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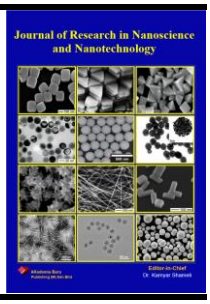

Cross-linked Chitosan-Based Hydrogels Nanocomposites for Treatment of Disease

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ABSTRACT

Chitosan nanoparticles can be used in many types of applications such as food packaging, cosmetics and biomedical field. Chitosan can be modified to form chitosan-based hydrogels for antiviral purpose. Chitosan-based hydrogels are commonly used in wound healing, tissue engineering and drug delivery. This study explains about the properties and the applications of chitosan-based hydrogels since there were not much paper or research about chitosan-based hydrogels used as antiviral agent. Usually, many researchers did some studies about antiviral application focusing only on chitosan itself but not specifically into chitosan-based hydrogels. The properties of chitosan are also not well explained in some research. The purpose of this study is to investigate the antiviral application of chitosan-based hydrogels based on chitosan properties.

Keywords: Chitosan, nanoparticles, hydrogels, antiviral application.

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1. Introduction

Hydrogel characterised as a polymeric structure where the chains are connected by non-covalent and to form a three-dimensional network. These structures have the ability to absorb significant quantities of water, which causes the structure to swell. This induces electrostatic repulsions that can facilitate the swelling of its structure [1]. Hydrogels are formed primarily by electrostatic interactions with the hydroxyl groups located at carbon-3 and carbon-6 and the amine group located in the position carbon-2 of the one units. Therefore, it appears to form cross-linked three-dimensional structures with aldehydes which are used, for example, to grow proton conductivity membranes that may be used in fuel cells [2]. As chitosan is a biodegradable polymer, these hydrogels were used for the preparation of biodegradable sutures, hemodialysis membranes and wound healing [3].

Biopolymer are polymer of biomolecules that contain one units that are covalently bonded to become bigger molecules. Materials derived by synthetic chemistry from surrounding such as vegetable oils, sugars, fats, etc. can identified as biopolymers [4]. Based on application, biopolymers may be labelled as bioplastics, biosurfactants, biodetergents, bioadhesives, biofloculants, and so on [5]. Crosslinking is well known as the typical method to overcome the biomaterials limitations [6,7]. Cross-linkers bind molecules, enhance molecular weight, and give better mechanical properties and stability in general. However, crosslinking reduces degradability, deceases the availability of functional groups in the crosslinked polymer. Thus, resulting in subsequent processing challenges and a potential rise in cytotoxicity [7]. In 1811, chitin is considered as the second highest of polysaccharides on the Earth, being first describe by Henri Braconnot [8]. It occurs in Nature as ordered macrofibrils in the exoskeleton of mollusks and crustaceans, as well as in fungi and insect cuticles [9]. Its natural abundance that makes it possible to obtain more than 1000 tonnes per year and marine organisms are the major sources which is about 70% [3]. To form chitosan nanoparticles, there are three ways of chitosan crosslinking method which are chemical crosslinking, radiation crosslinking and physical crosslinking [10]. For chemical crosslinking method, chemical crosslinkers are glutaraldehyde, genipin, glyoxal, dextran sulfate, 1,1,3,3-tetramethoxypropane, among others [11]. Next, radiation crosslinking method does not need heat or catalyst which means no additional toxic is involved. Researchers have used radiation polymerization to create IPNs for drug delivery purposes [12-15]. By putting azide and lactose moieties on chitosan via a condensation reaction, photo-cross-linkable chitosan was created [16]. In contrast to chemical cross-linking, which is achieved through covalent bonding, physical cross-linking is achieved by the use of cross-linkers that establish ionic contacts between the polymer chains. Pentasodium tripolyphosphate (TPP) and calcium chloride are two well-known examples of chitosan physical cross-linkers [10].

The inclusion of nano-reinforcements into the chitosan matrix has shown to be a powerful strategy for overcoming biopolymer's traditional disadvantages. Food industries, medicine, biotechnology, agriculture, cosmetics, textiles, environmental protection, and other industries could benefit from chitosan-based nanocomposites [17-20]. Chitosan content in composites is often kept high, resulting in products with good bioactivity and biocompatibility. Improved mechanical and barrier qualities, thermal stability, and transparency are among the additional benefits [21]. New drug delivery methods have aimed to concentrate the quantity of chemotherapeutic drugs at the affected site (targeted delivery) while reducing their systemic dissemination. Chitosan nanocomposites have played a critical role in these sectors by providing a non-toxic, biocompatible, stable, target-specific, and biodegradable delivery system [22]. Antiviral and antibacterial polymers come in a variety of forms, ranging from natural biopolymers with inherent antiviral/virucidal capabilities to manufactured thermoplastic elastomers. Sulfonic functional groups in antimicrobial polymers and polysaccharides like glutamine and fluctosan perform well against virus adherence [23]. Hydrogels are frequently regarded as biocompatible, and they can be utilized to contain antiviral inorganic elements or synthetic polymers [24,25]. Nature can be an inspiration in the development of antiviral techniques, and naturally derived biopolymers could be an appealing choice as a complement to the finding of naturally occurring

antibiotics such as antimicrobial peptides (AMPs). Natural goods may be able to provide adaptable and tuneable designs to combat a wide range of viruses. Some natural bacteriostatic compounds have antiviral properties and can be employed as antiviral/virucidal drug carriers in an indirect manner [26-29].

In this review paper, the application of chitosan towards viral diseases treatment will be studied and also the properties of cross-linked chitosan nanoparticle based hydrogel as drug delivery system.

2. Chitosan sources and their properties

In 1811, chitin is considered as the second highest of polysaccharides on the Earth, being first describe by Henri Braconnot [8]. It occurs in Nature as ordered macrofibrils in the exoskeleton of mollusks and crustaceans, as well as in fungi and insect cuticles [9] and it is shown by Table 1. Its natural abundance that makes it possible to obtain more than 1000 tonnes per year and marine organisms are the major sources which is about 70% [3]. Although chitin can be found in huge amounts in nature from a variety of sources, chitosan can only be found in small amounts in nature, such as in some fungus. Chitosan is primarily produced from chitin through chemical or enzymatic treatments in industrial or research purposes [30].

Table 1. Chitin and chitosan resources [9].

