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Nutritional and Biomedical Applications of Chitin and Chitosan: A Mini Review

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A B S T R A C T

The marine biopolymers, chitin and chitosan are prospective candidates possessing a widespread application in the field of clinical nutrition and biomedicine. The primary amine moieties present in the structural skeleton of chitin and chitosan pose pronounced physio-chemical and biological properties, which are profound to exert anticancer, antioxidant and antimicrobial functions, exploited in pharmaceutical and biomedical industries for the formulations of drugs and biomaterials. As these natural biopolymers exhibit diverse properties, they find potential attention in tissue engineering, wound healing process, microencapsulation and sustained drug delivery applications. The main emphasis of the present mini-review is to highlight the recent advancements on functional properties of chitin and chitosan with respect to nutritional and biomedical applications.

Key words: Chitin, Chitosan, Biomedical and nutritional applications

Recently, there is growing scientific attention towards the utilization of the derivatives of the predominant natural basic polysaccharide, chitin, due to their potential roles in pharmaceutical and biomedical fields as a functional biomaterial with biocompatible and biodegradable properties (Kumar 2000). Apart from biodegradability and biocompatibility, many novel properties such as antibacterial, hypolipidemic, anticancer, wound-healing properties, etc. have been conferred upon chitin, chitosan and chitosan oligomers (Krajewska 2004, Kim *et al*. 2008). After the natural polysaccharide cellulose, chitin is considered as the 2nd most plentiful structural polysaccharide moiety originated naturally especially in the exoskeleton of insects, crustaceans. It is also present in significant proportions in sources like fungi, worms, diatoms, etc. Though, the major sources from which chitin is extracted includes crustaceans, insects and microorganisms, the commercial grade chitin and chitosan are obtained in huge quantities from the processing shell wastes derived from shrimps, crabs, lobsters and krill (Younes and Rinaudo 2015). In comparison with chitin, chitosan-the deacetylated derivative of chitin, is considered as a more valuable bioactive polymer due to the occurrence of numerous reactive amino groups, which offer options of chemical modification in the structural moiety to produce a wide-ranging variety of derivatives having potential applications in food, pharmaceutical and biomedical fields (Dutta *et al*. 2004). In this mini-review, an effort has been made to highlight some of the recent research advancements in modulating structural and functional properties of chitin and chitosan with special emphasis on nutritional and biomedical applications.

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Structure

In nature, chitin a major constituent present in the exoskeleton of insects, crustaceans and/or in the cell walls of fungi and yeast, is structurally arranged as ordered crystalline microfibrils made of poly β-(1-4)-N-acetyl-D-glucosamine (Fig 1). Chitosan, the N-deacetylated derivative of chitin is polycationic and its charge density is governed by the degree of acetylation and pH. Also, it can be dissolved in diluted aqueous acidic solvents due to the protonation of –NH2 groups at the C2 position (Fig 2) (Pillai *et al*. 2009, Aranaz *et al*. 2010).

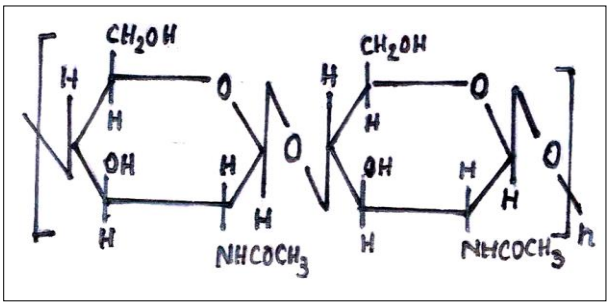

Fig 1 Chemical structure of chitin

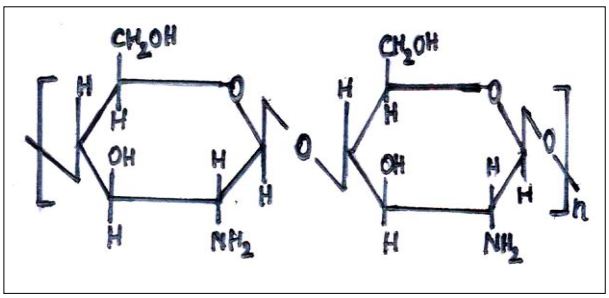

Fig 2 Chemical structure of chitosan

The X-ray diffraction analysis of chitin by scientific researchers has confirmed the polymorphic nature of chitin existing in crystalline structures, α, β and γ chitins. These three polymorphic forms are categorized with respect to (i) size (ii) number of chitin chains and (iii) degree of hydration. In the α form of chitin, the chains are oriented in anti-parallel fashion; whereas in the β form the chains are organized in a parallel orientation; and in the case of γ form the chains are arrayed in two parallel strands alternating with single antiparallel strand. The most prevalent α form occurs in chitinous cuticles of insects and crustaceans, while the β and γ forms of chitin are commonly present in cocoons (Merzendorfer and Zimoch 2003).

