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Chapter 1

Extraction of Fungal Chitosan and its Advanced Application

Sahira Nsayef Muslim[1]; Israa MS AL-Kadmy[1]*; Alaa Naseer Mohammed Ali[1]; Ahmed Sahi Dwaish[2]; Saba Saadoon Khazaal[1]; Sraa Nsayef Muslim[3]; Sarah Naji Aziz[1]

[1]*Branch of Biotechnology, Department of Biology, College of Science, AL-Mustansiryiah University, Baghdad-Iraq*

[2]*Branch of Fungi and Plant Science, Department of Biology, College of Science, AL-Mustansiryiah University, Baghdad-Iraq*

[3]*Department of Geophysics, College of remote sensing and geophysics, AL-Karkh University for science, Baghdad-Iraq*

*Correspondense to: Israa MS AL-Kadmy, *Department of Biology, College of Science, AL-Mustansiryiah University, Baghdad-Iraq. Email: israaalkadmy@gmail.com*

1. Definition and Chemical Structure

Biopolymer is a term commonly used for polymers which are synthesized by living organisms [1]. Biopolymers originate from natural sources and are biologically renewable, biodegradable and biocompatible. Chitin and chitosan are the biopolymers that have received much research interests due to their numerous potential applications in agriculture, food industry, biomedicine, paper making and textile industry. Chitin is a polysaccharide, made of N-acetyl-D-glucosamine units connected by β (1→4) linkage (**Figure 1A**). When the acetyl-D-glucosamine units in chitin lose acetyl groups, the molecule is called chitosan (**Figure 1B**) [2].

Chitosan is a natural co-polymers of chitin, composed by units of 2-amino-2-desoxi-Dglycopyranose and of 2-acetamide-2-desoxi-D-glycopyranose interconnected by glycosidic bonds β-1,4 in variable proportions. The first type of units is frequently present in chitosan. This polymer is naturally found in the cell wall of fungi, mainly in the Mucorales order [3,4]. Chitosan is formed by the chitin deacetylation, and the group N-acetyl can be suffer several degrees of deacetylation. Chitosan is characterized according to its deacetylation level and

molar mass, once such features may influence the degradability and in the polysaccharide hydrolysis [2,5]. According to the medium acetylation level (AL), chitosan may be obtained with physical-chemical properties differentiated regarding the solubility parameters, pKa and viscosity [5,6,7]. It is difficult to obtain chitosan with high deacetylation level as due long process of isolation, and the degradation of the polymer also increases [5,7].
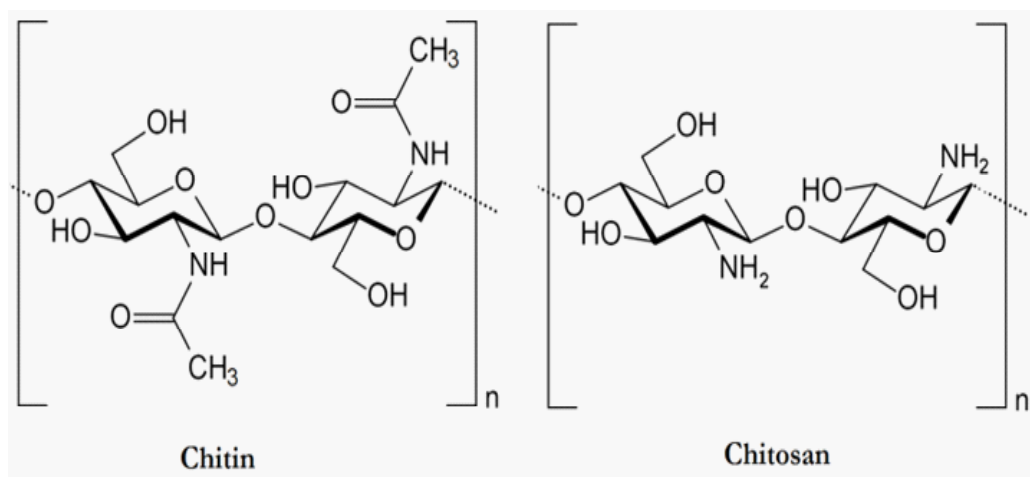
Figure 1: Chemical structure of chitin and chitosan