Microorganisms	Sea Animals	Insects
Yeast (β-type)	Crab	Beetles
Spores	Krill	Scorpions
Mycelia penicillium	Prawn	Spiders
Brown algae	Shrimp	Ants
Blastocladiacease	Lobster	Brachiopods
Green algae	Mollusca	Cockroaches
Fungi (cell walls)	Annelida	-
Chytridiaceae	Coelenterate	-
Ascomydes	Crustaceans	-

2.1 Chitin and Chitosan Molecular Structure

Chitosan is a chitin-derived polysaccharide. Depending on the source of chitin, its molecular weight ranges from 300 to 1000 kDa. Chitin is the world's second most abundant natural polymer after cellulose. It can be found in crustaceans like shrimp and crabs [31,32]. Chitosan is a copolymer comprising D-glucose amine and acetyl-D-glucose amine. Chitosan, a linear and semicrystal polymer [33, 34] with at least 60% of its glucose amine residue deacetylated (which corresponds to a deacetylation degree of 60). Chitin deacetylation is accomplished either chemical hydrolysis under harsh alkaline conditions or enzymatic hydrolysis in the presence of certain enzymes, such as chitin deacetylase [35, 36]. Figure 1 shows the chemical structure of chitin made up of 1-4 linked 2-acetamido-2-deoxy-β-D-glucopyranose.

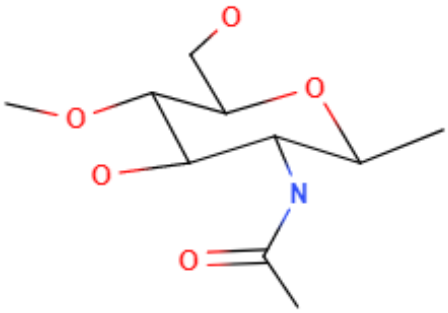

Figure 1. Chemical structure of chitin.

Chitin contain three different crystalline allomorphs which are α-, β- and γ-forms with each of these differ in micro-fibrils orientation [37]. The most typical type of chitin is α-chitin. Its unit cell made up of N, N′-diacetylchitobiose units arranged in two antiparallel strands. As a result, adjacent polymer chains are kept together by 06-H→06 hydrogen bonds, while the chains are maintained in sheets by 07→H-N hydrogen bonds [38-40]. This results in a probabilistic combination of -CH2OH orientations that can form intermolecular and intramolecular hydrogen bonds, corresponding to half of the oxygen atoms on each residue. This gives out in two types of amide groups which half of the amide groups act as acceptors for 06-H→O=C intramolecular hydrogen bonds, while the other half form interchain C=O→H-N bonds. If the polymer chains are allowed to crystallize, they ultimately self-assemble into microfibrils [41]. Chitosan is distinguished from chitin by the presence of amino groups, which is reflected in its solubility in dilute acids (pH 6), as well as its ability to form complexes with metal ions, allowing it to be used for waste water treatment and purification [33,34]. Chitin, on the other hand, has very few practical applications due to its low solubility, if any [42]. Surprisingly, chitosan's aqueous solubility is pH dependent, allowing it to be processed under mild conditions [43]. Chitosan is a deacetylated form of chitin that contains at least 50% free amine. It has a heterogeneous chemical structure that includes both 1-4 linked 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose (shown in Figure 2).

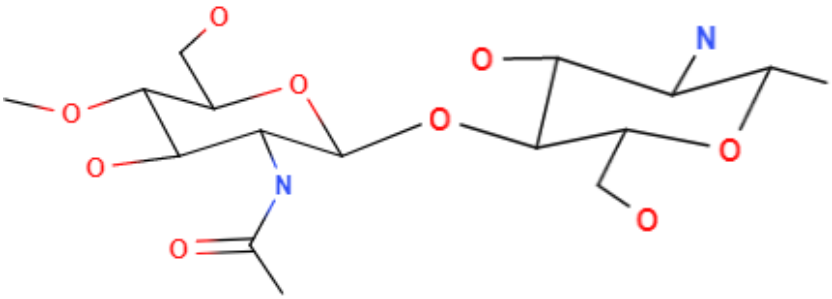

Figure 2. Chemical structure of chitosan

2.2 Chitosan Derivatives

Chitosan can undergo many types of chemical reactions including hydroxylation, carboxylation, alkylation, acylation and esterification due to active hydroxyl and amino groups. These chemical reactions add pendant groups into chitosan, disrupting its crystal

structure and boosting the solubility of the modified chitosan as a result. These chitosan derivatives have improved physicochemical and biological properties, making them more suitable for use as biomedical carriers [44]. Table 2 shows the chitosan derivatives and its cross linker. Chemical modification can easily improve the structural properties of chitosan for a specific application. Chitosan contains hydroxyl, acetamido, and amine functional groups which made chitosan is accessible to chemical modification. As a result, chemical alterations would not alter the chitosan skeleton, preserving the original physicochemical and biological features while introducing new or enhanced ones [45].

Table 2. Chitosan Derivatives.

Chitosan Derivatives	Cross-linker	Chitosan nanoparticles	References
ZnO-Chitosan	Zinc oxide	ZnO-Chitosan	[46]
Chitosan-triphosphate (SPIONPs-CS)	Pentasodium triphosphate (TPP)	Chitosan-triphosphate (SPIONPs-CS)	[47]
Chitosan esters	Sulfuryl chloride	Sulfated Chitosan	[48,49,50,51,52]
Carboxylated Chitosan	Monocholoroacetic acid	Carboxymethyl Chitosan	[53,54,55]
Alkylated chitosan	Sodium borohydride	N-alkylated Chitosan	[56,57]

2.2.1. ZnO-Chitosan Composites

In adsorptive separation and purification, commercial sorbents such as activated carbon, zeolites, activated alumina, and silica gels play key roles. Shafiq, et al. recently used the sol-cast transformation process to synthesize chitosan composites with various ZnO concentrations. Chemical interactions between chitosan and ZnO in composites become increasingly apparent at higher filler concentrations. Compared to chitosan, the composites had a much lower degradation rate and improved heat stability. Biocidal activity against gram positive and gram negative bacteria was observed in these mixtures [46].

2.2.2. Chitosan-triphosphate Nanoparticles

The most frequent approach for producing a medicinal product with desired properties is ionotropic gelation. Sanjai, et al. used the ionotropic gelation process to encapsulate superparamagnetic iron oxide nanoparticles (SPIONPs) at varied concentrations within chitosan-triphosphate (SPIONPs-CS). The capacity of polyelectrolytes counter ions to cross link to create hydrogels is the basis for ionotropic gelation. The utilization of naturally occurring polysaccharides such chitosan as biopolymers has risen in new areas such hydrogel sustained release formulation, resulting in an environmentally friendly pharmaceutical product development process [47].

