

Chitin and Chitinases: Biomedical And Environmental Applications of Chitin and its Derivatives

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Abstract

Disposal of chitin wastes from crustacean shell can cause environmental and health hazards. Chitin is a well known abundant natural polymer extracted after deproteinization and demineralization of the shell wastes of shrimp, crab, lobster, and krill. Extraction of chitin and its derivatives from waste material is one of the alternative ways to turn the waste into useful products. Chitinases are enzymes that degrade chitin. Chitinases contribute to the generation of carbon and nitrogen in the ecosystem. Chitin and chitinolytic enzymes are gaining importance for their biotechnological applications. The presence of surface charge and multiple functional groups make chitin as a beneficial natural polymer. Due to the reactive functional groups chitin can be used for the preparation of a spectrum of chitin derivatives such as chitosan, alkyl chitin, sulfated chitin, dibutyl chitin and carboxymethyl chitin for specific applications in different areas. The present review is aimed to summarize the efficacy of the chitinases on the chitin and its derivatives and their diverse applications in biomedical and environmental field. Further this review also discusses the synthesis of various chitin derivatives in detail and brings out the importance of chitin and its derivatives in biomedical and environmental applications.

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Introduction

Chitin is a natural polymer, first discovered in mushrooms by French Professor, Henrri Braconnot, in 1811. Chitin is the second most abundant biopolymer next to cellulose with an annual production of 10^{10} to 10^{11} tons¹. In many respects, chitin is similar to cellulose and is considered to be a derivative of cellulose where the C2 hydroxyl groups were replaced by acetamido residues^{1,2}. In nature, chitin is found as crystalline microfibrils which form the structural components of many organisms. Chitin serves as a structural and functional material wherever reinforcement and strength are required in a number of living organisms². The commercial value of chitin has dramatically increased recently due to the beneficial properties of its soluble derivatives, which are suitable for a wide variety of industrial applications in biotechnology, agriculture, food processing, cosmetics, veterinary, medicine, dentistry, environment protection, and paper or textile production³. Chitin is one of the ubiquitous polymers found in many organisms (Table 1) from cell walls of fungi and algae to cuticle of insect's, shells of mollusks (endoskeleton of cephalopods) and crustaceans⁴. Chitin is widely distributed in the invertebrates and in the lower forms of plants. Chitin is a well-known component in the fungi while it is a major component in the exoskeletons of arthropods such as crustaceans and insects. Approximately 75% of the total weight of crustaceans (shrimp, crabs, prawns, lobster, and krill) ending up as waste are mainly used for the isolation of chitin⁵. In fact more than 10,000 tons of shell fish waste is available every year, which would

provide sufficient raw material for the production of chitin⁶. Chitin contains amino sugars, comprising of two monomeric units namely N-acetylglucosamine and glucosamine. Chitin is a linear unbranched chains of β -(1 \rightarrow 4) linked 2-acetamido-2-deoxy-D-glucose (N-acetyl-D-glucosamine) residues of polysaccharides. The amount of glucosamine present in chitin is very low and hence it is less soluble in solvents and water³¹. The β -1,4-linkage between the monomeric units provides a linear structure, stability and rigidity to chitin. The abundant hydroxyl groups and amino groups of the polymer have the tendency for inter and intra molecular hydrogen bonds which resulted in the formation of linear aggregates with extensive crystallinity³². The molecular weight (Mw) of chitin can be as high as 10^6 Da and the structure of chitin is represented in Fig. 1. In nature, chitin exists in three different polymeric forms namely α , β and γ with different physical properties³³. The different forms of chitin differ in their arrangement of the polymeric chain (Fig. 2). In α -chitin, the chains are arranged anti-parallel to each other, in β -chitin, they are arranged parallel to each other and in γ -chitin the polymeric chains are arranged randomly in which two parallel chains and one anti-parallel chain forms the polymeric structure.

The main source of α -chitin is from crustaceans such as crabs and shrimp whereas β -chitin is derived from squids and γ -chitin is from loligo³². The characteristic features of chitin namely degree of deacetylation (DDA) and molecular mass can vary with the method of isolation, the process and origin of chitin. The degree of deacetylation can be defined as the molar

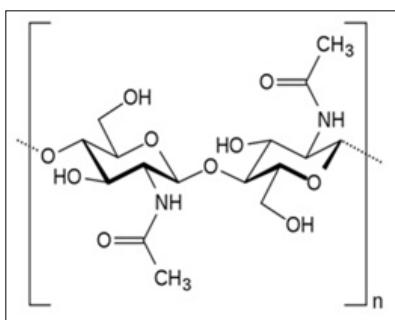


Figure 1. Chemical structure of chitin.

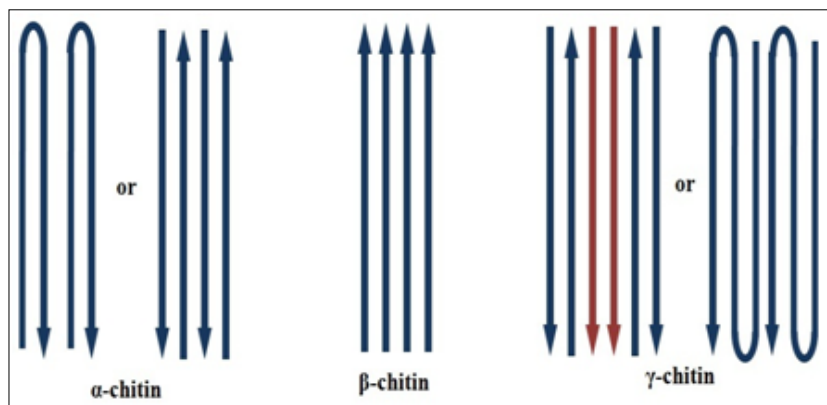


Figure 2. Schematic representation of three different polymeric configurations (α , β and γ) of chitin.

Table 1. Various sources of chitin

Groups/Species	References
Beetles (Insects)	7, 8
<i>Bombyx mori</i> (Insects)	7
Honeybees (Insects)	9
<i>Aedes aegypti</i> (Insects)	10
<i>Cancer pagurus</i> (Crab)	11
<i>Carcinus maenas</i> (Crab)	12
<i>Lithodes aequispinus</i> (Golden king crab)	13
<i>Chionoecetes opilio</i> (Snow crab)	
<i>Erimacrus isenbeckii</i> (Korean hair crab)	
<i>Paralithodes platypus</i> (Blue king crab)	
<i>Paralithodes camtchaticus</i> (Red king crab)	
<i>Chionoecetes bairdi</i> (Tanner crab)	
<i>Parapenaeopsis stylifera</i> (Shrimp)	14
<i>Penaeus carinatus</i> (Shrimp)	15
<i>Penaeus monodon</i> (Shrimp)	15,16
<i>Litopenaeus vannamei</i> (Shrimp)	17
<i>Jasus lalandii</i> (Lobster)	18
<i>Homarus americanus</i> (Lobster)	19
<i>Sepia officinalis</i> (Cuttlefish)	20
<i>Loligo vulgaris</i> (Squid)	20
<i>Absidia glauca</i> (Fungi)	21
<i>Absidia coerulea</i> (Fungi)	22
<i>Aspergillus niger</i> (Fungi)	23
<i>Mucor rouxii</i> (Fungi)	24
<i>Phycomyces blakesleeanus</i> (Fungi)	25
<i>Gongronella butleri</i> (Fungi)	26
<i>Absidia blakesleeana</i> (Fungi)	27
<i>Rhizopus oryzae</i> (Fungi)	28
<i>Trichoderma reesei</i> (Fungi)	29
<i>Lentinus edodes</i> (Fungi)	30

fraction of deacetylated monomer units present in the chitin polymer chain³⁴. The DDA content allow to differentiate between chitin and chitosan. If the DDA is less than 50%, it is then termed as chitin and if the DDA is greater than 50%, it is termed as chitosan³⁵. DDA is the most important factor which influences the properties of chitin, viz. solubility, flexibility, polymer conformation and viscosity³⁶.

