Biomedical Applications of Quaternized Chitosan

Subjects: Polymer Science

Contributor: Kamla Pathak

The natural polymer chitosan is the second most abundant biopolymer on earth after chitin and has been extensively explored for preparation of versatile drug delivery systems. The presence of two distinct reactive functional groups (an amino group at C2, and a primary and secondary hydroxyl group at C3 and C6) of chitosan are involved in the transformation of expedient derivatives such as acylated, alkylated, carboxylated, quaternized and esterified chitosan. Amongst these, quaternized chitosan is preferred in pharmaceutical industries owing to its prominent features including superior water solubility, augmented antimicrobial actions, modified wound healing, pH-sensitive targeting, biocompatibility, and biodegradability. It has been explored in a large realm of pharmaceuticals, cosmeceuticals, and the biomedical arena. Immense classy drug delivery systems containing quaternized chitosan have been intended for tissue engineering, wound healing, gene, and vaccine delivery.

quaternized chitosan antimicrobial biocompatibility biomedical vaccine wound healing

1. Introduction

Chitosan, an aminoglucopyran polysaccharide, is widely utilized in pharmaceuticals, cosmeceuticals, biomedicals, agricultures, foods and packaging sectors owing to its inherent properties including biodegradability, biocompatibility, and non-toxicity [1][2]. The natural biopolymer chitosan is predominantly obtained from the exoskeletons of marine crustaceans, mollusks, insects, and fungi through an alkali deacetylation process. **Figure 1** illustrated the schematic developmental process of quaternized chitosan from native chitin [3]. In contrast to natural occurring chitin, chitosan is very soluble in acidic solvents and fluroalcohols. Chitosan is a weak base, possessing pKa ranging from 6.2 to 7.0, and tends to be biodegradable in both in vitro and in vivo into non-toxic metabolites. It can be easily digested by lysozyme into non-active oligosaccharide and is thus a desirable component for designing absorbable sutures, osteoconductive implants and tissue scaffolds [4]. The biological functions of chitosan biopolymer are based on its molecular weight, degree of acetylation, charge density and extent of quaternization [5]. The physicochemical and biological properties of chitosan are primarily affected by the degree of deacetylation that directly impact on its molecular weight, pKa, crystallinity, hydrophilicity, degradation, and biological actions [6][7]. A degree of deacetylation value close to 0% or 100% prolongs biodegradation and cell adhesion, whereas transitional values of the degree of deacetylation display speedy degradation rates of chitosan. The physicochemical properties including the degree of acetylation and the molecular weight of chitosan and its derivatives are accountable for its biological responses [8]. USFDA-approved chitosan is highly utilized in tissue

engineering, skin regeneration and wound healing drug delivery systems [9]. The presence of inter- and intramolecular hydrogen bonds demonstrates crystalline behaviour, which accounts for its poor aqueous solubility. The limited solubility in a wide range of physiological solutions restricts the use of chitosan in designing drug delivery systems [10].

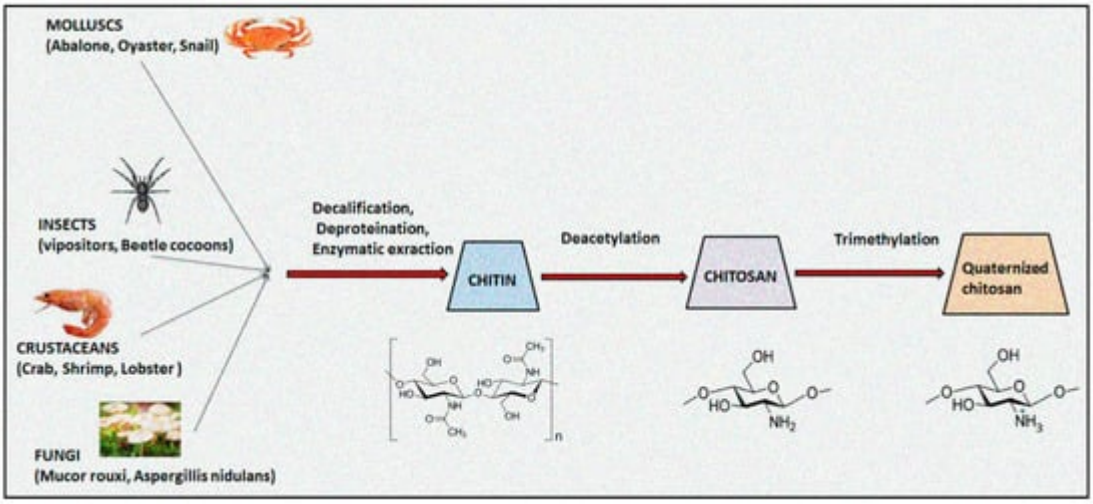

Figure 1. Schematic developmental process of quaternized chitosan from native chitin.

