



Review

Biofabrication of Chitosan-Based Nanomedicines and Its Potential Use for Translational Ophthalmic Applications

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Abstract: Drug delivery to the anterior and posterior segment of eye remains a challenge. Nanoparticle-mediated drug delivery has indicated some promise. The presented review aims to summarize recent advancements in chitosan-based nanotherapies for ocular drug delivery and the challenges encountered during the process. Significant research using chitosan, a cationic linear polymer, is being conducted for ocular drug delivery. A vast number of publications exploit the mucoadhesive properties of the polymer, which arise due to interactions between the amino acids of chitosan and the sialic acid residues in mucous. The high degree of crosslinking in chitosan nanoparticles facilitates a dramatic increase in ocular drug retention of the desired drug, which subsequently helps in ocular penetration and improving the bioavailability of the drugs. A noted decrease in the initial burst of the drug is the basis for developing sustained drug release formulation using biodegradable and biocompatible chitosan polymer. In vitro as well as in vivo studies have indicated enhancement in the uptake, accumulation, and removal of chitosan nanoparticles from the site of delivery. In summary, chitosan- or modified-chitosan-based nanoparticles are being widely tested as drug carriers for treatment of bacterial and viral infections, glaucoma, age-related macular degeneration, and diabetic retinopathy.

Keywords: chitosan; nanoparticles; ocular diseases; biofabrication

1. Introduction

In 1811, a French professor named Henri Braconnot conducted research on mushrooms that led to the discovery of ‘fungine’, which was later termed chitin. The name ‘chitine’ was put forward in the year 1823 by Odier when he extracted the same material from insects and plants [1]. Based on Greek etymology, the word chitin, which is derived from the word ‘chiton,’ translates into ‘A Coat of

Mail'. Chitin is chemically (β -(1-4)-poly-N-acetyl-D-glucosamine) and is the second most abundant biopolymer after cellulose. Being an aminopolysaccharide, it is obtained from the exoskeletons of crustaceans like lobster, shrimp, barnacles, crab, insects, and the cell walls of fungi [2]. In 1859, professor C. Rouget discovered chitosan, which is the deacetylated derivative of chitin [3]. Since then, the biomedical applications of chitosan polymer, including a carrier for targeted drug and gene delivery, as a component of artificial matrices used in tissue engineering, and as an antimicrobial agent for wound dressing, are explored to a great extent. Recently, in 2018, the global chitosan market size was valued at USD 5.71 billion. Due to high market demand and growing applications, it is anticipated that the chitosan market size will accelerate exponentially at a CAGR of 20.8% through 2025 [4].

2. Structure of Chitosan

One of the major sources of chitin occurs in nature as crystalline microfibrils that form structural elements of exoskeletons of crustaceans, arthropods, and fungal cell walls. The structure of chitin resembles the structure of cellulose, but it consists of monomers of N-acetyl D-glucosamine units which are linked to each other via β (1 \rightarrow 4) linkages. The major difference between cellulose and chitin structures is the substitution of the hydroxyl group (OH) on the glucose molecule in cellulose with an acetyl functional group in chitin Figure 1a. The presence of the acetyl amine group in the structure of chitin is responsible for increasing its capacity to form hydrogen bonds between adjacent polymers which imparts increased structural strength [5]. The chitin molecule can be converted into chitosan either by the action of enzymes or by the chemical hydrolysis method [6]. Chitosan is an N-deacetylated derivative of chitin, which is formed by the conversion of the acetamide groups in the chitin into a primary amine group in chitosan (Figure 1b). The molecular weight of chitosan is around 5.3×10^5 Daltons, with the nitrogen content varying from 5–8% [7,8]. To date, two polymorphs of chitosan have been discovered. The crystal structure of polymorphs can be either hydrated (tendon) form or anhydrous (annealed) form [9]. The degree of deacetylation of chitin governs the chemical reactivity of the chitosan molecule as the deacetylation process of chitin is never complete. The chitosan molecule is insoluble in water, which limits its usage, but it is soluble in dilute organic acids, and thus chitosan-acid salts are used extensively in industrial applications [10].

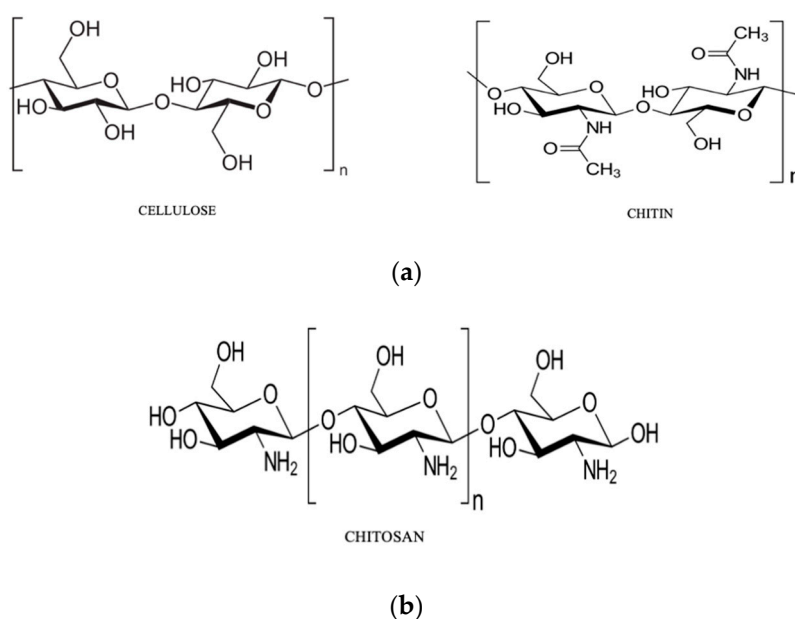


Figure 1. (a) Difference between cellulose and chitin polymers. (b) Structure of chitosan polymer.