2.2.3. Chitosan Esters

Some of the oxygen-containing inorganic acids (or their anhydrides) on the chitosan molecule are used for esterification of chitosan. In the realm of biology, sulfated chitosan has a wide range of applications as a substitute for heparin or heparin sulfate, including anticoagulant and antiviral medications, to stimulate osteogenic differentiation and particular protein binding [48-50]. Sulfated chitosan has the same regulatory mechanism as heparin. In vivo investigations reveal that violent reactions with specialized cells and biologically active chemicals affect the functioning of proteins and cells [51,52]. High-strength fibers can be made from chitosan derivatives produced through esterification.

2.2.4. Carboxylated Chitosan

Carboxylated chitosan processes usually involve both the –NH2 and –OH groups in order to obtain carboxyl-modified chitosan derivatives. Glyoxylic acid can be used to perform carboxylation. To obtain carboxymethyl chitosan, chitosan has been treated with monochloroacetic acid under various circumstances. The degree of carboxymethylation and the conditions of modification affect the water solubility of carboxymethyl chitosan [53]. Not only does carboxylation improve chitosan's water solubility, but it also produces amphiphilic chitosan derivatives with both –NH2 and –COOH groups. These compounds have strong water solubility and surface activity, as well as film formation, moisture absorption, moisture retention, antibacterial [54], antioxidant [55], and other biological properties, making them valuable in cosmetics, food, and medicinal applications.

2.2.5. Alkylated Chitosan

Chitosan alkylation can involve both the functional groups –NH2 (amino) and C3, C6–OH (hydroxyl). Reactions involving the amino group, on the other hand, occur at a faster rate than those involving hydroxyl groups, and they also protect particular functional groups better. As a result, chitosan alkylation takes place primarily through the amino group, resulting in N-alkylated chitosan derivatives [56]. Chen developed a variety of N-alkylated chitosan compounds. N-alkylated chitosan exhibits good biocompatibility, according to hemolysis and toxicity tests. N-alkylated chitosan demonstrated better hemostatic activity than unmodified chitosan in in vitro blood coagulation assays [57]. The addition of alkyl groups reduces hydrogen bonding between chitosan molecules, making the modified alkylated chitosan more water soluble and promising in biological applications.

2.3 Chitosan antiviral and biomedical application

Chitosan based derivatives were commonly used in biomedical application due to their biomedical properties. Table 2 shows the summary of biomedical application of chitosan based derivatives.

3. Application of Chitosan

3.1. Tissue Engineering

Tissue engineering is the process of fabricating tissue substitutes for implantation into the body using living cells that are altered and controlled by their extracellular environment.

[58,59]. Tissue engineering's primary goals are to repair, replace, maintain, or improve the function of a particular tissue or organ [60]. Chitin-based materials that can be manufactured into tubular forms have been successfully used as a template for cells in tissue engineering of nerves and blood arteries [61-64]. Chitin-based scaffolds are adaptable and can be used for a variety of regenerative applications [65]. Chitin and chitosan have been used to make polymer scaffolds in tissue engineering with great success. High porosity (with an acceptable pore size distribution); biodegradability (degradation rate should match the pace of neo-tissue synthesis); and structural integrity are some of the core parameters for designing polymer scaffolds (to prevent the pores of the scaffold from collapsing during neo-tissue formation); biocompatibility; interacting with cells to enhance cell adhesion; stimulating cell function (proliferation, migration, and differentiation) [66].

3.2. Wound Healing

Madhumathi et al. (2010) created a chitin/nanosilver composite scaffold for wound healing. These scaffolds were discovered to have antibacterial and blood-clotting properties against *S. aureus* and *E. coli*. These characteristics have made them useful nanostructures for wound healing. Sudheesh Kumar et al. (2010) used a -chitin hydrogel containing silver nanoparticles to build and characterize -chitin/nanosilver composite scaffolds for this application. Furthermore, Vero cells were used to test these scaffolds for cell adhesion capabilities, and the results suggested that nanosilver integrated chitin scaffolds were appropriate for wound healing applications [66].

3.3. Cancer Diagnosis

Semiconductor nanocrystals (or quantum dots) can be bioconjugated to a variety of biological recognition ligands and used in immunostaining and bioimaging of malignant cells and tissues to replace standard organic fluorescent dyes. Quantum dots, on the other hand, contain heavy metals such as cadmium sulphide, cadmium selenide, zinc selenide, and can be cytotoxic and even dangerous. For targeted cancer imaging, a heavy-metal-free luminous quantum dot (QD) based on doped-zinc sulphide (ZnS) was created and coupled with a cancer-targeting ligand, folic acid (FA). Folate receptors are overexpressed on many cancer cells, and when they interact with folate-conjugated nanoparticles, they can promote receptor-based endocytosis, allowing for intracellular uptake. Mannose receptors, on the other hand, have been utilized to diagnose cancer [66]. Manjusha et al. created a unique FA-conjugated carboxymethyl chitosan (CMCS) that was coordinated to manganese doped zinc sulphide (ZnS:Mn) quantum dots (FA-CMCS-ZnS:Mn) to generate composite nanoparticles [67].

3.4. Antitumor activity

In vitro experiments and in vivo animal models, chitosan and its derivatives have shown anticancer efficacy. Tokoro et al. [68] proposed that this activity was induced by an increase in interleukin-1 and 2 secretion, which resulted in the maturation and infiltration of cytolytic T-lymphocytes into the tumor. Dass and Choong [69] also showed that chitosan

could boost lymphokine synthesis and boost the proliferation of cytolytic T-lymphocytes [70].

3.5. Vaccine Adjuvant

Intranasal chitosan glutamate has been shown to greatly improve antibody responses to a diphtheria antigen (a non-toxic cross-reacting variant of diphtheria toxin, CRM197) in mice and guinea pigs [71]. Mills et al. looked at how this chitosan adjuvant affected the effectiveness of a diphtheria vaccine in healthy human volunteers [72]. Mann et al. used ferrets as a preclinical model for human influenza and conducted a live-virus challenge investigation in them. Both chitosan glutamate (CSN) and N, N, N-trimethylated chitosan (TM-CSN) were tested as adjuvants for an inactivated NIBRG-14 (H5N1) subunit antigen (derived from influenza antigen A/Vietnam/1194) [73,74].

3.6. Antibacterial/antimicrobial properties

In the presence of chitosan at concentrations greater than 0.025 percent, E. coli growth was reduced. Other microbial species, such as Fusarium, Alternaria, and Helminthosporium, are also inhibited by chitosan. Chitosan's cationic amino groups bind to anionic groups in microbes, preventing their development [75]. After entering bacterial cell walls, low-molecular-weight chitosan can bind to DNA and impede DNA transcription and mRNA production [70]. Chitosan derivatives are used in commercial disinfectants and topical antimicrobials due to their natural antibacterial properties [76,77].

