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Review Article

Study on the Physicochemical Properties of Chitosan and their Applications in the Biomedical Sector

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Chitosan is a natural polymer derived from the deacetylation of chitin. It is mainly derived from crustaceans and fungal sources. It has many intrinsic properties, such as biocompatibility, biodegradability, cationic nature, and nontoxicity. These features of chitosan have made it an attractive material for various applications. Furthermore, these unique properties have found significant biomedical applications, such as in drug delivery, tissue engineering, antimicrobial agent, and wound healing. However, it has its drawbacks, such as the raw material source being seasonal and localized, the extraction procedure being time-consuming, costly, and involving the use of harsh chemicals in substantial amounts, and the quality of chitosan obtained from marine sources being variable. Furthermore, studies are needed to increase the yield and utilization of chitosan for various industrial purposes. Technological improvements, such as gene modification will enhance the yield and application of chitosan. This review focuses primarily on the numerous applications of chitosan in the biomedical field, including tissue engineering, wound dressing, drug delivery, and others.

1. Introduction

A naturally occurring polymer called chitosan is derived from chitin, a homo polysaccharide consisting of repeated units of *N*-acetyl-D-glucosamine residues that are held together by *β*-(1–4) linkage [1]. Invertebrates, such as insects, shrimp, and crabs as well as microorganisms, such as fungi, yeast, algae, and bacteria, all naturally contain the biopolymer chitin in their structure. In addition to being present in the cell walls of some fungi, particularly those belonging to the zygomycetes class [2], chitosan is a chitin derivative polymer that can be produced by partial deacetylation. Chitosan is a copolymer of D-glucosamine and *N*-acetyl-D-glucosamine, in which the number of D-glucosamine and *N*-acetyl-D-glucosamine residues varies depending on the degree of deacetylation [3].

After cellulose, chitosan is the second most abundant biopolymer [4]. Its structure is similar to cellulose's, with the exception that chitosan has an amino group in place of the hydroxyl group at position C-2 (Figure 1) [5]. Unlike cellulose, it has a positive ionic charge that allows it to bind to other molecules that have a negative charge, such as negatively charged proteins, lipids, ions, fats, and ions [6]. Chitosan is non-toxic, biodegradable, non-allergenic, bioactive, biocompatible, and has good adsorption capabilities. These properties of chitosan make it an attractive material for various applications [1, 7]. Moreover, it can be produced as flakes, beads, powders, membranes, gels, and sponge forms [8]. It has been employed in a variety of industries owing to its desirable properties, including the medical, biotechnological, and agricultural sectors [3]. Chitosan was approved by the Food and Drug Administration as a feed additive in 1983 [1].

Commercial production of chitosan involves the deacetylation process, which involves treating the chitin polymer with alkali to remove the acetyl groups [9]. The

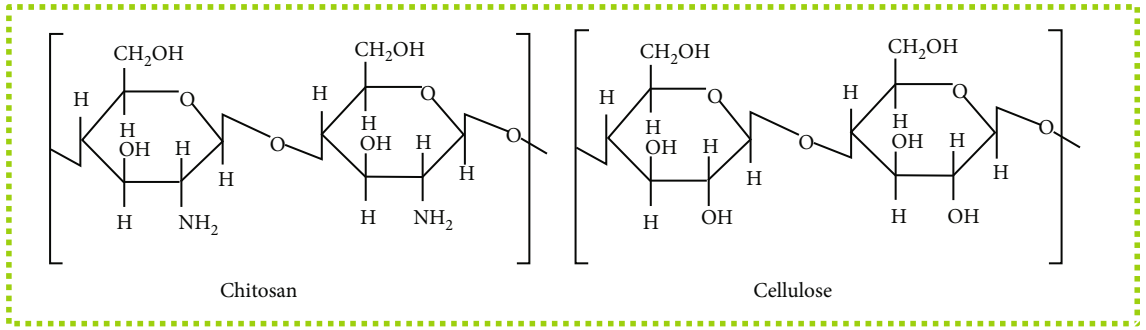

Figure 1: Schematic diagram of chemical structures of cellulose and chitosan.

general extraction process involves the deacetylation of chitin using strong alkali at high temperatures (Figure 2).

In this review, the natural occurrence, biosynthesis process, and physicochemical properties, that is, their solubility, molecular weight, degree of deacetylation, and viscosity of chitinous polysaccharides, will be discussed. Furthermore, their commercial extraction process along with their extraction from fungal biomass will be addressed. The biological properties and their applications in biomedical sectors will be highlighted. The main aim of this review study is to overview the state of the art of chitosan science and its advanced biomedical application.

2. Occurrence and Biological Function of Chitosan in Nature

Chitin is a very abundant biopolymer, which is the main structural component of shells of crustaceans (crab, shrimp, and lobster), exoskeletons of insects and mollusks, and the cell walls of some fungi. Chitosan, which occurs in some classes of fungi, such as zygomycetes, is less common in nature [10]. Chitin is often found in a variety of species along with other macromolecules. Nevertheless, higher animals and higher plants do not contain chitin or chitosan in their structure [11]. Shrimp and crab shell wastes have been used as a primary industrial feedstock for the large-scale production of chitin and chitosan. According to reports, marine organisms contain 20–30% of chitin, 30–40% of proteins, 0–14% of lipids, and 30–50% of minerals [12, 13]. On the other hand, chitin is mostly found in the fungal cell walls and septa of ascomycetes, zygomycetes, basidiomycetes, and deuteromycetes. Among them, the zygomycetes class of fungi contains substantial chitosan along with chitin in their cell walls. The fungal cell wall is made up of 10–20% chitin, 50–60% glucans, 20–30% glycoproteins, and minor proportions of lipids, pigments, and inorganic salts [14].