3. Production of Chitosan

The major source of chitosan production is chitin found abundantly in nature. Chitin extraction on an industrial scale is done from remnants of crustaceans, especially shrimp shells. Based on a country-level analysis, Japan is the highest producer of chitosan. On average, the waste generated from the crustacean shell consists of 30–40% chitin and the rest comprises proteins and calcium carbonate [2]. The first process is to isolate chitin from the crustacean shells, obtained from varied marine sources, by use of chemical extraction. The shells are washed, dried and crushed into a fine powder. This is followed by the process of demineralization that involves acidic treatment of shells, which leads to the dissolution of calcium carbonate. It is carried out at room temperature with the aid of a dilute HCl solution. An increase in temperature can lead to the degradation of polymeric units, thus a proper control as well as temperature must be maintained throughout treatment depending upon the source of extraction [11,12]. Modification to the treatment consists of demineralization with milder acids, ethylenediaminetetraacetic acid (EDTA), and lactic acid [13,14]. This is followed by the deproteinization step, which involves treatment with an alkaline solution (1M NaOH) for a duration of 2 h at 95 °C temperature conditions. The solid obtained is washed continuously to achieve a neutral pH and decoloration is carried out by treatment with an organic solvent, or H₂O₂ which breaks down various pigments like carotenoids. The chitin is oven-dried at a temperature of around 70–80 °C [15]. In a study conducted to determine the correlation between the seasonal changes and its effect on the quality and chemical composition of chitin, it was found that no significant seasonal variations occur. Thus, chitosan producers can rely on chitin as a raw material for synthesis [16].

The second process for extraction is dependent on the use of specific enzymes for extraction. The bacterial protease enzymes from *Pseudomonas aeruginosa* can be used to remove 72% of the proteins from shrimp, but the efficiency as compared to the chemical extraction process is 5–10% lower [17,18]. All these processes require high energy consumption and are not cost-effective. Thus, a greener approach for the extraction of chitosan from fungal sources is being investigated. Chitosan can be extracted in an eco-friendly and cleaner method from the hyphal walls of fungus *Mucor rouxii* which, on analysis, was revealed to contain 35–40% of glucosamine content [19]. The extracted chitin can be converted into chitosan by the process of deacetylation (Figure 2). The glycosidic bonds in the chitin are more susceptible to the treatment of alkali. The process of deacetylation can be conducted either homogeneously or heterogeneously. In homogenous deacetylation, alkali chitin is obtained, which is then suspended on crushed ice. This leads to the formation of soluble chitosan with an average 48–55% degree of deacetylation. In contrast, in the heterogenous process chitin is exposed to hot and concentrated NaOH, which forms insoluble chitosan with a higher 85–99% degree of deacetylation [15]. During the process of deacetylation of chitin, the acetyl functional group located on the C₂- position of D-glucosamine is removed and an amine is formed [5,20].

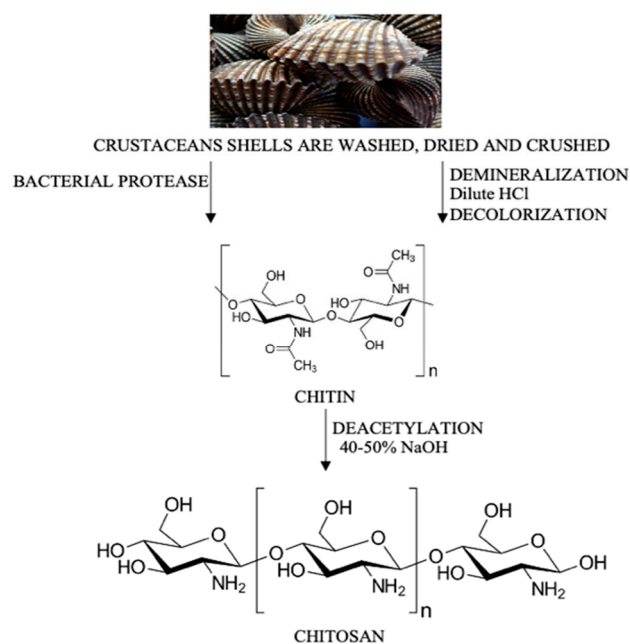


Figure 2. Chitosan production.

