal of pharmaceutics* (2014) **465** (1), 413
197. Vepari, C., and Kaplan, D. L., *Progress in polymer science* (2007) **32** (8-9), 991
198. Buitrago, J. O.*, et al.*, *Acta Biomaterialia* (2018) **69**, 218
199. Chen, J. L.*, et al.*, *Biomaterials* (2010) **31** (36), 9438
200. Yeo, I.-S.*, et al.*, *Biomacromolecules* (2008) **9** (4), 1106
201. Huang, D.*, et al.*, *European Journal of Pharmaceutics and Biopharmaceutics* (2014) **88** (1), 104
202. Dong, Y.*, et al.*, *European Journal of Pharmaceutics and Biopharmaceutics* (2015) **91**, 82
203. Pan, X.*, et al.*, *Journal of Drug Delivery Science and Technology* (2020) **60**, 102086
204. Nagaraj, R.*, et al.*, *Journal of Drug Delivery Science and Technology* (2019) **52**, 334
205. Yavuz, B.*, et al.*, *The Scientific World Journal* (2013) **2013**, 732340
206. Kalomiraki, M.*, et al.*, *Int J Nanomedicine* (2015) **11**, 1
207. Kambhampati, S. P., and Kannan, R. M., *Journal of Ocular Pharmacology and Therapeutics* (2013) **29** (2), 151
208. Mastorakos, P.*, et al.*, *Nanoscale* (2015) **7** (9), 3845

