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Review Article

Competitive Biological Activities of Chitosan and Its Derivatives: Antimicrobial, Antioxidant, Anticancer, and Anti-Inflammatory Activities

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Chitosan is obtained from alkaline deacetylation of chitin, and acetamide groups are transformed into primary amino groups during the deacetylation. The diverse biological activities of chitosan and its derivatives are extensively studied that allows to widening the application fields in various sectors especially in biomedical science. The biological properties of chitosan are strongly depending on the solubility in water and other solvents. Deacetylation degree (DDA) and molecular weight (MW) are the most decisive parameters on the bioactivities since the primary amino groups are the key functional groups of chitosan where permits to interact with other molecules. Higher DDA and lower MW of chitosan and chitosan derivatives demonstrated higher antimicrobial, antioxidant, and anticancer capacities. Therefore, the chitosan oligosaccharides (COS) with a low polymerization degree are receiving a great attention in medical and pharmaceutical applications as they have higher water solubility and lower viscosity than chitosan. In this review articles, the antimicrobial, antioxidant, anticancer, anti-inflammatory activities of chitosan and its derivatives are highlighted. The influences of physicochemical parameters of chitosan like DDA and MW on bioactivities are also described.

1. Introduction

Natural polymers are considered environmentally friendly alternatives widely used in medical, agricultural, food, and environmental industries and so on due to their especially renewable, sustainable, and nontoxic properties [1]. Especially in biomedical filed, the natural polymers play very important role. Polysaccharide polymers are the most efficient applicants for the preparation of biomedical products. There are mainly two types of polysaccharides: (i) homopolysaccharides, one type of monomer unit; (ii) heteropolysaccharides, two or more types of monomer unit [2]. They possess a wide range of molecular weights and a significant number of functional groups that give a rise to chemical modification availability [3]. Among the many different sorts of polysaccharides, cellulose (bacterial cellulose and nanocellulose) [4–9], starch [10–14], seaweed (alginate, carrageenan, fucoidan, and ulvan) [15–18], chitin, and chitosan are mainly studied. Due to their attractive abilities to improve the pharmacokinetics and pharmacodynamics of small drug, protein, and enzyme molecules, macromolecular polysaccharides have been receiving significant attention [2, 3]. Polysaccharide polymers demonstrated very efficient attachments of bioactive therapeutic agents, which leads to an increase in the duration of activity [2]. The bioactive agents can bind covalently to polysaccharide backbone structures.

Chitosan is a biopolysaccharide obtained by a de-N-deacetylation process of chitin which is the primary structural polymer in arthropod exoskeletons [19–22]. Chitosan contains three types of reactive groups which are the primary amine group and the primary and secondary hydroxyl groups at C-2, C-3, and C-6 positions, respectively [15]. Among the three reactive groups, the primary amine at the C-2″ position of the glucosamine residues is the most considerable functional groups for biological activities of chitosan [23]. Chitosan has received a significant attention for several

decades due to their unique biological activities. This review aims to supply the recent information about the competitive biological activities of chitosan and its derivatives for medical and pharmaceutical applications. Among many biological activities of chitosan and its derivatives discovered so far, antimicrobial, antioxidant, anticancer, and anti-inflammatory activities were described with recently published outcomes.

2. Chitosan

After cellulose, chitin is the most abundant natural mucopolysaccharide and commonly found as constituent of the exoskeleton in animals, particularly in crustaceans, mollusks, and insects [19–22]. Chitosan is derived from alkaline deacetylation of chitin composing of 2-amino-2-dedoxyd-glucose and 2-acetamino-dedoxy-d-glucose units linked with b-(1 → 4) bonds (Figure 1) [19, 20]. In the process of deacetylation of chitin, the acetamide groups are transformed to the primary amino groups, which are the principal functional groups of chitosan. Chitosan possesses 5–8% nitrogen in the molecules in form of the primary aliphatic amine groups, which makes chitosan proper for typical reactions of amines [19, 23]. The degree of deacetylation of chitosan is referred to the molar fraction of N-acetylated units (DA) or percentage of acetylation (DA%). The high viscosity and low solubility of chitosan limit its biological applications since the attractive biological properties of chitosan are strongly depending on the solubility in water and other commonly used solvents [24]. The degree of deacetylation (DDA) makes an important role to decide its bioactivities as they are directly related to the cationic behavior of chitosan, and the protonation of the amino groups occurs in aqueous acidic solutions [21, 22, 25–28].