3.7. Antiaging cosmetics

Morganti et al. [78] created block-copolymer nanoparticles (BPN) that were made up of phosphatidylcholine and linoleic acid nanocomplexed with hyaluronan and chitin nanofibrils (PHHYCN) Thee nanoconstructs will encapsulate cargos containing cholesterol, creatine, caffeine, melatonin, vitamins E and C, and the amino acids glycine and arginine. Because all of the individual chemicals had shown some activity in this area, the goal was to use these nanocarriers for skin rejuvenation. After one month of therapy, skin treated with active chitin nanofibrils carrying loaded BPN was shown to be softer and more moisturized. Both fine wrinkles and crease lines were reduced during the first 15 days of therapy with injectable active chitin nanofibril containing BPN, as well as the presence of telangiectasia, resulting in a significant improvement in the overall appearance of the face during the regression period [78].

Table 3. The biomedical application of chitosan.

Application	Chitosan	Ref
Tissue engineering	Chitin-based scaffolds	[58-66]
Wound healing	Nanosilver integrated chitin scaffolds	[66]
Cancer diagnosis	FA-conjugated carboxymethyl chitosan (CMCS)	[66,67]
Antitumor activity	hexa-N-acetylchitohexaose (NACOS-6)	[68,69,70]

Vaccine adjuvant	Chitosan glutamate (CSN) and N,N,N-trimethylated chitosan (TM-CSN)	[71,72,73,74]
Antibacterial/antimicrobial properties	Water-soluble chitosan (chitosanglucosamine derivative)	[70,75,76,77]
Antiaging cosmetics	Block-copolymer nanoparticles (BPN)	[78]

4. Cross-linked Chitosan Nanoparticles

4.1. Significance of Crosslinking

Depending on the degree of cross linking and the presence or lack of crystallinity, adding cross-links between polymer chains changes the physical properties of the polymer. According to Maitra & Shukla (2014) [79], the outcome of cross-linking is:

i) Elasticity

Limited cross-linking produces elastomers, which are elastic polymers. However, when the number of cross-links rises, the polymer becomes more rigid and can no longer stretch; the polymer becomes less viscous, less elastic, and possibly brittle. The introduction of short chains of sulfur atoms that join the polymer chains in natural rubber, for example, causes vulcanization or sulfur curing. Short sulfur atom chains act as bridges, connecting one polyisoprene chain to the next until all of the chains are linked into one enormous super molecule. Vulcanization is a sort of cross-linking that increases the strength of rubber through a chemical process. It makes rubber a tough and long-lasting substance used in automotive and motorcycle tires.

ii) Decrease in the Viscosity of Polymers.

Cross-linking stops the chains from moving past each other, which is required for polymers to flow. The creep behaviour improves as a result of the restriction in flow.

iii) Insolubility of the Polymer.

Cross linking results in insolubility as the chains are tied together by strong covalent bonds. Crosslinked materials can't dissolve in solvents, but can absorb solvents. Crosslinked material after absorbing lot of solvent is called a gel. For example crosslinked polyacrylamide gel. Uncrosslinked polyacrylamide is soluble in water, and crosslinked polyacrylamides can absorb water but is insoluble. Water-logged gels of crosslinked polyacrylamides are used to make soft contact lenses.

iv) Increased T_g and Increase Strength and Toughness.

Crosslinking alters local molecule packing, resulting in a reduction in free volume and a rise in T_g. The glass transition temperature of PVA crosslinked with boric acid was enhanced [80]. PVA molecular mobility is slowed by cross-links, which must not be present in the crystalline domains.

v) Lower Melting Point

There is a decline in crystalline behaviour for crystalline polymers with low degrees of cross linking, as cross linking causes impediment to chain orientation, resulting in softer, elastic polymers with lower melting points.

vi) Transformation of Thermoplasts into Thermosets

Thermoplasts become thermoset plastics after extensive cross-linking. The shape of the polymer cannot be modified once the cross-links have formed without harming the plastic. Unlike thermoplastic polymers, the process cannot be reversed by reheating; instead of being moldable and pliable, thermoset plastics begin to degrade. Polyisoprene was the first thermoset. The more sulfur crosslinks that are added to polyisoprene, the stiffer it becomes. It's a flexible rubber with a light crosslinking. When heavily crosslinked, it hardens into a thermoset.

4.2 Preparation of Cross-linked Chitosan Nanoparticles

Chitosan NPs can be made in a number of ways, including the 'bottom up' method, the 'top down' method, or a mixture of the two. Ionotropic gelation, microemulsion method, solvent evaporation, polyelectrolyte method are examples of "bottom up" procedures, while milling, high pressure homogenization, and ultrasonication are examples of "top down" methods [81].

4.2.1. Ionotropic Gelation Method

The cationic chitosan amino groups are crosslinked to a polyanionic crosslinker in this approach. Aqueous acidic chitosan solution is made and dropped into a tripolyphosphate (TPP) solution while stirring continuously at a consistent rate. Because TPP is anionic, it forms crosslinks with chitosan, resulting in chitosan-TPP NPs. This complex entraps and transports pharmaceuticals, and these nanocarriers can then be evolved into effective delivery systems. This method has been reported to produce chitosan-NaF NPs [82]. The use of an aqueous medium reduces the dangers and toxicities associated with the use of an organic solvent in this approach. The mechanical strength of the NPs produced by this approach is limited [83].

4.2.2. Reverse Micellar Method/Micro-emulsion Method

A polymer, surfactant, crosslinker (the most usually used is glutaraldehyde), and an organic solvent are employed in this process (n-hexane, toluene). It entails preparing a surfactant solution in an organic solvent of choice, as well as preparing a polymer and crosslinker blend to be added to the surfactant combination. The Schiff reaction is used to crosslink the two solutions in the solvents, followed by the elimination of excess surfactant with calcium chloride, resulting in the desired polymer-crosslinker NPs [83].

4.2.3. Co-Precipitation Method

In this process, chitosan is produced in a low pH acidic solution, then a high pH solution, such as ammonium hydroxide, is added, yielding extremely monodisperse NPs. This approach has been used to make chitosan-coated iron oxide NPs [85].

4.2.4. Emulsion-Droplet Coalescence Method

Crosslinking and precipitation are both used in this process. Two emulsions are made here: (a) To make a water/oil emulsion, aqueous chitosan solution with the medication is added to liquid paraffin oil and swirled at a steady speed. (b) A second water/oil emulsion is created by mixing an aqueous solution of chitosan in sodium hydroxide with paraffin oil. The two emulsions are then combined with high-speed stirring, resulting in emulsion droplet collisions and coacervates, which are then centrifuged and filtered to generate chitosan-drug NPs [86]. This approach has been described to produce dexibuprofen NPs for the treatment of rheumatoid arthritis [87].