2. Occurrence and Biological Functions in Nature

Chitin is a characteristic compound found in fungi and some animals. In animals, chitin mainly exists in the shells of crustaceans and mollusks, in the backbone of squids and in the cuticle of insects. Long chitin molecules are associated with proteins by covalent bonds and together they form a complex structural network. On crustacean's shells, calcium carbonate deposits into the network contributing to strength of the shells and protection of the organisms [2]. In fungi, chitin exists in the cell wall of spores and hyphae. It is associated with glucan molecules in form of microfibrils, which are embedded in an amorphous matrix and provide the framework in cell wall morphology [3]. Chitosan is not native to animal sources, but a small number of fungi, such as *Mucor, Absidia* and *Rhizopus* species have chitosan as one of the structural components in the cell wall [3].

The amount of chitin in animal and fungi is specific to species, age and environmental conditions where the organism exists. Chitin content in the dry shells of crabs, lobsters and shrimps ranges from 14 to 27 % [4], while in the fungal cell wall it varies from 2 to 42 %, the lowest value corresponding to yeasts, and the highest values to Euascomycetes [3].

2.1. Properties of chitosan

Chitosan is a weak base insoluble in water but soluble in dilute aqueous solutions of various acids, the most widely used is acetic acid [8]. The acid solubility is explained by the protonation of the free amino group, characteristic in the chitosan *in natura*, which change to NH_2 to NH_3^+, whereas in alkaline condition, the hydro solubility is due to the formation of

carboxylate, from the introduced carboxylic group [9,10]. The possibility to obtain a variety of polymer derivatives with differences solubility, thermal stability, reactivity with other substances and specificity regarding the binding site, providing several biological applications of the chitosan [11]. Some applications of the chitosan, it is highligh it's the use in the pharmaceutical industry, more specifically related to dental clinic [12]

3. Production of Chitosan from Fungal Sources

Production of chitin and chitosan from fungal mycelium has recently received increased attention due to significant advantages. For example, while crustacean waste supplies are limited by seasons and sites of fishing industry, fungal mycelium can be obtained by convenient fermentation process that does not have geographic or seasonal limitations [10]; fungal mycelia have lower level of inorganic materials compared to crustacean wastes, and thus no demineralization treatment is required during the processing [11]; crustacean chitin and chitosan may vary in the physico-chemical properties, while fungal chitin and chitosan have relatively consistent properties because of the controlled fermentation conditions [12]; fungal chitin and chitosan are apparently more effective in inducing the plant immune response and are potentially more suitable for agricultural applications [13].

Many fungal species, including *Absidia glauca, Absidia coerulea, Aspergillus niger, Mucor rouxii, Gongronella butleri, Phycomyces blakesleeanus, Absidia blakesleeana, Rhizopus oryzae, Trichoderma reesei* and *Lentinus edodes* have been investigated for the production of chitin and chitosan [10-12,14-19]. Among all investigated species, the most commonly researched one is *M. rouxii* [10,14,15] and quantities of chitin and chitosan in its mycelia can reach 35% of cell wall dry weight [16].

Fungi are usually harvested at their late exponential growth phase to obtain the maximum yield for chitin and chitosan. Although fungi can be grown on solid media, cultivation for chitin and chitosan isolation is usually carried out in the yeast peptone glucose broth (YPG), potato dextrose broth (PDB) or molasses salt medium (MSM), the performance of different media and didnot find significant difference in the yield and physico-chemical properties of chitosan and chitin obtained [14].

Extraction process from fungal sources is similar to industrially utilized except that no demineralization treatment is required due to low mineral content in fungal mycelia [16]. Generally, the extraction procedure consists of three steps:

(1) alkaline treatment to remove protein and alkali soluble polysaccharides;

(2) acid reflux to separate chitin and chitosan

(3) precipitation of chitosan under alkaline conditions.