The functional amino groups in chitosan are easily modified by chemical reaction and that results in the changes of the mechanical and physical properties. High molecular weight of chitosan allows less availability for its bioactivities, and thus, depolymerization by hydrolysis of polymer chains is frequently performed to acquire low molecular or oligomers of chitosan. In acid hydrolysis, temperature and acidic concentrations were critical factors affecting on the results [29]. The enzymatic degradation of chitosan is getting an attention since it possesses many advantages like milder condition, high specificity, no modification of sugar rings, and mass production comparing to chemical hydrolysis [30]. Common nonspecific enzymes like lysosome, chitinase, pectinase, and cellulose are employed [21]. Proteolytic enzymes, such as pepsin, papain, pronase [31, 32], hepatopancreas [33], and chitosanase [30], were also studied to obtain the low molecular weight of chitosan. Chitosan oligosaccharide (COS) is an oligomer of chitosan, which usually has a degree of polymerization (DP) < 50–55 and an average molecular weight (MW) < 10,000 kDa [34]. COS has good water solubility and low viscosity and thus has more favorable applicant than chitosan in biomedical applications. Aranaz and his colleagues well reported the relations between the biological characteristics and MW and the deacetylation degree of chitosan [21]. When the DDA increases, the solubility of

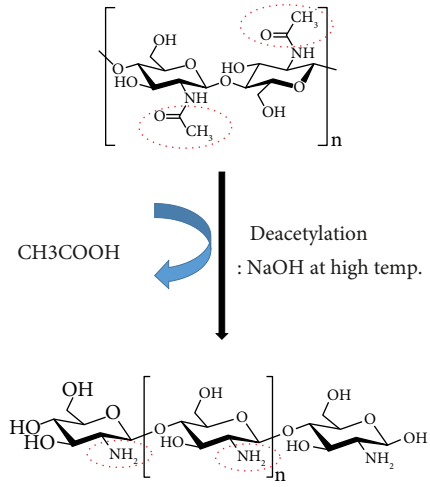

Figure 1: Schematic presentation of chitin deacetylation with alkaline.

chitosan also increases and the more possible interactions are permitted between the available sites of chitosan and other molecules. Thus, the mucoadhesive capacity of chitosan polymers increases with an increase of DDA by providing higher numbers of reactive amino groups available for interaction with other molecules [35, 36]. The cationic characteristic of chitosan is pH dependence (pKa 6.3) and makes it ready to interact with negatively charged molecules such as proteins, therapeutic DNA or RNA, fatty acids, bile acids, phospholipids, and anionic polyelectrolytes [35, 37, 38]. Besides the MW and DDA of chitosan, other physicochemical properties like polydispersity (MW/MN) and crystallinity or the pattern of acetylation might be also considered since they affect on mechanical and biological activities of chitosan [24].

Chitosan and its derivatives are extensively studied in medical and pharmaceutical fields due to their competitive biological properties like biocompatibility, biodegradability, nontoxicity, and analgesic, antitumor, hemostatic, hypocholesterolemic, antimicrobial, and antioxidant properties and so on [35, 39]. These properties are very advantageous in biomedical applications of tissue engineering, wound healing, excipients for drug delivery, and gene delivery [37, 38, 40–42]. The preparations of chitosan-based biomedical materials are varied such as finely divided powders, films, membranes, gels, coatings, nanoparticles, suspensions, and hydrogels, and they can influence their biomedical activity [24]. Depending on the operation purpose, types of drug, and healing target, the preparation manner can be varied.

3. Antimicrobial Activity

The antimicrobial activity has been considered the most essential and influential bioactivity of chitosan and employed not only to the preparation of biomedical materials but also to the functionalization of other polymeric materials including fibers and food conservation [43–51]. The most concerned problem found in hospital and healthcare institutions is infections by microorganism, and thus the

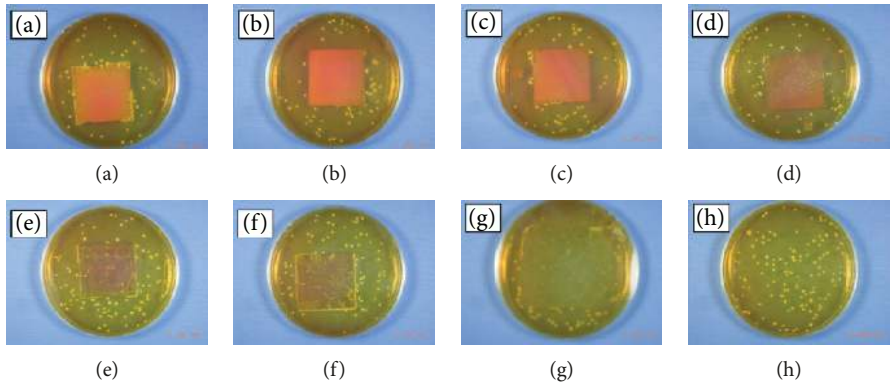

Figure 2: Effect of the DDA of chitosan on the growth inhibition of *S. aureus*. Higher DDA was more effective on inhibiting the growth of *S. aureus*: (a) DD 92.2%; (b) DD 90.1%; (c) DD 88.0%; (d) DD 83.9%; (e) DD 79.7%; (f) DD 75.5%; (g) PVC; (h) control (adapted from [59]).