Conformational changes in the chitosan skeleton are reliant on local environmental conditions including the pH, pKa, N-substitution group, process temperature and types of acids. The functional groups (hydroxyl and amino) contribute an essential role for imparting solubility to the chitosan molecule. At low pH, the attached amino group undergoes protonation, solubilizes and provides positive charge to the medium, offering strong electrostatic interaction with negative charge cell components. Moreover, the pKa of amino group highly depends on the degree of acetylation (DA); hence, the solubility of chitosan is also reliant on DA [11].

Various chemical modification such as acetylation (insertion of anhydride and acyl chloride) [12], alkylation (alkyl group) [13], carboxylation (glyoxylic acid, chloroalkanoic acid) [14], quaternization (quaternary ammonium salts) [15], esterification (sulphuric acid, phosphoric acid and chlorosulfonic acid) [16] and etherification (chloroacetic acid, ethylene oxide, dimethyl sulphate) [17] at the C2, C3 and C6 position of chitosan are carried out to improve its physicochemical (enhanced aqueous solubility, improved absorption, high bioavailability, etc.) and biological properties (improved antimicrobial action and antioxidant, high penetration across cell membrane, mucoadhesiveness, etc.) **Figure 2** displays the structural differentiation between chitosan and quaternized chitosan [18].

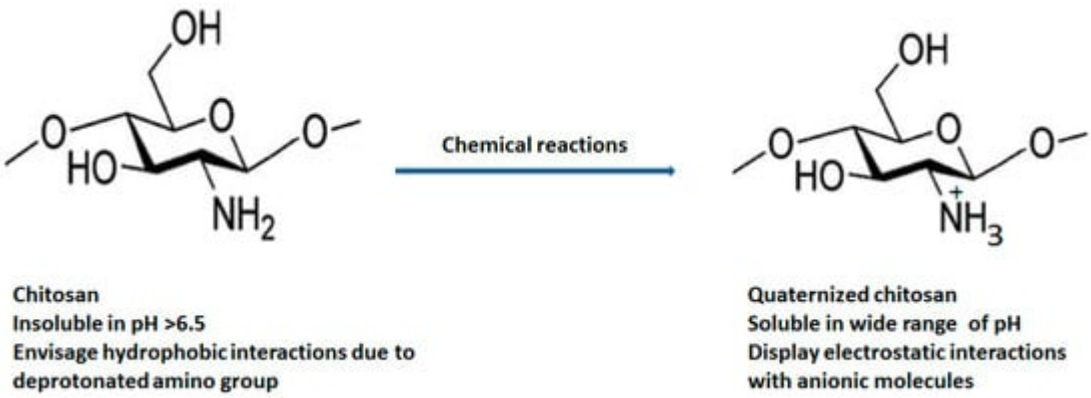

Figure 2. Structural differentiation between chitosan and quaternized chitosan.

2. Quaternized Chitosan Derivatives and Physicochemical Properties

The quaternization of chitosan involves the insertion of a hydrophilic group via any of three methods: direct quaternary ammonium substitution, epoxy derivative open loop and N-alkylation. A high degree of substitution provides better aqueous solubility and enhanced antimicrobial action, and lessens cytotoxicity with innate mucoadhesiveness and efficient penetration. The degree of quaternization and molecular weight are a few essential parameters that elicit physicochemical and biological actions (**Table 1**). **Figure 3** compiles drug delivery approaches and biomedical applications of quaternized chitosan [19].

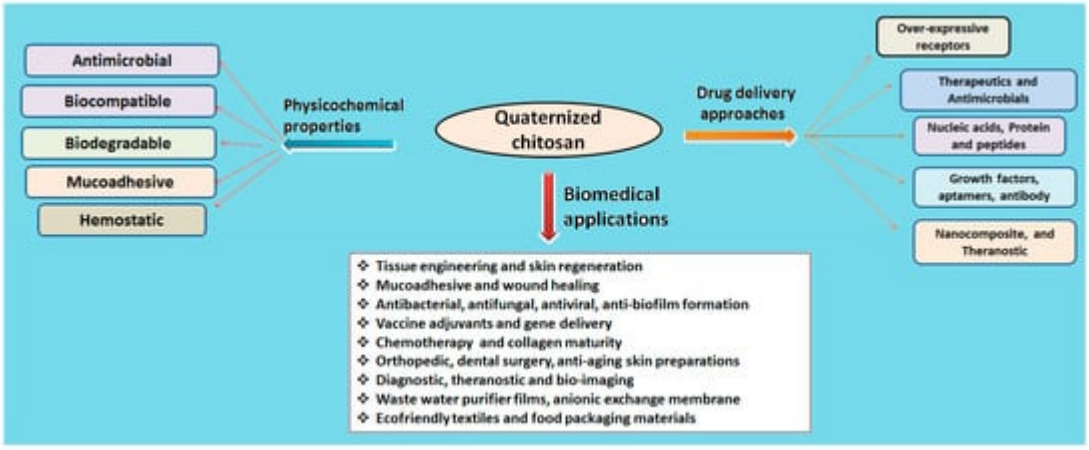

Figure 3. Quaternized chitosan and its physicochemical and drug delivery approaches.

Table 1. Effect of parameters influencing the biological responses [20][21].