Removal of proteins by alkaline treatment is commonly performed with 1N NaOH at 95 °C from 1 to 6 h or at 121 °C from 0.25 h to 1 h [17]. Separation of chitosan by acid treatment is usually carried out by 2 to 10 % acetic or hydrochloric acid at 95 °C for 3 to 14 h. For example, Synowiechi et al. [16] used 2 % NaOH at 90 °C during 2 h for alkali treatment and 10 % acetic acid at 60 °C during 6 h for acid reflux during extraction of chitin and chitosan from *M. rouxii.* Hu et al. [18] adopted autoclaving at 121°C in both alkaline and acid treatments of *Absidia glauca mycelia*. However, the temperature and time of acid treatment had to be reduced to 25 °C and 1 h to avoid the depolymerization of chitosan during extraction from zygomycetes strains [19].

Most of the studies in this field concentrate on the fermentation processes to produce fungal mycelia for chitin and chitosan extraction [10-12,14-19]. Relatively few studies have focused on the fungal waste from industrial fermentations or mushroom industry [17]. However, considering the amount of waste that accumulates during processing, citric acid industry and mushroom industry, specifically from *Agaricus bisporus* growing practices, can provide plenty of raw materials for fungal chitin and chitosan production.

Citric acid is the most widely used organic acid in food, beverage and pharmaceutical industries. The industrial production is based on *A. niger* submerged fermentation. The current world requirements for citric acid are estimated to be 400,000 tons per year [20]. Taking into account that 20 % dry mycelium waste is produced under industrial fermentation conditions, approximately 80,000 tons of *A. niger* mycelium waste accumulates every year [21]. Managing this waste presents an extra expense for the producers and alternative solutions for mycelium disposal have been evaluated. One of the potential outputs for the spent mycelia is in feed supplements. However, this type of feed seems to be difficult to compete with the other low price feeds.

White common mushroom, *Agaricus bisporus*, is the most consumed mushroom in the U.S. In last several years the production has been relatively constant and sales totaled 382 million kilograms in the 2002/03 season [22]. The waste accumulated during mushroom production and harvest consists mainly of stalks and mushrooms of irregular dimensions and shape. Depending on the size of the mushroom farm, the amount of waste ranges between 5 and 20% of the production volume. This waste material results in approximately 50,000 metric tons per year that currently has no application (personal communication).

4. Purification of Chitosan

4.1. Chemical methods

The obtained chitosan has to be purified to make it suitable for the pharmaceutical use. The purification process was designed in three steps [23]:

1) Removal of insolubles with filteration

2) Reprecipitation of chitiosan with 1 N NaOH

3) Demetallisation of retrieved chitosan

4.1.1. Removal of insolubles with filtration

One mg/ml chitosan acetic acid 1% (v/v) solution is prepared by a magnetic stirrer until an homogenous solution is obtained. The insolubles were removed by filteration through Whatman filter paper 22μm.

4.1.2. Reprecipitation of chitosan with 1N NaOH

Chitosan was precipitated from filtered chitosan solution by titration with 1 N NaOH until pH value of 8.5. The chitosan obtained is washed several times with distilled water by centrifuging at 8,000 to10,000 xg. All the above steps were carriedin the presence of reducing agent Dithiothreitol, (DTT) in order to provide more consistency and reproducibil-ity between chitosan batches for biomedical applications (any other hydroxides other than NaOH are reactive which would another step in purification if such materials are used).