Chitin exists in nature in three crystalline forms: α-, β-, and γ-chitin, each with specific physicochemical properties depending on their degree of hydration, unit cell size, and number of chitin chains per cell [2]. The variations between these polymorphs are related to the manner, in which crystalline regions' chains are reciprocally arranged. α form arranged in an antiparallel, β form arranged in parallel, and γ form alternates between sets of two parallel strands and single antiparallel strands [15]. α-Chitin, which is found in crustaceans and the cell walls of fungi, is the only extractable and most widespread type of chitin among them.

2.1. Major Sources of Chitosan

2.1.1. Crustaceans as Large-Scale Production of Chitosan. The industrial production of chitosan relies mainly on the deacetylation of its parent polymer (chitin). Chitin is a fibrous substance consisting of polysaccharides, which is the major constituent in the exoskeleton of arthropods and the cell walls of fungi [16]. Hence, commercial chitosan has been extracted from crustaceans' chitin by deacetylation using strong alkalis (Figure 3). In addition to chitin, the main structural components of a crustacean shell include proteins, lipids, and inorganic salts. As a result, the production of chitin and chitosan from these sources involves a stepwise chemical extraction process [8].

However, the extraction of chitosan from crustaceans has drawbacks, such as the raw material source being seasonal and localized [17]. Furthermore, the extraction procedure is time-consuming, costly, and involves the use of harsh chemicals in substantial amounts [18]. In terms of physicochemical qualities, the quality of chitosan obtained from marine sources is variable [19]. In this regard, looking for other viable feedstocks is important for sustainable chitosan production.

2.1.2. Fungus as Large-Scale Production of Chitosan. Fungi are employed in numerous biotechnology industries processes, including baking, brewing, antibiotic, organic acid, and enzyme manufacturing industries, resulting in the discharge of fungal biomass wastes. These wastes together with those from the mushroom industry could be a potential feedstock for the extraction of chitinous polysaccharides. This makes extracting chitosan from fungal cell walls a viable alternative to overcome the limitation of extracting chitosan from marine sources [20].

Most fungi species belonging to ascomycetes, zygomycetes, basidiomycetes, and deuteromycetes possess chitin in their cell wall [21]. Chitosan-producing fungi could be a promising feedstock for commercial production [22]. The zygomycetes have been investigated as an alternate source of chitosan since they contain a large quantity of chitosan. Furthermore, the physicochemical properties of chitosan can be manipulated and standardized by controlling the

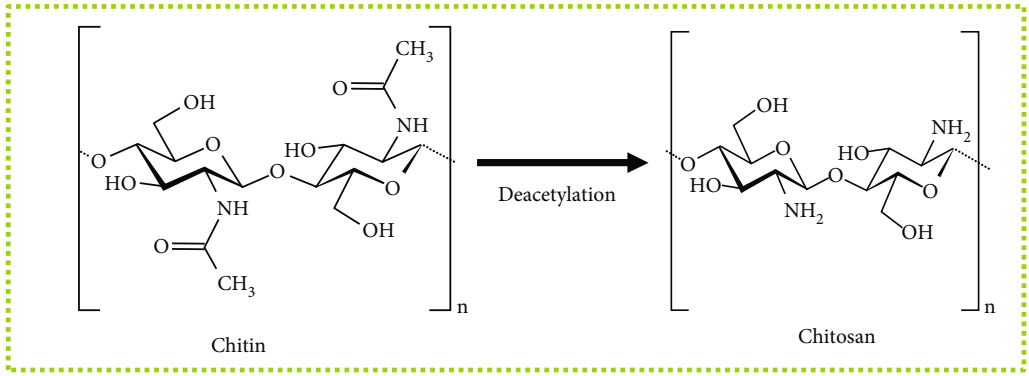

Figure 2: Structure of chitin and chitosan.

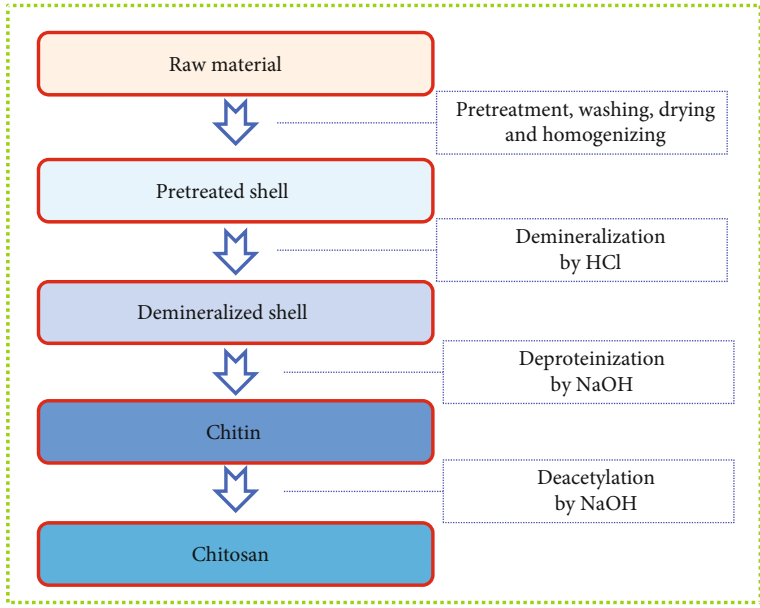

Figure 3: Scheme for the steps in deacetylation of chitin to form chitosan.

parameters of growth conditions [23]. Mucorales, such as *Cunninghamella*, *Rhizomucor*, *Gongronella*, *Mucor*, *Absidia*, and *Rhizopus*, species have been studied for chitosan production [24]. Mucolares fungus has chitin deacetylase, which catalyzes the deacetylation of chitin to chitosan, resulting in chitin and chitosan in their cell wall [24].

Utilizing fungal biomass wastes as a raw material is advantageous in terms of cost reduction, non-allergic polymer production, reduction of environmental contamination and related disposal problems, and value addition to existing Mycotech-products [25, 26]. There are also other advantages no seasonal variations, depigmentation, and demineralization steps are also eliminated during the extraction process, and fewer chemicals are utilized, different species and growth conditions can be used to produce chitosans with different properties [24].