4.2.5. Polyelectrolyte Complexation Method

Charge neutralization is achieved through electrical contact between positively charged amine groups of chitosan and negatively charged anions such as the carboxyl group of alginate or the dextran group of dextran sulphate. Charge neutralization and self-assembly of the polyelectrolyte complexes occur when chitosan solution is produced in acetic acid and combined with the anionic solution at room temperature under continuous stirring [88,89]. This approach has been reported to produce chitosan-guar gum NPs for use in bone regeneration therapy [90]. Alginate ionotropic pre-gelation followed by polyelectrolyte complexation with chitosan are claimed to be used to make insulin-loaded NPs for diabetes [91].

4.2.6. Spray Drying Method

This is a well-known method for producing powders, agglomerates, and granules from a solution or suspension containing medication and excipients. In this process, chitosan is dissolved in acetic acid solution, an appropriate crosslinking agent is added to this drug solution, and the resulting solution/dispersion is then atomized with a hot stream of air, resulting in the creation of minute droplets, which evaporate to generate NPs. This approach has been described to produce spray dried inhalable powder containing nanoaggregates for pulmonary administration of anti-tubercular medicines [92]. Ionotropic gelation (according to recent literature, the most favoured process for preparing NPs) is based on an electrostatic contact between the amine group of chitosan and a negatively charged group of anionic polymers. This charge-based interaction of negatively charged drug payloads is more amenable to the ionic gelation method, resulting in high drug encapsulation efficiency, low polydispersity index, and optimal drug release, with entrapment efficiency ranging from 32% to 97% and drug release profiles ranging from 44% to 80% [93]. Polyelectrolyte

complexation, which includes crosslinking chitosan with medicines and leads in delayed drug release, is the second most popular approach. Due to poor entrapment efficiency and low cargo release profile, other methods employed to a lesser extent based on literature data include solvent evaporation, coprecipitation, and emulsion droplet method.

4.3 Properties of Cross-linked Chitosan Nanoparticles

Particle size, polydispersity index (PDI), zeta potential, surface charge, and shape are all morphological parameters that influence the usefulness spectrum of chitosan NPs. Their biological functions are likewise influenced by these characteristics. Their entrapment efficiency and loading capacity aid in the modulation of drug payloads and, as a result, drug-related toxicity. The drug-related toxicity is influenced by the particle size of the NPs. The method of production is important in managing these qualities and even tailoring them to create chitosan NPs for specific applications in a variety of industries. The end-product requirements of particle shape, size, PDI, stability, release kinetics, and toxicity guide the technique selection. The negatively charged crosslinking agent sodium tripolyphosphate interacts electrostatically with chitosan to produce amphoteric ion-pair in the ionotropic gelation technique. This characteristic improves the produced NPs' protein adhesion properties. The chitosan-crosslinker molar ratio regulates the mean diameter of the NPs and, as a result, the drug cargo release kinetics. The size distribution is influenced by the use of a weak acid such acetic acid as a solvent to dissolve the chitosan and the temperature of crosslinking, while the stirring speed helps modify the yield of NPs. Ionotropic gelation is a straightforward approach that uses mild reaction conditions that have little influence on the payload, with researchers tweaking the particle size of the NPs using this method. The limited stability of the NPs in acidic medium and poor amenability for high molecular weight medicines are two drawbacks of this approach [94-96].

Chitosan's physicochemical properties, such as crystallinity, solubility, and degradation, are dictated by its molecular weight and degree of deacetylation, which are determined by the method and process parameters utilized during its manufacture from chitin. Chitosan that has been completely deacetylated has the highest crystallinity, while chitosan that has been partially deacetylated has a semi-crystalline appearance. A higher degree of deacetylation corresponds to a larger positive charge density, which promotes solubility. A higher positive charge also increases interaction with the negatively charged mucin in the mucus membrane, enhancing its mucoadhesive property. Mima et al. found a direct link between the degree of deacetylation and chitosan tensile strength in the wet state, but no effect in the dry condition. In comparison to low molecular weight chitosan, films made from high molecular weight chitosan have better tensile strength and elongation. The rate of degradation is inversely proportional to the degree of deacetylation, which is an important factor to consider when developing chitosan-based scaffolds for tissue engineering applications [97].

4.4 Biomedical application of Cross-linked Chitosan Nanoparticles

The features of chitosan NPs overcome the limits of traditional drug delivery methods, and their ease of synthesis allows for targeted drug distribution with better efficacy. Table 3 shows the biomedical/antiviral application of cross-linked chitosan loaded with drugs.

5. Antiviral Vaccines

Chitosan NPs-based vaccines are transferred by mucosal and systemic routes, and are particularly helpful in mucosal administration because they increase absorption and a mucosal immune response, while chitosan works as an adjuvant in systemic administration. The reason behind chitosan is commonly used as vaccine nanocarrier due to its resistant against enzymatic degradation in the mucosal tissues which then enhances antigen uptake by mucosal lymphoid tissue. Therefore, DNA mucosal vaccines are widely explored. It also shows promise in terms of triggering humoral and cellular immune responses. An attempt was made to construct a vaccine for leishmaniasis by using chitosan NPs containing whole leishmania lysate antigen (WLL) and soluble leishmania antigens (SLA). The aim of this study was to vanquish lack of an appropriate adjuvant which resulted in low efficacy. The study, on the other hand, found no significant difference in the activity of the NPs formulation in inducing a pure Th1-type immune response, and hence did not achieve the targeted result. Fernando et al. found that the produced IBV vaccine (including BR-I genotype strain encapsulated in chitosan NPs (IBV-CS)) was a suitable option for inducing marked IFN gene expression and generation of IgA and IgG anti-IBV antibodies across avian infectious bronchitis virus strains (IBV) [98,99].

Dhakal *et al*. was using pigs to test mucosal immunity and influenza vaccine protective effectiveness. The researchers used chitosan NPs to incorporate dead SwIAV H1N2 (-lineage) antigens (KAg). Pigs vaccinated with this nanovaccine produced more IgG antibodies, as well as robust cross-reactive mucosal IgA and cellular immune responses, as evidenced by enhanced cytotoxic T-lymphocyte, IFN-secretion, and lymphocyte proliferation. This was seen against all strains of the Influenza A virus [100]. Poly-caprolactone/chitosan NPs were tested for hepatitis B by Jesus et al. By secreting powerful anti-hepatitis B surface antigen IgG1 isotype triggered IFN- and IL-17 secretions, NPs demonstrated an adjuvant effect. The induction of a cellular immune response driven by Th1/Th17 was also seen [101].