antimicrobial activity should be primarily considered in biomedical materials. The exposure of subcutaneous tissue caused by wounds like cut, surgery, burn, and so on provides a moist, warm, and nutritious environment that is very suitable for growing the microorganisms [52]. The wound infections are seriously considered since they can cause an increase of trauma and a burden on financial resources to the patients. The mechanism of antimicrobial activity of chitosan is not yet fully understood although numerous researches have been carried out so far. The antimicrobial effect of chitosan is much higher comparing to chitin due to the numbers of the amine groups that is responsible for cationic property of chitosan. Positively charged chitosan at acidic condition might interact with negatively charged residues of carbohydrates, lipids, and proteins located on the cell surface of bacteria, which subsequently inhibit the growth of bacteria [21, 22]. Thus, the electronic property of chitosan plays a very important role in the inhibition mechanism of microorganisms. The high density of positive charge on the structure of chitosan or its derivatives generates strong electrostatic interaction that is affiliated with DDA. With this theory, chitosan is more promising for the inhibition of Gram-negative than Gram-positive bacterium since the negatively charged cell surfaces interact more with positively charged chitosan [22, 43, 47, 53]. However, many researches demonstrated that the chitosan was a more efficient inhibitor against Gram-positive compared to Gram-negative microorganism in their experimental results [44, 45, 54–58].

Takahashi and his colleagues tested the influence of DDA of chitosan on the antimicrobial activity against *Staphylococcus aureus* using two different testing methods, that is, incubation using a mannitol salt agar medium and a conductimetric assay [59]. In both testing methods, the DDA of chitosan played a dominant role in the inhibition of *Staphylococcus aureus* growing (the higher DDA showed the higher rate of inhibition) (Figure 2).

Jung et al. and Younes et al. also achieved similar results about the antimicrobial activity depending on chitosan DDA [60, 61]. When the DDA was nearly 100% (99%), chitosan inhibited almost all types of bacteria tested at the minimum inhibitory concentration (MIC).

There is another theory proposed about the inhibition mechanism of chitosan, that is, an inhibition of RNA and protein synthesis by permeation into the cell nucleus and eventually rupture and leakage of intracellular component. In this theory, the MW is the most decisive factor on the activity [20–22]. The low MW of chitosan was found that easily penetrates into the cell wall of bacteria, combining with DNA and inhibiting the synthesis of mRNA and DNA transcription. With the increase of MW, the permeation into the cell nucleus capacity is decreased. In the case of high MW chitosan, it binds to the negatively charged components on the bacterial cell wall forming an impermeable layer around the cell and consequently changes the cell permeability and blocks transport into the cell [38, 62].

Apart from the MW and DDA, the solubility, pH, and temperature environment are also affecting on the antimicrobial activity of chitosan. At a lower pH, the positive ionic charge increases and chitosan is more absorbed by bacterial cells [20–22]. Benhabile et al. experimented the antimicrobial potential of chitin, chitosan, and its N-acetyl chito- and chito-oligomers against four Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and ATCC 43300, *Bacillus subtilis*, and *Bacillus cereus*) and seven Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Vibrio cholerae*, *Shigella dysenteriae*, *Prevotella melaninogenica*, and *Bacteroides fragilis*) [63]. In this publication, both N-acetyl chito- and chito-oligomers were more effective on the inhibition activity against all tested microorganism than chitosan and chitin, and the decisive effect of DDA and MW on antimicrobial activity was well proved. When DDA is the same (~80%), the effect of MW on the inhibition capacity against *Escherichia coli* was studied by Liu et al. [64]. The authors tested the MW from 55 to 155 kDa, and the lower MW has the higher activity of inhibition against *Escherichia coli*.

Jeon and his colleagues presented the antimicrobial potential of chitosan microparticles against *Escherichia coli*, *Salmonella enterica*, *Klebsiella pneumonia*, and *Streptococcus uberis* [65]. The chitosan microparticles showed a broad-spectrum antimicrobial activity, and when high concentration of chitosan microparticles was applied, the activity

increased. Despite the many studies realized so far, still there is a limitation to conclude about the clear relation between antimicrobial capacity of chitosan and its MW and DDA. It might be due to many other factors affecting the inhibition rate such as sorts of bacterial strains and conditions of biological testing [66]. To expect the synergetic effect of the antimicrobial activity, the incorporation with other promising compounds [43, 44, 67–69] and the modification of structure of chitosan molecules are attempted [26, 70–72]. The phytochemicals like phenolic compounds are broadly attempted to improve antimicrobial activity of chitosan by grafting into the structure [44, 73]. Kim et al. reported the antibacterial effect of chitosan-phytochemical (caffeic acid, ferulic acid, and sinapic acid) conjugates on acne-related bacteria *P. acnes*, *S. epidermidis*, *S. aureus*, and *P. aeruginosa*, and the results exhibited higher (synergetic) antimicrobial effects than that of unconjugated chitosan [73]. Eom et al. prepared the conjugates of chitosan and ferulic acid in the presence of β-lactam antibiotics, and their synergetic antibacterial effect against methicillin-resistant *Staphylococcus aureus* was achieved [74].