2.1.3. Chitosan from Plants. Plant-derived chitosan has another source of chitosan and has not been industrialized to date even though it was discovered by French botanist Braconeau [27]. The authors conclude that, currently, industry-based chitosan is made mainly from cuticles of crustaceans, such as crabs and shrimps. It is mainly due to the content of the chitin and alkali treatment process. In the shrimp and crab, the content of chitin is high and the alkali removal treatment, such as washing with water, is very easy after a high concentration alkali treatment of chitin. Whereas, in the case of plant-derived chitin, after high-concentration alkali heat treatment the structure of basidiomycetes becomes extremely weak, which requires high-capacity high-speed centrifugation in the removal and cleaning of residual alkali. In addition, it contains more than 50% of high molecular weight polysaccharides. As a result, chitosan production from plants is very difficult to industrially mass-production. The higher alkali treatment can reduce their higher-order structure, so when washed with water to remove alkali, glucan and fine fibers dissolve, viscosity increases, and fine particles are generated [27].

2.2. Mechanism of Chitosan Extraction. All species, including algae, crustaceans, fungi, and insects, have a highly conserved pathway for synthesizing chitin [13]. The biosynthesis pathway is common for arthropods and fungi, which involves the

conversion of a carbohydrate, such as glucose, glycogen, or trehalose to chitin. The first step is the synthesis of glucose-6-phosphate (G6P) by hexokinase from glucose, which can be obtained free or through the hydrolysis of trehalose by trehalase. Glycogen phosphorylase will depolymerize glycogen if the starting material is glycogen, yielding glucose-1-phosphate, which will subsequently go through isomerization via phosphoglucomutase. The end product will be G6P. G6P is formed and further converted to fructose-6-phosphate by phosphoesoisomerase. After that, fructose-6-phosphate is transformed into *N*-acetyl-D-glucosamine-6-phosphate (GlcNAc-6-P), which involves amination and acetylation [11].

Phospho-*N*-acetyl glucosamine mutase catalyzes the isomerization of GlcNAc-6-P to a1-phospho-*N*-acetyl-D-glucosamine. Uridine triphosphate is used in further interconversion to generate uridine-5′-diphosphate (UDP), which is then converted into *N*-acetyl glucosamine (uridine-5′-triphosphate). Subsequently, in the presence of chitin synthase, chitin is produced from UDP *N*-acetylglucosamine. The linear chains spontaneously assemble to form microfibrils with varying diameters and lengths [12]. The chitin deacetylase enzyme (EC 3.5.1.41), which is found in the cell wall of some fungi catalyzes the deacetylation of chitin to chitosan [28]. The biosynthetic pathway of chitin is graphically represented in Figure 4. After synthesis, chitin is organized as a microfibre and then structured into an extracellular matrix.

Extraction and purification of chitosan from crustaceans generally involve three main steps: demineralization, deproteinization, and deacetylation steps (Figure 3). Demineralization is a process of converting the insoluble calcium carbonate into soluble calcium chloride using hydrochloric acid (HCl), which can be easily removed by water. Then deproteinization step is performed, where sodium hydroxide (NaOH) removes protein and other organic components other than chitin in the shell. The deacetylation step is the final process of converting chitin to chitosan using 40–50% (w/w) heated NaOH solution [5]. Despite the increasing need for chitin and chitosan across the world, the amount produced could not keep up with the high demand [29].

The typical chitosan extraction from fungi begins with a heated alkaline treatment of the dry fungal biomass. This causes the fungal cell to be disrupted and solubilizes several of its constituents, including proteins, alkali-soluble glucans, and mannans. This process results in alkali-insoluble matter (AIM), which is readily separated from a soluble fraction that contains the cell's alkali-soluble matter by centrifugation and/or filtering. The chitosan from the AIM can be extracted from the other polymers by bringing the AIM's pH level down to 4.0 by adding an organic acid, such as acetic acid. Chitosan can then be separated from other polymers that are insoluble at acidic pH by centrifugation or filtering. Since chitosan is insoluble in alkaline pH, increasing the pH to 9.0 results in free chitosan to be precipitated from the supernatant. The product is then washed with water, ethanol, and/or acetone before being dried.

2.3. Factors Affecting Chitosan Extraction. The chitin and chitosan yield can vary depending on species type, the nutritional source, incubation conditions and period, fermentation state, and chitosan extraction procedure. The maximum yield of chitosan can be at the late exponential phase [30–32]. Another factor that determines chitosan yield is the fermentation state. In submerged fermentation (SMF), the fermentation parameters, including pH, temperature, and nutrients, can be readily regulated, and fermentation scale-up and recovery of fungal biomass are also easier [11]. On the one hand, cultivating fungal biomass through solid state fermentation could produce a high yield of chitosan compared with submerged fermentation [8].

The molecular weight and degree of deacetylation are important factors that greatly impact the functional properties of biopolymers and their solubility. Even though many fungi, such as the *Gongronella* species, *Absidia* species, *Rhizopus* species, and *Aspergillus* species, contain chitosan in their cell walls [33], the majority of chitosan is extracted by deacetylating chitin with extremely alkaline conditions at high temperatures.

Temperature, alkali concentration, and reaction duration that are utilized during the extraction processes have also a significant impact on the yield and purity of chitinous polymer and alter molecular weight and degree of deacetylation [34].

2.4. Physicochemical Properties of Chitosan. Chitin and chitosan are composed of thousands of D-glucosamine residues linked together by β-(1–4) linkage. Depending on the degree of deacetylation of chitin, chitosan contains 15–50% *N*-acetyl-D-glucoseamine units. Since chitin and chitosan contain amino groups and have nitrogen contents that vary from 5% to 8%, they exhibit distinct biological roles [15]. Chitosan is an *N*-deacetylated derivative of chitin produced by changing the acetamide groups into primary amino groups. Chitosan is more water-soluble and chemically reactive compared with chitin due to the presence of primary and secondary hydroxyl groups on each repeat unit as well as the amine group on each deacetylated unit [35]. These reactive groups in chitosan are easily susceptible to chemical modification, changing its mechanical and physical properties and making it a desirable material for various applications. Most of the properties of chitosan emerge from their physicochemical features, including solubility, deacetylation degree, viscosity, and molecular weight.