5.1. Central Nervous System (CNS) Diseases

Chitosan nanoparticles are commonly employed to encapsulate hydrophilic medicines, nucleic acids, proteins, and peptides due to their poor permeability as well as medications that are cleared by the mucosa. Chitosan's cationic nature opens tight junctions of the blood brain barrier (BBB) via two pathways: extracellular and intracellular, allowing medicines to pass through the BBB and blood cerebrospinal fluid barrier. Chitosan's mucoadhesive property improves retention time, which boosts absorption and treatment efficacy. Intranasal delivery of CNS disease medications is usually chosen since it allows for higher drug penetration in the brain and less systemic exposure [102].

According to Jhaveri et al., there are several characterization of chitosan that affect the efficiency of drug delivery to the brain which are:

- The particle size which may affect the endocytosis rate across the brain endothelial cells.
- High positive zeta potential which can leads to better stability and no particle accumulation.
- High efficiency of drug entrapment which give good interaction between chitosan matrix and the drug.
- High resistance to phagocytosis which can reduced toxicity.

Rotigotine-loaded chitosan NPs (RNPs) were fabricated in a study for Parkinson's disease, and an in-vivo and ex-vivo investigation was conducted to compare the effectiveness of RNPs vs. the rotigotine solution. RNPs were found to have enhanced bioavailability and nasal residence. RNPs exhibited a consistent drug release profile, allowing for a reduction in the dosing regimen, which was previously one of the key limitations of rotigotine solution (low plasma half-life) [103]. According to Liu et al., the intranasal drug delivery for epilepsy, developed by carboxymethyl chitosan NPs as a carrier for carbamazepine as an alternative to the conventional intravenous and oral administration of CBZ, showed effective penetration through the BBB and a sustained drug release with pronounced absorption [104]. Galantamine-loaded thiolated chitosan NPs (GNPs) were generated and tested across nasal and orally administered galantamine solutions in a study by Sunena et al. to expand the therapeutic range of galantamine for Alzheimer's disease. When GNPs were tested in mice with scopolamine-induced amnesia, they demonstrated remarkable recovery results [105].

5.2. Infectious Diseases

Antimicrobial indiscriminate usage has been linked to the emergence of drug resistance, and the paucity of new drug candidates in the drug discovery pipeline has fueled substantial efforts to improve the efficacy of existing medications. Current medications have a decreased cellular absorption and a fluctuating plasma drug concentration, resulting in greater dose frequency and side effects. To circumvent these disadvantages, researchers are attempting to encapsulate antimicrobial medicines in chitosan NPs. By enhancing intracellular uptake, minimizing drug efflux, and avoiding biofilm development, this encapsulation helps to decrease the occurrence of drug resistance. Because of its polycationic composition, chitosan's natural antibacterial capability allows it to interact with negatively charged bacterial cell walls and cytoplasmic membranes, resulting in osmotic stability, membrane breakdown, and intracellular content release. It has the ability to penetrate bacteria and inhibit m-RNA and protein synthesis as a result of its affinity for bacterial DNA [102].

Kumar et al. investigated the efficacy of rifaximin-chitosan NPs in the treatment of inflammatory bowel disease. Rifaximin chitosan NPs were found to have enhanced solubility, colon target selectivity, and therapeutic efficacy in this investigation [106]. Sharma et al. created ketorolac tromethamine-loaded chitosan NPs to increase the drug's ocular delivery, which is used to treat post-operative eye irritation, allergic conjunctivitis, and other conditions. The eye's protective mechanism lowers the amount of bioavailable dosage

delivered by traditional administration techniques. Increased bioavailability and ocular residence duration were seen in in vitro drug release, stability tests, release kinetics, and surface morphology, resulting in improved efficacy and therapeutic response [107]. Xu et al. developed vancomycin-loaded N-trimethylchitosan NPs (VCM/TMC NPs) for chronic osteomyelitis, and the results of an in-vivo study showed that osteoblasts increased alkaline phosphatase activity (a biomarker for osteoblast differentiation), indicating target specific delivery and long-term drug release [108].

Table 4. The biomedical/antiviral application of cross-linked chitosan loaded with drugs.

Applications	Diseases	Cross-linked chitosan with drugs	Research finding	Ref
Antiviral vaccines	Leishmaniasis	Chitosan NPs loaded with whole and soluble Leishmania antigens	Found no significant difference in the activity of the NPs formulation	[98,99]
	Influenza	Killed SwIAV H1N2 (δ-lineage) antigens (KAg) encapsulated in chitosan NPs	Produced more IgG antibodies, as well as robust cross-reactive mucosal IgA and cellular immune responses	[100]
	Hepatitis B	Polycaprolactone/chitosan NPs	Provide strong adjuvant effect for hepatitis B antigen	[101]
Central Nervous System Diseases	Parkinson's disease	Rotigotine-loaded Chitosan-TPP NPs	RNPs were found to have enhanced bioavailability and nasal residence	[103]
	Epilepsy	Carboxymethyl chitosan NPs as a carrier to deliver carbamazepine (CBZ-NPs)	Effective penetration through the BBB and a sustained drug release with pronounced absorption	[104]
	Alzheimer	Galantamine-loaded thiolated chitosan NPs	Remarkable recovery results	[105]
Infectious Diseases	Inflammatory Bowel Disease	Rifaximin-chitosan NPs	Enhanced solubility, colon target selectivity, and therapeutic efficacy	[106]
	Post-operative eye inflammation and allergic conjunctivitis	Chitosan-loaded ketorolac tromethamine NPs	Increased bioavailability and ocular residence duration, resulting in improved efficacy and therapeutic response	[107]
	Chronic Osteomyelitis	Vancomycin-loaded N-trimethylchitosan NPs	Osteoblasts increased alkaline phosphatase activity, indicating target specific delivery and long-term drug release	[108]

6. Cross-linked Chitosan-Based Hydrogels

6.1 Hydrogels and its Classification

The word "hydrogel" refers to a water-insoluble polymeric network with a high capacity for water absorption [109-113]. A macromolecular polymer gel made up of a network of crosslinked polymer chains is known as a hydrogel. Hydrogels are made from hydrophilic monomers and a functional crosslinker to facilitate network development. Through molecular entanglements or chemical cross-linking, the polymers such as synthetic polymer, natural polymer, homopolymer or copolymer are employed to create three-dimensional networks [114]. Hydrogels are a promising class of materials for biomedical applications such as drug administration and tissue engineering because of their ability to swell under biological circumstances [79]. Hydrogels have a 3D network structure, which makes them insoluble. Cross connecting can be done physically or chemically. This insoluble cross-linked structure allows active agents and biomolecules to be effectively immobilized and released. Because of their high water content, hydrogels resemble real soft tissues.

