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NATURES GIFT CHITOSAN ITS PRODUCTION AND BIOLOGICAL APPLICATION-REVIEW ARTICLE

G.M.Mir

Department of chemistry, Govt. Womens College Anantnag J&K (India)

Abstract:

Chitosan is a natural polycationic linear polysaccharide derived from chitin. The low solubility of chitosan in neutral and alkaline solution limits its application. Nevertheless, chemical modification into composites or hydrogels brings to it new functional properties for different applications. Chitosan are recognized as versatile biomaterials because of their non-toxicity, low allergenicity, biocompatibility and biodegradability. This review presents the recent research, trends and prospects in chitosan. Some special pharmaceutical and biomedical applications are also highlighted.

Keyword: Chitosan, polycationic, biomedical applications, biocompatibility

Address for Correspondence:
G.M.Mir,

Department of chemistry,

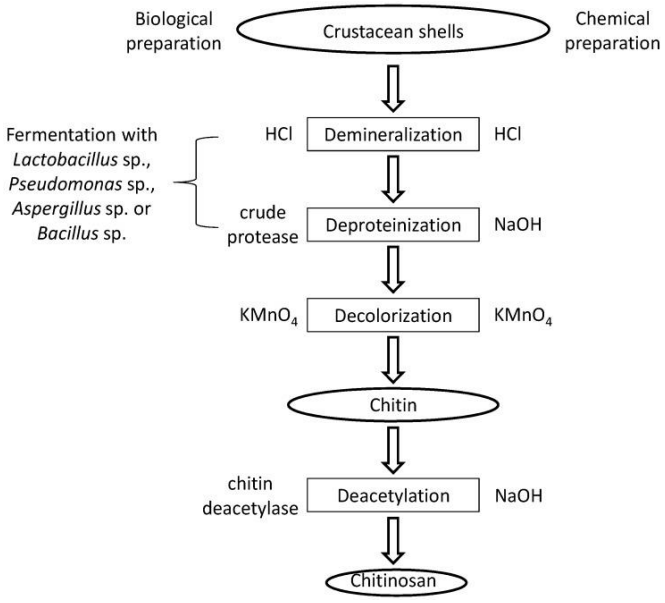
Govt. Womens College, Anantnag, J&K (India).

INTRODUCTION:

Chitosan is composed of β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine randomly distributed within the polymer. The cationic nature of chitosan is rather special, as the majority of polysaccharides are usually either neutral or negatively charged in an acidic environment. This property allows it to form electrostatic complexes or multilayer structures with other negatively charged synthetic or natural polymers [1-2]. The interesting characteristics of chitosan such as biocompatibility, non-toxicity, low allergenicity and biodegradability allow it to be used in various applications [3]. Besides, chitosan is reported to have other biological properties, such as antitumor [4], antimicrobial [5], and antioxidant [6] activities. The degree of deacetylation, which is described by the molar fraction of deacetylated units or percentage of deacetylation, and the molecular weight of chitosan, were found to affect these properties [7]. Chitosan has been widely used for different biological and biomedical applications recently due to its unique properties. For instance, it can be used in water treatment [8], wound-healing materials pharmaceutical excipient or drug carrier [9], obesity treatment [10] and as a scaffold for tissue engineering . There is increased interest in pharmaceutical as well as biomedical applications of chitosan and its derivatives and significant development has been achieved. It can be reflected in the increasing number of related publications throughout the years.

Production of chitosan

The raw material for the production of chitosan is chitin. The main sources are the shells of crustaceans, mainly crabs and shrimps. The purification process is easier for shrimp shells which are thinner. Usually, shells of the same size and species are grouped, then cleaned, dried and ground into small shell pieces [11]. There is no standard purification method as different chitin sources require different treatments due to the diversity in their structures. Conventionally, the protocol is divided into demineralization, deproteinization and decolorization steps which can be carried out using chemical or biological (enzymatic treatment or fermentation) treatments. The end-products need to be highly purified if they are to be used for biomedical or pharmaceutical purposes, as residual proteins, minerals or pigments can cause serious side effects. Conversion of chitin to chitosan can be achieved by enzymatic or chemical deacetylation. Chemical deacetylation is more commonly used for commercial preparation because of economic issues and feasibility for mass production. No matter which method is used, depolymerization is inevitable [12]. The processes involved in chemical and biological preparation of chitosan from crustacean shells are illustrated in Figure 1 .