Traditionally chitin is extracted from the exoskeletons of crustaceans by chemical methods which include a combination of three basic steps viz. (i) deproteinisation, (ii) demineralization and (iii) bleaching. Deproteinisation is performed by treating the crustacean shells in alkaline solutions such as NaOH and KOH³⁷. Demineralisation is generally performed by treating the shells in acidic solutions like HCl, HNO₃, H₂SO₄, CH₃COOH and HCOOH at a high temperature of 90-100°C³⁸. Finally bleaching is carried out to get colourless chitin. Alternatively chitin can also be extracted by using biological methods; in particular the deproteinisation is performed by using microbial extracellular proteases instead of alkaline solutions³⁹.

Biological demineralization of the crustacean shells is performed by enzymatic and microbiological methods by using natural probiotic organisms. Extraction of chitin by microbiological method is the most effective technique than the extraction of chitin by chemical methods⁴⁰. In biological process of chitin extraction, demineralization and deproteinisation occur simultaneously. Fermentation of shell wastes of shrimp (*Penaeus monodon*) were carried out with lactic acid bacteria where chitin was recovered by adding carbohydrates as a natural energy source⁴¹. The chemical and biological (enzymatic) methods used for extraction of chitin is schematically represented in Fig. 3.

In the production of chitin derivatives, the roles of chitinase enzymes are also remarkable in recent decades. Chitinase enzymes have specific molecular structure and function besides and exhibit substrate specificity and catalytic mechanisms. Chitinases also promote degradation of chitin into novel products having industrial applications⁴¹⁻⁴⁴.

Derivatives of Chitin

Chitosan

The utilization of chitin may be restricted due to its poor solubility, low porosity, and surface area⁴⁵. Hence to overcome the limitations and control the properties of chitin, various significant derivatives are produced. Chitosan is the one of the most important derivatives of chitin in terms of applicability⁴⁶. The chitin undergoes extensive deacetylation process to produce chitosan. The deacetylation is carried out using sodium hydroxide solution at 100 °C. The concentration of sodium hydroxide and variation in temperature influence the variation in DDA content during the process of production of chitosan. Depending upon the DDA content, the chitosan can be soluble in water or mild acidic solution⁴⁷.

Alkyl Chitin

Alkyl derivatives of chitin are known to significantly enhance the solubility and applicability of the chitin⁴⁸. For the production of N-alkyl-chitin, chitosan molecules are initially deacetylated completely and further treated with three kinds of aldehydes, namely formaldehyde, acetaldehyde, and pentanal to form Schiff bases of chitosan which in turn are reduced with sodium cyanoborohydride to form N-alkylated chitosans. The N-alkyl-chitosans are then transformed into the corresponding N-alkyl-chitins by acetylation with acetic anhydride followed by transesterification (process of exchanging the organic group R'' of an ester with the organic group R of an alcohol) to remove partly formed O-acetyl groups⁴⁹. The amorphous alkyl chitin (N-methyl-, N-ethyl- and N-pentyl) produced at C₂-carbon of the monomer show an enhanced affinity towards the organic solvents. Hence, the alkyl derivatives of chitin showed an excellent solubility and applicability.

N and O-Sulfated Chitin

Sulfated derivatives of chitin have attracted perennial research interests due to their functional similarity to heparin and hence the sulfated derivatives of chitin are used as an anticoagulant agent. Moreover attempts are made to prepare N- and/or O-sulfated-chitin using various reaction conditions and sulfating agents. Zou and Khor (2009), prepared

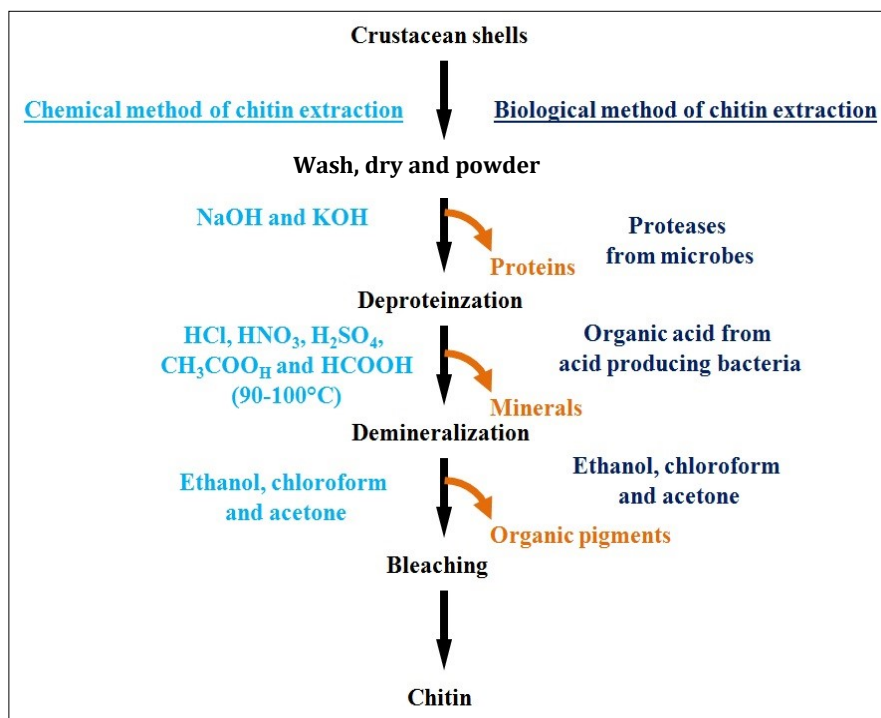


Figure 3. A schematic representation of the chemical and biological (enzymatic) methods for chitin extraction

sulfated-chitins of varying degree of sulfation (DS) by the reaction of chitin with sulfur trioxide-pyridine complex under homogeneous conditions in 5% LiCl/DMAc solvent system. Sulfation at 8°C or room temperature was regio-selective for the C₆-OH position with the DS ranging from 0.53 to 1.00 depending on the reaction time. When the reaction temperature was elevated, sulfation at the C₃-OH position also occurred. The degree of substitution and position of sulfation led to structure-activity relationship ambiguities⁵⁰.

Dibutyl Chitin

Dibutyl chitin or ester derivative of chitin is known to be an easily soluble derivative of chitin which binds with butyryl groups at C-3 and C-6 positions⁵¹. These chitin derivatives exhibited some desirable qualities like bioactivity, biocompatibility, biodegradability along with film and fiber-forming properties and also the derivatives have a huge potential for manufacturing a wide range of materials suitable for biomedical and industrial applications⁵². Dibutyl chitin was obtained by reaction of the chitin with butyric anhydride, and by using perchloric acid as catalyst and also from butyric

anhydride and butyric acid using methanesulfonic acid as catalyst and solvent. Bhatt et al., (2011) reported the occurrence of synthesis of chitin butyrate by reaction chitin with butyric acid in the presence of TFAA/H₃PO₄⁵³. Dibutyl derivatives of chitin are known to provide potential biomedical and industrial applications and they could also be used as intermediates for further chemical modifications under mild conditions.