4. Biofabrication Considerations

The physicochemical and biological properties of chitosan rely on the degree of acetylation (DA) and degree of polymerization (DP), which also determines the molecular weight of the polymer. Chitosan is the deacetylated form of chitin, but the deacetylation process is incomplete or partial. This causes chitosan to exist as a copolymer of N-acetylglucosamine units and D-glucosamine units. Chitosan differs in molecular weight, sequencing of the acetylated residues in the structure, degree of acetylation, and pK_a properties. As chitin deacetylation progresses, the important change that occurs is that chitosan becomes polycationic in acidic media. This is due to the exposure of the amino groups on the molecule that are ionizable. The molar fraction of N-acetylglucosamine units present in the chain are defined in the terms of degree of deacetylation (DDA). In the literature, degree of acetylation (DA) has been used, $DA = 100 - DDA$. The physicochemical properties of chitosan such as solubility [21], viscosity [22], and biocompatibility [23] are suggested to increase as the DA decreases. On the other hand, properties like crystallinity and biodegradability have a direct relation to the DA. The faster rate of degradation causes the breakdown and accumulation of amino sugars which are responsible for eliciting inflammatory responses. This faster degradation rate also affects the biocompatibility of chitosan [24]. Biological properties such as mucoadhesive, antimicrobial, hemostatic, antioxidant, and analgesic properties increase once the degree of acetylation is lower [25]. The degree of acetylation is a fundamental feature that needs to be accurately estimated for the characterization of chitosan. The most widely used techniques to determine DA include H-NMR, UV-Vis spectroscopy, infrared spectroscopy and elemental analysis [26].

A facile and efficient deacetylation method of chitosan using 1-butyl-3-methylimidazolium acetate (BMIMOA) as the reaction medium and catalyst has been studied in the literature. The process involves hydrothermally treated chitosan in BMIMOA which is then recovered by dialysis followed by repetitive washings with methanol and acetone. Degree of deacetylation (DDA), estimated by colloid titration technique, was enhanced from 77% to 86% for commercial chitosan. The extent of DDA enhancement was significantly higher than the conventional deacetylation method using aqueous sodium acetate. The DDA of chitosan treated with acetate (CH_3COO^-) and formate ($HCOO^-$) anions suggested that the acetate anion catalyzes the hydrolysis of the residual acetamido group in chitosan. In contrast, the formate anion did not act as the deacetylation catalyst [27].

The molecular weight is a fundamental characteristic of chitosan polymer and has a great effect on its biomedical properties. It has a partial influence on hydrated colloidal forms and the viscoelastic properties of solutions. Techniques like gel permeation chromatography (GPC), size exclusion chromatography, static light scattering (SLS), intrinsic viscosity measurements can aid to determine the molecular weight distribution [28]. Lower molecular weight chitosan is generally preferred, as it increases the transfection efficiency of chitosan pDNA complexes and permeation characteristics. This is usually achieved by physical, chemical or enzymatic degradation [29]. The viscosity development of the aqueous solution is impacted by the molecular weight, thus playing a significant role in the biopharmacological aspect of chitosan [30].

The chemical structure elucidation of chitosan suggests the presence of reactive hydroxyl and primary amino groups which are less crystalline in nature compared to chitin. The pKa value of primary amino groups is 6.3, so it is considered a strong base. The amino groups of chitosan become deprotonated at pH above 6; the polymer tends to lose its charge and solubility. In contrast, at lower pH value, the amino groups get protonated with positive charge, making chitosan a cationic polyelectrolyte soluble in water. This transition between solubility and insolubility of chitosan occurs at its pKa value between pH 6 and 6.5. This supports the nature of chitosan as readily soluble in mild acids like hydrochloric acid, acetic acid, and insoluble at neutral and alkaline pH values [31]. Owing to all the aforementioned properties, chitosan is considered a biodegradable, biocompatible, and non-immunogenic polymer which has been extensively studied as a drug and gene delivery system. Table 1 summarizes the desired characteristics of chitosan polymer for medical applications [32–34].

Table 1. Summary of Chitosan characteristics for medical use.

Variables	Desired Characteristics
Organoleptic properties	White or almost white fine powder No taste, no odor
Degree of deacetylation	>80%
Molecular weight	Low molecular weight or oligomers
Viscosity (1% in 1% AcOH, 20 °C)	<5 cps
Moisture content	5–15%
Ash content	<1%
Protein content	<1–0.2%
Insolubility	<0.5–0.1%

5. Fabrication Techniques for Chitosan-Based Nanoparticles

The fabrication techniques for the development of chitosan nanoparticles depend upon the chosen target for delivery and desired nanoparticle characteristics. Figure 3 summarizes the techniques heavily explored in the literature for the development of chitosan nanoparticles.

1. **IONIC GELATION:** At the nanoscale level, the physical, optical and mechanical properties of the polymer may differ as compared to the macroscopic level. The fabrication of chitosan-based nanoparticles by ionic gelation method was first reported by professor Calvo and his group, and is by far the most widely used technique [35]. In short, the method is based on the spontaneous interactions between the cationic chitosan (CS) polymer in the presence of an anionic crosslinking agent. Sodium tripolyphosphate (TPP) is commonly used anionic crosslinker which initiates the formation of a polyelectrolyte complex named TPP/CS. Under appropriate conditions, the complex is stabilized due to the electrostatic interactions created between the CS-NH₃⁺ and TPP-O⁻ groups. This results in a three-dimensional entanglement from sol to dispersed gel-like nanoparticles. The drugs or genes added during the process are encapsulated to form nanoparticles. The process results in nanoparticle formation in the size range of 200–300nm [36];
2. **POLYELECTROLYTE COMPLEX FORMATION:** This method is based on similar lines to ionotropic gelation, with the difference that it employs a polyanionic polymer. The most widely used polymers include poly (γ-glutamic acid), poly (aspartic acid) and hyaluronic acid which

have opposite charges to those on chitosan derivatives. Polycationic chitosan derivatives like trimethyl chitosan (TMC), glycidyl trimethyl chitosan interact with the aforementioned polyanions, resulting in nanoparticles. As compared to the conventional polyelectrolyte complex formation, thiolated trimethyl chitosan (TMC-SH) and thiolated hyaluronic acid (HA-SH) demonstrated better stabilization due to the intermolecular covalent disulfide bonds [37];