Parameters	Responses
Degree of quaternization (<65%)	Increased cytotoxicity

Parameters	Responses
Degree of quaternization (≥20%)	Increased mucoadhesiveness
	Decreased anticoagulation effect
	Increased antimicrobial action in pH = 7.2
	No effect on antimicrobial action in acidic pH
Degree of substitution (<1%)	Increased antioxidant property
Degree of substitution (<25%)	Increased antithrombin action and acid-binding capacity
Degree of substitution (>1%)	Decreased moisture absorption and retention ability
High concentration	Increased particle size, aggregation, zeta potential, cytotoxicity
	Decreased knockdown efficiency and poor transfection efficacy

Quaternization not only enhances solubility but also escalates chargeability and antibacterial efficacy [22].

The mechanical property plays a vital role in imparting biomedical application. It is observed that mechanical property of chitosan enhances on increasing its concentration owing to high crystallinity. Jana et al. (2012) investigated the comparative tensile strength of scaffolds fabricated from chitosan solution (4–12%). As the concentration of chitosan increased in scaffold, augmented mechanical strength (from 1.74 MPa to 17.99 Mpa) was displayed through XRD patterns. The degree of protonation and extent of crystallinity mediated the high strength of chitosan [23][24][25]. Britto et al. (2007) synthesized quaternary salts of chitosan through dimethylsulphate reaction. The mechanical strength, Young's modulus and maximum strain were compared to the unmodified, N-alkylated and quaternized chitosan films, which are well depicted in **Figure 4**. Unmodified chitosan film exhibited higher mechanical strength compared to quaternized (N-dodecyl chitosan) and alkylated (butyl chitosan) in order of 44.0 Mpa > 38.3 Mpa > 13.4 Mpa, respectively [26][27][28].

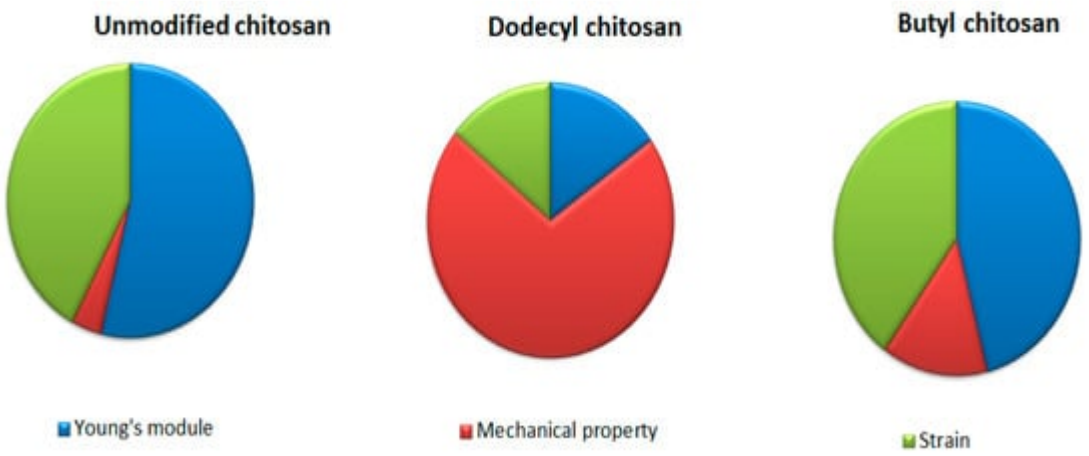

Figure 4. Comparative mechanical properties of chitosan and its derivatives.

3. Biomedical Applications of Quaternized Chitosan Derivatives

3.1. Antimicrobial

The chemical configuration of polycationic QCh is a desirable requisite for the antimicrobial action. Electrostatic interaction between cationic QCh and anionic microorganisms plays an important part for antibacterial activity. A higher degree of substitution of ammonium groups adorned on the backbone of QCh imparts a positive charge that neutralizes cell surfaces of bacteria and disturbs cytoplasmic integrity [29][30]. Moreover, hydrophobic alkyl substitutions on QCh stimulate significant bacterial death as they preferably interact with the inner surface of the bacterial cell wall. The antibacterial effect is prominent in neutral and high pH rather than acidic. The literature envisages that high-molecular-weight and solid QCh derivatives are unable to pass through the cell membrane. They are only adsorbed at the microbial cell surface, channelize electrostatic interactions, hamper nutrient transport, and cause alteration in cell permeability. Conversely, low-molecular-weight water-soluble QCh particles penetrate the bacterial cell wall, intercalate with DNA, and inhibit the transcription process [31]. QCh derivatives always remain positively charged and are soluble at all physiological pH.