2.4.1. Solubility. Chitosan is soluble in an acidic solvent but insoluble in a neutral or alkaline solvent. Although chitin is generally insoluble in solvents, deacetylating chitin results in soluble chitosan that has primary amino groups with a pK_a value of 6.5 [36]. When chitosan is dissolved in acidic solvents, the amine becomes protonated and becomes positively charged resulting in soluble chitosan. However, when the pH rises to 6 or higher, they lose their charge and become insoluble [36]. In addition to pH, the solubility of chitosan is affected by its molecular weight degree of deacetylation, temperature, and polymer crystallinity [10].

2.4.2. Molecular Weight. Molecular weight highly affects the physicochemical and biological properties of chitosan. Chitosan's molecular weight varies depending on the source material, as well as how it is prepared and extracted [11].

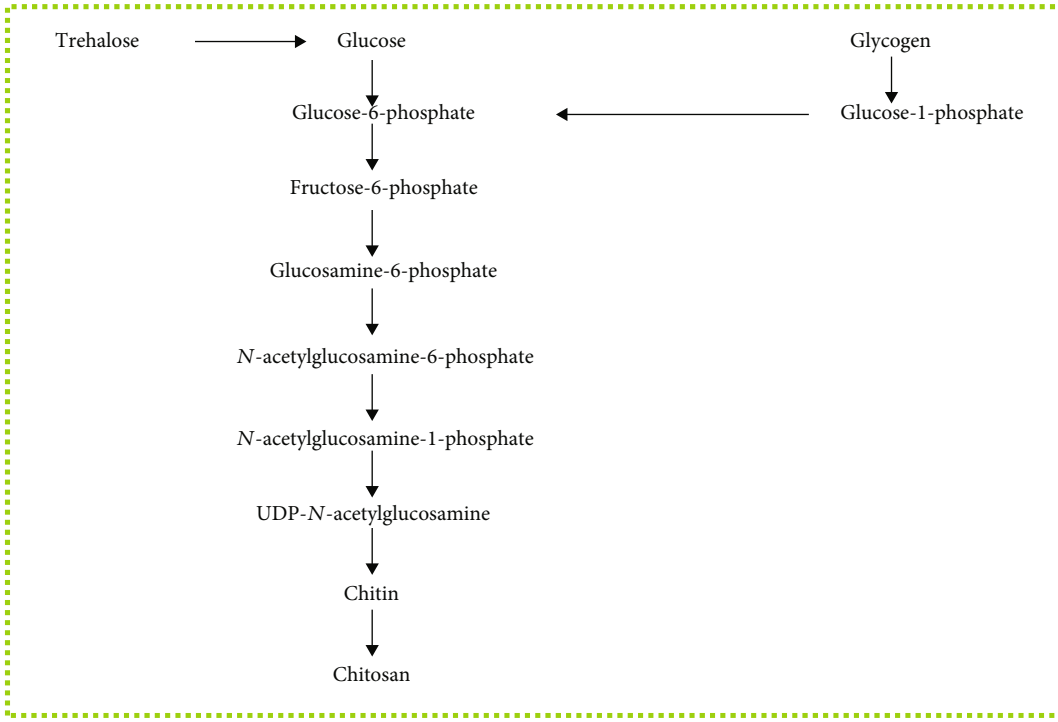

Figure 4: Biosynthesis of chitin and chitosan [12].

Chitosan can be classified as high, medium, or low molecular weight depending on its molecular weight range [37]. Chitosan becomes more viscous and less soluble as the molecular weight increases, which is undesirable for a variety of industrial applications. Given its better solubility and stability, low molecular weight chitosan is preferred for use in biological and industrial applications [9, 38].

2.4.3. Degree of Deacetylation. The degree of deacetylation is another factor that determines the physicochemical properties of chitosan, its activity, and its application. The degree of deacetylation refers to the distribution of amino groups along the polymer chain [37]. The cationic nature of chitosan in acidic media resulted from the amino group in the polymeric chain. Thus, the solubility and degree of viscosity are highly influenced by the degree of deacetylation [39]. The degree of deacetylation represents the molar fraction of *N*-acetylglucosamine units in the chain and can be defined in equation (1) below.

$$\mathrm{DD} = \frac{\mathrm{nGl_cN}}{\mathrm{nGl_cN} + \mathrm{nGl_cNA_c}}, \quad (1)$$

where DD is the degree of deacetylation, $\mathrm{nGl_cN}$ is the average number of D-glucosamine units, and $\mathrm{nGl_cNA_c}$ is the average number of *N*-acetyl glucosamine units.

The degree of deacetylation determines whether a polymer is chitin or chitosan. A deacetylation degree above 50%, often suggests the production of successful conversion of chitin into chitosan [29].

2.4.4. Viscosity. Viscosity is one of the factors that determine chitosan's industrial applicability, and it is highly dependent on the degree of deacetylation and the molecular weight of the chitosan. Viscosity increases as the degree of deacetylation increases and molecular weight decreases [10]. It can be also depending on the particle size and storage time of the chitosan [40]. Nanochitosan has lower viscosity about 30% for normal chitosan solution at the same concentration level. Storage time also affects about a 10% drop in the viscosity of normal chitosan for a storage time of 24 hours, whereas nanocolloids dropped by 17% for the same storage time [40]. Aranaz et al. point out in their study that, viscosity is a good determinant for the stability of the polymer in solution, as a reduction is observed during polymer storage due to polymer degradation [10].

