Volume 12 • Number 4 • December 2021 • em00779

JOURNAL OF CLINICAL AND EXPERIMENTAL INVESTIGATIONS

REVIEW ARTICLE

Chitosan and its Broad Applications: A Brief Review

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ABSTRACT

Objective: This review summarizes the methods of chitosan production as well as its various potential applications.

Materials and Methods: This study has been performed by literature review.

Results: Chitin is a natural compound that is the second most abundant biological compound in nature. Chitin is found in many fungi species and aquatic crustaceans like crabs, shrimps, and many insects. Shrimp is the source of one of the most rapidly increasing businesses in the world. However, during shrimp processing, the hard exoskeleton of shrimps, like shrimp skin and head portions, is discarded as bio-waste. This exoskeleton of shrimp contains a considerable amount of chitin. Chitosan is a bio-product that is produced from chitin by the deacetylation process. Either chemical or biological processes can carry out the deacetylation process. The massive number of chitin treated as bio-waste can be used to produce chitosan. Chitosan is a biocompatible compound, naturally biodegradable, and non-toxic, and this compound can be used in various applications. Chitosan has potential antimicrobial and antioxidant activities. Moreover, it can also be used in drug delivery, biotechnology, bio-nanotechnology, food technology, regenerative medicine, medicine, numerous industrial applications, gene therapy, cancer therapy, agriculture, environmental protection, and so on.

Conclusion: Chitosan can be used in almost all fields of biology. Although chitosan is not still used in all the mentioned fields shortly, chitosan should significantly impact these areas. More researches should be performed to make chitosan a compound of many applications and possibilities.

Keywords: chitin, chitosan, deacetylation, nanotechnology, regenerative technology

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Received: 19.05.2021,
Accepted: 05.08.2021
https://doi.org/10.29333/jcei/11268

INTRODUCTION

Chitin is a biological material that is one of the most ubiquitous natural compounds in nature. It is a high molecular weight linear homopolysaccharide, composed of repeating units of N-acetyl-D-glucosamine residues, held together by β-(1-4) linkage [1]. Chitin can be found in invertebrates like insects, shrimps, crabs, and many fungi. Chitosan is a derivative compound of chitin that can be obtained by partial deacetylation (**Figure 1**). Chitosan is composed of a co-polymer of D-glucosamine and N-acetyl-D-glucosamine. The number of D-glucosamine and N-acetyl-D-glucosamine residues in the co-polymer varies depending on the varying degree of deacetylation [2].

Aquaculture is one of the fastest-growing food business in the world. In recent years, the shrimp business has increased dramatically, especially in Asia [3]. However, in the shrimp industries, head and skin portions (exoskeleton) of the shrimp are separated from the flesh, and only the meat is taken. The exoskeleton is treated as bio-waste. Approximately 45-55% of the raw shrimp is discarded as a waste product during the processing steps [4,5]. Moreover, according to a study, crustaceans like shrimps may contain a very significant portion of the total weight of the shell or exoskeleton. For this, a considerable amount of shells and chitin compounds are being wasted every year. First identified and observed in the mushrooms by French Professor Henri Braconnot in 1811, it took 140 years for a book to be published based on chitosan, in 1951. Since then, lots of researches are being conducted on chitosan technology [6]. Chitin can be quickly processed into chitosan, which is a.

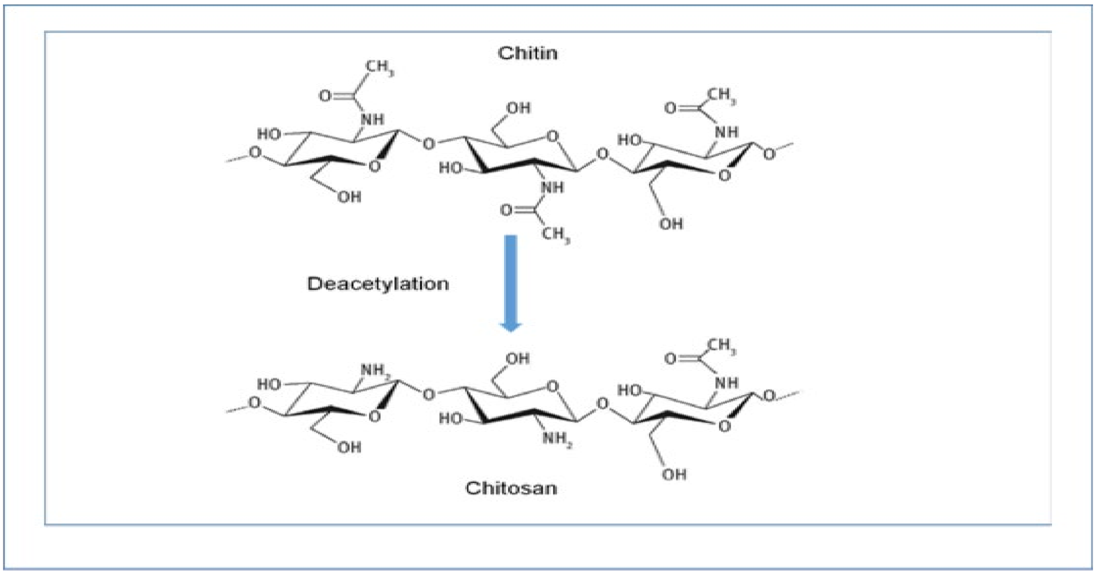

Figure 1. The conversion of chitin to chitosan by deacetylation

fiber-like substance. However, chitosan is not like plant fiber (like cellulose). Chitosan contains a positive ionic charge; for this reason, it can bind with negatively charged substances like negatively charged fat, ions, lipids, cholesterol, proteins, etc.