Antibacterial Activity

Many reports have shown that chitosan exhibited antimicrobial activity, but the actual mechanism has not yet been fully elucidated. Several hypotheses have been proposed based on its cationic nature. Low-molecular-weight chitosan can penetrate bacterial cell walls, bind with DNA and inhibit DNA transcription and mRNA synthesis [13], while high-molecular-weight chitosan can bind to the negatively charged components on the bacterial cell wall. It forms an impermeable layer around the cell, changes cell permeability and blocks transport into the cell [14-15]. This hypothesis was further supported by the studies from Muzzarelli et al. [16]. The hydrophilicity and negative charge on the cell surface were higher on gram-negative bacterial cell walls than those of gram-positive bacteria. Thus the gram-negative bacteria showed a stronger interaction with chitosan, which resulted in stronger antibacterial activity against them. It was also reported that the amount of chitosan binding to the bacterial cell wall was dependent on the environmental pH value, molecular weight and degree of acetylation of chitosan. Low environmental pH increases the positive charge in the chitosan polymer, which favors binding to the bacterial cell wall [17]. Younes et

al. [18-19] reported that a lower degree of chitosan acetylation and a lower pH are favorable to the antibacterial activity of chitosan. A reduction in the molecular weight of chitosan increases the antibacterial activity of chitosan toward Gram-negative bacteria and reduces the activity on the Gram-positive bacteria. Chitosan has a wide spectrum of activity and high killing rate against Gram-positive and Gram-negative bacteria. The activity is the result of interactions between chitosan and its derivatives with bacterial cell wall molecules. The studies mentioned in this section demonstrated a close relationship between the antibacterial activity and the hydrophilicity of the cell wall, thus the action is specific and showed lower toxicity toward mammalian cells.

Antifungal Activity

The effects of molecular weight and degree of acetylation of chitosan on its antifungal activity vary with the fungus. Chitosan was also found to exhibit antifungal activity against several phytopathogenic fungi such as Penicillium sp. in citrus fruit. Botrytis cinerea in cucumber plants , Phytophthora infestans, Alternaria solani and Fusarium oxysporum in tomatoes. The suggested mechanism involved a permeable chitosan film formed on the crop surface which interfered with the fungal growth and activated several defense processes like chitinase accumulation, proteinase inhibitor synthesis, callus synthesis and lignification. Silver nanoparticles distributed superficially on and internally in chitosan spheres demonstrated a macroporous feature, and could find applications such as fungicidal agents.

Chitosan applied at a dosage of 100 μg/mL killed the bulk of fungal species pathogenic to human pathogens examined but did not express toxicity to HEK293 and COS7 mammalian cells. Survival of Galleria mellonella larvae after infection with C. albicans was elevated by chitosan .

Water-soluble chitosan was fungi static to Macrophomina phaseolina. Fungal infection was suppressed and the activities of enzymes associated with defense including chitosanase and peroxidase in infected seedlings were up regulated following exposure to water-soluble chitosan the existence of cranberry and quince juice in the composition of chitosan and whey protein-chitosan films reinforced the elasticity and reduced the tensile strength of the films. Chitosan and whey proteins-chitosan films with quince and cranberry juice added are potentially useful for increasing the shelf life of apples.

The fungal inhibition indices of deacetylated chitosans generally increased with a rise in molecular weight. Nevertheless, high-molecular-weight chitosan derivatives with a low hydrophobicity and low-molecular-weight derivatives with a high hydrophobicity displayed the highest potency in suppressing growth in Aspergillus flavus in vitro.