209. Janaszewska, A.*, et al.*, *Biomolecules* (2019) **9** (8)
210. Zhu, S.*, et al.*, *Biomaterials* (2010) **31** (6), 1360
211. Menjoge, A. R.*, et al.*, *Drug discovery today* (2010) **15** (5-6), 171
212. Murugan, E.*, et al.*, *RSC Advances* (2015) **5** (129), 106461
213. Mishra, V., and Jain, N. K., *International journal of pharmaceutics* (2014) **461** (1), 380
214. Cheng, L.*, et al.*, *Colloids and Surfaces B: Biointerfaces* (2015) **136**, 37
215. Lancina, M. G., 3rd, and Yang, H., *Can J Chem* (2017) **95** (9), 897
216. Rajala, A.*, et al.*, *Nano Letters* (2014) **14** (9), 5257
217. Mandal, A.*, et al.*, *Journal of controlled release : official journal of the Controlled Release Society* (2017) **248**, 96
218. Terreni, E.*, et al.*, (2020) **12** (3)
219. Momekova, D. B.*, et al.*, *ACS Omega* (2021) **6** (49), 33265
220. Zhang, Z.*, et al.*, *Biomacromolecules* (2016) **17** (3), 798
221. Lu, Y.*, et al.*, *Nano Res* (2018) **11** (10), 4985
222. Custer, G. S.*, et al.*, *Langmuir* (2018) **34** (42), 12590
223. Cyphert, E. L.*, et al.*, *Exp Biol Med (Maywood)* (2017) **242** (7), 692
224. Sun, F.*, et al.*, *Drug Deliv* (2019) **26** (1), 575
225. Sai, N.*, et al.*, *Molecules (Basel, Switzerland)* (2019) **25** (1)
226. Weng, Y.*, et al.*, *Acta pharmaceutica Sinica. B* (2017) **7** (3), 281
227. Li, T.*, et al.*, *Drug Deliv* (2020) **27** (1), 410
228. Daza, J. H. U.*, et al.*, *Journal of Biomaterials Applications* (2020) **35** (3), 301
229. Chee, P. L.*, et al.*, *ACS Biomaterials Science & Engineering* (2021) **7** (9), 4659
230. Sorushanova, A.*, et al.*, *Advanced Materials* (2019) **31** (1), 1801651
231. Dhand, C.*, et al.*, *Biomaterials* (2017) **138**, 153
232. Chen, Y.*, et al.*, *RSC Advances* (2018) **8** (32), 18153
233. Palchesko, R. N.*, et al.*, *Advanced Healthcare Materials* (2018) **7** (16), 1701434
234. Kim, H.*, et al.*, *Journal of tissue engineering* (2019) **10**, 2041731418823382
235. Silva, N. C.*, et al.*, *Drug Deliv* (2015) **22** (7), 885
236. Mitra, R. N.*, et al.*, *ChemMedChem* (2014) **9** (1), 189
237. Nagarwal, R. C.*, et al.*, *Chemical & pharmaceutical bulletin* (2011) **59** (2), 272
238. Lin, H. R.*, et al.*, *Biomacromolecules* (2004) **5** (6), 2358
239. Tan, G.*, et al.*, *Acta Biomaterialia* (2021) **134**, 605
240. Argenziano, M.*, et al.*, *Gels* (2017) **3** (2)
241. Grimaudo, M. A.*, et al.*, *International journal of pharmaceutics* (2020) **576**, 118986
242. Sharma, P. K., and Chauhan, M. K., *Journal of Sol-Gel Science and Technology* (2020) **95** (1), 190
243. Dong, Y.*, et al.*, *Eur J Pharm Biopharm* (2015) **91**, 82
244. Davis, B. M.*, et al.*, *Small* (2014) **10** (8), 1575
245. Ding, Y.*, et al.*, *Acta Biomaterialia* (2021) **126**, 433
246. Zhou, X.*, et al.*, *Biomaterials* (2021) **268**, 120600
247. Kim, J.*, et al.*, *Journal of tissue engineering* (2021) **12**, 2041731421999750
248. Spater, T.*, et al.*, *Journal of tissue engineering* (2021) **12**, 20417314211000304
249. Li, J., and Mooney, D. J., *Nat Rev Mater* (2016) **1** (12), 16071
250. O'Shea, T. M.*, et al.*, *Adv Mater* (2015) **27** (1), 65
251. Schoenmakers, D. C.*, et al.*, *Nature Communications* (2018) **9** (1), 2172
252. Zhu, M.*, et al.*, *Scientific Reports* (2019) **9** (1), 6863
253. Cooper, R. C., and Yang, H., *Journal of Controlled Release* (2019) **306**, 29
254. Kushwaha, S. K.*, et al.*, *Int J Pharm Investig* (2012) **2** (2), 54
255. Fedorchak, M. V.*, et al.*, *Scientific Reports* (2017) **7** (1), 8639
256. Neamtu, I.*, et al.*, *Drug Deliv* (2017) **24** (1), 539
257. Onaciu, A.*, et al.*, *Pharmaceutics* (2019) **11** (9)
258. Argenziano, M.*, et al.*, *Gels (Basel, Switzerland)* (2017) **3** (2), 22
259. Singh, R. K.*, et al.*, *ACS Applied Materials & Interfaces* (2014) **6** (4), 2201
260. Neamtu, I.*, et al.*, Chapter 11 - Nanogels Containing Polysaccharides for Bioapplications. In *Polymeric Nanomaterials in Nanotherapeutics*, Vasile, C., (ed.) Elsevier(2019), pp 387
261. Grimaudo, M. A.*, et al.*, *International journal of pharmaceutics* (2020) **576**, 118986