Moreover, chitosan has excellent bioavailability, biodegradability, and adsorption properties, as well as non-toxicity. These properties make it very useful in various types of applications in multiple fields like wound treatment, drug carrier, food packaging, dietary supplement, chelating agent, pharmaceutical and biomaterial purposes, etc. [7,8]. Chitin and chitosan can be extracted from the exoskeleton by chemical and biological methods [9,10]. The biological process of chitin production is more environmentally friendly, cost-effective (depending on the microorganism used), has excellent decalcification efficacy (up to 86%), and high viscosity than the chemical method. The natural way also decreases the residual proteins in the shrimp shell. Moreover, studies have confirmed that biological processes produce chitosan with quality higher than the chemical method [11]. The Food and Drug Administration (FDA) has approved chitosan as a feed additive in 1983. Chitosan is now widely applied in functional food, environmental protection, and biotechnology [12].

This review aims to briefly discuss the various methods of chitosan production and the wide variety of chitosan applications.

Either chemical or biological methods can be applied to produce chitosan.

Chitosan Production by Chemical Method

The main steps involved in chitosan production is the extraction and purification of chitin from the shrimp exoskeleton. The main stages of chitosan production are demineralization, deproteinization, and deacetylation steps. For chitosan production from shrimp shells, at first, the shrimp wastes are taken from the shrimp processing industries, and washing and drying are done to get the shrimp waste powder. Next, pre-treatment by salicylic acid is carried out for several hours. After the pre-treatment step, the demineralization step is performed. The demineralization step is carried out using hydrochloric acid (HCL). However, the concentration may vary based on shrimp, and the degree to demineralization is required. This step is performed for converting the insoluble calcium carbonate into soluble calcium chloride, which can be easily removed by water in the later phases. After the demineralization step, the deproteinization step is performed, where sodium hydroxide (NaOH) is used. The concentrations of NaOH may also vary depending on the shrimp species. This step removes the protein content from the shrimp waste, and protein hydrolysate is produced, separated using filtration. This protein hydrolysate can be used as a protein supplement. After demineralization and deproteinization, chitin and a minimal amount of proteins and other substances remain. Now, the chitin is ready to deacetylate into chitosan. In the deacetylation step, extreme chemical conditions like 50% NaOH and very high temperatures, like 70-90% temperature or even higher, are required. About 70-90% of chitin deacetylation is necessary to produce a fair amount of chitosan (**Figure 2**). However, the chemical method is mainly limited by its hazardous effects on nature [13-15].

Chitosan Production by Biological Method

The biological process of chitosan production is superior to the chemical process because the natural process is eco-friendly, cost-effective, and ideal for the chemical method. In the biological approach, two ways can be used. One way is the enzymatic method, and the other one is the fermentation

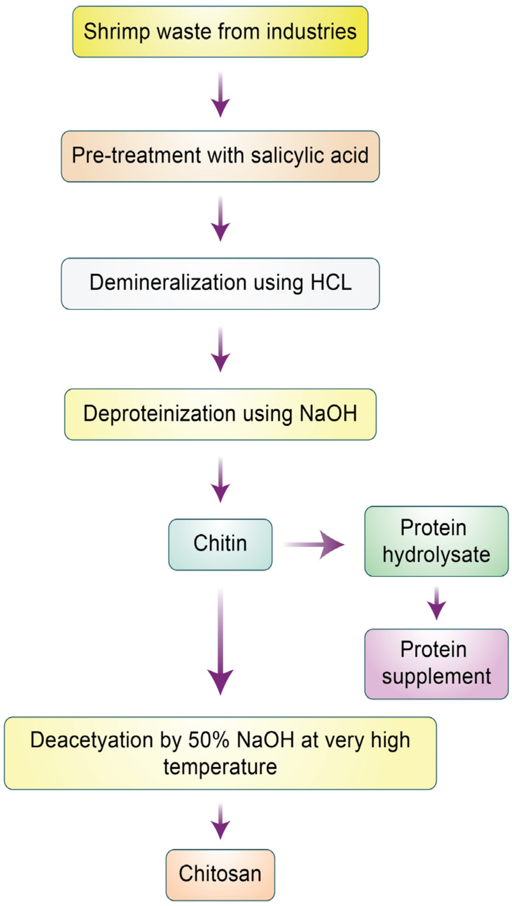

Figure 2. Production flowchart of chitin and chitosan by chemical method. In the production of chitin and chitosan by chemical method, the basic steps of chitosan production, demineralization and deproteinization are carried out by different types of chemicals like HCL, NaOH, etc.