Anti-HIV-1 Activity and for Construction of Nanoparticles Loaded with Anti-HIV Drugs

QMW-chitosan oligomers and WMQ-chitosan oligomers (in which Q, M and W stand for glutamine, methionine and tryptophan, respectively) exerted a protective action on C8166 cells against cytolytic effects of HIV-1RF strain. The oligomers suppressed syncytium formation induced by HIV, and reduced the HIV load without any inhibitory effects on activities of HIV-1 reverse transcriptase and protease in vitro. Syncytium formation was suppressed when HIV-infected and uninfected C8166 cells were co-cultured. QMW-chitosan oligomers and WMQ-chitosan oligomers inhibit HIV-induced cytopathic effects by exerting their effects on HIV entry stage [56]. Chitooligosaccharides are nontoxic and water-soluble compounds derived from chitosans by enzymatic degradation. Sulfated chitooligosaccharide III with a molecular weight of 3–5 kDa potently suppressed HIV-1 replication, HIV-1-induced syncytium formation, lytic action, and p24 antigen production. Sulfated chitooligosaccharide III obstructed viral entry and virus-cell fusion probably by interfering with the binding of HIV-1 gp120 to CD4 cell surface receptor. Unsulfated chitooligosaccharides did not have similar actions.

Antioxidant Activity

Antioxidants are well-known for their beneficial effects on health. They protect the body against reactive oxygen species, which exert oxidative damage to membrane lipids, protein and DNA. Much effort has been invested to investigate the antioxidant activity of chitosan and its derivatives in recent years. Park et al. [20] reported the in vitro oxygen radicals scavenging activity in chitosan and its derivatives. Low–molecular-weight chitosans are more active in scavenging free radicals, such as hydroxyl, superoxide, alkyl and 2,2-diphenyl-1-picrylhydrazyl radicals. It was proposed that the mechanism is due to the reaction of unstable free radicals with amino and hydroxyl groups on the pyranose ring, which form the stable radicals.

Nine kinds of hetero-chitooligosaccharides with relatively higher molecular weights, medium

molecular weights, and lower molecular weights have been prepared from partially deacetylated hetero-chitosans (90%, 75% and 50% deacetylated chitosan), and their scavenging activities against 1,1-diphenyl-2-picrylhydrazyl, carbon-centered hydroxyl, superoxide and radicals were studied by employing electron spin resonance spin-trapping technique. Carbon-centered, hydroxyl, and superoxide radicals were generated from 2,2-azobis-(2-amidinopropane)-hydrochloride, hydrogen peroxide-ferrous sulfate (Fenton reaction), and hypoxanthine-xanthine oxidase reaction. The electron spin resonance data demonstrated that medium-molecular-weight hetero-chitooligosaccharides prepared from 90% deacetylated chitosan manifested the highest radical scavenging potency. The radical-scavenging activity of hetero-chitooligosaccharides was related to the degree of deacetylation values and the molecular weight. Chitosan was prepared by alkaline N-deacetylation of â-chitin from squid pens, and N-carboxyethylated derivatives (N-CESC) with different degrees of carboxyethyl group substitution (N-CESC3 possessed the highest degree of substitution while N-CESC1 possessed the lowest degree of substitution) were synthesized. All three N-CESC samples displayed good water solubility at pH above 6.5. They manifested potent 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity, with EC_{50} values under 2 mg/mL. The ABTS radical scavenging activities of N-CECS with different degrees of substitution and concentrations are shown in Figure 3. It showed that the activity of N-CECS toward ABTS increased with concentration. Besides, the addition of carboxyethyl groups to chitosan enhanced its radical scavenging activity against ABTS. The scavenging ability of N-CESC against superoxide radicals showed a good correlation with the degree of substitution and concentration of N-CESC. The data suggested that N-CESC can be utilized to produce chitosan derivatives with good biochemical characteristics in vitro.

Tissue Engineering

Tissue engineering describes the use of a combination of cells, engineering materials, and suitable biochemical factors to improve or replace biological functions. It includes a wide range of applications such as repair or replacement of part of or whole tissues (for example, bone, cartilage, blood vessels, bladder, skin and muscle). Chitosan-based biomaterials have become a popular target in development for tissue engineering and significant progress has been made recently. It provides certain mechanical and structural properties for proper functioning for the repaired tissues.