3. **COMPLEX COACERVATION:** The origin of the term coacervation derives from Latin root “coacervare”, meaning to crowd or pile. The basic concept of complex coacervation involves liquid–liquid phase separation which is due to the mixing of two oppositely charged macroions. It is one of the easily applied techniques for nanoparticle formation which depends upon electrostatic interactions between two oppositely charged biopolymers. This method can be employed for the packaging of different plasmids [38]. Recently, zein-chitosan nanoparticles formed by complex coacervation were examined to encapsulate curcumin, which is known for its antioxidant properties. The nanoparticles that were formed possessed greater encapsulation efficiency and were affected with multiple factors like pH and zein to chitosan ratio [39]. The nanoparticles reported having improvised stability, controlled release of bioactive compounds, and biodegradability;
4. **POLYMER–DRUG COMPLEX FORMATION:** When active principles like insulin or genes consisting of an inherent negative charge are added in adequate proportions to cationic chitosan, they undergo nanoparticle formation. These insoluble complexes have higher encapsulation efficiency, stability, and a smaller particle size for better cell internalization. One of the major risk factors associated with diabetes mellitus is the development of cataract and diabetic retinopathy. The presence of insulin receptors on the ocular surface has indicated a promising strategy for treating these complications. Peptides like insulin undergo rapid degradation when administered orally, thus the encapsulation of insulin in chitosan nanocarriers can achieve sustainable release with small initial burst profiles in the eye [40,41];
5. **EMULSIFICATION SOLVENT EVAPORATION:** The technique of ESE is a well-defined method that comprises two steps: the process of emulsification of the solution consisting of the polymer and the substance to be encapsulated. This is followed by solvent evaporation or precipitation of polymer that results in particle hardening. The application of high sheer due to high-intensity sonification or homogenization during the emulsification process results in the breakdown of polymer into micro- or nanodroplets in the presence of surface-active agents. The solvent evaporation process majorly influences the morphology, encapsulation, and release properties of the encapsulated moiety. The single emulsion consists of the oil–water phase, while a double emulsion consists of the water–oil–water phase [42]. After the FDA approval of a cyclosporine A as an anti-inflammatory agent in the treatment of dry eye syndrome, attention has shifted to explore its uses in ophthalmology [43]. The encapsulation of cyclosporine A in poly(lactide-co-glycolide) nanoparticles coated with chitosan was prepared with single-emulsification solvent evaporation and demonstrated efficient ocular binding and prolonged anti-inflammatory properties [44,45];
6. **SELF ASSEMBLY:** Amphiphilic derivatives of chitosan are formed by attaching hydrophobic groups like cholesterol, cholic acid, deoxycholic acid, alkyl, acyl, or 5 β -cholanolic acid on chitosan backbone. At critical aggregation concentration, the amphiphilic derivatives of chitosan undergo spontaneous nanoparticle formation. The nanoparticles are characterized by the core-shell structure, as they consist of a hydrophobic core in a hydrophilic shell. Hydrophobic drugs with considerable water solubility have been loaded into the nanoparticles by direct addition to aqueous polymer dispersion [46,47].

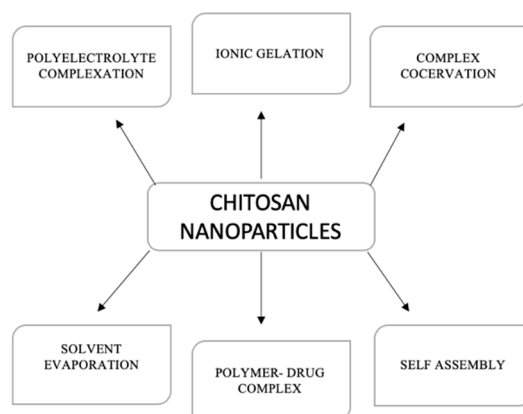


Figure 3. Chitosan-based nanoparticles' synthesis.