3. Biomedical Application of Chitosan

Chitosan has several properties to be used in biomedical applications. For instance, it has positive charges in an acidic medium, due to the protonation of amino groups, and it can bind with negative residues in the mucin, which lead to improved mucoadhesive properties. Furthermore, chitosan is a biocompatible, biodegradable, and non-toxic polymer that finds in various biomedical applications. These include antimicrobial and wound-healing biomaterial, drug carriers, and scaffolding material.

3.1. Antimicrobial Agent. Antibiotic resistance of bacteria is a major public health problem, thus finding an alternative to antibiotics is important. Chitosan and chitosan derivatives showed antibacterial activity against a variety of microorganisms, including bacteria, filamentous fungi, and yeast (Table 1) [41]. The exact mechanism of antibacterial activity is yet to be fully understood. However, different hypotheses

Table 1: Antibacterial activity of chitosan and its derivatives.

Material	Form	Type of microorganism	Species	References
Chitosan/polyvinyl alcohol /starch	Membrane	Gram-negative bacteria	*Escherichia coli*	[48]
		Gram-positive bacteria	*Staphylococcus aureus*	
Chitosan/β-cyclodextrin polymer	Sponge	Gram-positive bacteria	*Staphylococcus aureus*	[49]
Chitosan/PVP/nanocellulose	Film	Gram-positive bacteria	*Staphylococcus aureus*	[50]
Chitosan nanofiber mesh–gentamicin-loaded liposomes	Membrane	Gram-negative bacteria	*Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*	[51]
		Gram-positive bacteria		
Chitosan–vancomycin	Aerogel	Gram-positive bacteria	*Staphylococcus aureus*	[52]
Chitosan/sodium alginate–Cu	Hydrogel	Gram-negative bacteria	Methicillin-resistant *Staphylococcus aureus* and *Escherichia coli*	[53]
		Gram-positive bacteria		
Chitosan		Fungi	*Candida albicans*	[54]
Ag@CS/An		Fungi	*Phytophthora capsici*	[55]
Chitosan–capsaicin		Fungi	*Aspergillus parasiticus*	[56]
CS/AgSD	Sponge	Gram-negative bacteria	*Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis*	[57]
		Gram-positive bacteria	*Candida albicans*	

have been proposed, with the majority of these mechanisms relying on the polycationic characteristic of chitosan (Figure 5). The first proposed mechanism is that chitosan causes cellular permeability and induces intracellular component leakage as a result of its interaction with anionic components of the cell membrane, ultimately leading to cell death [42]. Another possible mechanism is chitosan penetration through the cell membrane followed by binding to DNA, which inhibits DNA replication and eventually leads to cell death [43]. Chitosan also seems to have a growth-inhibitory effect because it has a high ability to chelate several metal ions, such as Ni^{2+}, Zn^{2+}, Co^{2+}, Fe^{2+}, and Cu^{2+}, when the pH value exceeds its pKa value. As a result, microbial growth is inhibited [37]. Chitosan can also inhibit the growth of microbes by forming a dense polymer film on the surface of the cell and preventing nutrient and oxygen uptake.

Chitosan was shown to have a wide range of inhibitory efficacy against various Gram-positive bacteria, Gram-negative bacteria, and fungi. Gram-negative bacteria have lipopolysaccharides in their outer membranes, which give them hydrophilic surface characteristics. The outer membrane acts as a defense against hydrophobic toxins and macromolecules. On the other hand, the surface of Gram-positive bacteria is composed of peptidoglycans and teichoic acid, which are required for the activity of several membrane-bound enzymes. The method of antibacterial activity varies between Gram-positive and Gram-negative bacteria due to changes in cell structure (Figure 6). Non-crosslinked chitosan scaffolds were found to be effective against Gram-negative *Porphyromonas gingivalis* and Gram-positive *Streptococcus mutans* [45]. By adsorbing bacteria and then inducing the formation of clusters over some time, the developed chitosan scaffold was able to kill both pathogens in 6 hours.

Chitosan has strong antifungal action against a variety of fungi, including *Rhizopus oryzae, Aspergillus niger*, and *Alternaria alternate* [46]. Meng et al. investigated the antifungal mechanism of chitosan against *Aspergillus ochraceus* at the microstructure and transcriptome level. Chitosan was found to hinder spore germination and mycelia growth. Fungal mycelia displayed shriveling, abnormal branching, and vacuolation after chitosan treatment. Furthermore, chitosan disrupts ribosome biogenesis and glycerophospholipid metabolism at the molecular level [47]. This finding suggested that chitosan can disrupt the integrity of cell surface architecture and protein biosynthesis.

Chitosan also demonstrates a high efficacy against pathogenic microorganisms at low doses yet minimal toxicity towards mammalian cells compared with other compounds, giving it several benefits over other synthetic antimicrobials [8]. Chitosan antimicrobial activity highly depends on the degree of deacetylation, molecular weight, polymer viscosity, and polymer concentration [42].

3.2. Drug Delivery. The clinical phase of drug discovery and development is typically impeded by drugs failing to reach the target site of action, resulting in various side effects rather than favorable therapeutic effects. To improve health and extend life, various mechanisms have been developed for the targeted delivery and/or controlled release of therapeutic medications. Regarding this, the cationic polysaccharide chitosan has attracted great attention in the pharmaceutical and biomedical industries due to its wide availability and intrinsic pharmacological properties. Furthermore, biological characteristics like biocompatibility, biodegradability, nontoxicity, and low-immunogenicity lead chitosan to be involved in designing carriers for the controlled and targeted release of various drugs [35].