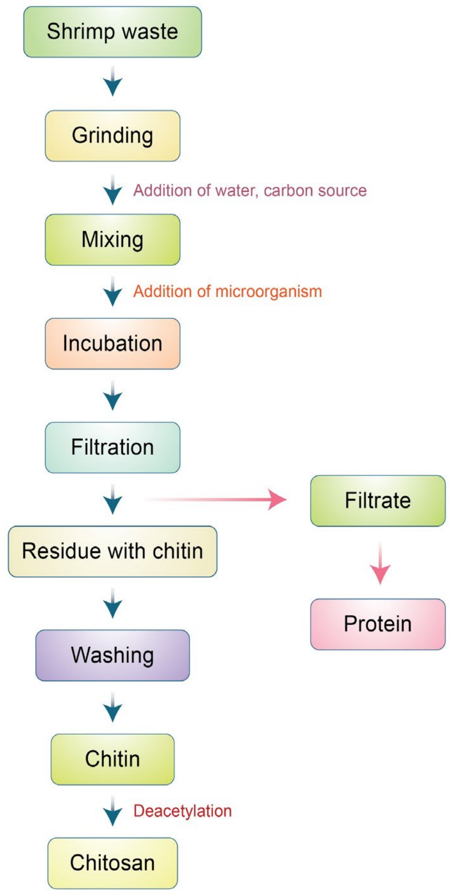

Figure 3. Production flowchart of chitin and chitosan by enzymatic method. In production of chitin and chitosan by chemical method, the basic steps of chitosan production, demineralization and deproteinization are carried out by various enzymes like trypsin, alcalase, pepsin, etc.

method. The enzymatic process is just like the chemical method described above. However, the only difference is that enzymes like papain, trypsin, alcalase, and pepsin are used instead of various chemicals in the deproteinization and demineralization steps [16]. However, additional steps, such as enzyme inactivation, followed by centrifugation, should be carried out. The centrifugation will separate the protein in the supernatant and the chitin in the pellet. The pellet is washed by water, ethanol, and acetone, sequentially, to get the pure chitin. On the other hand, the supernatant is first decolorized using charcoal, next filtration, neutralization (by NaOH), and lyophilization steps yielding protein hydrolysate. The protein hydrolysate can produce high-value peptides (**Figure 3**) [17].

The production of chitosan through the fermentation method utilizes various microorganisms like *Lactobacillus plantarum*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, etc. [18-19]. In the fermentation method, the shrimp waste is first collected and ground. Next, the ground substance is mixed with distilled water and appropriate carbon source and incubated at 370C temperature with an appropriate microbe for fermentation. After incubation of 2-3 days, filtration is used to separate the filtrate. The filtrate contains the proteins.

On the other hand, the residue is collected, and the residue contains mostly chitin. Next, after washing, the chitin is deacetylated to produce chitosan (**Figure 4**). In the fermentation process, the compounds produced by fermenting microorganisms are responsible for the shrimp waste's demineralization. For example, in the lactic acid fermentation, the lactic acid produced by the lactic acid fermenting bacteria reacts with calcium carbonate of the shrimp waste and produce calcium lactate. The calcium lactate then gets precipitated and can be removed easily by washing. However, the deproteinization of the chitin waste in the fermentation method occurs mostly by autolysis, a phenomenon which is common in fishes and shrimp waste [15,17,20].

POTENTIAL APPLICATIONS OF CHITOSAN

Chitosan has applications in a variety of fields, from agricultural sector to more advanced, biotechnology and nanotechnology disciplines (**Table 1**).