6. Anatomy of Eye

The human eye, from a lateral view, can be divided into anterior and posterior segments. The cornea is located at the outermost of the anterior segment followed by the anterior chamber, pupil, iris, lens, and conjunctiva. The vitreous humor, retina, macula, optic nerve, choroid, sclera together consist of the posterior segment of the eyeball, Figure 4. The most commonly occurring ocular diseases can be divided into two broad categories: the diseases associated with the anterior segment and the diseases associated with the posterior segment of the eye. In the anterior segment, cataract causing clouding of the lens is the leading cause of blindness in developed as well as developing countries. Other major diseases affecting the anterior segment are conjunctivitis, corneal ulcers, and keratitis (fungal infections). Usually, topical administration of the drugs or surgical management are conventional methods to address the above-mentioned disease conditions. However, due to several limitations like poor bioavailability of the drug, poor uptake, loss of drug due to mechanical blinking action and invasive procedures, the field of nanotechnology is widely being explored. Major diseases like glaucoma, age-related macular degeneration, diabetic retinopathy, bacterial or fungal endophthalmitis are associated with defects in the posterior chamber of the eye. Based on the literature, the trend has shifted to explore a variety of biodegradable polymers to fabricate nanoparticles that can be administered to the posterior segment of the eye [48].

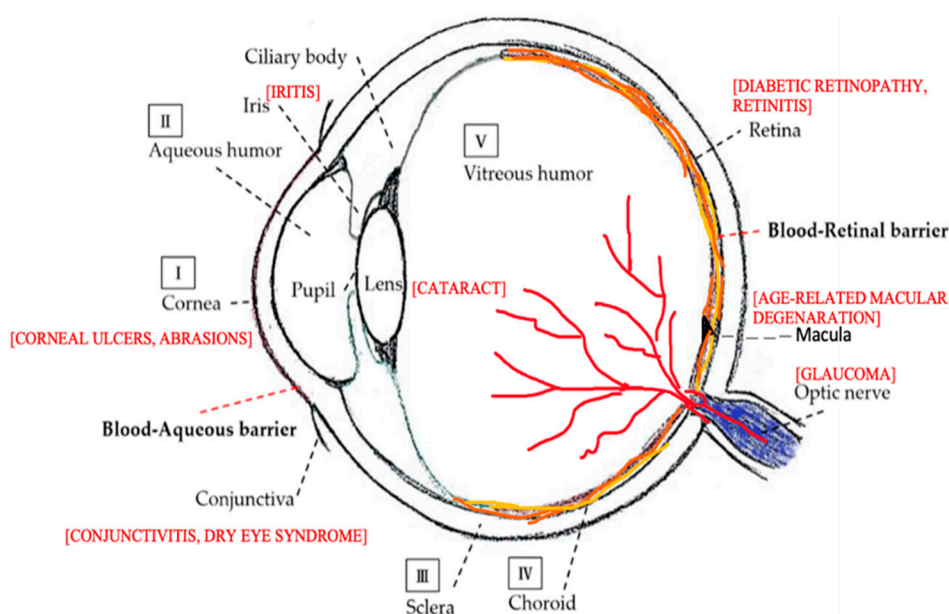


Figure 4. Diseases associated with anterior and posterior segment of eye.

7. Chitosan Based Nanoparticles for Anterior Segment of Eye

• Corneal keratitis

Fungal keratitis is the infection that arises in the cornea due to the commonly occurring fungal species of *Candida*, *Aspergillus*, and *Fusarium*. The predisposing factors include prolonged use of contact lenses, ocular trauma, vegetative ocular trauma, low socioeconomic status, and poor sanitation [49]. Corneal fungal infections are rare but severe, as they cause blindness if not treated. Fungal infection of the cornea is known as keratitis, while fungal infection located at the interior of the eye is known as endophthalmitis. Amphotericin B is a leading antibiotic which has proved its efficiency as a broad-spectrum anti-fungal agent. This macrocyclic polyene antibiotic has low bioavailability when administered as a topical formulation. This is due to effective removal mechanisms like constant blinking action and tear turnover. Along with the high molecular weight of Amphotericin B, its hydrophobicity majorly limits its penetration across the corneal epithelium [50]. Chitosan is a hydrophilic biopolymer that, when grafted with poly (lactic acid), imparts hydrophobicity to the molecule, making it an efficient delivery model for a hydrophobic drug like Amphotericin B. The PLA-g-CS copolymer is reported to be formed by the protection-graft-deprotection technique which undergoes self-assembly with Amphotericin B [51]. The nanoparticle acts as an effective drug carrier system for hydrophobic drugs with improvised water solubility and mucoadhesive strength [52].

• Corneal/Conjunctival cancer

Conjunctival/corneal squamous cell carcinoma is one of the common malignant tumors that affect the ocular surface. Recent investigations have suggested promising results using 1% 5-Fluorouracil a pyrimidine analog topically [53]. The topical dose of 5-Fluorouracil requires repeat treatment cycles and long-term follow-up in the patients. Acute and chronic side effects may occur because of the high concentration of the drug [54]. To get around these issues, the development of chitosan-based nanoparticles that are capable of forming a sustained drug delivery system with no side effects is the need of the hour. Chitosan, a cationic polymer, when reacted with sodium alginate which is an anionic polymer, can lead to the formation of a polyionic hydrogel system that aims to achieve a lower dose requirement of 5-Fluorouracil as well as prolonged release from the nanoparticle [55].

• Dry eye syndrome or Keratoconjunctivitis sicca

Keratoconjunctivitis sicca, commonly known as dry eye syndrome, is linked to an autoimmune condition that results due to the imbalance of protective immunoregulatory and proinflammatory pathways [56]. Extensive investigations have suggested the use of cyclosporin A, a local immunosuppressing agent, for treatments of keratoconjunctivitis sicca. Despite the vast investigations to incorporate the drug in collagen shields, emulsions or liposomes, significant therapeutic efficiencies have not been established. This is due to the limitation of cyclosporin's slow partition rate in corneal epithelium. When the hydrophobic peptide of cyclosporin A was associated with cationic chitosan polymer, a dramatic increase in the potential to deliver cyclosporin A to the outer ocular surface occurred [57].