Carboxymethyl Chitin

Soluble carboxymethyl chitin (CMCH) is one of the most attractive derivatives of chitin for biomedical applications⁵⁴. Traditional method of synthesis of carboxymethyl chitin involve mixing of chitin slurry in the presence of concentrated NaOH (40-60% w/w) and isopropanol under the heterogeneous reaction conditions at 100°C. Huang et al., (2012) prepared CMCH by using a mixture of NaOH, 2-propanol and monochloroacetic acid⁵⁵. Recently, Liu et al., (2015) synthesized novel homogeneous carboxymethyl chitin with a broad range of degree of substitution (0.035 to 0.74), high DA and little de-polymerization in aqueous NaOH/urea solution. Homogenous carboxymethylation

of natural chitin offers an advantage of a fair structural control⁵⁶. Based on the carboxymethylation percentage CMCH could be used as excipients (inert substances used as vehicles and diluents for drugs), especially for oral drug delivery.

Chitoligosaccharides

Chitoligosaccharides (COS) are partially hydrolyzed products of chitin, and have been recently focused for their solubility in acid-free aqueous media⁵⁷. The COS have been shown to possess more potential than chitin nutraceutical additive, since COS are easily absorbed through the intestine, quickly transported into the blood flow and are shown to exhibit systemic biological effects in the organism⁵⁸. Acid hydrolysis (hydrochloric, nitrous, phosphoric acid, hydrogen fluoride) and oxidative reductive depolymerization (mediated by peroxide, ozone, and persulfate) are important routes for synthesis of COS. Depolymerization under high energy impact (using ultrasound, microwave, etc.) and recombinant approaches (using enzymatic and microbial depolymerization) are also being tried for production of COS⁵⁹. Due to its low molecular weight chitoligosaccharides are thought to have several interesting bioactivities and applications.

Chitin Nanofibers

Chitin nanofibers (CNF) are biodegradable chitin derivatives, having typical width of 10-20 nm and large surface-to-mass ratio. The CNF are being prepared, and studied, more recently worldwide for various applications⁶⁰. When the CNF are blended with inorganic metals to prepare advanced hybrid organic-inorganic composites, they can have applications in electronics, electrical, optical devices and much needed solar energy production⁶¹. CNF was prepared from the shrimp and crab shells by various chemical treatments. In brief minerals were removed by HCl treatment, removal of proteins was done by refluxing the suspension with NaOH, pigments and lipids were removed by ethanol. After completion of above treatments, suspension was filtered washed with distilled water and kept wet for mechanical grinding for fibrillation, this wet slurry was made to a concentration of 1% and called chitin slurry. Chemical treatment loosened the tightly bonded fibrils bundles to larger extent apart from removal of minerals, proteins,

pigments, and lipids⁶². CNFs have successfully been used in many applications, including tissue engineering, wound dressing, cosmetic and skin health, stem cell technology, anti-cancer therapy, drug delivery, anti-inflammatory treatment, and obesity management⁶⁰.

Chitin Nanowhiskers

Chitin nano-whiskers (CNW) of slender parallelepiped rods have been successfully prepared from chitin, which has been recently explored in nanotechnology application. CNWs are currently being studied and used as reinforcing additives for high performance environmentally friendly and biodegradable nanocomposite materials, as biomedical composites for drug/gene delivery or nanoscaffolds in tissue engineering^{64,64}. Sriupayo et al., (2005) reported the chemical preparation of CNW from chitin. They treated the chitin with 3 N HCl at 100°C for 90 min under vigorous stirring. The ratio of 3N HCl to chitin was 100 mL/g. After treatment, the suspension was diluted with distilled water, followed by centrifugation at 10 000 rpm for 5 min. This process was repeated three times and the suspension was then transferred to a dialysis bag and dialyzed against deionized water up to neutral pH. The CNW suspension was sealed and preserved by storing in a refrigerator at 4°C⁶⁵. Qin et al., (2016) have used 3 M H₂SO₄ solution, for the hydrolysis of chitin in the preparation of CNW⁶⁶. CNWs have drawn attention in various applications due to their properties like nanosized dimensions, high surface area, high absorbability, biodegradability, nontoxicity, renewability, low density and easy modification⁶³. The schematic representation of the difference between CNF and CNW is represented in Fig. 4.

Chitin Nanoparticles

Chitin nanoparticles (CNP) with larger surface area are synthesized from powdered chitin and such CNP is known to have varied applications⁶⁷. CNP was isolated from the purified chitin by repeated acid hydrolysis. Chitin powder was soaked in 3 M HCl for 1.5 h at 90 °C in a water bath. The sample was centrifuged at 6000 rpm for 10 min and the pellets were collected. The acid hydrolysis step was repeated thrice and the pellets were suspended in distilled water to dilute the acid concentration. The suspension was dialyzed against distilled water until it reaches pH 6 and was

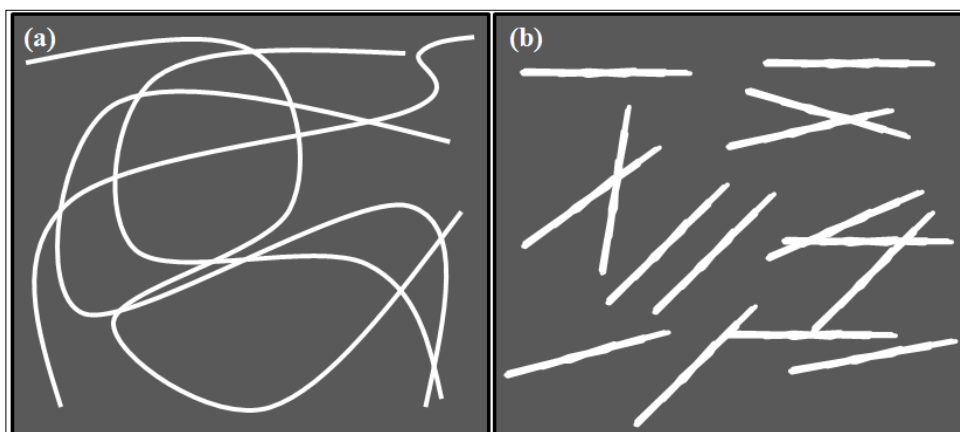


Figure 4. Schematic illustration of morphological difference of a) CNF and b) CNW.

homogenized using a tissue homogenizer. The homogenized sample was collected and lyophilized at $-60\text{ }^{\circ}\text{C}$ to get the powder form of CNP. Mechanical disruption and ultrasonication were carried out to cut down the size of nanoparticles¹⁷. SEM and TEM micrographs of prepared CNP from the shells of *Penaeus monodon* was displayed in Fig. 5. Smitha et al., (2013, 2015) have prepared chitin nanoparticle by cross linking the chitin using tripoly phosphate solution (TPP)^{68,69}. CNPs have been widely used in various applications due to its biocompatible, biodegradable and non-toxic nature.