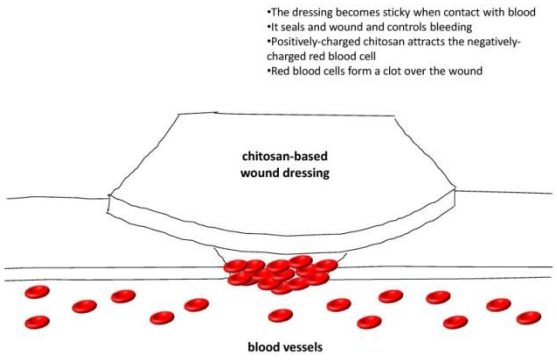
N-methacryloyl chitosan, produced as a result of a single-step chemoselective N-acylation reaction, acquires the desirable features of hydrosolubility, UV crosslinkability and injectability. It facilitates quick and cost-effective construction of patterned cell-loaded polysaccharide microgels with distinctive amino groups as building materials for tissue engineering and quick transdermal curing hydrogel in vivo for localized and sustained protein delivery. Chitosan-â-tricalcium phosphate composite exhibited histocompatibility with Beagle mesenchymal stem cells and was devoid of an effect on cellular growth and proliferation. It manifested efficacy in enhancing osteogenesis and vascularization and repair of bone defects in conjunction with mesenchymal stem cells. Reinforcement of silk matrix with chitosan microparticles (silk:chitosan 1:1, 1:2 and 2:1) produced a visco-elastic matrix that promoted re differentiation of caprine chondrocytes and retained more glycosaminoglycan which enhanced the aggregate modulus of the construct similar to native tissue. The data revealed one step forward in optimizing the construction of biomaterial scaffolds for cartilage tissue engineering. Bone morphogenic protein 2/Chitosan microspheres were successively loaded onto a deproteinized bovine bone scaffold. BMP-2 underwent an initial burst release followed by a sustained release. The encapsulated bone morphogenetic protein 2 possessed biological activities. Biocompatibility was good. The microsphere scaffold system may find applications in tissue engineering. A chitosan hollow tube employed for regeneration of the injured rodent transected sciatic nerve yielded results comparable to autologous nerve graft repair. Implantation of chitosan nanofiber tube could partially restore the function of a damaged phrenic nerve in beagle dogs as seen in improvement of diaphragm movement, slow phrenic nerve conduction, connection of the damaged nerve by newly regenerating nerve fibers surrounded by granulation tissue within the chitosan nanofiber tube . The loss of spinal cord tissue and cavity formation impedes the repair of damage to the spinal cord. The scaffold of chitosan+ECM+SB216763 enhanced neural stem cell differentiation into neurons, oligodendrocytes and astrocytes and hence is promising for repair of damaged spinal cords. Chitosan/bioactive glass nanoparticles scaffolds possess the shape memory characteristics of chitosan and the biomineralization activity of bioactive glass nanoparticles for applications in bone regeneration [21].

Drug Delivery System

Chitosan has been widely used in pharmaceutical industry in drug delivery systems in different forms, like tablets, microspheres, micelles, vaccines, nucleic acids, hydrogels, nanoparticles and conjugates. Chitosan and its derivatives can be used in drug delivery systems in both implantable as well as injectable forms through oral, nasal and ocular routes. Besides, they facilitate transmucosal absorption which is important in nasal and oral delivery of some polar drugs like peptides along with protein vaccines for their administration. It is commonly used as an excipient in tablet formulation for oral medication. High-molecular-weight chitosan is more viscous and delays the release of the active ingredient, prolongs the duration of drug activity, improves therapeutic efficiency as well as reducing the side effects of oral tablets. Chitosan microspheres have been extensively investigated for controlled release of drugs and vaccines through oral and nasal delivery. They were prepared by complexation between the cationic chitosan in addition to the anionic compounds such as tripolyphosphate or alginates. Different drugs or vaccines were loaded in the microspheres, they were protected, especially drugs which are protein in nature, in the digestive tract and absorbed through the paracellular route on the epithelial layer. The surface activity of chitosan is low as it does not possess any hydrophobic portions. It can be improved by chemical modifications at its glucosidic group with a hydrophobic substituent. The chitosan form micelles with an external hydrophilic shield and an internal hydrophobic center. The hydrophobic drugs were protected in the center with improved solubility and bioavailability. The chitosan micelles were formed by the electrostatic repulsions between oppositely charged polymers. The chitosan hydrogels are three-dimensionally structured hydrophilic polymers which can absorb and hold up to thousands of times more fluids than their dry weights and use in drug delivery. The drugs are loaded in the hydrogels by diffusion, entrapment, and tethering. Usually, the loaded hydrogel is injected into the body and the drug can diffuse into the neighboring tissues. The recent development of in situ forming depots using chitosan-based hydrogel has attracted much attention as a new method for controlled drug release. The original thermo sensitive chitosan-based polymer is in solution form at room temperature. When it is injected into the body, it forms semi-solid hydrogels at the physiological temperature. It showed protection for the drugs from physiological degradation, combined with prolonged and steady release of drugs. Chitosan nanoparticles exhibit outstanding biodegradable and biocompatible properties which have been studied extensively as drug carriers.