4. Antioxidant Activity

Free radical reaction is considered the major cause of several specific human disease and has become an intense interested theme to scientists. Due to its atomic or molecular structure, free radicals are unstable and very reactive. Thus, they tend to pair up with other molecules and atoms to be more stable state [75]. Phaniendra and his colleagues defined free radical as an atom or molecule containing one or more unpaired electrons in a valence shell or outer orbit and is capable of independent existence [76].

In human body, reactive oxygen species (ROS) are produced during the normal metabolism and they oxidize biomolecules, such as lipids, proteins, carbohydrates, and DNA, ultimately leading to oxidative stress [20]. The term of ROS is used not only for oxygen-derived free radicals like superoxide, hydroxyl radical, and nitric oxide but also for nonradical oxygen derivatives of high reactivity like singlet oxygen, hydrogen peroxide, peroxynitrite, and hypochlorite [75, 77]. In biological system, mitochondria are the main responsible for ROS generation during physiological and pathological states and their own ROS scavenging mechanisms required for cell survival [78]. Besides the normal cellular metabolism, there are many exogenous sources to generate ROS such as ozone exposure, hyperoxia, ionizing radiation, and heavy metal ions [79]. In cell metabolism, various enzymes such as catalase, superoxide dismutase, and glutathione peroxidase are involved as a part of the cellular defense system against ROS-mediated cellular injury [80]. When excessive ROS are generated in cellular metabolism, the defense mechanism is not able to protect cellular system and thus the oxidative stress is caused. The oxidative stress in the human body can cause various pathogenic processes including aging, cancer, wrinkle formation, rheumatoid arthritis, inflammation, hypertension, dyslipidemia, atherosclerosis, myocardial infraction, angina pectoris, heart failure, and neurodegenerative diseases such as Alzheimer, Parkinson, and amyotrophic lateral sclerosis [80–84]. In this aspect, an increasing interest in antioxidant agents is very natural.

Therefore, the antioxidant activity of chitosan has been getting high attention from many scientists. Chitosan has shown a notable scavenging activity against different radical species presenting a great potential for an extensive applications. The scavenging activity of chitosan derivatives against free radicals comes through donating hydrogen atom, and several theories were proposed by Xie et al. [85]:

(i) The hydroxyl groups in the polysaccharide unit can react with hydroxyl radicals by the typical H-abstraction reaction.

(ii) OH can react with the residual-free amino groups NH_2 to form stable macromolecules radicals.

(iii) The NH_2 groups can form ammonium groups NH_3^+ by absorbing H^+ from the solution, and then they react with OH through addition reactions.

The DDA and MW of chitosan are also the major factors deciding the scavenging capacity of chitosan [21]. Different with chitosan, chitin is an insoluble polymer in water and thus the major limitation exists for being a useful antioxidant agent.

The NH_2 groups in chitosan are responsible for free radical scavenging, and they can be protonated in acidic solution. There are many publications about the effect of MW and DDA on the scavenging capacity of chitosan. Mahdy Samar and his colleagues experimented an antioxidant activity with various chitosan samples with different DDA and MW and obtained results as high rate of DDA and low MW of chitosan has higher antioxidant activity [27]. Hajji et al. studied three types of chitosan obtained by deacetylation of chitin extracted from Tunisian marine sources shrimp *(Penaeus kerathurus)* waste (DDA: 88%), crab *(Carcinus mediterraneus)* shells (DDA: 83%), and cuttlefish *(Sepia officinalis)* bones (DDA: 95%) [86]. In the test of antioxidant activity, chitosan from cuttlefish with 95% DDA showed the highest value of scavenging effect on DPPH-free radical. Kim and Thomas evaluated the antioxidant activity of chitosan with different MW like 30, 90, and 120 kDa and proved that higher antioxidant activity acquired with lower MW of chitosan (30 kDa) [87]. Sun and his colleagues studied about chitosan oligomers with different MW and tested the scavenging capacity against superoxide anion and hydroxyl radical [88]. In both superoxide anion and hydroxyl radical, the chitosan oligomers presented relative stronger scavenging activity with lower MW. The antioxidant activity of enzymatically degradated chitosan against hydrogen peroxide, 2, 2-diphenyl-1-picrylhydrazyl radical, and chelating ferrous ion was reported by Chang et al. [89]. The results showed that lower MW of chitosan (~2.2 kDa) has the highest impact on the scavenging capacity. Li et al. prepared the low MW of chitosan by oxidative degradation using hydrogen peroxide and tested scavenging capacity against hydroxyl radical [90]. The results indicated that the MW of chitosan (lower

MW has better activity) and concentration were attributed to free radical scavenging effect.