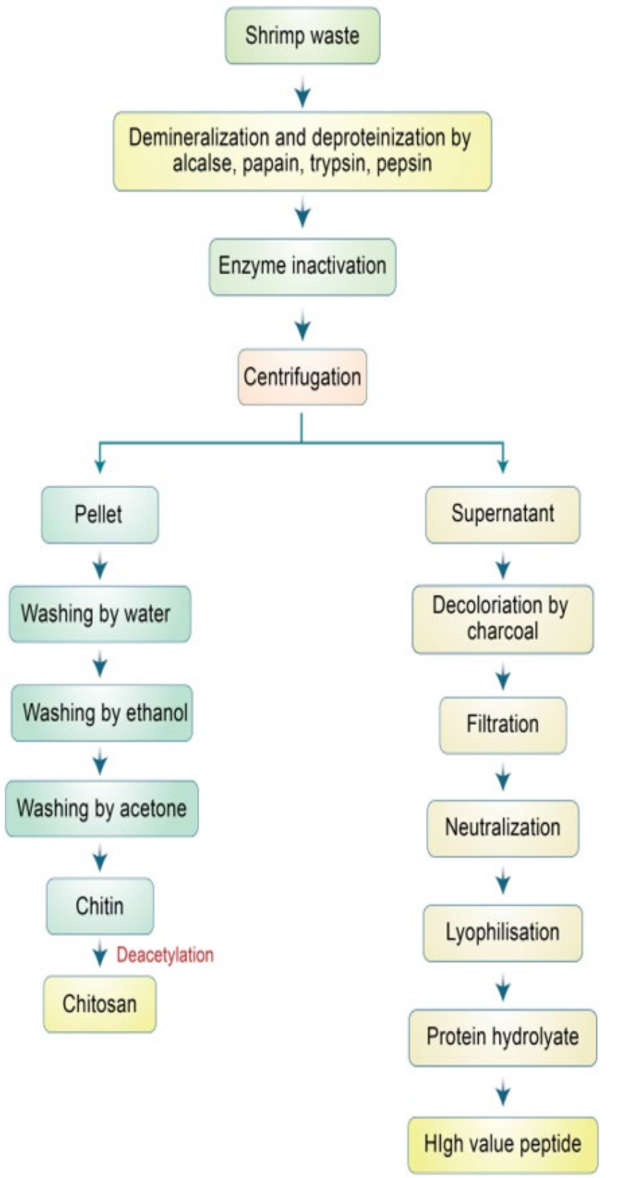

Figure 4. Production flowchart of chitin and chitosan by fermentation method. In the production of chitin and chitosan by chemical method, the basic steps of chitosan production, demineralization and deproteinization are carried out by fermentation by various microbes, instead of using enzymes and chemicals

Antimicrobial Agent

It has been reported from several studies that chitosan possesses good antimicrobial activity. Vital antibacterial activities have been observed against various types of bacteria like *B. cereus*, *Staphylococcus aureus*, *Lactobacillus plantarum*, *Bacillus megaterium*, *L. bulgaris*, *Salmonella typhymurium*, *E. coli*, *Pseudomonas fluorescens*, *Vibrio parahaemolyticus* [21-23]. The mechanisms of antimicrobial activities of chitosan as well as chitin are still mostly unknown; however, several hypotheses shed light on a different mechanism. One of the most accepted assumptions describes that a positively charged amino group is responsible for rendering the antimicrobial activities of chitosan. The positively charged amino group interacts with the negatively charged microbial cell membrane. This interaction causes the leakage of various proteins and other cellular components of the microbes [24,25]. The quaternized chitosan had shown more significant antimicrobial activities than the chitosan in multiple studies since quaternized chitosan has a higher degree of substitution of the quaternary ammonium. Therefore, it can interact very strongly with the cell membrane and exhibits more astonishing antimicrobial activities [26]. Anti-fungal chitosan activities have been observed on *Botrytis cinerea*, *Piricularia oryzae*, *Fusarium oxysporum*, *Trichophyton equinum*, and many other fungi. Chitosan may show the different mechanisms in inhibiting the fungi. For example, chitosan may inhibit the protein synthesis of *Saccharomyces cerevisiae* and affect the intracellular ultrastructures and membrane integrity of *Candida albicans*, thus inhibiting the two fungi [27]. The antimicrobial agent is affected by pH and molecular weight. The pH has an inverse effect on the antimicrobial properties of chitosan. Moreover, studies found that chitosan with a molecular weight below 300 kDa, the antimicrobial effect on *S. aureus* was strengthened when the molecular weight was increased; however, the impact on *E. coli* was weakened [28].

Table 1. Potential applications of chitosan and its derivatives

No	Area of application	Mechanism of action / Used as	References
01	Antioxidant	Antioxidant activities against hydroxyl, superoxide free radicals and DPPH	[89]
02	Antimicrobial agent	The positively charged molecules of the chitosan interact with the negatively charged microbial cell membrane and tend to pull apart the cell membrane. Thus, chitosan exerts its antimicrobial effects.	[90]
03	Drug delivery	Delivery of various drugs through various routes inside the body like oral, nasal, etc.	[34,35]
04	Food technology	Used as a flocculating and adsorbing agent and various chitosan-based films are used in food coating as antimicrobial agent.	[91-94]
05	Gene therapy	Delivering various genes which are used in gene therapy, cancer therapy, and siRNA technology	[39,44-46]
06	Bio-nanotechnology	Graphitic carbon nanocapsules/composites, tungsten carbide chitin whiskers, etc. are used in the production of micro-electrochemical systems and 3D networks.	[95-97]