Smart drug delivery systems can release drugs in response to environmental changes, such as temperature, pH, electric field, light, and some chemicals [58]. For instance, by combining chitosan with extremely hydrophilic polymers like polyvinyl alcohol,

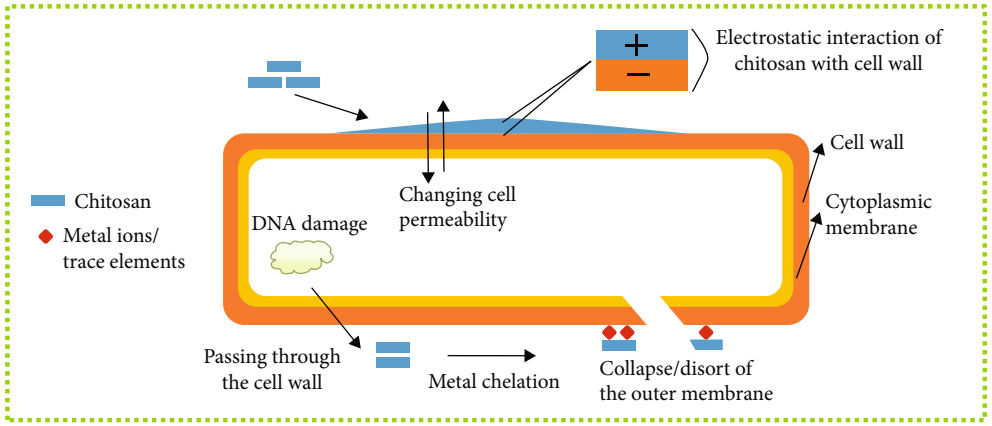

Figure 5: Antimicrobial mechanisms of chitosan [44].

polyvinylpyrrolidone, or gelatin, membranes or films with various hydrophilic behaviors with controlled swelling can be prepared for the regulated release of drugs [5]. The same authors developed hydrogels based on chitosan and polyvinylpyrrolidone with aminopropyletriethoxysilane for pH-sensitive drug release and tested it with the cefixime drug [59].

The hydrogel exhibited maximal swelling at pH 2 and decreased as the pH increased. In a simulated gastric fluid, drug release was 81.6% in 12 hours. A thermosensitive chitosan-based drug delivery system was also developed by Nawaz et al. A chitosan–gelatin-based hydrogel containing 5-fluorouracil (5FU)–alginate nanoparticles was shown to suppress the premature release of 5FU at the surface of the skin [60]. For a ultraviolet (UV) and pH-responsive drug delivery system, dual stimuli-responsive (ONB–chitosan) hydrogel was synthesized by Nisar et al. The hydrogel was synthesized by combining a photocleavable crosslinker, 4-formylphenyl 4-((4-formylphenoxy)methyl)-3-nitrobenzoate (CHO–ONB–CHO) with chitosan (Figure 7). The crosslinker's photocleavable activity was observed in the 310–340 nm UV absorption band. The hydrogel exhibited maximal swelling at pH 5.7 at 37°C and decreased as the pH increased.

Chitosan has been used in a variety of drug delivery applications, including ocular drug delivery, per-oral delivery, pulmonary drug delivery, nasal drug delivery, mucosal drug delivery, gene delivery, buccal drug delivery, vaccine delivery, and cancer therapy. It can be used in drug delivery in various forms like hydrogels, nanoparticles, nanofibers, and films. Chitosan is also used in drug delivery in the form of aerogel. Aerogel is a porous and ultra-light material that depends on the precursor materials, the material mixing ratio, the preparation method, and additives. Chitosan-based aerogel is a material of ultra-lightweight composed of 99.98% air by volume and possesses extremely high porosity and excellent strength [62].

Table 2 shows chitosan and its derivatives for drug delivery. Hence drugs with chitosan matrix have been used to develop different pharmaceuticals, including coated tablets, beads, films, and microcapsules.

3.3. Tissue Engineering. The basis of tissue engineering is designing and developing appropriate materials that can substitute or trigger regeneration processes in damaged tissues. Chitosan, a cationic polymer, is a promising biopolymer because it has several desirable properties, such as biological activity, widespread availability, biocompatibility, and structural resemblance to extracellular matrix components. Given that, significant effort has been put into developing novel chitosan-based materials that closely resemble the structure and functionality of tissues required for effective regeneration [63]. Chitosan's applicability as biomaterials for advancing advancement in several tissue engineering fields (Table 3), including tendon [64] and vascular replacement [65], skin [66], and nerve [67] regeneration, was reported by different scholars.

Bombaldi de Souza et al. [68] developed a chitosan-based scaffolding for periosteal tissue engineering. First, chitosan was chemically modified to phosphorylated chitosan, and subsequently, a chitosan xanthan-based scaffold was developed as a periosteal substitute. The developed material was able to stimulate osteogenesis, whereas being non-toxic to adipose tissue-derived stem cells. Chitosan in tissue engineering is involved in promoting cell adhesion, cell proliferation, and cell differentiation. By incorporating a methacrylated gelatin network into a nanocomposite hydrogel made of methacrylated chitosan and polyhedral oligomeric silsesquioxane, Zhang et al. developed a biodegradable hybrid double-network hydrogel. They observed that the hydrogel could preferentially guide the mesenchymal stem cells towards osteogenic differentiation in vitro and accelerate new bone regeneration in situ using a rat of calvarial defects [69].

Moreover, the polycationic nature of chitosan in a moderately acidic environment facilitates the immobilization of negatively charged enzymes, proteins, and DNA for gene delivery [63].

3.4. Wound Healing. Cuts, grazes, and other breaks in the skin can become infected pathogenic bacteria enter the wound and begin to multiply. The improper treatment process can lead to loss of skin, and initiation of an infection, which might spread to other organs. Therefore, it is necessary to develop wound dressings functionalized with antimicrobial agents since it is important to appropriately treat and