Although the antioxidant activity of chitosan has been proven through many researches, the level of activity is not very satisfactory due to the lack of a H-atom donor to serve as a good chain-breaking antioxidant [91]. The scavenging capacity of free radicals is related to bond dissociation energy of O–H or N–H and the stability of the formed radicals. Due to strong intramolecular and intermolecular hydrogen bonds in chitosan molecules, the OH and NH_2 groups are difficult to dissociate and react with hydroxyl radicals [85]. The various modifications of chitosan molecules to improve the activity were accomplished by grafting functional groups into molecular structure. Among the many tries, the grafting of polyphenols onto chitosan was the most actively studied. Most of polyphenols are found from natural sources and considered safe and environmentally benign materials. After recognizing their strong antioxidant activity, polyphenols have been extensively studied in the area of nutrient, food manufacturing, pharmaceuticals, and medicals [92–98]. The grafting reaction of chitosan and polyphenols was mostly assisted by enzymes [72, 99–101]. In the enzyme-catalyzed reaction, phenolic compounds are oxidized to *o*-quinones which are highly reactive electrophilic compounds further covalently graft to nucleophilic amine groups in chitosan through Schiff-base and/or Michael-type addition reaction [45, 72]. After modification of chitosan by grafting polyphenols, the antioxidant activity was remarkably increased due to the synergetic effects obtained from both chitosan and polyphenols. Figure 3 shows the grafting mechanism of chitosan and catechin by laccase-mediated oxidation reaction (Figure 3(a)) and an increase of antioxidant activity on the chitosan film after grafting catechol (Figure 3(b)).

5. Anticancer Activity

The general cancer treatments performed clinically using chemotherapy, radiotherapy, and surgery have considerably extended the life expectancy of patients. Many current anticancer drugs have nonideal pharmacological properties such as low aqueous solubility, irritating nature, lack of stability, rapid metabolism, and nonselective drug distribution, and they can cause several adverse consequences, including suboptimal therapeutic activity, dose-limiting side effects, and poor-patient quality of life [102, 103]. Thus, many scientists are inspired to search for more effective and harmless medication for cancer-suffering patients. Chitosan and its derivatives are considered the potential anticancer polysaccharide naturally obtained. Many efforts on searching an efficient anticancer agent from natural products lead an increasing interest in polysaccharides. Zong et al. published a review article about the anticancer activity of polysaccharides from fungi, plants, algae, animals, and bacteria [104]. They resumed the inhibition mechanism of tumor growth by polysaccharides as the following:

(i) Prevention of tumorigenesis by oral consumption of active preparations

(ii) Direct anticancer activity, such as the induction of tumor cell apoptosis

(iii) Immunopotentiation activity in combination with chemotherapy

(iv) Inhibition of tumor metastasis

An intrinsic antitumor activity of chitosan and its derivatives with low MW was verified through *in vitro* and *in vivo* experiments [105]. Along with antimicrobial and antioxidant activities, the DDA and MW of chitosan and its derivatives are also the major factors deciding antitumor activity. The effects of the DDA and MW of chitosan oligomers on antitumor activity *in vitro* were investigated by Park et al. [106]. The lower MW and higher DDA (higher solubility) are promising factors for the development of antitumor agents derived from chitosan in *in vitro* tests with Human PC3 (prostate cancer cell), A549 (carcinomic human alveolar basal epithelial cell), and HepG2 (hepatocellular carcinoma cell). Azuma and his colleagues well reviewed about the antitumor activity of COS *in vivo* and *in vitro* cell models showing an effectiveness on tumor growing, reduction of the number of metastatic colonies, suppressing cancer cell growing, and enhancement of acquired immunity [107]. COS has comparatively short chain length and readily soluble in water. Jeon and Kim examined the antitumor activity of COS with different molecular weight against S180 (sarcoma 180 solid) and U14 (uterine cervix carcinoma number 14) tumor cell-bearing mice [108]. The results proved that the antitumor activity was clearly dependent on MW and the range of MW 1.5 to 5.5 kDa effectively inhibited the growth of both tumor cells S180 and U14 in the mice. At the same time, the mice survived more days without weight loss. In several studies, nanoparticles prepared with chitosan showed direct inhibition activity to the proliferation of human tumor cell by inducing apoptosis and growth suppression without signs of neurological toxicity or weight loss proving the safeness of chitosan nanoparticles in the mouse model [109–111]. Xu et al. described that the antitumor activity of chitosan nanoparticles might be related to anti-angiogenic activity that is correlated with vascular endothelial growth factor receptor (VEGFR2) production and subsequent blockage of vascular endothelial growth factor-(VEGF-) induced endothelial cell activation [109]. The stearic acid-g-chitosan oligosaccharide (CSO-SA) micelles were studied for antitumor drug or gene delivery carriers [112, 113]. Hydrophobic drug, podophyllotoxin, was successfully loaded in the CSO-SA micelles demonstrating a sustained release and *in vitro* anticancer effects for suppressing against human breast carcinoma (MCF-7) cells, human lung cancer cells (A549), and human hepatoma cell line (Bel-7402) [112]. Polyethylenimine-conjugated stearic acid-g-chitosan showed good DNA-binding capacity (formation of gene delivery complex) with effectively suppressing the tumor (above 60% tumor inhibition) without systematic toxicity [113]. There are also many other studies about the chitosan and chemically/physically