Hydrogels can be classified into two types which are natural and artificial polymeric based networks. Polysaccharide or protein chains are frequently used in natural hydrogel constructions. Polysaccharides contain one unique structure which is a hydrophilic structure that makes them ideal for making hydrogels [115]. The example of polysaccharide-based hydrogels is chitin, dextran, hyaluronic acid, cellulose, pectin, alginate hydrogels and chitosan [116-121]. There are also natural hydrogel lattices are formed by protein chains such as silk, collagen, elastin, keratin, resilin and gelatin [122-124]. On the other hand, polyacrylamide, poly (ethylene glycol), poly (vinyl alcohol) and poly (ethylene oxide) are artificial polymers that have been utilized for hydrogel formations [125]. Natural polymers are usually more biocompatible than synthetic polymers because they go through enzyme-controlled biodegradation by human enzymes like lysozyme and form biocompatible byproducts [126]. Synthetic polymers, on the other hand, are chemically stronger than natural polymers due to hydrolysable moieties that degrade at a slower pace. This trait allows for a longer lifespan in the human body [127].

6.2. Synthesis of Chitosan-Based Hydrogels

Chitosan polymers required cross-linking to enhance chitosan features such as stability and durability for pharmaceutical application. The process of chitosan cross-linking and preparation is used to classify chitosan-based hydrogel networks. Chemical and physical cross-linking are the two methods that can produce chitosan hydrogels.

6.3. Chemical Cross-Linking Method

Chemically cross-linked hydrogels are made by covalently connecting chitosan macromers in an irreversible bonding process. There are four states of formations found in chemical cross-linked hydrogels and shown in bellow. When chitosan conducts a cross-linking reaction with another polymeric chain, the most basic type of chemical hydrogel is formed. In derivation, the second chain might be similar to or different from the initial structural unit. Chemical cross-linking is caused by amines and hydroxyl groups found on chitosan chains. Cross-linking can be accomplished via cross-linkers or a photo polymerization reaction.

- *Cross-linking by Cross-Linkers*

Polymer cross-linking can occur between polymers or polymers and a cross-linker [128]. The cross-linking reaction between chitosan chains is started by cross-linkers [110,129]. Dialdehyde compounds like glutaraldehyde and other reagents like palladium cation, genipine, acrylic acid and diisocyanate are some of the most common cross-linkers [130-134].

- *Cross-linking by Photopolymerization*

Photopolymerization is another way to make covalently cross-linked chitosan hydrogels [135]. Photopolymerization is the conversion of a liquid precursor solution to a gel using photoinitiators and visible or UV light. This method is applied both in vivo and in vitro [109,136]. Adjusting the distance and time of exposure controls the polymeric reaction. UV or visible light, when combined with photoinitiators, produces free radicals, which activate radical polymerization and result in the formation of cross-linked hydrogels. A photocrosslinkable derivative of chitosan was created by adding azide and lactose moieties to the polymer.

6.4. Physical Cross-Linking Method

The other way of chitosan-based hydrogel crosslinking is physical cross-linking. Ionic contacts, such as ionically cross-linked chitosan hydrogels and polyelectrolyte complexes, or secondary interactions, such as grafted chitosan hydrogels and entangled chitosan hydrogels, are examples of physical interactions [129].

- *Ionically Cross-Linked Chitosan Hydrogels*

Anions are frequently used as ionic cross-linkers to build ionically cross-linked chitosan hydrogels because chitosan is a cationic polyelectrolyte polymer with ionizable amine groups [137]. Multivalent counter ions, such as phosphate-bearing compounds like tripolyphosphate, are one example (TPP). This ionic cross-linking procedure, also known as chitosan ionic gelation, is typically used to load tiny molecular weight medicines, although it has recently been utilized to load macromolecules as well [138].

- *Polyelectrolyte Complexed Chitosan Hydrogel*

Ionic interactions between two oppositely charged polymers generate polyelectrolyte complex networks. Because of their biocompatibility and biodegradability, polysaccharides are a desirable candidate for producing polyelectrolyte complexes [139]. The charge density of the polymers, mixing ratio, amount of each polymer, and other factors all play a role in the formation of these polyelectrolyte complexes. The net charge affects the solubility of the resultant complex. The complex will usually be insoluble and precipitate if the net charge is zero [140]. The stability of hydrogels is determined by the type of cross-linking used. Permanent properties of covalently cross-linked hydrogels with covalent cross-linkers include resistance to environmental factors. These systems require an additional purification

process to eliminate hazardous unreacted cross-linkers. Due to lack of chemical cross-linkers, physically cross-linked hydrogels are better in biocompatibility than chemically cross-linked. The surrounding conditions such as temperature, pH or ionic strength may effect the mechanical stability of the cross-linked chitosan hydrogels [126]. This unique property of physically cross-linked hydrogels is particularly beneficial for the development of stimuli-responsive systems that are sensitive to environmental factors and can be employed for medication delivery in specific situations [141]. Chitosan derivatives have been produced and tested in order to improve the characteristics of chitosan-based hydrogels. The functional amino groups on chitosan chains aid the polymer's entry into chemical processes, resulting in derivatives with better features such as mucoadhesion, drug loading, and gene transfer ability [142]. Other chemical changes have piqued interest as a way to make photopolymerizable chitosan derivatives or increase chitosan's water solubility.

7. Antiviral / Pharmaceutical Applications of Chitosan-Based Hydrogels

According to Li et al., (2009) and Tamura et al., (2011), chitosan is reported to be biocompatible and biodegradable with non-toxic and non-immunogenic degradation products [143,144]. Chitosan can be bacteriostatic and bioadhesive, as well as antioxidant, hemostatic agent and chelating agent [145,146]. This polymer can help control bleeding by including a procoagulant that aids in faster clotting [147]. Chitosan has attracted interest from a variety of industries, including pharmaceutical, cosmetics, food, medical, and agricultural [148]. Drug and gene delivery [149,150], wound dressing [151], tissue repair [109,152], and tissue engineering are some of the medicinal applications of chitosan [153]. Table 4 shows the applications of chitosan-based hydrogels in treatment of antiviral diseases.