The antibacterial activity of the very first N,N,N-trimethyl chitosan was reliant on the degree of quaternization from the [−$N(CH_3)_3$] structure present in the molecule. A higher degree of quaternization improved its aqueous solubility. Additionally, the presence of hydrophobic methyl groups increased the interaction with the lipoidal cell membrane of the microorganism and depicted superior antimicrobial action [32]. Another derivative, N,N-di-ethyl-N-methyl quaternized chitosan (DMCHT) was synthesized via reductive alkylation of aldehydes, through the formation of Schiff base. The developed QCh exhibited antimicrobial efficacy owing to the formation of polyelectrolyte complexes between QCh derivative and peptidoglycan of bacterial cell, which, in turn, inhibited the growth of bacteria [33]. The antimicrobial efficacy of TMC and DMCHT was compared at 50% degree of quaternization. The results showed superior antibacterial activity of TMC against *S. aureus* owing to the presence of smaller alkyl

groups that enabled easy interaction with the cell wall of the bacteria. Voluminous DMCHT was comprised of heavy N-ethyl functional groups on its structure [34].

Furthermore, comparative antimicrobial efficiency was assessed among DMCHT (N, N-di-ethyl-N-methyl quaternized chitosan), BZDCHT (N-benzyl-N-N-di methyl chitosan) and BDCHT (N-butyl-N,N-dimethylchitosan) against *S. aureus* and *E. coli* at pH 7.4. The outcomes displayed prominent antimicrobial activity of these QCh as compared to chitosan. DMCHT and BDCHT showed higher hydrophilicity required for better antimicrobial efficacy in comparison to BZCHT. Insertion of hydrophobic groups (phenyl and benzyl) decreased the antimicrobial action by virtue of shielding interaction between N-quaternized site and the cell wall of the bacteria [35]. Reports cite that the protonated amine groups including $+NH_3$ and +ND/+NM (non-quaternized amine group) are highly effective antibacterials compared to +NT (N-trimethylated) ones. However, the chains of TMC derivatives are more flexible and readily interact with the bacterial cells than chitosan at pH ≥ 5.5 suggesting promising antibacterial potential where neutral pH required [36].

3.2. Antiproliferative

Biocompatible quaternized chitosan displays adequate antimicrobial and antiproliferative activity both in vitro and in vivo. For instance, QCh-coated sutures exhibited comparatively high anti-infection and cytocompatibility compared with triclosan-coated sutures, owing to the presence of ample positive charge on decorated quaternary ammonium functional groups that interact with the negatively charged phosphoryl groups of microorganisms [37]. Triclosan, a widely used antibacterial agent, has a significant advantage over other antibiotics by virtue of its low drug resistance and potent inhibition of biofilm formation. However, triclosan-coated sutures are effective in controlling surgical site infections, but the occurrence of tissue toxicity and induction of tumor proliferation and endocrine disorders limit its practice in surgical operation. The above investigation favored the usage of broad spectrum antibacterial QCh as an alternative to triclosan in orthopedic surgery [38].

3.3. Antibiofilm

Biofilms, the complex colonies of living microorganisms developed at the infection site, are extremely resistant to antibiotics and antimicrobials. These biofilms consist of a well-recognized self-produced extracellular matrix made up of protein, polysaccharide, and DNA [39]. Bacterial adherence and the biofilm formation at the implantation site involve two typical processes. Initially, the accumulation of microbial community starts, which releases polysaccharide intracellular adhesion, an extracellular substance. Furthermore, PIA is mediated through intracellular adhesion that comprised of different core genes such as ica A, B, C, ica D and one regulatory gene icaR. The icaA gene is an index of biofilm preparation. A high concentration of QCh is capable of preventing icaA transcription, thus limiting biofilm formation or microbial viability [40].

Biofilms produced at the post-surgical infection sites are mostly associated with the use of medical devices including catheters, implants, endotracheal tubes, valves, etc. Both Gram-positive (*S. aureus*, *S. epidermis*) and Gram-negative (*P. aeruginosa*) bacterial communities grow on healthcare devices [41]. These pathogenic bacteria can survive even in the presence of high doses of antibiotics (1000 times high) than required to eliminate their

planktonic population. Once well-organized biofilm is formed around the surgical implants surface, it becomes difficult to completely rule out through antibiotic therapy [42].

Most of the existing antibiotics and antimicrobials are unable to eradicate biofilms, owing to their similar activity spectrum and common modes of action. QCh can be employed for the elimination of biofilm by virtue of its anti-infective property against bacteria, viruses, and fungi [43][44]. Both high- and low-molecular-weight QCh functionalized with hydrophobic residues (thiol protected 6-mercaptonicotinamide and methylated cyclodextrins) are reported as unconventional antimicrobial agents as they display improved wound healing, mucoadhesiveness and antibiofilm potential [45].

3.4. Antifungal Activity

As with antibacterial activity, the occurrence of ample positive charge on QCh supposed to interact with the anionic residues of macromolecules present on the fungal surface resulted in the leakage of intracellular electrolytes and nutrients. Reports cite that QCh affects morphogenesis of the fungal cell wall and controls the functions of enzymes accountable for growth [46]. Guo et al. (2007) synthesized different QCh derivatives for the study of antifungal activity against *Botrytis cinerea* and *Colletotrichum lagenarium*. Four QCh derivatives, i.e., N-(2-hydroxyl-phenyl)-N,N-dimethyl CS, N-(5-bromic-2-hydroxyl-phenyl)-N,N-dimethyl CS, N-(2-hydroxyl-5-nitro-phenyl)N,N-dimethtyl CS and N-(5-chloro-2-hydroxy-phenyl)-N,N-dimethyl CS, were developed, which exhibited better antifungal effects compared to the unmodified chitosan [47]. The result emphasized that high molecularity of QCh is accountable for stronger antifungal efficacy [48].