• Bacterial conjunctivitis

Antibiotic administration is performed in multiple ocular disease conditions. Bacterial conjunctivitis, informally known as 'pink eye,' causes irritation, watering, itching, and discharge of the eye. For treating eye infections, penicillin, aminoglycosides, fluoroquinolones and tetracyclines are the common class of antibiotics administered. Eye drops are always the choice of formulation for the ocular delivery because they are easy to administer and noninvasive. Nanoparticles made up of chitosan and sodium tripolyphosphate-hyaluronic acid were loaded with ceftazidime, which belongs to the class of cephalosporin antibiotics that have indicated promising results [58]. These nanoparticles, when added to polymer (hydroxypropyl) methylcellulose (HPMC) in an isotonic

solution, lead to the formulation of eye drops that do not immediately clear off from the eye and thus increase the contact time and prolonged release of the drug [59].

• Ocular inflammations

Inflammation of the eye involves a combination of symptoms like eyelid ptosis, a reduction in ocular motility, discomfort in the eye, proptosis, and edema. Anti-inflammatory agents have been explored for treating ophthalmic conditions, macular edema post-cataract-surgery, manage scleritis. By far, anti-inflammatory agents like prednisolone, dexamethasone and nonsteroidal anti-inflammatory drugs (NSAIDs) administered topically or orally are the choice of treatment in ophthalmology. However, due to the poor bioavailability issues associated with the topical treatment, the formation of chitosan nanoparticles could be a promising solution to treat ocular inflammations. Glucocorticoids like dexamethasone have been reported to bind corticosteroid receptors present in the cells of the trabecular meshwork. They act by inhibiting phospholipase-A2, thus hindering the synthesis of prostaglandins and other agents which play a role in an inflammatory response. The aim of developing a chitosan-based nanoparticle is to circumvent the systemic toxicity associated with these particles. The nanoparticles can assist in delivering a drug in a controlled and continuous fashion to the surface of the eye. The use of hyaluronic acid in the formulation has been indicated to act as a protein repellent. The hyaluronic acid-coated chitosan nanoparticles have demonstrated better cellular targeting, improving corneal and conjunctival epithelial cell regeneration and mucoadhesive properties of the nanoparticles [60]. Dexamethasone sodium phosphate was encapsulated in chitosan nanoparticles, which were then impregnated into contact lenses. The prototype lenses were formed by using poly (2-hydroxyethyl methacrylate) (pHEMA) and demonstrated the conjunction of biodegradability of the polymer and contact lens to entrap hydrophilic drug [61].

Sodium deoxycholate acts as an endogenous surfactant, which initiates the self-aggregation of the chitosan nanoparticles. This carrier system has been exploited to carry hydrophobic and higher molecular weight prednisolone to treat ophthalmic infections [62]. Chitosan, when combined with chondroitin sulfate, a component abundantly present in the extracellular matrix of cartilage, resulted in development of drug carrier system for NSAIDs. The novel complex nanoparticle formed is being investigated for the delivery of bromfenac sodium, which is a potent anti-inflammatory drug with enhanced ocular penetration properties [63].

8. Chitosan-Based Nanoparticles for Posterior Segment of Eye

• Glaucoma

It is estimated that approximately 80 million people worldwide will suffer from irreversible blindness due to glaucoma by 2020 [64]. One of the major risk factors of glaucoma is the increase in intraocular pressure (IOP) which is due to the disparity between the secretion and drainage mechanism of aqueous humor. Timolol maleate is one of the FDA-approved topical formulations used as an anti-glaucoma agent. Glycosylated chitosan, which is synthesized by covalently linking D-galactose units to chitosan, exhibits better cell compatibility, water-solubility, ocular tolerance, drug loading, and release characteristics, and the pH of the formulation was closer to the physiological pH of the eye, making it a promising approach for administering an array of beta-adrenergic blockers [65]. A novel colloidal system with a diameter ranging between 100–1000 nm solid lipid nanoparticles consisting of inner solid lipid core are being explored as drug carrier systems for ocular delivery. This carrier system can encompass lipophilic drugs, which struggle to penetrate the corneal barrier. Solid lipid nanoparticles (SLN), when loaded with methazolamide, have significantly reduced the intraocular pressure, thus acting as effective treatment as an anti-glaucoma agent [66]. However, despite the potential of SLN to act as drug carriers, the major challenge to address is poor retention characteristics. This is due to the negative charge present on SLN which limits its interaction with the negatively charged corneal surface. The rationale is to invert the charge of SLN by applying chitosan coatings, which is a cationic polysaccharide. During the fabrication process, it was observed that factors like

the amount of methazolamide loaded, phospholipid content, and emulsifier concentration can affect the nanoparticle formulation [67]. Carteolol, a beta blocker, is a drug of choice in the treatment of glaucoma but is limited due to its low bioavailability. Chitosan nanoparticles loaded with carteolol showed better spread and retention properties as compared to the conventional aqueous carteolol solution in the precorneal space [68].