8. Drug delivery

Several drug delivery applications are being researched using hydrogels based on chitosan and its chemically modified forms [154]. Because chitosan contains an amine group, it is cationic, whereas mucosal glycoproteins are negatively charged [155,156]. As a result, it can act as a bioadhesive substance on negatively charged biological surfaces. The introduction of bioadhesive polymers like chitosan extends the drug-loaded system's residence period and allows for localized drug delivery [157]. Chitosan also facilitates drug paracellular transport, which has a significant impact on the efficacy of drug delivery systems [158]. Chitosan has been employed as a drug carrier for several routes of administration since it is biocompatible and biodegradable with a structure that can be easily manipulated.

8.1. Oral Drug Delivery

Drug distribution to the mouth cavity, stomach, intestine, and colon can all be done with hydrogel scaffolds. Drug delivery to the oral cavity can be used to treat mouth illnesses without risking a first-pass effect. The pH-sensitive hydrogels direct medication distribution to specific regions in the body, such as the stomach or intestine, and so improve drug bioavailability. Irritable or inflammatory bowel disease can be relieved using chitosan-based

hydrogels as a drug delivery mechanism in the colon [159,160]. Next, in order to improve the systemic bioavailability of nifedipine and propranolol, buccal tablets with chitosan as the mucoadhesive layer have been developed [161]. Colon-specific medication delivery has been explored using chitosan-polyacrylic acid hydrogels. The presence of polyacrylic acid segment's pH and the biodegradability properties, make it a potentially appropriate carrier for medication release in the colonic region [162].

8.2. Ocular Drug Delivery

The main disadvantage of traditional ocular formulations is that they only last a limited time in the affected area. Drug administration via hydrogel systems may increase drug retention in the site, increasing the likelihood of higher bioavailability. Ocular hypertension has been controlled using a thermosensitive chitosan-gelatin based hydrogel filled with latanoprost [163]. When compared to diclofenac eye drop, diclofenac micelles put into nano-composite hydrogel improved medication residual time [164]. Chitosan glycerophosphate hydrogel (thermosensitive) enhanced the penetration and corneal bioavailability of ofloxacin compared to aqueous solution. When compared to an aqueous solution, a thermosensitive chitosan-glycerophosphate hydrogel enhanced ofloxacin penetration and corneal bioavailability. For ocular delivery of timolol, an in-situ thermosensitive hydrogel of chitosan and isopropyl acrylamide was utilized, and the method quadrupled the drug release [154].

8.3 . Nasal Drug Delivery

Chitosan has the ability to open tight connections between epithelial cells in mucosal membranes, allowing medication molecules to move more freely [165]. Furthermore, chitosan's high water absorption and mucoadhesive properties make nasal medication distribution easier [166]. Chitosan and PEG were used to make a thermosensitive hydrogel. At body temperature, the fluid formed a gel after being sprayed into the nasal cavity (Table 5). This hydrogel technology has a reduced mucosal clearance and a longer-lasting drug release [167]. Nasal administration of vaccinations and peptide medications using chitosan hydrogels showed promising results for delivering vaccines and peptide drugs when oral administration is not possible [168,169].

Table 5. Antiviral application of chitosan-based hydrogels.

Applications	Findings	References
Oral drug delivery	• Chitosan-based hydrogel can be designed for inflammatory bowel diseases • Drugs have been formulated with chitosan to enhance the systemic bioavailability of the drugs. •Biodegradability of chitosan along with pH sensitivity provide a potential suitable drug carrier.	[159-162]

Ocular drug delivery	• Diclofenac micelles loaded into nano-composite hydrogel improved drug residual time. • The chitosan-based hydrogel has been used for controlling ocular hypertension. • The thermosensitive hydrogel of chitosan doubled the drug release for ocular delivery of timolol.	[163.164,154]
Nasal drug delivery	• Nasal administration using chitosan hydrogels was promising for delivery of vaccines and peptide drugs. • This hydrogel system presented lower mucosal clearance and sustained drug release in site	[165-169]
Wound healing	• Increases macrophage activation that results in further events such as release of biological mediators • Chitosan can activate the complement system and stimulate fibroblasts.	[170-173]
Tissue engineering	• Allow host cells migration and proliferation and finally replacing the malfunction organs. • Scaffolds can be used for other tissue regeneration such as bone, cartilage, skin and nerve.	[174-180]

8.4. Wound Healing

Chitosan itself can directly use for wound healing. Due to the permeation of inflammatory cells like polymorphonuclear leukocytes, releasing inflammatory mediators such as migration of macrophages, rise in the amount of collagen and tumor necrosis factor-α are all possible mechanisms for healing. GlcNac (N-acetyl-D-glucoseamine), a component of chitosan, binds to particular receptors in the body, causing macrophage activation and subsequent actions such as the release of biological mediators [170]. Chitosan also activates the complement system [171] and increases the release of IL-8 and other cytokines by fibroblasts [172]. The most common application of chitosan hydrogels for wound healing is as a wound dressing and hemostatic agent to speed up the healing process. HemCon bandage is one of the commercially available chitosan-based hemostatic products. HemCon works by binding to negatively charged tissue cells, attracting negatively charged red blood cells, and producing a tight seal over the incision to stop excessive bleeding [173].

9. Tissue Engineering

In the last two decades, chitosan hydrogels have been employed as tissue engineering scaffolds. These systems are built around two components: cells and hydrogel polymeric strands. One of the benefits of chitosan as a scaffold is its biodegradability. Human enzymes such as lysozyme can breakdown chitosan [174]. Additionally, N-acetylation can be used to improve the biodegradability and biocompatibility of chitosan for tissue engineering applications. Chitosan with a high deacetylation degree near 100 has been shown to have a higher rate of breakdown, cell biocompatibility, and cell adhesion potential [175]. The

biodegradation rate of the scaffold should match the time it takes to heal malfunctioning tissue. Chitosan scaffolds can be utilized to regenerate cartilage, bone, skin and nerves, among other tissues [176-179]. Because neural cells have a limited potential to regenerate, treating central nervous system problems is difficult. Neural stem cells, such as embryonic, fetal, or adult stem cells, are required for nerve tissue engineering [180].

10. Conclusion

In the conclusion, chitosan has different types of applications based on the cross-linkers that cross-linked with the chitosan itself. The modification of chitosan is depending on the diseases or the applications. Like chitosan-based hydrogels, hydrogels enhanced the biodegradability, biocompatibility, and other properties of chitosan in antiviral applications as mentioned above. There are other types of applications by using other chitosan nanoparticles such as food packaging, cosmetics, act as drug carrier, etc. The factor that determine whether the chitosan used in which applications is the cross-linker. The cross-linker will have modified the chitosan structure, thus it also modified the properties of chitosan. Chitosan-based hydrogels used in pharmaceutical applications are likely to evolve significantly in the future. Future breakthroughs in antiviral treatment may be enabled by a better knowledge of chitosan's underlying characteristics.

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