Table 1 (continued). Potential applications of chitosan and its derivatives

No	Area of application	Mechanism of action / Used as	References
07	Regenerative technology	Used in bone regeneration, neural regenerative technology, cornea regenerative technology, cardiac regeneration therapy, and skin regenerative technology	[58,62,63,66-69,71]
08	Electrolyte	Sulfuric acid and chitosan combination has the ability to discharge high voltage.	[98]
09	Effluent treatment	Due to the coagulating treatment of chitosan, chitosan can be used to remove effluents like suspended solids from various processing plants like whey, dairy, poultry, and seafood processing plants.	[99]
10	Paper manufacture	Production of filter papers, water-resistant papers, biodegrading packages, water-resistant papers	[100]
11	Photography	Used as a fixing agent for color photography and developing color films	[100]
12	Wood industries	Used as wood adhesive, fungicide, wood quality enhancer, and preservative	[100]
13	Cosmetics	Due to the fungicidal activities, UV absorbing ability, and biocompatibility, chitosan can be used in various cosmetics.	[101]
14	Absorption enhancer	Superporous hydrogel-based systems and other chitosan complexes can increase the absorption of drugs across the Caco-2 and intestinal cells.	[102]
15	Agriculture	The antimicrobial activities of chitosan have made it useful for inhibiting various plant pathogens. It also causes an increase in the auxin concentration and urea release in the soil, germination capacity, root length and activity, and seedling height. In animals, chitosan by-product can be used as a protein supplement.	[27,73,74,76-83]
16	Environmental protection	Used in removal of various organic and inorganic pollutants from the environment as well as heavy metals and harmful pesticides	[84-88]
17	Obesity treatment	The lowering effect of cholesterol and LDL is exploited in obesity treatment using chitosan.	[56]
18	Permeation enhancer	Chitosan and its derivatives can increase the permeation of intestinal, nasal, and buccal epithelial cells, and for this reason, it can be used in the drug delivery system as a permeation enhancer.	[103]
19	Catalyst research	Used in liquid phase chemical and biochemical transformations	[104]
20	Immune therapy	Chitosan has the ability to activate humoral immunity, complement system, and CD4+ cells.	[47,49,50]
21	Energy production	Due to the ability to provide ionic conductivity in acetic acid solution, chitosan can be used in the production of solid-state batteries.	[105]
22	Immobilization of cells	Cells like *E. coli* can be immobilized using chitosan beads.	[106,107]

Drug Delivery

One of the most striking and promising aspects of chitosan is its efficiency in the drug delivery system. The process of safe transportation of the desired therapeutic effect of the pharmaceutical compound in the body based on nanotechnology is called "Drug Delivery". The process mainly facilitates site-targeting in the body and systemic pharmacokinetics using nanoparticles or several methods. Since the unique features of Chitosan have been discovered that are favorable for the pharmaceutical fields, scientists have started thinking about its use in the upgrading drug delivery process. The property of showing solely cationic character among all other biodegradable biopolymers cited to be used in the arena of pharmaceutics makes chitosan unique. Due to the presence of an amine group (positively charged), it can adhere to negatively charged biological surfaces (mucosal glycoproteins) as a bioadhesive material [29,30]. Moreover, it is a biocompatible, biodegradable, bioactive, and non-toxic amino polysaccharide. Also, it is soluble in aqueous solutions at pH<6.5. Therefore, it can be considered a favorable material in developing drug delivery processes because of its excellent exceptional mucoadhesive properties [31].

Along with the mucoadhesive character, chitosan has some other features which make it favorable for the drug delivery system. It contains a great controlled drug releasing ability. In the case of a sustained drug, the drug-releasing process is predetermined, and it works for a specific time. Moreover, it proceeds by a simple drug dissolution process or diffusion, or by the osmotic system, which is membrane controlled and often creates obstacles like ionic interaction. Cationic drugs can ensure the controlled release using the anionic polymeric excipients like polyacrylates, sodium carboxymethylcellulose or alginate [32,33]. The mucoadhesive properties mentioned before, which is based on the cationic character of chitosan, works in a specific way. The mucus gel layer comprises sialic acid and sulfonic acid, which is the reason for exhibiting the anionic substructure.

Chitosan's cationic primary amino groups interact with the anionic gel layer; thus, the mucoadhesiveness can be achieved. Using these various exceptional properties of chitosan drugs can be delivered through the parenteral route and different non-invasive routes like oral, nasal, and ocular mucosa routes along with intravesical mucosa via chitosan nanoparticles in a proper way. The chitosan nanoparticles can be made using several methods, including the ionotropic gelation method, microemulsion method, and polyelectrolyte complex method, etc. [34,35].

Chitosan exhibits some more excellent properties like *in situ* gelling properties efflux pump inhibitory properties due to the cationic character. This process executing via nanoparticles is a promising technology in various pharmaceutical applications like tissue engineering or controlled drug release carriers and the oral drug delivery system. It opens the tight junctions of the mucosal membrane and enhances absorption. Their positive charge makes them favorable in pulmonary drug delivery. Nasal drug delivery systems are also based on these properties; chitosan can be used here as an auxiliary agent [36,37]. Methyl-pyrrolidinone chitosan exhibited the most mucoadhesive and permeation enhancing properties in vaginal are and buccal environments. Even in ocular drug delivery, the hydrogel nanoparticles of the chitosan system based on non-toxic and permeation enhancing effects works excellently [38]. With all of these phenomenal approaches in drug delivery of chitosan, it has been considered a potential biomaterial in developing drug delivery systems [33].

Gene Therapy

Whenever it comes to the strategy of treating non-submissive disease by gene therapy, it raises a big concern about immunogenicity and toxicity. Ensuring these two terms can lead to the proper use of this far-reaching effective viral system. However, this sector coming up with non-viral vector instead of a viral vector for assuring gene delivery precisely undoubtedly plays a promising role. Cationic polymer like chitosan can form a complex form of DNA, and it is a safe and feasible vector for a non-viral gene therapy. Chitosan, for having a positive charge, can bind the negatively charged DNA and protect it from nuclease degradation [39].