262. Khan, K. U., *et al.*, *AAPS PharmSciTech* (2020) **21** (5), 141
263. Torres-Luna, C., *et al.*, *European Journal of Pharmaceutical Sciences* (2020) **154**, 105503
264. Sharifi, S., *et al.*, *Bioactive Materials* (2021) **6** (11), 3947
265. Sharifi, S., *et al.*, *ACS Applied Bio Materials* (2021) **4** (10), 7682
266. Jumelle, C., *et al.*, *Acta Biomaterialia* (2022) **137**, 53
267. Kong, B., *et al.*, *Nature Communications* (2020) **11** (1), 1435
268. Kurian, A. G., *et al.*, *Bioactive Materials* (2022) **8**, 267
269. Andreadis, I. I., *et al.*, *Molecular Pharmaceutics* (2021)
270. Zhou, Q., *et al.*, *Acta Biomaterialia* (2021) **134**, 177
271. Mensah, R. A., *et al.*, *Journal of Biomaterials Applications* (2021) **36** (5), 912
272. Liu, H., *et al.*, *Acta Biomaterialia* (2021) **131**, 162
273. Yu, X., *et al.*, *Nanomedicine: Nanotechnology, Biology and Medicine* (2018) **14** (1), 185
274. Agarwal, P., *et al.*, *European Journal of Pharmaceutics and Biopharmaceutics* (2019) **142**, 83
275. Khosravimelal, S., *et al.*, *Small* (2021) **17** (30), 2006335
276. Yang, H., *et al.*, *ACS Nano* (2012) **6** (9), 7595
277. Sun, X., *et al.*, *ACS Omega* (2020) **5** (1), 674
278. Haagdorens, M., *et al.*, *Regenerative Engineering and Translational Medicine* (2021)
279. Ahearne, M., *et al.*, *Advanced Functional Materials* (2020) **30** (44), 1908996
280. Fernandes-Cunha, G. M., *et al.*, *Scientific reports* (2020) **10** (1), 16671
281. Xeroudaki, M., *et al.*, *Scientific Reports* (2020) **10** (1), 16936
282. Song, Y., *et al.*, *Frontiers in Surgery* (2021) **8** (344)
283. Chen, F., *et al.*, *Investigative Ophthalmology & Visual Science* (2020) **61** (7), 1208
284. Chen, F., *et al.*, *Biomaterials* (2020) **255**, 120176
285. Feng, L., *et al.*, *ACS Applied Materials & Interfaces* (2021) **13** (41), 49369
286. Koivusalo, L., *et al.*, *Biomaterials* (2019) **225**, 119516
287. Chen, F., *et al.*, *Chemistry of Materials* (2020) **32** (12), 5208
288. Fernandes-Cunha, G. M., *et al.*, *The Ocular Surface* (2021)
289. Xu, Q., *et al.*, *Materials Science and Engineering: R: Reports* (2021) **146**, 100641
290. Chaidaroon, W., *et al.*, *Drug Des Devel Ther* (2021) **15**, 4435
291. McTiernan Christopher, D., *et al.*, *Science Advances* **6** (25), eaba2187
292. Kapoor, Y., and Chauhan, A., *J Colloid Interface Sci* (2008) **322** (2), 624
293. Song, Y., *et al.*, *Materials science & engineering. C, Materials for biological applications* (2018) **88**, 1
294. Jung, H. J., and Chauhan, A., *Biomaterials* (2012) **33** (7), 2289
295. Huang, J.-F., *et al.*, *ACS Nano* (2016) **10** (7), 6464
296. Perez Navarro, I., *et al.*, *Acta Ophthalmologica* (2016) **94** (S256)
297. Kamiya, K., *et al.*, *Contact Lens and Anterior Eye* (2020) **43** (3), 218
298. Peng, C. C., *et al.*, *Journal of controlled release : official journal of the Controlled Release Society* (2012) **162** (1), 152
299. Kim, H.-J., *et al.*, *ACS Nano* (2014) **8** (3), 2998
300. Lee, D., *et al.*, *Scientific Reports* (2016) **6** (1), 34194
301. Zhu, D., *et al.*, *Acta Biomaterialia* (2021) **127**, 242
302. Fan, X., *et al.*, *Acta Biomaterialia* (2020) **115**, 60
303. Ma, L., *et al.*, *Journal of Colloid and Interface Science* (2021)
304. Maulvi, F. A., *et al.*, *International journal of pharmaceutics* (2021) **608**, 121090
305. Lu, D., *et al.*, *Acta Biomaterialia* (2021)
306. Behtaj, S., *et al.*, *Acta Biomaterialia* (2021) **126**, 238
307. Khan, S. A., *et al.*, *Acta Biomaterialia* (2021) **128**, 262
308. Peng, C.-C., *et al.*, *Biomaterials* (2010) **31** (14), 4032
309. Bengani, L. C., and Chauhan, A., *Biomaterials* (2013) **34** (11), 2814
310. Hao, X., *et al.*, *Biomaterials* (2012) **33** (23), 5659
311. Ciolino, J. B., *et al.*, *Biomaterials* (2014) **35** (1), 432
312. Thissen, H., *et al.*, *Biomaterials* (2010) **31** (21), 5510
313. White, C. J., *et al.*, *Biomaterials* (2011) **32** (24), 5698
314. Qin, G., *et al.*, *Biomaterials* (2017) **124**, 55
315. Ross, A. E., *et al.*, *Biomaterials* (2019) **217**, 119285