4.1.3. Demetallisation of retrieved chitosan

Reprecipitation precedes demetallisation by the addition of 1 ml of 10% w/v Aqueous solution of sodium dodecyl sulfate (SDS) and stirring for 30 min for dissolving the protein left over finally. After leaving the solution stirring at room temperature overnight, 3.3 ml of 5% w/v ethylenediaminetetraa-cetic acid (EDTA) was added and stirred at room temperature for 2 additional hours for precipitation of heavy metals with EDTA. The water insoluble chitosan precipitate was collected by centrifugation at 5000xg for 30 min using REMI and washed several times with distilled water by resuspending and re-centrifugation for 30 min. the residue obtained is dried in hot air oven at 60 gently to prevent physical damage in the chain structure. The obtained dried chitosan is stored in the dessicator.

4.2. Biological methods

An alternative way to solve chemical extraction problems is to use biological methods. The use of proteases for deproteinisation of crustacean shells would avoid alkali treatment. Besides the application of exoenzymes, proteolytic bacteria were used for deproteinisation of demineralised shells [24]. This approach allows obtaining a liquid fraction rich in proteins, minerals and astaxanthin and a solid chitin fraction. The liquid fraction can be used either as a protein-mineral supplement for human consumption or as an animal feed [25]. Deproteinisation processes have been reported for chitin production mainly from shrimp waste using mechanical [26], enzymatic [27,28] and microbial processes involving species like *Lactobacillus,*

Pseudomonas aeruginosa K-187 and *Bacillus subtilis* [29]. Biological demineralisation has also been reported for chitin production from crustacean shells; enzymatically, using for instance alcalase, or by microbial process involving species like *L. pentosus* 402 or by a natural probiotic (milk curd). In these biological processes, demineralisation and deproteinisation occur mainly simultaneously but incompletely [18,24,29].

4.2.1. Use of lactic acid bacteria for chitin recovery

Fermentation has been applied to fish for many years and represents a low-level (artisanal) and affordable (neither capital nor energy intensive) technology [25]. It consists in the ensilation of crustacean shells and a low-cost in situ production of lactic acid from by-products such as whey, lignocellulose and starch. Lactic acid production by lactic acid bacteria induced a liquefaction of the semi-solid waste and led to a low pH and activation of proteases [28]. The protein-rich liquid could be separated from the chitin, which remained in the sediment [24]. This method might offer a commercial route for the recovery of chitin [26].

Lactic acid is formed from the breakdown of glucose, creating the low p^H, which improves the ensilation that suppresses the growth of spoilage microorganisms. Lactic acid reacts with the calcium carbonate component in the chitin fraction, leading to the formation of calcium lactate, which precipitates and can be removed by washing. The resulting organic salts from the demineralisation process could be used as de- and anti-icing agents and/or preservatives [26]. Deproteinisation of the biowaste and simultaneous liquefaction of the shrimp proteins occurs mainly by proteolytic enzymes produced by the added *Lactobacillus*, by gut bacteria present in the intestinal system of the shrimp, or by proteases present in the biowaste [25]. It results in a fairly clean liquid fraction with a high content of soluble peptides and free amino acids [26].

Lactic acid fermentation combined with chemical treatments has been studied as an alternative to chemical extraction of chitin, reducing the amount of alkali and acid required [27]. It was considered as a pretreatment of shrimp waste followed by demineralisation and deproteinisation using low concentrations of HCl (0.5 M) and NaOH (0.4 M).

4.2.2. Use of non-lactic acid bacteria for chitin recovery

Non-lactic acid bacteria have also been tested for chitin recovery. Fermentation of shrimp (*Metapenaeopsis dobsoni*) shell in jaggery broth using *Bacillus subtilis* for the production of chitin and chitosan showed that the level of acid produced as well as the proteolytic activity of *B . subtilis* allowed shell demineralisation and deproteinisation [29]. About 84 % of the protein and 72 % of minerals were removed from the shrimp shell after fermentation Pseudomonas aeruginosa K-187 strain isolated from the soil of northern Taiwan is a producer of protease and chitinase/lysozymes when cultured in a medium containing shrimp and crab shell wastes

as the sole carbon sources [30]. It was shown that *P. aeruginosa* K-187 is capable of shell waste deproteinisation in either solid-state, liquid- -solid or liquid fermentation. Higher deproteinisation yield was recorded in solid-state fermentation, 82 % after 5 days, showing that *P. aeruginosa* K-187 is more efficient than the proteolytic bacterium *P. maltophilia*, known to be highly efficient in the deproteinisation of prawn shell waste. The use of protease produced by *P. aeruginosa* K-187 was therefore promising in deproteinisation of crustacean wastes [29].