Wound Healing

Chitosan and its derivatives exhibit biodegradable, biocompatible, antimicrobial activity and low immunogenicity which are advantageous for development as biomaterials for wound healing. They provide a three-dimensional tissue growth matrix, activate macrophage activity and stimulate cell proliferation. Chitosan promotes activity of polymorpho nuclear leukocytes, macrophages and fibroblasts that enhance granulation as well as the organization of the repaired tissues. It will be slowly degraded into N-acetyl-β-D-glucosamine which stimulates fibroblast proliferation, aids regular collagen deposition in addition to stimulating hyaluronic acid synthesis at the wound site. It accelerates the healing progress along with preventing scar formation. Nano fibrous and adhesive-based chitosan have been developed as wound dressing materials recently. The electrospun chitosan nano fiber mats were found to be porous, have a high tensile strength, high surface area of the mats combined with ideal water vapor and oxygen transmission rate. It also showed compatibility with adipose derived stem cells, which is considered beneficial for wound healing. The adhesive-based wound dressing was usually applied in surgery to enhance wound healing. The chitosan adhesive shows strong sealing strength as well as not requiring sutures or staples. It can effectively stop bleeding from blood vessels along with air leakage from the lung [22].



Cardiovascular Diseases Treatment

Administration of chitosan-oligosaccharides by gastric gavage to apolipoprotein E deficient mice (apoE-/-) fed a high fat diet for 12 weeks lowered triglyceride and cholesterol levels in non-high density

lipoprotein fractions, undermined atherosclerosis, increased atherosclerotic plaque stability, up regulated hepatic expression of low density lipoprotein receptor, scavenger receptor BI and also the expression of macrophage scavenger receptor BI and ATP binding cassette transporter A1. There was no effect on the plasma lipid level in LDL-R mice with a deficiency of low density lipoprotein receptors and cholesterol absorption in wild-type mice.

Chitosan is a biodegradable and inexpensive polymer which has numerous applications in biomedical as well as pharmaceutical industries. A large amount of research work has been done on chitosan and its derivatives for the purpose of tissue engineering, drug delivery, wound healing, water treatment, antitumor and antimicrobial effects.

CONCLUSION:

Chitosan and its derivatives have been widely studied for potential tissue engineering biomaterials as they will be degraded at a reasonable rate without causing any inflammatory reaction or producing any toxic end-products when the new tissues are formed. They are porous in nature for diffusion of gases, nutrients, and metabolic wastes for the seeded cells along with increasing the surface area for cell attachment, migration and differentiation. They can be molded easily into anatomical shape and volume; are biocompatible with the surrounding biological fluids and tissues; as well as providing temporary mechanical support. All these properties fit the special properties use as tissue engineering scaffold. Chitosan demonstrated antitumor activity in terms of as a therapeutic agent and as a drug carrier. As a therapeutic agent, it has been suggested that the antitumor activity related to their ability to induce cytokines production through increased T-cell proliferation. Other investigators reported that it involved MMP-9 inhibition and strong pro-apoptotic effects against tumor cells.

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