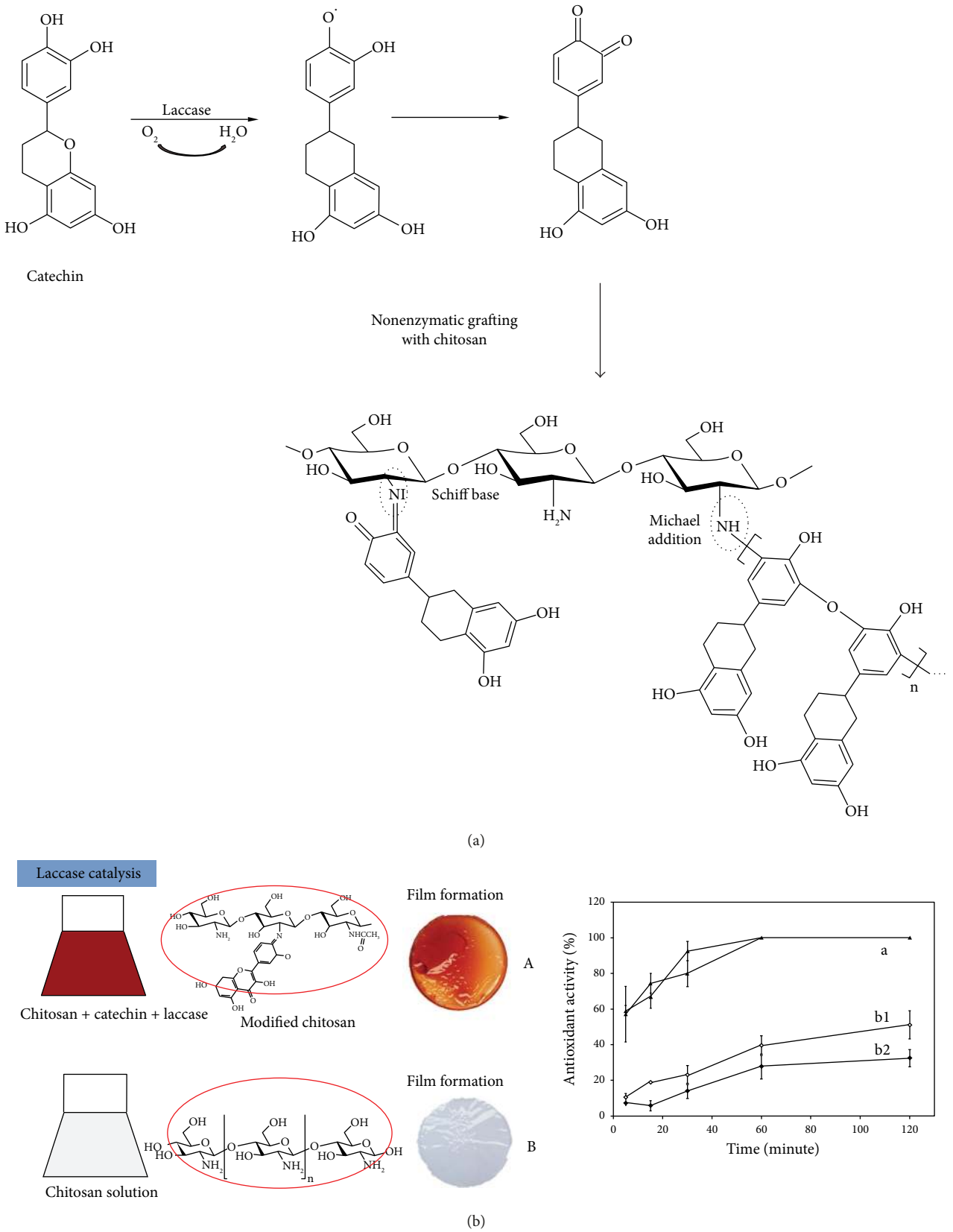

Figure 3: (a) Schematic presentation of enzymatic oxidation of catechin by laccase and nonenzymatic grafting with chitosan and (b) enzymatically modified chitosan film with catechin flavonoids (A) and unmodified chitosan film (B). After modification with catechin, the antioxidant activity of films reached to 100% in 60 minutes while chitosan film (b) showed less than 40% of radical reduction. b1: 95% of DDA; b2: 64% of DDA (adapted and modified from [26]).