The conjugation process (electrostatic interaction) of DNA and chitosan nanoparticles is clutter-free, and it remains very stable during storage. The DNA-chitosan micro nanoparticle has a perfect size (20nm-500nm), which can easily enter the cells through endocytosis or pinocytosis and enhance transfection rate. The transfection can be occurred via both in vivo and in vitro, but the in vitro process can give an effective transfection and ensure well productivity [40,41]. The amino groups of chitosan help to ignore the "proton sponge effect" [42,43]. Chitosan has been modified to several compounds for gene therapy such as:

1) Lactosylated Chitosan
2) Galactosylated chitosan-graft-polyethyleneglycol (GCP)
3) Quaternization of oligomeric Chitosan
4) Chitosan/DNA/ligand complexes
5) Dodecylated chitosan vector
6) Deoxycholic acid modified-chitosan vector

Chitosan-based gene therapy went through several experiments and developed depending on the transfection efficacy. The maximum modified chitosans mentioned here show a low transfection rate than the standard DNA-Chitosan formulations and have chance of harming the DNA [39]. Chitosan-coated poly-isohexylcyanoacrylate (PIHCA) nanoparticles are used for intravenous delivery of small interfering RNA (siRNA). The developed method of interfering small RNAs (siRNAs) and silencing a specific gene to inhibit the associated protein is widely used in several diseases treatment, including cancer therapy [39,44]. Moreover, mannosylated Chitosan with a plasmid can express interleukin-12 (IL-12), which might be used effectively in cancer immunotherapy. Scientists came up with some proof that chitosan can inhibit tumor cell proliferation along with tumor growth by inducing apoptosis and reducing glycolysis gradually [45,46]. Chitosan also shows potential in the delivery of the renowned CRISPR/Cas9 system. And already, carboxymethyl chitosan and PEGylated chitosan have been used in CRISPR/Cas9 system delivery. Along all of these, chitosan has a great promising factor in furthermore development of gene therapy systems.

Immunity

Chitosan has the ability to boost the immune system. To fight diseases, harnessing immune systems' methods daily using the most probable components, is the key to the highest development. Suppressing secondary tumor or adjuvants increase the body's immune response to vaccination. Working on that, it has been reported that the vaccine adjuvant chitosan raises cellular immunity by DNA Sensor cGAS-STING-Dependent Induction of Type-I Interferons which can be base of developing vaccines that trigger cell-mediated immunity (Chitosan could boost body's immune response to vaccines (Retrieved December 06, 2020, from https://www.medicaleconomics.com/view/chitosan-could-boost-bodys-immune-response-vaccines). It has been found that Chitosan increases antigen-specific antibody titers over 5-fold and antigen-specific splenic CD4+ proliferation over 6-fold. Antibody titers and strong, delayed-type hypersensitivity (DTH) responses proved that chitosan induced both humoral and cell-mediated immune responses as an adjuvant. Chitosan activates humoral immunity like the serum and interstitial fluid [47,48].

It also activates complement like the activation of anaphylatoxins such as C3a and C5a except for C4 by an alternative pathway. Among them, C3a activates the mast cells to secrete histamine, and C5a functions in the activation of phagocytic cells [49]. Due to chitosan's high viscosity than water, chitosan can be used effectively as an antigen depot. And for this reason, chitosan can be used with vaccination to generate adaptive immune response over a long period, which substantiated that chitosan can increase the immune system as an adjuvant [47,50]. Moreover, the unique characteristics of chitosan have made it able to be used in various vaccine constructions and delivery, such as influenza vaccine delivery, preparation of hepatitis B vaccine and polio vaccine, etc. [51-53]. Furthermore, due to having immunostimulatory properties, chitosan (Ch)/poly (γ-glutamic acid) (γ-PGA) nanoparticles (NPs) assimilates IFN-γ for immune response modulation in the sector of colorectal cancer [54].

Obesity Treatment

Scientists have confirmed that chitosan can be effectively used to treat obese patients. When studying the obesity lowering activity of chitosan on 3T3-L1 cells, chitosan showed significant anti-obesity activity. The 3T3-L1 cell line was converted into adipocytes and treated with chitosan. This treatment decreased the lipid accumulation activity of the adipocytes and down-regulated adipogenic marker proteins such as leptin, resistin, etc. Chitosan mediated the anti-obesity activities by interfering with the adipocyte differentiation and inhibiting the adipogenic transcription factors and genes [55]. Chitosan also has the capability of lowering cholesterol and low-density lipoprotein (LDL), thus aids in obesity treatment [56].