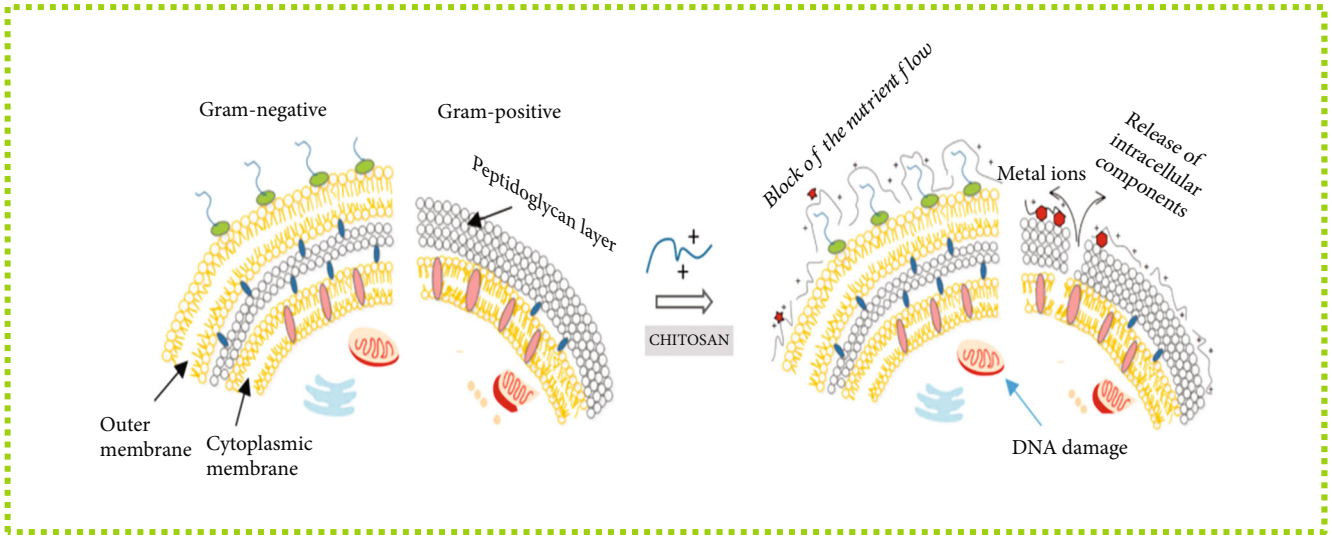

Figure 6: Models for the action of chitosan on Gram-positive and Gram-negative bacteria [43].

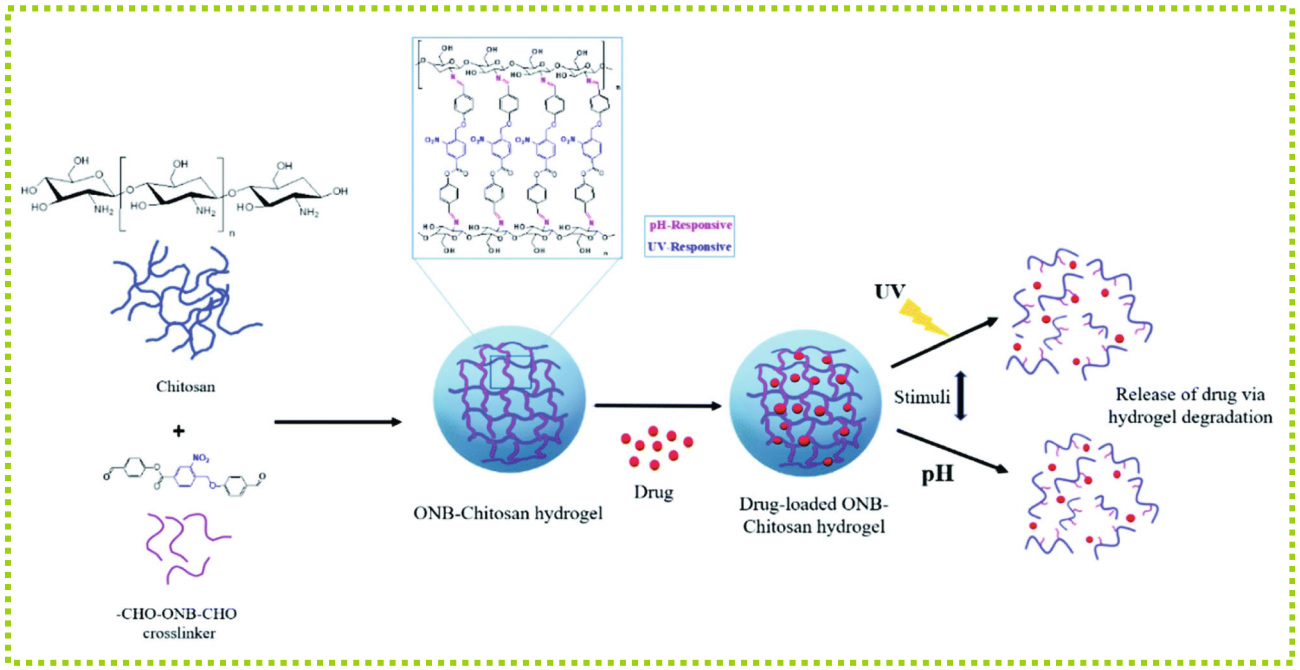

Figure 7: Photocleavable and pH-responsive hydrogel for drug delivery applications [61].

protect the wound to reduce the risk of infection [79]. Since the number of novel antibiotic classes has declined and no new classes have been developed after daptomycin and linezolid in the 1980s, new commercial drugs are based on optimizations of existing molecules or combinations of multiple compounds [80].

Hemostasis, inflammation, proliferation, and skin remodeling are the four phases of wound healing. Due to its capacity to speed up wound healing, chitosan has been investigated as a wound-healing material. The ability of wound healing of chitosan-based material is related to their ability to activate polymorphonuclear cells, and fibroblasts, produce cytokines, migrate giant cells, and stimulate type IV collagen formation. Furthermore, their vulnerability to degradation by bodily fluid enzymes, such as lysozyme into chito-oligomers that excite macrophages and increase collagen deposition speeds up the wound-healing process [81]. The commercially available wound dressings of chitosan are in the form of non-wovens, hydrogels, films, and sponges [55].

3.5. Other Applications of Chitosan. Chitosan and its derivatives are applied in a wide range of biological activities, such as immunity-enhancing, antitumor and anticancer effects, acceleration of calcium and iron absorption in vivo, antiinflammatory effects, and repair of arthritic tissue, antioxidant

Table 2: Chitosan and its derivatives for drug delivery.