Chitin Nanocomposite

Chitin nanocomposites are multiphase materials consisting of a chitin matrix and nanosized fillers to alter the stability and the mechanical properties of the chitin⁷⁰. Polymer nanocomposites can be produced by introducing a crosslinking agent into the polymer matrix. Chitin whisker and tannic acid cross link chitosan composite which was synthesized and the mechanical and physicochemical properties of such nanocomposites were studied by Rubentheren et al., (2015)⁷¹. Chitin nanocomposites can also be produced by introducing chitin nanofibers with high aspect ratio, high strength and high modulus into synthetic polymer matrices like polyacrylic acid (PAA). Bogdanova et al., (2016) has shown exfoliation of the squid β -chitin in aqueous acrylic acid (AA), after which a composite film of chitin microfibrils in polyacrylic acid (PAA) has been prepared by *in situ* polymerization of the AA⁷². Also chitin nanocomposites can be produced by incorporating metal

nanoparticles into chitin matrix. In our recent study, we reported the synthesis of α -chitin/silver nanocomposite (CNP/AgNP) by incorporating α -chitin nanoparticles isolated from a mixture of the shrimp shells and silver nanoparticles (AgNP)⁷³. The TEM micrograph of CNP/AgNP displayed in Fig. 6 shows CNP nanocomposite which prevented the agglomeration of AgNP by stably encapsulating the AgNP.

Chitin Hydrogels

Hydrogels are three-dimensional hydrophilic polymer-based networks with high water content resembling the native extracellular matrix⁷⁴. Kawata et al., (2016) prepared calcium phosphate cross linked chitin nanofiber hydrogel and it used for bone tissue regeneration applications⁷⁵. Similarly, Liu et al., (2016) prepared CMCH hydrogel by simple NaOH treatment and it used for three-dimensional cell culture⁷⁶. Due to hydrogels shared resemblance with natural soft tissue (high water content, controllable porosity and generally acceptable biocompatibility) for the past several decades, hydrogels have been widely explored as promising biomaterial candidates for cell scaffolds and drug delivery vehicles⁷⁷.

Biomedical Applications

Chitin and its derivatives are biodegradable and biocompatible natural polymers, safe and non-toxic, and bind to mammalian and microbial cells potentially. Here, we discussed some of the potential biomedical applications of chitin and its derivatives (Fig. 7).

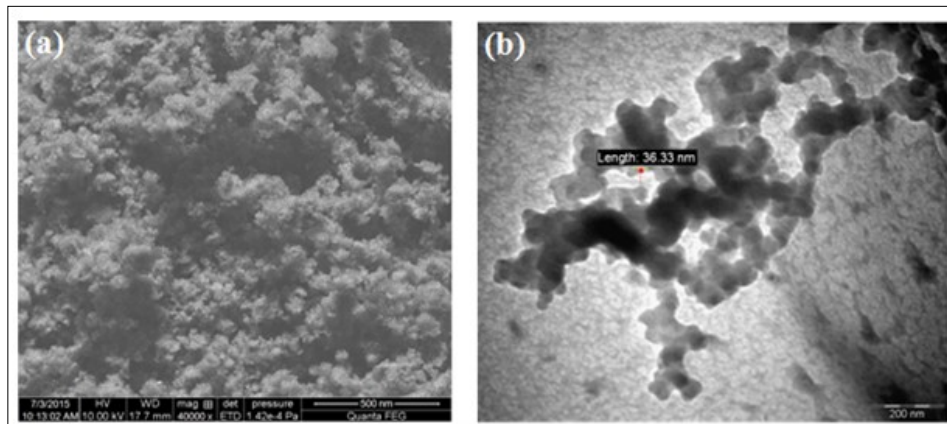


Figure 5. a) SEM and b) show TEM micrograph of chitin nanoparticles synthesized from the shells of *Penaeus monodon* Fabricius (Reprinted from [17]).

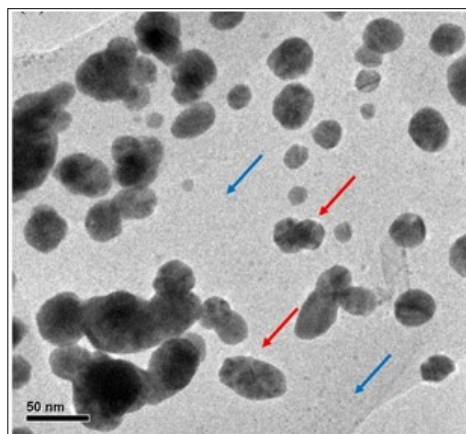


Figure 6. TEM micrograph of CNP/AgNP; Blue arrow indicates the surface of CNP; Red arrow indicates AgNP (spherical shaped spots) embedded in the surface of CNP (Reprinted from [73]).

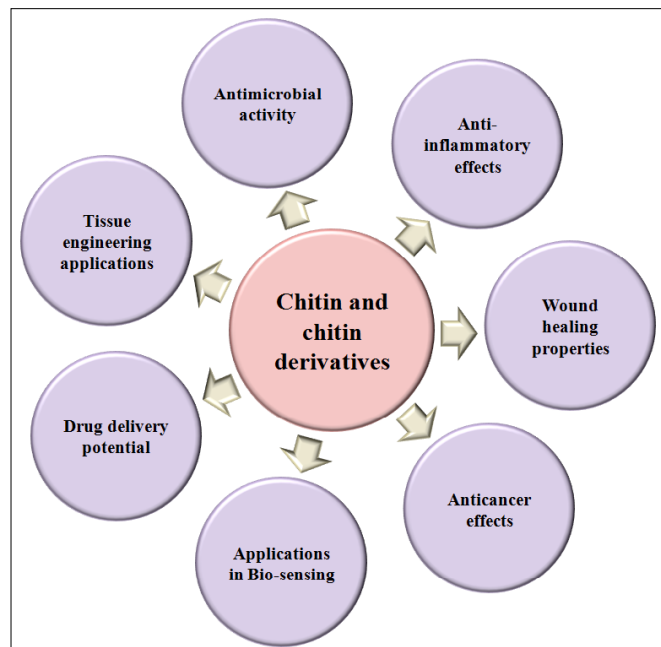


Figure 7. Schematic representation of the biomedical applications of chitin and its derivatives

Antimicrobial Activity

The increasing antibiotic resistance pattern exhibited in majority of the pathogenic microorganism is a major problem throughout the world⁷⁸. In recent years, there has been an increased interest in the development of antimicrobial substances from natural products.. Abdel-Rahman et al., (2015) studied the antibacterial activity of chitin and chitosan, isolated from shrimp shell by chemical treatments, these products were tested against *E. coli* strains and it was concluded to exhibit antibacterial activity. The chitosan had high DDA content as well as antibacterial activity than chitin⁷⁹. The antimicrobial activity of chitin and chitosan extracted from *Parapenaeus Longirostris* shrimp shell waste was studied against four different genera of bacteria viz. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* and two fungi viz. *Candida albicans* and *Candida parapsilosis*. The results of the study further confirmed that generally the antimicrobial activity seem to be related with DDA⁸⁰. Jiang et al., (2016) investigated the antibacterial activity of lysozyme immobilized on CNW and the results of the study provided evidences so that the lysozyme immobilized CNW system exhibited greater antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* when compared with free lysozyme⁸¹. Sahraee et al., prepared corn oil emulsified nanocomposite gelatin film with chitin nanofiber to study the antifungal activity and showed improved physical, mechanical, thermal and antifungal properties⁸². In addition, the α -chitin nanofiber processed by dynamic high pressure homogenization exhibited a significant antifungal activity against *Aspergillus niger*⁸³. Similarly the enzymatically deproteinized chitin, chitosan and its by-products isolated from Norway lobster exhibited very good antimicrobial activity against bacterial and fungal strains⁸⁴. Likewise, CNF included carrageenan films exhibited strong antimicrobial activity against *Listeria monocytogenes*⁸⁵. Sun et al., (2017) synthesized a novel water-soluble sulfonated chitosan, as a kind of linear sulfated polysaccharide, by introducing 1,3-propane sulfone to the amino group of chitosan under mild acidic conditions. They also studied the antimicrobial activity of the sulfonated chitosan against bacterial and fungal strains and concluded that the