Regenerative Therapy

Chitosan in bone tissue engineering

Currently, different types of bone disorders and injuries are treated by bone grafting. In bone grafting, a bone from another source is taken and grafted at the injured site. Bone grafting can be of two types: autograft and allograft. In autograft, the bone is taken from another part of the body of the patient; for this reason, it is histocompatible and non-immunogenic. Autografts also provide the necessary signals for osteoinduction like bone morphogenetic proteins (BMPs). On the other hand, the allografts are taken from another person's body; for this reason, allografts can cause different types of infections and immune reactions and have lower osteoinduction efficiency [57]. Chitosan can be used in bone regeneration processes. Chitosan, hybridized with silica xerogel, were tested for their efficacy to be used in guided bone regeneration (GBR). The chitosan-silica xerogel hybrid showed promising results in bone regeneration and produced almost no adverse effects like inflammation [58]. On the other hand, novel chitosan nanofibre membrane and chitosan-nanocrystalline calcium phosphate composite scaffold have also proved to be effective in bone regeneration [59,60].

Chitosan in neural regenerative technology

The field of regenerative technology in neural science has received a lot of attention in recent years since such treatments affect a patient's life. Such treatments are complicated to carry out. Like the bone regeneration system, conventional processes used in neural regeneration involve autografts and allografts. Since the autografts are taken from the particular patient, there is less chance of immune-rejection; however, this process is limited by a shortage of nerve cells in the patient and size differences of the donor site nerve cells and receptor size nerve cells. On the other hand, the allografts are taken from another person, mainly from a cadaver, and could meet the demand of nerve cells; however, there is a potential chance for immune-rejection [61]. Chitosan can be the right solution for nerve treatment and regeneration procedures. The chitosan-apatite composites are proved to be effective in nerve regeneration. Other chitosan complexes like chitosan-polyglycolic acid (PGA) nerve guidance conduit, collagen-chitosan complex, cross-linked carboxymethyl chitosan, etc., also showed their efficacy in nerve regeneration treatments [62-64].

Chitosan in corneal regenerative technology

The cornea is one of the most critical parts of the eye. It is a transparent structure of the visual system, and without cornea, the optical functions would be significantly hampered [65]. Injuries in the eye often lead to corneal degeneration. In such cases, corneal transplantation is carried out. However, due to the limitation in the supply of cornea tissues from the donor individuals, researchers are currently developing artificial cornea tissues. Chitosan technology can be used effectively in such areas. Researchers used chitosan-polyethylene glycol (PEG) hydrogel films to cultivate corneal endothelial cells (CEC). The chitosan performed well in the experiments providing mechanical, optical, and proliferative support to the CEC. Moreover, a complex made from chitosan and sodium alginate also showed promising efficacy in CEC proliferation and regenerative technology [66,67].

Chitosan in cardiac regenerative technology

Chitosan regenerative technology can be used effectively in treating various cardiac injuries. Such injuries are currently treated with transplants and artificial devices because the human heart has a low capability to repair itself naturally [68]. However, this practice is limited by the deficient availability of hearts from donors. Therefore, scientists have focused on developing novel regenerative treatments to treat various types of heart diseases. Chitosan hydrogels can be used effectively in such treatments as potent cardiac regeneration therapy [69,70].

Skin regeneration using chitosan regenerative technology

Skin is the largest and one of the most complex organs of the body. Since it is always exposed to the external environment, there is a high risk of skin damage by harmful external factors like trauma, injury, and especially fire. In the case of burning, severe alteration of biomolecule homeostasis and tissue architecture occurs. Continuous production of fluid and attack of pathogens make the wound healing process very challenging. Conventionally, autologous transplantation is carried out for treating the burned, injured sites. However, due to the limitation of enough skin to be implanted from the autologous source, research is carried out for new, biological compounds to be used as implantation. Chitosan technology can have a significant impact in this field. Recently, a silver nanoparticle decorated chitosan scaffold has been developed that can be effectively used in wound healing and tissue regeneration therapies. Such technologies will help treat the wound caused by burning in the recent future [71,72].

Agriculture

The antimicrobial, anti-insecticidal, non-toxic, and biodegradability properties of chitosan can be used effectively for agricultural purposes. Chitosan-based nanoparticles have been developed, which can be used to deliver essential agrochemicals and genetic materials to the plants. Chitosan nanoparticles can provide pesticide and herbicide for crop protection, deliver fertilizers, deliver nanosensors for crop monitoring, and improve soil health. Furthermore, studies have confirmed that using chitosan nanoparticles can increase the soil's auxin and urea release. Chitosan also inhibits the growth of various plant pathogenic bacteria and fungi like *Botrytis cinerea*, *Fusarium oxysporum*, *Micronectriella nivalis*, *Rhizoctonia solani*, *Erwinia carotovora*, *Agrobacterium tumefaciens*, etc. [27,73,74]. Chitosan biopolymers help plants to defend the pathogen attacks by several mechanisms. Chitosan can activate some pumps of the plant cells that may secrete anti-pathogenic compounds. Chitosan can induce the production of key enzymes of the phenylpropanoid pathways. It can also stimulate the plant to produce various types of immune-enhancers and elicitors and activate the Ca2+ dependent defensive pathways like deposition of Ca2+ dependent callose synthase. All these responses have antiviral, innate immune-stimulatory effects. Moreover, these responses make the plants more resistant to secondary infections and help the plants to produce more secondary metabolites [75]. Chitosan also can improve germination capacity, root length, and activity, and seedling height. Studies have confirmed that chitosan can form a semi-permeable film on the film surface due to its film-forming capability, which helps maintain the plant seeds' moisture [76,77]. Moreover, the chitosan coating also helps the plants become tolerant to the drought stress by developing the root system and increasing the plant roots' capability to absorb more water [78]. There is also evidence that chitosan increases the production of abscisic acid that decreases the rate of transpiration and maintains the closure of stomata in low water environments such as drought [79,80].