Insertion of an arylfurfural group to the N-quaternized chitosan indicated amplified antifungal effects compared to unmodified chitosan. Moreover, N-quaternized arylfuran chitosan (QACHT) substituted with chlorine and nitrogen oxide further modified the antimicrobial and the antifungal effects that interacted with anionic macromolecules of fungal cell wall and mediating seepage of intracellular electrolytes [49]. Deacetylated chitosan functionalized with propyl and pentyl trimethylammonium bromide exhibited increased antifungal activity, i.e., three- and six-fold higher for *A. flavus*. In vitro antifungal assay evaluated the minimum inhibitory concentration for 72 h by varying the QCh derivatives concentration (0.5–16 g/L). The outcomes revealed that QCh derivatives inhibited mycelium growth even at one quarter of the concentration of deacetylated chitosan [50].

The antifungal activity of QCh can also be improved by the addition of more quaternary ammonium groups such as N-trimethyl. Increased molecular weight, positive charge, degree of quaternization and hydrophobic functional moieties intensify antifungal action. Il'ina et al. (2017) synthesized synthetic metal (Cu II) complexes of QCh (N-propyl chitosan derivative) and evaluated the antifungal efficacy against yeast (*C. albicans* and *R. rubra*) and mycelial fungi (*F. oxysporum* and *C. herbarum*). N-propyl-derived QCh was developed through treatment with glycidyl trimethyl ammonium chloride under controlled conditions. Amalgamation of N-propyl QCh (53%) and copper ions (13%) proved efficient against common fungal plant pathogen *F. oxysporum* [51]. Viegas de Souza et al. (2017) synthesized low-molecular-weight QCh derivative (dodecyl aldehyde-treated propyl trimethyl-ammonium bromide chitosan) to investigate the antifungal effect on *A. parasiticus* and *A. flavus*. The outcomes revealed that

amphiphilic QCh derivatives exhibited amplified inhibition indices that were reliant on hydrophobicity and polymer concentrations. These QCh derivatives opened novel avenues for the development of chitosan-based biofungicides [52].

3.5. Mucoadhesiveness

N,N,N-trimethyl chitosan is widely utilized as a penetration enhancer for the delivery of peptides and macromolecular compounds across the mucosa in alkaline and neutral pH medium. Here, the degree of quaternization has a direct impact on the property of mucoadhesiveness and penetration across the membrane. Snyman et al. (2003) studied the effect of extent of quaternization (22–48%) and molecular mass (100,000 g/mole) on the biological activity of synthesized TMC polymers. The outcomes suggested decreased mucoadhesiveness on increased quaternization of TMC polymers [53][54].

The ocular or ophthalmic drug delivery system encounters several route barriers related to nasolacrimal drainage, stimulated lacrimation, blinking, blood ocular barrier and corneal impermeability. The use of mucoadhesive polymers such as chitosan derivatives facilitates effective ocular drug delivery owing to their elite interaction with the mucosal membrane. The presence of positive charge mediates the electrostatic interaction with the anionic mucin of the mucosal layer [55]. The ocular drug absorption of therapeutics depends on their aqueous solubility and mucoadhesiveness. Quaternized chitosan shows better candidature for enhanced permeation across ophthalmic tissues owing to their improved solubility and mucoadhesive property. However, beta-cyclodextrin conjugated chitosan has been employed for the ocular delivery of dexamethasone but restricted mucoadhesive limited their use.

3.6. Drug Carriers

3.6.1. Nanofibers

Nanofibers have always been a lucrative tool for the preparation of scaffolds, either as topically applied dressing material or in the membrane unit for filtration. Water purification systems enclosing membrane filtration units are widely accepted strategies for the retention of pathogens including bacteria and viruses according to their size. **Table 2** compiles few novel biomedical usages of QCh-based nanofibers. Reports cite that holding small-sized viruses (<25 nm) necessitates enormous membrane surface area along with low water flux and high transmembrane pressure. Moreover, the filtration membrane unit has to be replaced with another new membrane frequently. To overcome this downside, Bai et al. (2013) fabricated an electro spun nanofibrous membrane comprised of quaternized chitosan polymer (HTCC) and graphene allotrope. HTCC has the proficiency to adsorb pathogenic non-enveloped porcine parvovirus on its surface. Distinctive attributes of graphene hydrophobicity and ionogenic HTCC enhanced the functional efficiency of developed nanofibers (95% virus retention). The developed nanofibers embedded with a blend of HTCC/graphene depicted effective microfiltration membrane water purification systems featured with low pressure technology for the significant removal of pathogens [56].

Table 2. Quaternized chitosan-embedded nanofibers in diverse biomedical arena.