microbial inhibition was dependent on the type of chitosan used and the type of microorganisms⁸⁶. Zhang et al., (2016) reported that chitin enhances the biocontrol activity of *Rhodotorula mucilaginosa* against blue mold and *Rhizopus* decay of peaches⁸⁷. Gelatin nanocomposite film containing 0, 3, 5, and 10 % concentrations of chitin have been synthesized and the antifungal property was evaluated. The study confirmed that, incorporation of chitin with gelatin films not only improved physical properties of the film, but also can develop a functional nanocomposite biopolymer with potential antifungal activity⁸⁸. Solairaj & Rameshthangam (2016) have prepared CNP/AgNP and evaluated the antimicrobial activity against bacterial and fungal strains. They demonstrated that the prepared composite to exhibit potential antimicrobial activity, which is higher than the pure AgNP. The CNP/AgNP has also tested for mosquito larvicidal activity and reported that, the composite have potential larvicidal activity against *Aedes aegypti*⁷³. In a similar research, AgNPs-loaded chitin nanocrystal nanocomposites were produced and coated on a cellulose paper which showed potential antimicrobial activity against *E. coli* and *S. aureus*⁸⁹.

Anti-Inflammatory Effects

Synthetic anti-inflammatory agents possess some side effects such as gastric irritation, ulceration and decreased host resistance in the patients. In order to find some natural anti-inflammatory agents with biocompatibility and biodegradability property, extensive research works have been carried out in chitin and its derivatives. Khanal et al., (2000) studied the potential usefulness of phosphated chitin (P-chitin) as an anti-inflammatory agent in a mice model of acute respiratory distress syndrome. The research group reported that P-chitin with a molecular weight of 24000 D, 58% degree of substitution and 4% degree of deacetylation was found to be the most effective in blocking the lung injury when administered at 8 mg/kg level⁸⁹. Lee et al., (2009) prepared two kinds of COSs (90-COSs and 50-COSs) from 90% and 50% deacetylated chitosan and evaluated their anti-inflammatory activity. The results evidenced that; 90-COS has showed potential anti-inflammatory effect via down-regulation of (both transcriptional and

translational expression) tumor necrosis factor (TNF)- α , interleukin (IL)-6, inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 genes⁹⁰. Another study suggests that, COS possess anti-inflammatory activity, which is dependent on dose and molecular weight. A single dose of 500 mg/kg body weight may be suitable to treat acute inflammation cases⁹¹. El-Badry and Fetih (2011) studied the anti-inflammatory activity of the celecoxib loaded chitosan formulations. The results suggested that chitosan concentration and molecular weight are very crucial factors on the release of celecoxib from gel formulations and also confirmed that the chitosan gel formulations have significant anti-inflammatory activity⁹². Another similar research evidenced an increased anti-inflammatory activity of rutin encapsulated in chitosan microspheres. The researchers also suggested that, rutin loaded chitosan microspheres could be used in the treatment of mucosa inflammation, such as in the synovial, lung and bowel compartments⁹³. Wei et al., (2012) demonstrated that, COS could inhibit the inflammatory responses in N9 microglial cells through the suppression of nitric oxide (NO) production and down regulation of iNOS production at both transcription and translation levels⁹⁴. In various similar studies COS was proved for its anti-inflammatory property in uveitis rats and asthmatic models^{95,96}. The recent reports state that, chitin and its derivatives may act as a promising candidate material for treating and preventing inflammation.

Wound Healing Potential

The wound healing is a complex process, which includes hemostasis, inflammation, proliferation, and remodeling. A successful wound healing needs an appropriate treatment to modulate a series of complex interactions between different cells and cytokine mediators throughout the all phases of healing⁹⁷. There are numerous biomaterials used as wound dressings viz. alginates, polyurethane, hydrocolloids, collagen, pectin, hyaluronic acid and chitin derivatives for enhancing the wound healing process. Among them chitin derivatives have attractive wound healing properties. Minagawa et al., (2007) studied the effect of molecular weight and DDA of chitin/chitosan in wound healing. They found that, higher the DDA and low molecular weight of chitin/chitosan to shows the highest wound healing property⁹⁸. Abdel-Mohsen et al., (2016) reported the

use of chitin/chitosan-glucan complex for the preparation of micro and non-wovenfiber/nonwoven sheets after dissolution in urea/sodium hydroxide aqueous solution at -15°C. They further reported that the prepared wound dressing sheets have shown excellent wound healing ability and promoted accelerated wound closure of the rat skin⁹⁹. Marei et al., (2017) compared the wound healing properties of chitosan isolated from locust (*Schistocerca gregaria*) and shrimp (*Penaeus monodon*). The research group found that chitosan isolated from locust has showed a better wound healing ability. The histopathological studies of the healed wounds showed earlier granulation as well as dermis active angiogenesis with a significantly higher count with early marked epithelization and formation of thicker epidermis with minimal inflammation¹⁰⁰. Likewise, Aragão-Neto et al., (2016) prepared a wound healing hydrogel combined with policaju (POLI) from cashew tree (*Anacardium occidentale* L.) gum and chitosan. They reported that the POLI-CS hydrogel contributed for a most effective wound healing and modulation of the inflammatory process. The research group also reported that the combined use of POLI-CS hydrogel with low level laser therapy showed a better wound contraction, larger collagen presence, minor focal necrosis and early epithelization¹⁰¹. Chitosan hydrogel in combination with nerolidol, superficially deacetylated chitin nanofibrils are also reported for their wound healing properties^{102,103}. Moreover, the composite materials such as, sulfanilamide and silver nanoparticles-loaded polyvinyl alcohol-chitosan composite, chitosan-based copper nanocomposite and chitosan-Ag/ZnO composite have shown synergistic mechanism and an enhanced wound healing process^{104,105,106}. Freeze dried surface-deacetylated chitin nanofibers reinforced with sulfobutyl ether β -cyclodextrin are also reported as a new beneficial biomaterial for the treatment of wounds¹⁰⁷. Moura et al., (2014) reported that 5-methyl pyrrolidinone chitosan (MPC) wound dressings loaded with neurotensin could be used for the healing of diabetic foot ulcers. MPC foam combined with neurotensin can promote an anti-inflammatory response and stimulate re-epithelialization, which are important phases in the wound healing process¹⁰⁸. An ideal wound dressing should be able to absorb exudates and toxic

components from the wound surface, maintain a high humidity at the wound/dressing interface, allow gaseous exchange, provide thermal insulation, and protect the wound from bacterial penetration and it must be non-toxic¹⁰⁹. The above studies proved that chitin based nanostructures, nanocomposites and hydrogels have all the beneficial characteristics and could be used as wound dressings and an effective wound healing material.