4.3. Physicochemical properties and analysis

Properly processed, highly purified chitin and chitosan are white and odorless. Their chemical structures are similar to those of cellulose. The only difference is that the 2-hydroxy group of the cellulose has been replaced with an acetamide or amino group in chitin or chitosan, respectively [28]. Therefore, the physicochemical properties and research methodology for all three biopolymers are presumably similar. For example, chitin and chitosan are insoluble in the common organic and inorganic solvents, but soluble in salt organic mixtures of LiCl-N,N-DMAc, which is a common solvent for cellulose [30].

4.4. Applications of chitin and chitosan

Natural and non-toxic biopolymers chitin and chitosan are now widely produced commercially from crab and shrimp shell waste. During the past few decades, chitin and chitosan have attracted significant interest in view of a wide range of proposed novel applications [19]. Their unique properties, biodegradability, biocompatibility and non-toxicity make them useful for a wide range of applications Chitin is mainly used as the raw material to produce chitin-derived products, such as chitosans, oligosaccharides, and glucosamine [1]. There are now over 2000 concrete applications, and the field of nutrition is the largest user of chitosan with 1000 tonnes consumed in 2000. The worldwide industrial production of these derivatives in year 2000 is estimated to be above 10 000 tonnes [18].

4.4.1. Antimicrobial activity

It has been shown that chitosan posses strong antimicrobial activity against both gram-positive and gram-negative bacteria, including the foodborne pathogens, such as *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus,* and *Listeria monocytogenes* [31-33].

In its free polymer form, chitosan exhibits antifungal activity against *Alternaria alternata, Rhizopus oryzae, Aspergillus niger, Phomopsis asparagi,* and *Rhizopus stolonifer*. The antifungal activity of chitosan depends on its concentration, molecular weight, degree of substitution, and the type of functional groups added to the chitosan, as well as the type of fungus [32]. Whilst derivatives of the polymer can be created to target specific pathogens, chitosan shows natural antifungal activity without the need for chemical modification.

Two theories have been proposed for the antimicrobial mechanism of chitosan. Based on one, interaction between positively charged chitosan molecules and negatively charged microbial cell membranes results in the disruption of the cytoplasmic membrane and, ultimately, leakage of intracellular constituents [32,33]. By the other theory, chitosan oligosaccharides easily permeate into the nucleus of eukaryotic cell and interfere with the transcription of RNA and the synthesis of proteins [34]. However, chitosans with high molecular weight (above 100 kDa) generally express stronger antibacterial activity than chitosan oligomers [35].

Recent studies on chitosan depolymerisation have drawn considerable attention, as the products obtained are more water-soluble. Beneficial properties of chitosan and its oligosaccharides include: antitumour; neuroprotective; antifungal and antibacterial and anti-inflammatory [16,31,33].

The antimicrobial activity of chitin, chitosan, and their derivatives against different groups of microorganisms, such as bacteria, yeast, and fungi, has received considerable attention in recent years. Two main mechanisms have been suggested as the cause of the inhibition of microbial cells by chitosan [34]. The interaction with anionic groups on the cell surface, due to its polycationic nature, causes the formation of an impermeable layer around the cell, which prevents the transport of essential solutes. It has been demonstrated by electron microscopy that the site of action is the outer membrane of gram negative bacteria [35]. The permeabilizing effect has been observed at slightly acidic a condition in which chitosan is protonated, but this permeabilizing effect of chitosan is reversible [34]. Chitosan has been confirmed to possess a broad spectrum of antimicrobial activities [35]. However, the low solubility of chitosan at neutral pH limits its application. In this study H_2O_2 was taken to degrade the chitosan into water soluble chitosan. Several studies prove that an increase in the positive charge of chitosan makes it bind to bacterial cell walls more strongly [36]. The molecular weight is the main factor affecting the antibacterial activity of chitosan, from the results obtained. In contrast, some authors have not found a clear relationship between the degree of deacetylation and antimicrobial activity [37]. These authors suggest that the antimicrobial activity of chitosan is dependent on both the chitosan and the microorganism used [38-40,41]. studied the antimicrobial activity of hetero-chitosans with different degrees of deacetylation and Molecular weight against three Gram negative bacteria and five Gram-positive bacteria and found that the 75% deacetylated chitosan showed more effective antimicrobial activity compared with that of 90% and 50% deacetylated chitosan [39,40].