316. Shen, H. H.*, et al.*, *Nanomedicine (London, England)* (2015) **10** (13), 2093
317. Gorantla, S.*, et al.*, *RSC Advances* (2020) **10** (46), 27835
318. Patel, A.*, et al.*, *World J Pharmacol* (2013) **2** (2), 47
319. Wadhwa, S.*, et al.*, *Curr Pharm Des* (2009) **15** (23), 2724
320. Suresh, P. K., and Sah, A. K., *Expert Opinion on Drug Delivery* (2014) **11** (11), 1747
321. Lynch, C.*, et al.*, *Polymers (Basel)* (2019) **11** (8)
322. Li, Z.*, et al.*, *Nanoscale Advances* (2021) **3** (18), 5240
323. Bhattacharjee, A.*, et al.*, *European Journal of Ophthalmology* (2018) **29** (1), 113
324. Ghasemiyeh, P., and Mohammadi-Samani, S., *Trends in Pharmaceutical Sciences* (2019) **5** (1), 7
325. kour, J.*, et al.*, *Asian Journal of Pharmaceutical Sciences* (2021) **16** (2), 175
326. Sawadkar, P.*, et al.*, *Journal of tissue engineering* (2021) **12**, 20417314211019238
327. Rykowska, I.*, et al.*, *Molecules (Basel, Switzerland)* (2021) **26** (18)
328. Nguyen, D. C. T.*, et al.*, *Contact Lens and Anterior Eye* (2021) **44** (6), 101487
329. Li, C.-C., and Chauhan, A., *Industrial & Engineering Chemistry Research* (2006) **45** (10), 3718
330. Chatterjee, S.*, et al.*, *RSC Advances* (2020) **10** (60), 36751
331. Lancina, M. G.*, et al.*, *Investigative Ophthalmology & Visual Science* (2016) **57** (12)
332. Vega-Vásquez, P.*, et al.*, *Frontiers in Bioengineering and Biotechnology* (2020) **8** (79)
333. Cheng, Y.*, et al.*, *Journal of Pharmaceutical Sciences* (2008) **97** (1), 123
334. Kim, J.*, et al.*, *Journal of tissue engineering* (2021) **12**, 2041731421999750
335. Desai, R. M.*, et al.*, *Biomaterials* (2015) **50**, 30
336. Dillinger, A. E.*, et al.*, *Small* (2018) **14** (50), 1803239
337. Urtti, A., *Advanced drug delivery reviews* (2006) **58** (11), 1131
338. Macwan, J. S.*, et al.*, Challenges in Ocular Pharmacokinetics and Drug Delivery. In *Nano-Biomaterials For Ophthalmic Drug Delivery*, Pathak, Y.*, et al.*, (eds.) Springer International Publishing, Cham, (2016), pp 593

Highlights

- Requirements of nanocarriers for ocular drug delivery
- Drug formulations in diverse nanocarriers
- Diverse nanoplatform for drug delivery in glaucoma treatment
- Promising directions for hydrogel and contact lens-based treatment of glaucoma
- Applications and clinical challenges in glaucoma treatment

Conflicts of interest

The authors declare no competing financial interest.