Anticancer Effects

The mortality rate due to cancer still remains very high. Chemotherapy still remains one of the popular treatment options for cancer treatment; however, a low level of drug accumulation in and around the cancerous cells and a high accumulation of anticancer drugs in the healthy tissues limits its potential clinical applications¹¹⁰. The biocompatibility of chitin nanogels has been studied and reported by Rejinold et al., (2012) on an array of cell lines¹¹¹. The anticancer property of chitooligosaccharides with the highest degree of DA and lowest molecular weight has been reported by Kim et al., (2012) in human myeloid leukemia HL-60 cells¹¹². Huang et al., (2006) reported that highly charged COS show cell specific anticancer activity against HeLa, Hep3B and SW480 cell lines. They reported that highly charged COS derivatives could significantly reduce cancer cell viability, regardless of the positive or negative charges¹¹³. Salah et al., (2013) studied the active mechanism of chemically prepared low molecular weight chitin against human monocyte leukaemia cells (THP-1) and human monocytic cells (MRC-5). They speculate that low molecular weight chitin inhibited the action of YKL-40, a glycoprotein with anti-apoptotic effect. Consequently THP-1 cancer cells, which express YKL-40 undergoes mortality and noncancerous cell (MRC-5) which could not express YKL-40 can proliferate¹¹⁴. In addition, Gibot et al., (2014) studied the cell line dependent anticancer property of chitosan in human melanoma cell lines. The research group reported that chitosan could trigger both mitochondrial and death receptor mediated apoptosis signaling pathways in melanoma cell line¹¹⁵. The synergistic anticancer activity of chitosan combined with silver nanoparticles was also reported in human cervical cancer HeLa cells and human lung cancer A549

cells^{116,117}. The anticancer potential of β -chitosan nanoparticles was studied in human hepatocellular carcinoma cells and reported as promising anticancer agent¹¹⁸. The anticancer responses displayed by the chitin and its derivatives can be attributed to the beginning of development of new anticancer agents.

Bio-Sensing

Electrochemical methods have shown the remarkable advantages in the analysis of components or ingredients in pharmaceutical preparations and other biological molecules in human body fluids. The advantages of electrochemical sensing are mainly due to the sensitivity, low cost and relatively short analysis time of the biological compounds as compared to the other routine analytical techniques including chromatography, ELISA and Western blot¹¹⁹. The protonation of acetylamide group in chitin is effective for accumulating and separating some anions from a sample matrix, based on electrostatic interaction. This beneficial property makes chitin possible for immobilization of enzyme(s) and other materials that will provide effective sensing application. Sugawara et al., (2000) have demonstrated the glucose sensing ability of carbon-paste electrode which was modified with immobilization of glucose oxidase (GOD), demonstrated the electrostatic interactions of the chitin and GOD and they determined the amount of glucose present in the sports drinks¹²⁰. Chen et al., (2006) prepared chitosan membranes from the carapace of the soldier crab *Mictyris brevidactylus* and studied its application. The chitosan membrane was used to immobilizing enzymes for biosensor construction owing to their good electrochemical characteristics and excellent mechanical properties¹²¹. Kumar et al., (2010) demonstrated that, polymer nano-composites synthesized by the implementation of carbon nanotubes (CNT) in chitosan matrices that exhibited better mechanical property and electrical conductivity. The research groups also established that the composite material is very useful in designing electrochemical biosensor for the detection of organic vapours¹²². Immobilisation of functionalised carbon nanotubes into chitosan matrices using crosslinkers was performed and applied to sense organic molecules such as hydroquinone, dipyrone, and glucose¹²³. Liu et al., (2012) electrodeposited chitosan and silver nanoparticles to

form a positively charged surface on the glassy carbon electrode and used for the detection of the trichloroacetic acid (TCA). A sensitive amperometric sensor for trichloroacetic acid was constructed with low detection limit of 1.1 μM by using the fast diffusion and electron transfer process of the negatively charged TCAA in the positively charged silver nanoparticles doped chitosan hydrogel film¹²⁴. In a similar study, Liu et al., (2012) electrodeposited and prepared molecularly imprinted polymers (MIP) using combination of chitosan and graphene. These MIPs have been recognized as optimal elements to construct sensor with specific binding sites to target molecule. The researchers developed a sensor for dopamine detection based on the CS dispersed with graphene mixture as the functional matrix. Dopamine is a naturally occurring catecholamine which is an important neurotransmitter of mammals and it becomes a key marker for schizophrenia and Parkinson's disease¹²⁵. Similarly, Palanisamy et al., (2017) prepared a novel hybrid hydrogel composite of chitin stabilized graphite for selective and simultaneous electrochemical detection of dihydroxybenzene isomers in water¹²⁶. A electrochemical biosensor was fabricated using copper immobilized chitin nanostructures which exhibited rapid and sensitive detection of 0.776 μM glucose¹²⁷. All these demonstrate that chitin and its derivatives could be used for the development of sensors for sensing various chemicals, biomolecules and drugs.

Drug Delivery Potential

Nowadays, polymer based materials are known to act as a very promising platform for delivery of bioactive macromolecular drugs. A number of interesting properties of drug carriers include muco- and bioadhesiveness, a high capacity to associate and release therapeutic macromolecules, as well as their ability to enhance the transport of bioactive compounds across the epithelial barriers, such as the ocular, nasal and intestinal routes. Among the polymers used in drug delivery platform, chitosan (CS) is one of the well known molecules because of their biocompatibility, low toxicity, biodegradability, and muco- and bioadhesiveness¹²⁸. Cover et al., (2012) studied the effect of transcervical administration of doxycycline-loaded chitosan nanoparticles (DCNPs) for the treatment of uterine

infections. The DCNPs showed improved and sustained delivery of doxycycline, thereby minimizing the adverse effects and improved the drug efficacy¹²⁹. In various studies, CS scaffolds were fabricated and used for the delivery of therapeutic agents such as docetaxel, curcumin, 5-fluorouracil, pentoxifylline, ampicillin, dexamethasone, tetracyclinehydrochloride, amikacin, vancomycin and ketoprofen^{109,130,131}. Apart from drug molecules, chitin derived nanoparticles was used for the delivery of RNA, proteins and peptides. Nascimento et al., (2014) formulated epidermal growth factor receptor targeted CS nanoparticles loaded with small interfering RNAs (siRNAs) against mitotic arrest deficient 2 (Mad2) gene. Mad2 is an essential mitotic checkpoint component required for accurate chromosome segregation during mitosis and its complete abolition leads to cell death. The study confirmed that EGFR targeted CS loaded with Mad2 siRNAs was a potent delivery system for selective killing of cancer cells¹³². In another study, CS nanoparticles modified with T cell-specific antibodies were used for the delivery of siRNA to T cells. CD7-specific single-chain antibody was chemically conjugated to CS by carbodiimide chemistry, and nanoparticles were prepared by a complex coacervation method in the presence of siRNA. The results showed that the expression levels of CD4 receptors on T cells were greatly reduced by the delivery of CD4 siRNA using antibody-conjugated chitosan nanoparticles¹³³. Development of therapeutic peptides and its clinical use has been restricted to non-central nervous system diseases due to the poor permeation of peptides across the gastrointestinal mucosa and the blood-brain barrier. To overcome such restrictions, Lalatsa et al., (2012) fabricated the quaternary ammonium palmitoyl glycol chitosan nanoparticles (GCPQ) that facilitated delivery of orally administered peptides such as leucine-enkephalin (neurotransmitter) into the brain. The research concluded that GCPQ particles facilitated absorption of the oral mucus adhering drug peptide by protecting the peptide from gastrointestinal degradation, and by increasing the drug gut residence time and transporting GCPQ associated peptide across the enterocytes and to the systemic circulation, enabling the GCPQ stabilized peptide to be transported to the brain¹³⁴. With these recent research

findings, this section briefly revisited the application potential of chitin and its derivatives in drug, RNA and peptide delivery.