These important properties of chitosan are believed to have many commercial applications of high economic interests [19]. The antifungal and antibacterial activities of chitosan can be employed in production of biofertilizers and biopesticides of economical benefits [20]. Likewise the radical scavenging or the anti-oxidant activity of chitosan is of great interest in food industries and its possible use as natural additives has lead to a great interest in replac-

ing synthetic additives [41]. The use of the antimicrobial activity of chitosan has been used for development of antimicrobial films intended for use in packaging materials for foods, medical supplies and so on, or as laminated coating on items for which surface colonization is undesirable. Chitosan used as coating on fruits and vegetables is almost as effective as the fungicide TBZ at preventing spoilage during storage at proper conditions. Chitosan activity as anti-coagulant is useful in biomedical applications [42] like wound dressing, surgical sutures and for other treatments like reducing oxidative stress in live cells [23], Antitumor activity anti-inflammatory, effect HIV-1 inhibitors, antihypertensive, Hypoglycemic and hypolipidemic effect [40-43] etc. still research is going on. Many studies have been conducted to explore the many possibilities of utilizing the various properties of chitosan and research is still going on these aspects. Chitosan as a commercial chemical has promising range of applications [44].

4.4.1.1. Antimicrobial activity and applications in food preservation

It has been shown that chitosan posses strong antimicrobial activity against both gram-positive and gram-negative bacteria, including the foodborne pathogens, such as Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, and Listeria monocytogenes [44-47]. Two theories have been proposed for the antimicrobial mechanism of chitosan. Based on one, interaction between positively charged chitosan molecules and negatively charged microbial cell membranes results in the disruption of the cytoplasmic membrane and, ultimately, leakage of intracellular constituents [46]. By the other theory, chitosan oligosaccharides easily permeate into the nucleus of eukaryotic cell and interfere with the transcription of RNA and the synthesis of proteins [47]. However, chitosans with high molecular weight (above 100 kDa) generally express stronger antibacterial activity than chitosan oligomers [45].

4.4.2. Wastewater treatment with chitin and chitosan

Chitin and chitosan can be used for the adsorption or fixation of heavy metals [47] and dyes. Chitosan is a polycation polymer effective in coagulation, flocculation and dehydration of activated sludge, and hence used in wastewater treatment [16,18]. Another recent application is immobilization of microorganisms or sludge in chitosan matrices for wastewater treatment in extreme environmental conditions (extreme pH, presence of organic solvents), allowing the reuse of cells and hence their implementation in continuous process.

4.4.3. Applications of chitin and chitosan in food

Only limited attention has been paid to food application of these versatile biopolymers [48]. They offer a wide range of unique applications, which are non-exhaustively listed in **Table 4**. The use of chitosan in the food industry is related to its functional properties, and nutritional and physiological activities. Chitosan exhibits water-, fat- and dye-binding capacity, as well as emulsifying properties [49]; it was shown to be useful in the preparation of stable

emulsions without any other surfactant [45]. It has been used as a dietary supplement due to some interesting properties.