Components	Purpose	Research Outcomes	References
HTCC and PVA	Retention of non-enveloped virus on the highly charged HTCC/PVA nanofibers.	Nano-scaled HTCC/PVA nanofibers (100–200 nm) were developed, having the potential to adsorb mammalian virus porcine parvovirus (95%). The developed system followed Freundlich isotherm and showed fast adsorption kinetics (pseudo first order), which suggested the formation of efficient filter material for the purification of water	[57]
Doxorubicin, poly (L-lactide-coD, L-lactide) and QCh	Doxorubicin embedded poly (L-lactide-coD, L-lactide) mats were modified with QCh to enhance anti-proliferative activity.	Developed mats were evaluated against human breast carcinoma cell lines (MCF-7) and exhibited reduced cell viability and amplified antiproliferative activity. Fluorescent microscopy revealed that the presence of QCh induced apoptosis, which was the primary mechanism of MCF-7 cell death.	[58]
2,3-Epoxy-propyl trimethyl ammonium chloride	QCh fibres were designed using 2,3-epoxypropyl trimethyl. Ammonium chloride following ring open reaction to modify antibacterial and liquid absorption capacity.	Outcomes revealed excellent water retention capacity, modified swelling index and mechanical strength compared to bare chitosan. Superior antibacterial efficacy against *S. aureus* and lower cytotoxicity suggested its vital role in fabricating wound dressing materials.	[59]
Poly (lactic acid), QCh	Stereo complex crystallite (SC) membrane containing poly (lactic acid) QCh were employed to design disinfectant wound dressing material.	The enhanced thermal and mechanical properties of developed SC membrane owing to restricted mobility of lactide chains. They have better wound healing capacity (100% in 15 days). This biomass-based membrane was multifunctional as it has antioxidant, antibacterial and wound healing efficacy.	[60]
Silica coated poly (vinylidene)	High-performance anion exchange silica-coated	The surface of silica-coated poly (vinylidene) fluoride was grafted with quaternized chitosan to	[61]

Components	Purpose	Research Outcomes	References
fluoride and QCh	(vinylidene) fluoride along with QCh nanofibrous membrane were designed.	pursue dual action, i.e., ion exchange and strong reinforcement substrate. QCh-impregnated nanofibers showed superb mechanical strength (11.9Mpa). Adorned positive charges created channel-like ion transport channels that could efficiently serve as anion exchange membrane.	

that can sulphate due to swelling. Hydrogel-based dressing materials provide several advantages, including ample absorption of tissue exudate, maintenance of optimum moisture content at the site and encouraging cell proliferation (**Table 3**). Antibacterial hydrogels are highly required in the health care sector to accelerate the wound healing cure rate. In this context, Xiao et al. (2020) prepared hydrogel consisting of QCh, chemical cross-linker 'polyacrylamide', and silver nanoparticles. The developed hydrogel exhibited desirable tensile strength (approximately 100 kPa) with a shear stress of 10^4 Pa. Excellent swelling capacity, a synergistic antibacterial effect, and low toxicity of Ag-mediated hydrogel reinforced the designing of appropriate wound dressing materials [62].

Table 3. Biomedical utility of QCh-derived hydrogels.

Objective	Components	Research Highlights	References
Multifunctional QCh-based polyacrylamide hydrogel was developed that contained hemostatic and skin adhesive properties	QCh, Matrigel-polyacrylamide	The developed hybrid hydrogel had a three-dimensional microporous integrity and exhibited high mechanical strength and good adhesiveness with low toxicity. The outcomes from the histology study demonstrated improvement in wound healing, collagen deposition, and stimulation of skin adnexal regeneration. The developed QCh-based antibacterial hydrogel demonstrated promising potential for designing wound dressing materials.	[63]
Dual crosslinked QCh-clindamycin loaded	QCh, clindamycin	The developed nanocomposite-embedded hydrogel withstood sufficient mechanical	[64]

Objective	Components	Research Highlights	References
hydrogel was prepared to manage methicillin-resistant *S. aureus* (MRSA) bacteria		and injectable efficiencies. The system responded on variable pH that enabled maximum interaction with MRSA bacteria (90% killed) in acidic conditions and overcame the antibiotic resistance challenge.	
A novel wound dressing-based injectable hydrogel was designed employing QCh and PLEL (PLEL-nBG-QCS-C) hydrogel to promote angiogenesis.	QCh and PLEL [Poly (D, L-lactide)-poly (ethylene glycol)-poly (D,L-lactide)] and bioactive glass	PLEL hydrogels preloaded with bioactive glass ($CaO-SiO_2-P_2O_5$) could efficiently seal the broken skin and increase the cure rate of wounds. Additionally, they were thermosensitive, tissue adhesive, and antibacterial.	[65]
QCh-based timolol maleate thermosensitive hydrogel was prepared for improved ophthalmic disorders.	Timolol maleate, Sodium hydrogen carbonate, QCh	The developed transparent thermosensitive hydrogel presented desirable porosity, swelling index, and biodegradability. The addition of sodium hydrogen carbonate enabled enhanced thermosensitivity to the system. In vitro drug release revealed the initial burst release in early hours followed by controlled release of timolol maleate for a week. This supported the potential use of the developed hydrogel for glaucoma management.	[66]
Dopamine-gelatin-crosslinked QCh	Dopamine, QCh, Metronidazole, gelatin	The formulated injectable hydrogel exhibited sufficient rheological parameters.	[67]