Tissue Engineering

Tissue engineering is one of the basic approaches to recuperate/replace the tissues and organs that are damaged or diseased. However, limited availability of grafts, risk of disease transmission, pain at the graft site, lack of enough fusion, morbidity at the donor site and cost, are some of restraining factors of tissue engineering. Biomaterials with appropriate physio and biochemical properties thereby used to achieve successful survival rates over tissue engineering¹⁰⁹. Recently, chitin and its derivatives have shown remarkable promise in tissue engineering. Kumar et al., (2013) have developed a nanocomposite scaffold for use in tissue engineering, using a mixture of pectin, chitin and nano CaCO₃ by lyophilization, The research group evaluated the cytocompatibility of the scaffold on mouse fibroblast cell lines (NIH3T3 and L929) and human dermal fibroblast (HDF) cells. The results confirmed that the scaffold showed negligible toxicity towards cells. Cell attachment and proliferation studies were also conducted using these cells, which showed that cells attached onto the scaffolds and started to proliferate after 48 h of incubation¹³⁵. In another study, graphene oxide (GO)–chitosan (CS)-hyaluronic acid (HA) based bioactive composite scaffold containing an osteogenesis-inducing drug simvastatin was fabricated for bone tissue engineering application. The *in vitro* results showed that the scaffold material offered a significant influence on osteogenesis and biomineralization and it possess an excellent biocompatibility and to be used as a bone tissue engineering scaffold¹³⁶. Liu et al., (2016) prepared CS/chitin nanocrystals (CNC) composite scaffolds by a dispersion-based freeze dry approach which exhibited significant enhancement in compressive mechanical strength of the composite scaffolds which were successfully applied as scaffolds for MC3T3-E1 osteoblast cells, which in turn showed excellent biocompatibility and low cytotoxicity. The results of the study also revealed that CNCs can markedly promote the cell adhesion and proliferation of the osteoblast on CS and it can have potential application in bone tissue engineering¹³⁷. In a similar research, novel porous

composite scaffolds consisting of chitin, chitosan and nano diopside powder were prepared by using the freeze-drying method. Cytocompatibility of the scaffolds and cell attachment were studied by using human gingival fibroblast cells. The scaffolds demonstrated no sign of cellular toxicity and the cells were found to be attached to the pore walls within the scaffolds and the results suggested that the developed composite scaffolds could be a potential candidate for tissue engineering¹³⁸. Pangon et al., (2016) have used chitin whisker (CNW) to enhance the mechanical properties of chitosan/poly (vinyl alcohol) (CS/PVA) nanofibers and to offer osteoblast cells to grow with hydroxyapatite mineralization. The CNW combined with hydroxyapatite in bionanocomposite was shown to act as a key to promote osteoblast cell adhesion and proliferation¹³⁹. Although these research findings supported the use of chitin and its derivatives for tissue engineering, further studies on toxicity, degradation and *in vivo* effects of these chitin scaffolds are required before using them for clinical trials/human use.

Environmental Applications

Soil and water pollution by organic and inorganic contaminants is of a growing concern because of their potential detrimental effects on human health and the environment. As environmental protection is becoming an important global problem and industries pay attention to the development of technology which limits the environmental problems. Recently, the commercial value of employing chitin and its derivatives for environmental applications gathered considerable interests. CT and its derivatives have been used for several environmental applications, including remediation of both organic and inorganic contaminants from water and soil. Also biocompatible nature of CT and its derivatives making them suitable, for immobilizing sensing elements such as, enzymes and nanoparticles for the sensing of environmental hazardous chemicals. Especially, Chitinases are known to play different roles in various organisms, their induction in the sensor elements is not yet beneficially unified. In chitin-utilizing organisms, chitinases require the presence of an inducer in the medium. Expression of induced hydrolases, in general, is controlled by hydrolysis products which are synthesized in very low concentration in the absence of an inducer and this

allows for appropriate changes to be made in the composition of the medium for the generation of a signal to increase in the production of target enzymes¹⁴⁰. Herein, we have summarized the applications of CT and its derivatives in the removal of dyes, organic and inorganic pollutants, and remediation of metal pollution.

Removal of Dyes

Wastewater effluents in some industries, such as dyestuff, textiles, leather, paper, and plastics, contain several kinds of synthetic dyestuffs. A very small dye amount in water is highly visible and can be toxic to life in water and harmful to human beings. Hence, the removal of dyes from process or waste effluents becomes of fundamental importance to the environment. Chitin and its derivatives have excellent adsorption capacities and low cost when compared to activated carbon and therefore they received considerable interests for decontaminating the environment or removal of dyes and toxins¹⁴¹. Prado et al., 2004 compared the adsorption behavior of indigo carmine dye on chitin and chitosan. They reported that due to the presence of more basic nitrogen centers in chitosan, indigo carmine dye adsorbed more spontaneously in chitosan than chitin¹⁴¹. In a similar study, Dolphen et al., (2007) compared the adsorption behavior of chitin and chitin modified with sodium hypochlorite solution in Reactive Red 141 from wastewater. The hydroxyl group of the modified chitin was transformed into CH_2OCl that cannot react with the dye solution. Therefore, dye adsorption by modified chitin involves mainly physical adsorption and adsorption capacity was higher than that of chitin¹⁴². In another research, chitin was modified into pure chitin hydrogel (CG3), which showed excellent mechanical properties and biocompatibility, for wastewater treatment. CG3 exhibited microporous structure, large surface area and affinity on malachite green, leading to the high uptake capacity of dye¹⁴³. To extend the applicability of chitin as dye adsorbent, Dotto et al., (2015) used ultrasound-assisted technology to modify the chitin surface and investigated the adsorption of methylene blue. Ultrasonic surface modified chitin (USM-chitin) presented more adequate characteristics, such as higher surface area, higher porosity, lower crystallinity and a more rugged surface for adsorption purposes, than raw

chitin. Also USM-chitin can be reused for seven times maintaining the same adsorption capacity¹⁴⁴ [144]. The follow up of the same work was carried out as fixed bed adsorption of methylene blue by USM-chitin supported on sand. The optimal bed performance was attained with flow rate of 10 ml min^{-1} with initial MB concentration of 50 mg L^{-1} and also the bed performance was maintained after five adsorption-elution cycles¹⁴⁵. Wang et al., (2015) fabricated a sunlight photocatalyst by *in situ* synthesis of Cu_2O in the regenerated chitin (RC)/graphene oxide (GO) composite film, where the porous chitin film was used as the microreactor for the formation of nano Cu_2O . The Cu_2O /RC photocatalyst exhibited good photodegradation of dyes¹⁴⁶. In another study, chitin/graphene oxide (Chi:nGO) hybrid gels were prepared and investigated the biosorption property. Remazol Black (RB) and Neutral Red (NR) were used as an acid and basic dye model for adsorption study. The results revealed that the adsorption was dependent on both the solution pH and the Chi:nGO proportion¹⁴⁷. Chitin nano whiskers (ChNW) are obtained from native chitin by acid hydrolysis, and considered as a very attractive class of nanomaterial with high surface to volume ratio and with hydroxyl and acetamide functional groups. Gopi et al., (2016) reported enhanced adsorption of crystal violet achieved using ChNW isolated from shrimp shells¹⁴⁸. In a similar study, Solairaj et al., (2016) prepared chitin nanoparticles from shrimp shells and studied the adsorption property of methylene blue, bromophenol blue, and coomassie brilliant blue. The results evidenced that the chitin nanoparticle showed significant increase in mechanical, thermal stability and dye adsorption property¹⁷. The findings confirmed that CT and its derivatives are simple, fast reacting, low cost biodegradable materials that can be used for effective dye removal process.