4.4.4. Biomedical application of chitin and chitosan

Chitin and chitosan show excellent biological properties such as non-toxicity, which is illustrated by a dose limit per day of 17 g/kg [16], biodegradation in the human body, biocompatibility, and immunological, antibacterial, wound-healing and haemostatic activity, in cell culture, tissue engineering and drug delivery [50,51], since it is highly biocompatible and biodegradable in physiological environment [20,46]. Chitin is also used as an excipient and drug carrier in film, gel or powder form for applications involving mucoadhesivity.

4.5. Anti-infammatory effects

Inflammation is a physiological body immune response against pathogens, toxic chemicals or physical injury. While acute inflammation is a short-term normal response that usually causes tissue repair by recruitment of leukocytes to the damaged region, chronic inflammation is a long-term pathological response involving induction of own tissue damage by matrix metalloproteinases (MMPs) [52,53].

Although the anti-inflammatory effects of chitin and its derivatives have been rarely reported, in recent years data has been accumulating. First of all, it was found that chitin is a size-dependent regulator of inflammation [54]. While both intermediate-sized chitin and small chitin stimulates TNF production in murine peritoneal macrophages, large chitin fragments are inert, Furthermore, it was found that chitin stimulates the expression of TLR2, dectin-1, the mannose receptor and inflammatory cytokines, differentially activated NF-κB and spleen tyrosine kinase. Chitosan was confirmed to partially inhibit the secretion of both IL-8 and TNF-α from mast cells, demonstrating that water-soluble chitosan has the potential to reduce the allergic inflammatory response [55]. Since mast cells are necessary for allergic reactions and have been implicated in a number of neuroinflammatory diseases, chitosan nutraceuticals may help to prevent or alleviate some of these complications. Chitosan oligosaccharide may possess an anti-inflammatory effect via the inhibition of TNF-α in the LPS-stimulated inflammation. These functions of chitosan to exert anti-inflammatory effect could be unilized in the nutraceutical industry as well as in functional foods for prevention and alleviation of inflammatory diseases. In addition, it was reported that chitosan promotes phagocytosis and production of osteopontin and leukotriene B by polymorphonuclear leukocytes, production of interleukin-1, transforming growth factor b1 and platelet-derived growth factor by macrophages, and production of interleukin-8 by fibroblasts, enhancing immune responses [56].

4.6. Anticancer effects

In recent years, it was revealed that the tumor inhibitory effect of COS is probably related to their induction of lymphocyte cytokines through increasing T-cell proliferation. Basically, the antitumor mechanism of COS is enhanced by acquired immunity via accelerating T-cell differentiation to increase cytotoxicity and maintain T-cell activity [57]. The antitumor effects of various low-molecular weight chitosans, such as water-soluble 21- or 46-kDa molecules with low viscosity, produced by enzymatic hydrolysis of over 650-kDa chitosan, which displayed decreased tumor growth and final tumor weight in sarcoma 180-bearing mice due to increase of natural killer cell activity [58,59]. The results indicate the low-molecular-weight water-soluble chitosans and oligochitosans might be useful in preventing tumor growth, partly through enhancing cytotoxic activity against tumors as an immunomodulator [60].

4.7. Drug delivery system

To provide anticancer chemotherapy, chitosan is attracting increasing attention as drug and gene carriers due to its excellent biocompatibility, biodegradability, and nontoxicity [61]. Chitosan has an important role in delivery of drugs, with the potential to improve drug absorption and stabilize drug components to increase drug targeting. In addition, as a potential gene deliverer, chitosan can protect DNA and increase the expression period of genes. Chitin or chitosan derivatives, which were conjugated with some kinds of anticancer agents, can execute better anticancer effects with a decrease of side effects and gradual release of free drug in the cancer tissues. Furthermore, chitosan nanoparticles were synthesized and applied for in vivo antitumor activity [62]. On the other hand, for ocular drug delivery, liposomes coated with low-molecular weight chitosan may be potentially applicable to clinic uses [63].