Evidence has suggested that the inflammation and oxidative stress that arises due to the cell dysfunction of trabecular meshwork increases resistance to the outflow of aqueous humor [69]. This increased intraocular pressure is the major risk factor for glaucoma. In recent years, many antioxidants like quercetin and resveratrol, which act as free radical scavengers, have been tested as a therapeutic aid for the treatment of glaucoma [70,71]. Research findings have indicated that, due to a better penetration across the cornea, the efficiency of resveratrol encapsulated PEG-modified chitosan nanoparticles increases significantly. In addition, quercetin, which competitively inhibits the expression of P- glycoprotein in mucosa, increases the bioavailability of resveratrol. PEG-modified chitosan polymer was used to form a dual-drug delivery device for administration at the same time [72]. Naturally obtained from the roots of *Coleus Forskolii*, a diterpene alkaloid has been investigated to reduce the intraocular pressure in the eye. The use of Forskolol can prove an alternative for patients sensitive to treatment with conventional beta-blockers [73]. The use of chitosan nanoparticles coated with poly lactic-co-glycolic acid has improvised the entrapment efficiency of the hydrophobic drug along with increasing the ocular tolerance due to its safe use. These nanoparticles can be fabricated using one-step emulsification and sonification process and thus are cost-effective [74].

• Age-Related Macular Degeneration (AMD)

After cataract, AMD is the leading cause of blindness in the elderly population [75]. Various flavanones like naringenin have been tested to improve the prognosis of the disease. However, the poor solubility of the drug has led researchers to develop a nontoxic carrier system using sulfobutylether- β -cyclodextrin/chitosan nanoparticles, which demonstrated better in vivo corneal release and drug retention. Thus, the frequency of administration of a drug in AMD patients can be reduced to obtain better patient compliance [76]. The conventional therapy for age-related macular degeneration includes administration of various therapeutic proteins such as ranibizumab, directed to treat symptoms of wet AMD. Ranibizumab is a monoclonal antibody fragment that is anti-angiogenic, thus suppressing the action of vascular endothelial growth factor. The literature has suggested the administration of ranibizumab through biodegradable polymers like PLGA. The major issue associated with this is difficulty in protein loading, which can be circumvented by formulating system-within-system delivery devices. Chitosan-N-acetyl-L-cysteine nanoparticles were encapsulated in PLGA microparticle to enhance the entrapment efficiencies and slow-release profile with no initial burst. This may have promise to overcome the cost burden associated with intravitreal injections [77].

The etiology of degenerative diseases such as AMD is typically associated with photo-oxidative damage, fluctuations in the PUFA levels, physical trauma or environmental and chemical factors. Antioxidant therapy is emerging as a choice of supplementary treatment that focuses on the delivery of antioxidants like lutein, zeaxanthin, rosmarinic acid, beta-carotene, and vitamin supplements which can be potent inhibitors of retinal neovascularization and macular degeneration. However, there are several challenges associated with absorption and targeting of the therapy. In the literature, the encapsulation of rosmarinic acid in chitosan-TPP nanoparticles demonstrated better permeability into the cells and alleviation of AMD [78].

• Bacterial and Fungal endophthalmitis

The human eye is susceptible to various bacterial infections due to its constant exposure to the environment. Bacterial endophthalmitis is an ocular inflammation caused by bacterial species of *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA) and *Streptococcus* species that causes irreversible damage to the photoreceptor cells in the posterior segment of the eye, leading to blindness.

Daptomycin is a conventional lipopeptide antibiotic used against gram-positive bacteria, which is approved for the treatment of the bacterial endophthalmitis. Direct administration of daptomycin leads to an insufficient concentration of the drug at the site of action due to lacrimal fluid secretion and protective barriers. The development of chitosan [79] or chitosan-coated alginate nanoparticles [80], which can act as drug carriers for daptomycin, provides an alternative for improvising the efficiency and residence time. The favorable properties of chitosan and alginate contributing in the opening of tight junctions present between the epithelial cells allow enhanced intraocular drug transport.

Amphotericin B is widely explored for fungal endophthalmitis. Amphotericin B administration to the eye has been studied using various approaches including corneal shields, intravenous and intracameral injections. However all these approaches have certain limitations, like chances of expulsion, diminished ocular bioavailability, chances of developing cataract. Chitosan, when conjugated with lecithin, a phosphatidylcholine, helps to improvise the mucoadhesive properties and increases the delivery of Amphotericin B to eye [81]. Table 2 summarizes chitosan-based nanoparticles and the potential applications in ophthalmology.

Table 2. Summary of Chitosan-based nanoparticles.