Table 1: Anticancer and antitumor activity of chitosan involved preparations tested in various cancer cells.

Cancer types	Used CS form	Tested cells	Remarkable results	Ref.
Breast cancer	SCS[1], SBCS[2]	MCF-7, MDA-MB-231	Inhibition cell proliferation and inducing apoptosis	[114]
	CS[3]	MDA-MB-231, MCF-7, T47D	Inhibition cell proliferation, inducing apoptosis nontoxic to fibroblast L929 normal cells	[102]
	Docetaxel-CN[4]	MCF-7	Inhibition cell proliferation, nontoxic to normal cells	[115]
	FA-CS-UA-NPs[5]	MCF-7	Inhibition cell viability, inhibition tumor growth (reduction of size)	[116]
	MCN[6]	MCF-7	Inhibition cell proliferation	[117]
Prostate cancer	CA[7] scaffolds	LNCaP, C4-2, C4-2B, TRAMP-C2	Good interaction with immune cells, including tumor-infiltrating B cells	[118]
	CS-EGCG NP[8]	22Rv1	Inhibition tumor growth (reduction of size)	[119]
	GC-based CNPs[9]	PC-3	long-term tumor growth inhibition	[120]
	CHGC[10]	LNCaP, PC-3	Inhibition tumor growth (reduction of size)	[121]
	FA-CS PLGA NP[11]	DU145	Inhibition cell proliferation	[122]
	CS-AGR2 siRNA NP[12]	PC-3	Inhibition cell viability	[123]
Colon cancer	CSHA[13] membranes	HT29, DLD-1, HCT116, SW480,	In situ inhibitory effect on cancer cell	[124]
Liver cancer	Bio-CS NP[14]	SMMC7721	In situ inhibition cell proliferation, in vitro and in vivo efficient cell targeting	[125]
	CS, CSHA[13] membranes	Huh7, HepG2, Hep3B, SKHep-1	Inhibition cell proliferation	[124]
Esophageal cancer	CS NP	CAF cell from cancer patient	Inhibition cell proliferation, antimetastatic ability	[126]
Oral cancer	CLCS NP[15]	SCC-9	Reduction cell viability	[118]
	CS	HSC-3, HSC-4, Ca9-22, and HaCaT	Reduction cell viability	[127]

SCS[1]: sulfated chitosan; SBCS[2]: sulfated benzaldehyde chitosan; CS[3]: chitosan; docetaxel-CN[4]: docetaxel-loaded chitosan nanoparticle; FA-CS-UA-NPs[5]: folate-chitosan nanoparticles loaded with ursolic acid; MCN[6]: magnetic chitosan nanoparticles; CA[7]: chitosan-alginate; CS-EGCG NP[8]: chitosan nanoparticles encapsulating epigallocatechin-3-gallate; GC-based CNP[9]: glycol chitosan-based chitosan nanoparticles; CHGC[10]: glycol chitosan; FA-CS PLGA NP[11]: folic acid conjugated-chitosan functionalized poly (D,L-lactide-co-glycolide) nanoparticles; CS-AGR2 siRNA NP[12]: chitosan-based AGR2 siRNA nanoparticle; CSHA[13]: hyaluronan- (HA-) grafted chitosan; bio-CS NP[14]: biotinylated chitosan nanoparticles; CLCS NP[15]: curcumin-loaded chitosan-coated nanoparticles.

modified chitosan or chitosan derivatives for various types of cancer treatment *in vivo* and *in vitro*. Most of the studies commonly demonstrated that chitosan involved anticarcinogenic tools that are very efficient on the inhibition of cell proliferation, inducing apoptosis, cell viability, reduction of tumor size, cell targeting, less side effect, and low toxicity. In Table 1, the summarized several literatures about the anticancer effects of chitosan involved anticarcinogenic tools on breast, prostate, esophageal, liver, oral cancer cells, and so on.