Nanoparticles enable chitosan to elicit dose-dependent tumor-weight inhibition with highly impressive antitumor efficacy in vivo. The doses and particle quantum size have a great effect on their efficacy as drug carriers. In particular, with small particle size and positive surface charge, the complex could exhibit higher antitumor activity than other chitosan derivatives [64]. Smaller sized particles seem to enhance efficacy of the particle-based drug delivery systems. Basically, chitosan nanoparticles are produced with a mean particle size ranging from 40 to 100 nm and a positive surface charge of about 50 mV [65]. To introduce these products into in vitro cell culture systems, they should be filtered by a membrane with diameter of 0.45 μm and autoclaved. In in vivo animal models, different administration routes of chitosan nanoparticles, such as intravenous (i.v.) or intraperitoneal injection (i.p.) and oral administration (p.o.), could exhibit little difference in antitumor activities. However, because nanoparticulate systems have been developed to improve the blood circulating time and tumor targeting efficacy of vincristine, administration of chitosan nanoparticles i.v. can contribute in vivo efficacy to antitumor activities followed by a prolonged blood half-life of drugs [66].

4.8. other applications of chitin and chitosan derivatives

Chitin and chitosan derivatives may effectively reduce soil-borne diseases. In addition, chitin exhibits several functions, including retention of nutrients in the soil, and contributes to the nitrogen cycle [14,67-69]. Chitin and chitosan have a versatile application potential in agriculture. In addition, they have found various other applications [15]. Chitin can also be transformed into saccharides under certain conditions . It can also be used as a slowly degrading substrate in microbial fuel cells [69,70,].

5. Chitosan Nanoparticles

Chitosan is soluble in acidic conditions - in solution the free amino groups on its polymeric chains can protonate, giving it a positive charge. Chitosan nanoparticles are biocompatible, relatively non-toxic, biodegradable, and cationic in nature [71,72]. Chitosan nanoparticles can be formed by incorporating a polyanion such as tripolyphosphate (TPP) into a chitosan solution under constant stirring [73].

These nanoparticles can then be used for drug delivery and gene therapy applications. Due to its poor solubility at pH more than 6.5, a number of chemically modified chitosan derivatives with improved water solubility can be used as well [74,75]. Ionic gelation is the most commonly used method for synthesising chitosan nanoparticles [6]. In this method, chitosan precursors are cross-linked using sodium tripolyphosphate (TPP). The method typically yields large sized (100–300 nm) particles with a high degree of polydispersity. Even though ionic gelation is a widely used method and factors governing the size and dispersivity of chitosan nanoparticles (such as the concentration of reactants, temperature, pH, and the level of deacetylation) are well known [7] our basic understanding of the process at mechanistic level is poor. In the ionic gelation process, TPP cross links randomly oriented chitosan molecules, which, in turn, are connected to other similarly cross-linked moieties. Such intra- and intermolecular cross-linking is rather uncontrolled and leads to polydispersity in the synthesized preparation [76].

5.1. Applications of chitosan nanoparticles

The applications of chitosan nanoparticles are [74,75]:

• As antibacterial agents, gene delivery vectors and carriers for protein release and drugs

• Used as a potential adjuvant for vaccines such as influenza, hepatitis B and piglet paratyphoid vaccine

• Used as a novel nasal delivery system for vaccines. These nanoparticles improve antigen uptake by mucosal lymphoid tissues and induce strong immune responses against antigens.

• Chitosan has also been proved to prevent infection in wounds and quicken the wound-healing process by enhancing the growth of skin cells.

• Chitosan nanoparticles can be used for preservative purposes while packaging foods and in dentistry to eliminate caries.

• It can also be used as an additive in antimicrobial textiles for producing clothes for healthcare and other professionals.

• Chitosan nanoparticles show effective antimicrobial activity against Staphylococcus saprophyticus and Escherichia coli.

• These materials can also be used as a wound-healing material for the prevention of opportunistic infection and for enabling wound healing.

• The nanoparticles have also been proven to show skin regenerative properties when materials were tested on skin cell fibrocblasts and keratinocytes in the laboratory, paving the way to anti-aging skin care products.

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