Objective	Components	Research Highlights	References
injectable hydrogel was prepared to localize delivery for the combat of Parkinson and associated inflammation as well.		The cytocompatibility of hydrogel revealed the cell viability and proliferation of L929 fibroblast cells. In vitro study exposed localized release of both dopamine and metronidazole.	
QCh-based pH-sensitive veterinary hydrogel vaccine for improved cellular and humoral responses.	QCh, Montanide™ ISA206 and glycerophosphate	The developed hydrogel was biocompatible, safe, and had efficiencies to adsorb inactivated porcine reproductive and respiratory syndrome virus. Moreover, the system ruled out the downsides of mineral oil side effects and encouraged immunogenicity.	[68]
Development of NQC-loaded thermostable and multifunctional hydrogel	N-quaternized chitosan (NQC), poly vinyl alcohol, glutaraldehyde	Different hydrogels on varying concentration of NQC and PVA were designed to modify metal ion uptake, swelling capacity, compatibility, and antibacterial efficacy.	[69]

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exhibited an adsorption capacity for phosphate (97.5%) and nitrate ions (99%) that followed Freundlich isotherm model. The presence of common ions such as chloride, sulphate and bicarbonate did not alter the sorption capacity of quaternized chitosan beads [71].

The literature envisages that polycationic ammoniated polymers are fascinating options for the adsorption of negatively charged ions and sulphated polysaccharides through electrostatic interaction. In this regard, QCh has been explored for capturing anions, even at high pH. Eskandarloo et al. (2018) proposed the formulation of quaternized chitosan/polystyrene microbeads (CS/PS) for the selective adsorption of heparin, an anticoagulant from porcine intestinal mucosa sample. The comparative adsorption efficiency of CS/PS microbeads and marketed Amberlite FPA98 Cl resin was evaluated utilizing a heparin-bovine serum albumin model in pH range 4.1–9.2. The outcomes depicted superior adsorption efficiency of CS/PS micro beads (2.84 mg/g) and could be regenerated after treating with sodium chloride solution. Furthermore, the recovered microbeads can be reused for adsorption of heparin without any loss of adsorption capacity. Moreover, CS/PS microbeads could adsorb heparin from real biological sample containing heparin [72].

3.6.4. Nanoparticles

Splendid accomplishments have been anticipated through chitosan-based nanoparticles for the management of different diseases over past decade. These biodegradable and biocompatible nanoparticles are not only exhibit improved solubility, site specific/localized action but also minimize undesirable toxicity. Highly demanded chitosan is frequently explored as a carrier in drug delivery systems, fabrication of wound dressing materials, management of skin regeneration and tissue engineering. Quaternized chitosan-based nanoparticles (QCh NPs) have attracted wide interest owing to their exclusive physicochemical and biological features. Chemical modification such as grafting, functionalization, Schiff base formation and quaternization are few strategies that expand physicochemical and biological features of chitosan. Quaternization of chitosan significantly improves the aqueous solubility in neutral pH hence enhances the diffusion of drug moiety across biological membrane in neutral/alkaline physiological conditions. The positive charge facilitates pronounced mucoadhesiveness, antimicrobial activity, biocompatibility, and biodegradability as well as widening its biomedical applications. Numerous trimethyl, triethyl, dimethyl ethyl, and N-(2-hydroxy-3 trimethyl ammonium) propyl derivatives of quaternized chitosans have been discussed in **Table 4** as potential carriers for the transportation of proteins, genes, vaccines, and chemotherapeutics at the target site [73][74].

Table 4. QCh-based nanoparticles and their biomedical applications.

Objective	Components	Research Highlights	References
Ketoconazole was entrapped in QCh NPs for superior antifungal activity	Ketoconazole, QCh, sodium triphosphate	Nanoscaled KCZ-QCSNPs displayed superb entrapment efficiency (~90%). Performed tube dilution method revealed preeminent antimicrobial activity.	[75]
QCh derivative ‘HTCC’ NPs were embedded in various fabric materials to evaluate antimicrobial efficacy.	HTCC, cotton fabric, polyester, polyacrylic acid	The developed HTCC nanoparticles embedded in cotton fabric exhibited superior antimicrobial action against *Fusarium oxysporum* and *Bacillus subtilis* compared to polyester and mixture of cotton.	[76]
Anthrax vaccine adjuvant containing Fucoidan-HTCC nanoparticles were developed to improve rapid induction of immunity	Sulphated polysaccharide (Fucoidan, FUC) and HTCC	An active complexation between opposite-charged FUC and HTCC was conducted through varying their mass ratio. MTT assay on L929 or JAWS dendritic cells evaluated low cytotoxicity, improved cellular internalization and high cell viability.	[77]