In animal husbandry, chitosan can be used as protein supplementation of the feed. The protein residue produced as the by-product of chitosan can be used as a feed supplement, which can increase the immunity of animals and meet the demand for protein. Moreover, chitosan and its derivatives can be processed as animal feed [81-83].

Environmental Protection

Chitosan and its derivatives can be used to remove various environmental pollutants from the environment. These pollutants can be both inorganic and organic pollutants. For example, nitrate pollutants can be eliminated by polyethylene glycol (PEG)-chitosan and polyvinylalcohol (PVA)-chitosan, phosphates can be removed by chitosan on which Cu(II) is immobilized, etc. [84-85]. Moreover, pollution-causing dyes like methylene blue and methylene violate can also be removed using chitosan. Pesticides like glyphosate can be eliminated from the environment using the chitosan-alginate complex. Again, studies have confirmed that chitosan has the ability to remove heavy metal ions from the environment [86-88].

LIMITATIONS OF CHITOSAN

With a huge range of potentialities and a wide range of uses in biopharmaceutical, biomedical, and others, chitosan is also bound to some limitations. For belonging some intrinsic macromolecular characteristics, it has become the most commonly cited polymers in scientific research. From controlled drug release to gene therapy and working in cancer treatment it has been the ultimate light of hope in the biomedical sector.

Chitosan needs a high cleansing and modification for proper usage. Basically, chitosan is known as a marine polysaccharide acquired from the hard outer skeleton of shellfish, including crab, lobster, and shrimp. Therefore, it is considered to be a very easily accessible substance from marine or food industry waste. Genuinely, the samples of chitosan are completely raw materials. After some purification process, it gets ready to be used in medical and other sectors. As a result, this creates issues related to the presence of impurities, proteins, pyrogenic, endotoxic, and cytotoxic agents, bioburden, metals, and other inorganics, which are must to ensure safety for final human use. Chitosan studies working in drug delivery due to its proper uptake, targeted release, distribution, and toxicity show reconnoiter of chitosan interacting with some mutual solutes or other biomolecules. And these may express some subtle effects both in classical solution thermodynamics and in biological applications. These unwanted and often hidden or unexpressed effects throw the whole process to the wind with uncertainty [108]. In siRNA delivery, chitosan has been noticed for its high buffer ability in the endosomal pH range. Due to unfavorable physicochemical properties, such as poor solubility, unionized nature, colloidal instability, and weak binding with nucleic acids at physiologic pH (leads to premature release of nucleic acids, poor cell uptake and transfection), its applications are limited in various sectors, although scientists are trying to overcome these problems [109]. Instead of having many biological and physicochemical properties, it's not been gone under much clinical use due to low solubility and poor mechanical characteristics. So, in the case of the drug delivery process, the low solubility of chitosan in neutral and alkaline pH and the process of preparation must be changed according to the drug that is to be delivered [110]. As a versatile biopolymer, industries have an onward demand for chitosan in developing countries. Products produced from chitosan have a positive impact on the market. But due to the extreme difficulty to access enough purity and source authenticity of the biopolymer, it has some limited use in the biomedical area. Moreover, the development of new materials is also limited due to their higher cost, whereas petroleum-based polymers with similar properties cost less than that. On the other hand, in vivo studies are recently limited. Finally, there is a relentless competition with the synthetic polymers out there that are relatively cheap and viable for commercial use.

CONCLUSION

Chitosan is a biological compound that can be produced from chitin. It is naturally biodegradable, eco-friendly, biocompatible, and non-toxic material, making it a potent compound of interest. Chitin is deacetylated to varying degrees to produce chitosan. Either chemical or biological methods can carry out the production of chitin. However, chitosan is a compound that has many possibilities. Chitosan can be used in almost all fields of biology. Chitosan has potent antimicrobial properties, making it useful for antimicrobial agents in food, agriculture, and medical treatments. Chitosan can significantly impact regenerative technology, for example, cornea, skin, cardiac regenerative technologies, etc. Moreover, chitosan can be used in obesity treatment, immune therapy, gene and cancer therapy, and various other fields like agriculture, environment protection, wood industry, cosmetics, energy production, etc. Chitosan in nanotechnology is also an emerging field. Although chitosan is not still used in all the mentioned fields shortly, chitosan should significantly impact these areas. More researches should be performed to make chitosan a compound of many applications and possibilities.

Author contributions: All authors have sufficiently contributed to the study, and agreed with the results and conclusions.

Funding: No funding source is reported for this study.

Declaration of interest: No conflict of interest is declared by authors.

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