6. Anti-Inflammatory Activity

Inflammation is the first protective response to infection or injury of human body driven in a tissue compartment by a specific set of immune and inflammatory cells with the aim of restoring its structural and functional integrity after exposure to an adverse stimulus [128]. Numerous researches have carried out about the anti-inflammatory and proinflammatory properties of chitosan and its derivatives. Davydova and his colleagues tested the anti-inflammatory activity of chitosan with high (MW: 115 kDa) and low molecular weight (MW: 5.2 kDa), and both chitosan samples presented an intensified induction of anti-inflammatory IL-10 cytokine in animal blood and suppression of colitis progress [129]. The authors concluded that the main contribution to anti-inflammatory activity of chitosan was driven by structural elements comprising its molecule, but not depending on MW. Friedman et al. reported the inhibition capacity of chitosan-alginate nanoparticles against inflammatory cytokines and chemokines induced by *P. acnes*, and the results showed that chitosan-alginate nanoparticles efficiently inhibited *P. acnes*-induced cytokine production in human monocytes and keratinocyte in a dose-dependent manner [130]. Besides inhibition capacity, they also showed high specificity of controlled drug delivery potential for topical therapeutics. Oliveira et al. examined the inhibition of proinflammatory cytokines and anti-inflammatory activities of chitosan film [131]. From the achieved results, a reduction of TNF-α (proinflammatory cytokines) in 3~10 days of cells cultured on chitosan film and significant increase of anti-inflammatory cytokines IL-10 and TGF-β1 are presented. Anti-inflammatory activities of COS were demonstrated by many scientists notwithstanding that the exact mechanism is not yet fully understood. Chung et al. studied two types of COS with high (70 kDa) and low molecular weight (MW:

<1 kDa), and their anti-inflammatory capacity was compared [132]. In low molecular weight COS, the significant inhibition effect against IL-4, IL-13, and TNF-α cytokines was found showing the potential in alleviating the allergic inflammation *in vivo*. Li et al. proposed the mechanism of the lipopolysaccharide-induced NF-κB-dependent inflammatory gene expression by COS, which was associated with reduced NF-κB nucleus translocation [133]. NF-κB is an important transcription factor in mediating the proinflammatory responses. Similar study was carried out by Ma et al. [134], and the positive effect of pretreatment with COS on the suppression of LPS-induced NF-κB and AP-1 activation in macrophages was explained. The results explained that COS is a potential inhibitor against NF-κB- and AP-1-mediated inflammation responses in macrophage by showing the suppression of the LPS-induced c-fos (proto-oncogene) expression in macrophages in a concentration-dependent manner. Yang and his colleagues reported COSs with different MW: COS-A (10 kDa < MW < 20 kDa) and COS-C (1 kDa < MW < 3 kDa) [135]. In both COS samples, the remarkable inhibition activity was observed against the LPS-induced nitric oxide production of RAW 264.7 cells by 50.2% and 44.1%, respectively, without cytotoxicity. Comparing to COS-A, COS-C (lower MW) has a higher level of inhibition activity at lower concentration applied. Li et al. reported the proinflammatory and inflammatory activities of COS (obtained by enzymatic hydrolysis using chitosanase) on cytokines [136]. The authors examined the level of proinflammatory cytokines like IL-1β, IL-6, and TNF-α and anti-inflammatory cytokine IL-2 in mouse osteoarthritis (OA) model. The reduction of serum expression of proinflammatory cytokines and enhancement of anti-inflammatory activity were achieved. Apart from that, the relief of knee joint swelling symptom of mouse model was observed by measuring the changes of the diameter of the knee joint.

7. Future Prospects

Chitosan and its derivatives are extensively studied for medical and pharmaceutical applications. Their unique and attractive bioactivities have been proved through in situ and in vitro experiments. They are easy to obtain in nature with low-cost processes via alkaline deacetylation of chitin. Besides that, the possible acquirement of raw materials by reusing of by-products from food processing industries is also very competitive. Taking into account their many advantages, the interest on the industrial applications of chitosan and its derivatives might be constantly increased. The commercialization of products prepared with chitosan and its derivatives is not yet very common and easy to find. In the future, the effort might be made for easier accessibility of costumers to commercial products in the market. To get more confidence on the chitosan-based commercial products from customers, more fundamental studies on the natural polysaccharides with useful bioactivities might be accomplished including the mechanism of bioactivities of chitosan molecules. This review might help to clarify what have been the most considered among many advantages of chitosan and its derivatives for medical applications in the literatures and will motivate many scientists to work on both fundamental studies and more variety industrial applications.

8. Conclusion

Chitosan and its derivatives possess very attractive biological activities. The potential availability of chitosan and its derivatives in biomedical applications was mainly focused in this review article like antimicrobial, antioxidant, anticancer, and anti-inflammatory activities. Countless researches have been carried out and have commonly reported excellent activities without toxicity. The MW and DDA of chitosan were the most decisive factors affecting on the biological activities mentioned in this review. Thus, in many cases, the hydrolysis of chitosan to reduce the MW to improve their functionality in diverse manners and its effects on biological activities were studied in parallel. The excellent results have been shown through the many scientists, but still there are many challenges required to be explored to explain their mechanism of bioactivities. This review will contribute to the authors working not only on the preparation of chitosan-based biomedical products but also on the evaluation of their specific biological activities.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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