Objective	Components	Research Highlights	References
		Combination of FUC-HTCCNPs and anthrax vaccine adsorbed (AVA) significantly improved magnitude of cellular/humoral immunity and mice survival rate compared to administration of AVA alone.	
Nanoparticles containing N-2-HTCC and N,O-CMC encapsulated vaccine antigens (IBV/H120) were developed for significant increments in lymphocytes, interleukins, and interferon in chicken	N-2-HTCC, N,O-carboxy methyl chitosan (CMC), infectious bronchitis virus (IBV)/H120 and Newcastle disease virus (NDV)	The developed nanoparticles, i.e., N-2-HTCC-CMC/NDV/IBV, predicted great stability and low cytotoxicity on storing at 37 °C for 3 weeks. In vivo assay on chicken revealed sustained release of both NDV and IBV with enhanced release of IgG and IgA that facilitated the proliferation of immune modifiers in chicken body. The developed QCh-based NPs showed the potential to combat respiratory diseases in chicken.	[78]
Ecofriendly QCh derivative HTCC nanoparticles were designed to increase the durability and microbial resistance of *Antheraea pernyi* silk fabric.	HTCC and 1,2,3,4 butane tetracarboxylic acid, sodium hypophosphite	The conventional dip-and-dry-cure method was applied to evaluate silk fabric durability (*A. pernyi*). Wrinkle resistance, microbial resistance (against *S. aureus* and *E. coli*) and shrinkage resistance were observed even after washing *A. pernyi* silk fabric more than 50 times.	[79]
5-flurouracil (5-FU) embedded HTCC NPs developed for improved entrapment efficiency and in vitro release	5-FU, HTCC, sodium tripoly-phosphate (TPP)	5-FU/HTCC NPs were prepared through ionic gelation method via electrostatic interaction between positive-charged HTCC and negative-charged TPP. Encapsulated 5-FU exhibited controlled release profile in pH 7.4 buffer.	[80]

superior mechanical properties (tensile strength), improved ionic absorption, enhanced antimicrobial action owing to their enormous surface-to-volume ratio (surface area), smaller size, and higher dispersion in given media. Abdel-Aziz et al. (2020) have developed a novel antituberculosis delivery system composed of N,N,N-trimethyl chloride (TMC)/Ag nanocomposite synthesized through a one pot green route. Synthesized nanocomposite (11–17 nm) system has exhibited promising antimycobacterial action (MIC 1.95 µg/mL). The observed antitumor activity displayed less toxicity (IC_{50} 357.2 µg/mL) for normal (WI 38) lung cells and preeminent growth inhibition for A549

cancerous cells (IC_{50} 12.3 µg/mL) [81]. Luo et al. (2015) initially synthesized nanocomposites from chitosan/montmorillonite resin and quaternized modifier 2,3-epoxypropyltrimethyl ammonium chloride chitosan/montmorillonite resin. The developed quaternized chitosan-containing nanocomposites were small, spherical, smooth, dense, and exhibiting good dispersibility in water. The adsorption study performed on methyl orange revealed that quaternized chitosan/montmorillonite was strongly adsorbed compared to without montmorillonite, and thus can be a prospective material for column packing and wastewater treatment [82]. In this series, mechanical and ionic conductive properties of QCH functionalized carbon nanotube membrane matrix were evaluated. Improved dispersion of carbon nanotube promoted the load transfer and assisted hydroxide ion exchange through the membrane matrix. Reduced ionic conductivity and modified tensile strength indicated the potential application in preparation of anionic exchange membrane fuel cells [83]. Similarly, Gong et al. (2019) developed a layered double hydroxide ion conductor composed of QCH/PVA and carbon nanotubes. The system exhibited 1.57-fold enhanced tensile strength, 47 mS/cm^2 ionic conductivity at 80 °C, and displayed a good reinforcing property [84].

QCh nanoparticles are highly acclaimed for designing oral drug delivery systems by virtue of significant drug diffusion and improved penetration across the epithelial barrier. Several ‘bottom up’ methods for nanoparticles development are widely emphasized, including emulsion droplet coalescence, ionic gelation, the reverse micelle method, self-assembly, chemical alteration, coacervation, and precipitation. Methods such as milling, ultrasonication, and high-pressure homogenization listed as ‘top down’ are also employed for nanoparticles synthesis [85]. Omar et al. (2021) developed novel oral drug nanocarriers composed of quaternized aminated chitosan and curcumin to enable the slow release of curcumin at the site of colon.

3.6.6. Vaccine Adjuvants

Vaccine adjuvants are essential components that modify vaccine potency through encouraging cell-mediated or humoral immune responses via vaccine antigens. An ideal adjuvant should have the efficacy to solubilize antigens, facilitate transportation across mucosal barrier and potential for encouraging systemic and mucosal immunity. Chitosan and chitosan derivatives are fascinating candidates for vaccine adjuvants by virtue of their remarkable physicochemical (solubility, stability, biodegradability) and biological values (cytocompatibility, non-toxicity, antimicrobial). Reports cited that quaternized chitosan have greater potential to induce antigen-presenting cells (APCs), encourage cytokine stimulation and produce preferred humoral, cellular, and mucosal immune responses [86].

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