Remediation of Inorganic Contaminants

Metals are the major inorganic contaminant worldwide and the removal of toxic metals from water is a matter of great interest in the field of water pollution control. Numerous metals such as chromium, mercury, lead, copper, etc., are known to be toxic which are a serious cause for water pollution. Chitin and its derivatives have been evaluated for remediating heavy

metals, such as Cu(II), Pb(II), Hg(II), Cd(II) and Zn(II) in recent years¹⁴⁰. Gandhi et al., (2010) prepared a composite material by combining nano-hydroxyapatite (n-HAp) with chitin and chitosan for the removal of copper(II) from aqueous solution. The adsorption capacity of n-HAp/chitin (n-HApC) and n-HAp/chitosan (n-HApCs) composite were found to be 5.4 and 6.2 mg g⁻¹ respectively with a minimum contact time of 30 min. The research group also confirmed that due to the presence of more numerous number of chelating reactive amino groups in chitosan than the acetamide groups present in chitin, the n-HApCs composite experienced a higher efficiency than the n-HApC composite¹⁴⁹. Kousalya et al., (2010) suitably modified the chitin for enhancing the metal sorption capacity and as an alternate for chitosan. The research group prepared protonated chitin (PC), carboxylated chitin (CC) and grafted chitin (GC) to study the metal sorption property using Cu(II) and Fe(III) ions. Among the modified forms of chitin, GC showed higher SC towards Cu(II) and Fe(III) than CC, PC and CT¹⁵⁰. Saravanan et al., (2013) have prepared chitin/bentonite composite for better metal adsorption capacity and resistance to acidic environment. They evaluated the chitin/bentonite as the adsorbent for the sorption process of chromium from aqueous solution. The results confirmed that the composite material can act as a biosorbent at the optimum pH of 4.0¹⁵¹. Similarly chitin nanofibrils (CNF) was evaluated for the removal of Cd(II), Ni(II), Cu(II), Zn(II), Pb(II), Cr(III) in aqueous solution. The CNF showed much higher adsorption behavior than chitin micro-particles¹⁵². Likewise various modifications such as functionalization of chitin using polypyrrole, irradiated grafting of acrylonitrile on to chitin, acetophenone derivative of nano-chitosan, crosslinking chitosan into poly(alginate acid) nanohydrogel and thiol-functionalization chitin nanofibers were used for the removal of Cr(VI), As (III), Cu(II), Cd(II), Hg(II) and Pb (II)¹⁵³⁻¹⁵⁷. The above findings summarize the use of chitin and modified chitin for the removal of metals from the aqueous environment.

Remediation of Organic Contaminants

Wastewater that was contaminated with organic contaminants can be remediated with chitin and its derivatives dependent on the characteristics of the contaminants. Yoshizuka et al., (2000) prepared

chitosan micro particles (CMs) and silver-complexed CMs (SCMs) by using different cross linking agents, i.e. glutaraldehyde and epichlorohydrin. The research group investigated the adsorption and release behaviors of CMs and SCMs towards a typical pesticide, methyl parathion (MP). The results of the study concluded that SCM cross linked with glutaraldehyde could be used for the removal of methyl parathion¹⁵⁸. Dolphen & Thiravetyan (2011) synthesized chitin nanofibers from shrimp shells and studied the adsorption of melanoidin, a food additive which cause some mutagenic, carcinogenic and cytotoxic effects. They exhibited a maximum adsorption capacities of melanoidins by chitin nanofibers and they were 131, 331 and 353 mg/g at 20 °C, 40 °C, and 60 °C, respectively. They also found that temperature could play a major role in the adsorption behavior of chitin nanofibers¹⁵⁹. Similarly, Lu et al., (2011) prepared the chitosan beads and porous crab shell powder from shrimp shells and studied the removal of 17 organochlorine pesticides (OCPs) from the polluted water solution. The study confirmed that the surface morphology of chitosan beads having a rough surface and pores, can serve as the adsorption site for pesticides¹⁶⁰. Chitosan-carbon based biocomposite are used for the efficient removal of phenols from aqueous solutions¹⁶¹. Recently Elanchezhiyan & Meenakshi (2016) studied the recovery of oil from oil-in-water emulsion by metal incorporated chitin using adsorptive method¹⁶².

Along with chitin and its derivatives, the environmental applications of chitinase enzymes are studied and reported. Chitinases can be used to convert chitinous waste of marine organisms into simpler useful depolymerized components, and thus promoting reduction of water pollution. Chitinases are also used in conversion of chitinous waste into biofertilizers¹⁶³. Chitinases can be used in the production of single cell protein by utilize the chitinous waste effectively¹⁶⁴. All together chitinases, CT and its derivatives can be used for the remediation of various organic contaminants from the environment.

Conclusion and Future Perspective

At the outset, this review focused on the recent developments related to biomedical and environmental applications of chitin, chitinases, and chitin derivatives.

In the first part of the review, various methods that have been employed to improve the functionality of chitin have been discussed. Chitin and its enzymes can be readily derivatized into various forms which can find applications in diversified fields. Chitin's biomedical applications are not only from its easy availability, but also from its inherent material and chemical properties such as degradability, mechanical strength and biological activity. The activities of chitin in specific applications greatly depend on its degree of acetylation, molecular weight and functionalization. CT and its derivatives provide highly valuable components with health benefits such as anti-microbial, anti-cancer, wound healing and anti-inflammatory effects. As chitin is an eco-friendly biodegradable material, the environmental remediation process using chitin and its derivatives, may lead to the development of futuristic methods and materials to reduce the environmental toxins. Though enzyme chitinase, chitin and its chitin derivatives showed potential applications, these biocompatible materials are underutilized and their use in all of the above applications need further research and validation to exploit their potential medical and environmental applications. With the recent advances in the applications of chitinases, chitin and its derivatives, it is hoped that this review will encourage aspiring researchers to use chitin and chitinases in various approaches for the development of valuable innovative biomaterials, technologies and methodologies for the benefit of mankind.

Abbreviations

AA – Acrylic acid

AgNP – Silver nanoparticles

Chi:nGO – Chitin/graphene oxide

CMCH – Carboxymethyl chitin

CMs – Chitosan microparticles

CNF – Chitin nanofibers

CNP – Chitin nanoparticles

CNP/AgNP – α -chitin/silver nanocomposite

CNW – Chitin nano-whiskers

COS – Chitoligosaccharides

COX – Cyclooxygenase

CS – Chitosan

DA – Degree of acetylation

DDA – Degree of deacetylation

DMAc – N,Ndimethylacetamide

DS – degree of sulfation

EGFR – Epidermal growth factor receptor

EIS – Electrochemical impedance spectroscopy

GCPQ – Quaternary ammonium palmitoyl glycol chitosan

GO – Graphene oxide

GOD – Glucose oxidase

H3PO4 – Phosphoric acid

HA – Hyaluronic acid

HAp – Hydroxyapatite

IL – Interleukin

iNOS – Inducible nitric oxide synthase

LiCl – Lithium chloride

Mad2 – Mitotic arrest deficient 2

MIP – Molecularly imprinted polymers

MP – Methyl parathion

MPC – Methyl pyrrolidinone chitosan

Mw – Molecular weight

NaOH – Sodium hydroxide

OCPs – Organochlorine pesticides

PAA – Polyacrylic acid

P-chitin – Phosphated chitin

POLI – policaju

scFvCD7 – CD7-specific single-chain antibody

siRNA – Small interfering RNA

TCAA – Trichloroacetic acid

TFAA – Trifluoroacetic anhydride

TNF – Tumor necrosis factor

USM chitin – Ultrasonic surface modified chitin

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