Chitosan-Based NPs	Drug	Route	Disease Targeted	Reference
Poly (lactic acid)-grafted-chitosan	Amphotericin B	Topical	Corneal keratitis	[52]
Chitosan/Sodium alginate	5-Fluorouracil	Topical	Corneal cancer	[55]
Chitosan nanoparticle	Cyclosporin A	Topical	Dry eye syndrome	[57]
Chitosan/sodium tripolyphosphate-hyaluronic	Ceftazidime		Bacterial conjunctivitis	[59]
Hyaluronic acid-coated chitosan	Dexamethasone			[60]
Chitosan nanoparticle	Dexamethasone sodium phosphate	Topical	Ocular inflammations	[61]
Chitosan/Sodium deoxycholate surfactant	Prednisolone	Topical		[62]
Chitosan/chondroitin sulphate	Bromfenac sodium	Topical		[63]
Glycosylated chitosan	Beta-adrenergic blockers (Timolol)	Topical		[65]
SLN-chitosan coating	Methazolamide			[67]
Chitosan nanoparticles	Carteolol	Topical	Glaucoma	[68]
PEG-modified chitosan	Quercetin			[72]
Chitosan nanoparticles coated with poly lactic-co-glycolic acid	Forskolin	Topical		[73]
Sulfobutylether- β -cyclodextrin/chitosan	β -cyclodextrin	Topical	Age-related Macular Degeneration	[76]
Chitosan-N-acetyl-L-cysteine nanoparticles encapsulated in PLGA microparticle	Ranibizumab	Prospective intravitreal		[77]
Chitosan-TPP	Rosmarinic acid			[78]
Chitosan nanoparticle	Daptomycin			[79]
Chitosan/Sodium alginate	Daptomycin		Bacterial and Fungal endophthalmitis	[80]
Chitosan conjugated with lecithin	Amphotericin B	Topical		[81]

9. Biodegradation of Chitosan

The study of the metabolic fate of chitosan nanoparticles is an important aspect, as it can modulate the bioavailability of these nanoparticles in the body. Biodegradation is the process of breaking down hydrophilic chitosan polymer into smaller fragments that are suitable for renal clearance. The rate of biodegradation process of chitosan is contingent on the degree of deacetylation in the molecule [82]. Failure in the biodegradation of chitosan nanoparticles can undergo retention in the eye, which can exert toxic effects. Basically, the process of hydrolysis of chitosan involves the action of various enzymes that break glucosamine–N-acetyl-glucosamine, glucosamine–glucosamine, and N-acetyl-glucosamine–N-acetyl-glucosamine linkages in the structure [83]. In vertebrates, the chitosan polymer is acted upon by various acids present in the stomach and is subjected to chemical degradation. The presence of rich microbial flora and lysozymes in the colon leads to the further degradation of the chitosan polymer [84]. In glycoside hydrolase, 18 families (GH18) and eight human chitinases have been identified. The enzyme chitinase in microorganisms is involved in the

hydrolysis of the acetyl group in chitin, which is the precursor of chitosan [85]. To promote better formulation characteristics, studies have indicated that N-substitution has a predominant effect on the rate of enzymatic biodegradation. The biodistribution of chitosan nanoparticles is dependent on the molecular weight, as an increase in the molecular weight decreases the plasma concentration and formulation modifications [86]. Various parameters, like type of dosage form, particle size, route of administration, and modifications in the functional groups can alter the biodistribution of chitosan nanoparticles in the body.

10. Toxicity of Chitosan

The major challenge in front of a clinical pharmacologist is to develop ocular drug delivery devices that would circumvent the intricate barrier without causing any toxicity or secondary complications in the eye. Ocular toxicity can be asymptomatic and cause complications like blurred vision, inflammation and unwanted lachrymal secretions, defective color vision, hemorrhage, ocular surface discomfort. The use of chitosan for drug delivery has not yet been approved by the FDA, as it is not classified as Generally Recognized as Safe (GRAS). However, the applications of chitosan in the dietary industry and as an aid for wound dressing have been approved by US-FDA and EU mainly because of their biocompatible and biodegradable nature [87]. The reason for its disapproval as a drug delivery device is still questionable, as no toxicity in the cell culture model and animal models has been reported to date. There have also been no published data demonstrating the toxicity of chitosan for human use. In general, cationic polymers demonstrate cell toxicity due to the presence of cationic charge and the level of toxicity is influenced by the molecular weight of the polymer. However, according to the cytotoxicity study on chitosan salts by MTT assay, chitosan hydrochloride salt (MW 45kDa) resulted in high toxicity with an IC₅₀ of 0.22 ± 0.06 mg/mL, whereas a salt formation with lactic, glutamic, and aspartic acid was demonstrated to be less toxic [88]. The cytotoxicity of chitosan-coated silver nanoparticles is induced in a dose-dependent manner in rats [89]. Approaches like derivatization of the molecule, the formation of soluble salts, copolymerization, complexations, and use of surfactants are some of the methods utilized to modify the chitosan for desired characteristics [90].

11. Conclusions

Current treatment modalities are associated with multiple limitations like poor retention, bioavailability, and the concomitant risk of contamination during invasive procedures. The literature has suggested a paradigm shift in the research focus on the utilization of nanoparticles. Based on the findings, polymeric nanoparticles are gaining a lot of interest and have demonstrated promising results in ocular therapy. The use of chitosan derivatives has been highly exploited to encapsulate drugs as well as genes and to act as carriers. Several advantages like biocompatibility, biodegradability, reducing the frequency of administration, better patient compliance, circumventing various ocular barriers, protecting drugs during transport, and drug release in a controlled and sustained fashion make a strong argument that chitosan-derivatives-based nanoparticles will emerge as promising drug and gene delivery devices.

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