Material	Form/delivery system	Stimuli	Targeted site	Drug	References
ONB–chitosan	Hydrogel	pH UV	Cancerous tissues Endosomes Lysosomes	Dox	[56]
Chitosan nanoparticles	Hydrogel	Electric field pH	Wound sites	Fluorescein isothiocyanate–dextran	[57]
Chitosan–Polylactide-co-glycolide	Microcapsule	pH	Gastritis	Oleophilic curcumin, hydrophilic catechin, and hydrophilic rhodamine B	[58]
Chitosan–poly(*N*-isopropylacrylamide)	Hydrogel	Temperature	Antibacterial	Levofloxacin	[59]
Cationic chitosan-based graphene oxide	Hydrogel	pH Glucose	Diabetes	Bovine serum albumin	[60]
Chitosan coated magnetic nanoparticle (Chitosan–MNP)	Microbeads	Electric field		Vancomycin	[61]
N-succinyl-*N*′-octyl chitosan	Micelles	pH	Colon	Curcumin	[63]
N-palmitoyl chitosan	Microparticle	pH	Hep G2 cell	Superparamagnetic iron oxide Doxorubicin	[64]

Table 3: Chitosan and its derivatives in tissue engineering.

Material	Activity	Application	References
Chitosan–copper nanoparticle	Osteogenesis and antibacterial Wound healing	Bone tissue engineering Skin tissue engineering	[70–72]
Chitosan–hydroxyapatite	Osteogenesis Bone regeneration	Regenerative tissue engineering Bone tissue engineering	[73–75]
Tripolyphosphate-crosslinked and chitosan/gelatin biocomposite	Regeneration of anisotropic tissues	Uniaxial tissue engineering	[76]
Chitosan/collagen type I/nanohydroxyapatite	Osteogenesis	Bone tissue engineering	[77]
Carboxymethyl, chitosan-amorphous, and calcium phosphate hydrogel	Osteogenesis	Bone tissue engineering	[78]
Chitosan–Zn oxide nanocomposite	Osteogenesis and wound healing	Tendon repair	[64]
Poly(ε-caprolactone)-carboxymethyl chitosan/poly(ε-caprolactone)–chitosan	Antithrombotic and antibacterial	Vascular tissue engineering	[65]
Chitosan/aloe film	Wound healing Skin regeneration	Skin tissue engineering	[66]

activity, angiotensin-I-converting enzyme inhibition, excluding toxins from the intestines, reducing heavy-metal poisoning in humans, radio-protective properties, preventing tooth decay and tooth diseases, as a bifidus factor to regulate microbial metabolism in intestines, and antimutagenic effects [82, 83].

The antiinflammatory mechanism of chitosan is due to the acid hydrolysis of chitosan to glucosamine hydrochloride or its sulfate, phosphate, and other salt preparation by salt conversion. In addition, also has free amino groups; can neutralize gastric acids and form a protective membrane in the stomach, so chitosan could be used to cure acid indigestion and peptic ulcer [83]. It is also an immune regulator that can activate macrophages and natural killer cells and improve the delayed-type hypersensitive reaction, increase cytotoxicity, and induce mitosis [84]. Chitosan at an addition of 0.02% had antioxidant effects in lard and crude rapeseed oil [85]. Chitosan can also accelerate the absorption of calcium and iron, and the chelation of metal ions may also be related to its drug-delivery characteristics [86].

Chitosan plays an important role in commercial wound dressings due to its hemostatic characteristics and wide availability [87–89]. For the development of hemostatic materials from chitosan, composite materials have been

prepared with a combination of chitosan and other chemicals. For example, a chitosan-based wound dressing loaded with inorganic additives, such as $AlCl_3$, $FeSO_4$, $Al_2(SO4)_3$, and levofloxacin, was manufactured [87]. In that system, inorganic additives can inhibit hemorrhage and levofloxacin can be released to supply antibacterial functions. The results showed that the chitosan-based materials with $Al_2(SO4)_3$ and levofloxacin had the highest blood absorption capacity and increased hemostatic capability in an in vivo mice injury model [87]. In addition, for interventional diagnosis, chitosan-based materials are widely used [90]. Due to its unique physicochemical properties and vast availability chitosan is a well-suited material for the interventional diagnosis system [91, 92].

4. Conclusions and Future Perspectives

Chitosan is a biopolymer that can be produced from chitin. Chitin and chitosan represent a variety of desirable properties due to high charge density, reactive hydroxyl, and amino groups, and as well as extensive hydrogen bonding capacity. The combination of versatile physicochemical and biological characteristics, allows them to have a wide range of biotechnological applications, including biomedical, industrial, and environmental areas. The main aim of this study was to assess various uses of chitosan and to fill different gaps in the literature so far. In addition, also it mainly focuses on the numerous applications of chitosan in the biomedical field, such as tissue engineering, drug delivery, and wound dressing, as a result of its physicochemical and biological features and antibacterial effect. The current topic is mainly significant due to their unique characteristics, such as cost reduction, non-allergic polymer production, eco-friendly, and is locally available. However, the extraction of chitosan from crustaceans has drawbacks, such as the raw material source, being seasonal and localized. Furthermore, the extraction procedure is time-consuming, costly, and involves the use of harsh chemicals in substantial amounts. More research should be performed to make chitosan a compound with many applications and possibilities. In addition, to make fungal chitosan an industrial feedstock further research and methodological improvement should be made. Moreover, strain improvement and metabolic engineering could increase the yield and quality of chitosan.

Data Availability

All data presented or analyzed during this study are included in this article.

Conflicts of Interest

The author(s) declare(s) that they have no conflicts of interest.

Authors' Contributions

Digafe Alemu: advising, editing, and manuscript preparation; Efrata Getachew: reviewing and writing; Ajoy Kanti Mondal: designing, reviewing, writing, and validating the manuscript. All authors have read and agreed to publish the manuscript.

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