Extraction

Generally, the exoskeleton/cuticles of crustaceans, such as shrimps, lobster, crabs, etc. are used as the raw materials for manufacturing chitin and chitosan. The rigid shell of crustaceans is composed of chitin embedded with protein network and calcium carbonate deposits. The chemico-biological interaction existing between the small fraction of protein and polysaccharide complex is very intimate. Apart from crustaceans, chitin can be extracted from insects and microorganisms. But for the commercial production, shells of shrimps, crab, lobsters and krill are employed. The extraction of chitosan is carried out by chemical and biological process which include demineralisation and deproteinisation followed by deacetylation (Gadgey and Bahekar 2017, El-Knidri *et al*. 2018).

Demineralization

The mineral solubilization is performed by acidic treatment which includes the major acids like HCl, HNO_3, H_2SO_4, CH_3COOH and HCOOH. The major inorganic constituents found in the exoskeleton of crustaceans are calcium carbonate and calcium chloride, which can be easily eliminated using dilute hydrochloric acid solution. The biological processing is carried out by lactic acid produced by bacteria through the conversion of an added carbon source. The calcium carbonate in the chitin fraction reacts with lactic acid, which precipitates as salt and can be removed by washing (Gadgey and Bahekar 2017, El-Knidri *et al*. 2018).

Deproteinization

The chemical method for deproteinization is usually achieved by treatment with alkali like dilute sodium hydroxide solution where as in biological method microorganisms are employed, which yields a molecule with a higher molecular weight. The methods are carried out by proteases secreted into the fermentation medium. In addition, deproteinisation can be carried out by adding exoproteases and/or proteolytic bacteria (Gadgey and Bahekar 2017, El-Knidri *et al*. 2018).

Deacetylation

The conversion process of chitin into chitosan essentially requires elimination of acetyl group, which is usually attained by treating with hot concentrated potassium or sodium hydroxide solutions. The material obtained after the completion reaction has to be washed with several volumes of distilled water to neutrality and finally dried overnight in an oven (El-Knidri *et al*. 2018).

Applications

Tissue engineering: The physiochemical characteristics like porous assembly, gel forming ability, chemo-modulation capability and high affinity towards bio-macromolecules have made chitin, chitosan and its derivatives as excellent candidates for the fabrication of supporting material in the field of tissue engineering (Kumar 2000). Recent studies about chitosan reveal that it can form polyelectrolyte complexes with chondroitin sulphate and hyaluronan and this interaction helps to develop enriched performances in regenerating hyaline cartilage. The scaffolds envisioned for the regeneration of cartilage is thus a preparation of chitosan and hyaluronan (Muzzarelli *et al.* 2012). The incorporation of hydroxyapatite on chitosan coated polylactic acid nanofiber mat followed by simulated body fluid immersion has resulted in a composite formation that mimic compositional, structural and biological functions of a native bone (Lin *et al.* 2014). The placement of a scaffold prepared using neural stem cells seeded to covalently immobilized interferon-γ and a hydrogel matrix consisting of methacrylamide chitosan within the subcutaneous region of rats has stimulated the extension of neurofilament fibers from the host tissue into the scaffold matrix. Since there is no direct recoupling of the transplanted cells with the host tissue noticed, the study has revealed that neural stem cells can be manipulated with the help of chitosan to aid regeneration in patients suffering with spinal cord injury (Ham *et al.* 2020). In tissue engineering, the bioceramics prepared using three-dimensional chitosan-based scaffolds in combination with

growth factors and stem cells are found to possess higher periodontal regenerative capacity as compared to other polymeric biomaterials (Lauritano *et al*. 2020).

Microencapsulation

In many nutraceuticals and pharmaceuticals applications, such as food and feed formulations, oral and parenteral delivery of drugs, chitosan have been broadly used for microencapsulation of nutrients and drugs. In order to achieve the delivery of nutrients and drugs into target specific locations, chitosan is usually combined with other polymeric substances to micro or nanoencapsulate nutrients and drugs (Abdelkader *et al.* 2018). Microencapsulation efficacy has been observed when chitosan grafted with ferulic acid was used to encapsulate pyridoxine and thiamine. This encapsulated microsphere has shown anti-inflammatory activity also (Chatterjee *et al.* 2016). Chitosan-encapsulated genistein serves as a carrier for the colon delivery of genistein, which aid in therapeutic approaches for the prevention of cancer (Rahmani *et al*. 2020). Another problem encountered in cancer therapy is the poor tumor selectivity which has become a major limitation for many anti-tumor drugs including doxorubicin. The selectivity of the drug enhanced by the preparation of drug delivery systems with chitosan-based pH-sensitive polymer improved the activity of doxorubicin (Li *et al*. 2020). The major advantage of encapsulation is that there is enhanced solubility of bioactive compounds, protection from degradation, and prolonged residence. A nanoparticle engineered with chitosan hydrochloride and sulphobutyl ether - β – cyclodextrin enhanced antibacterial effect of cinnamaldehyde. Consequently, the nanocarrier exhibited effective encapsulation and cinnamaldehyde was released in a continuous manner making it effective in the storage of food (Zhu *et al*. 2020).

Wound healing

A fundamental research in the medical field has frequently been in the area of wound healing which lead to several trials. The wound healing can be defined as a multifaceted process that are conceded by a numerous factor. Sometimes, even with appropriate care, some wounds fail to heal in a suitable fashion and may become chronic. For the wound healing applications, chitosan seems to be an exceptional candidate as dressing material (Paul and Sharma 2004). A compound dressing composed of collagen, chitosan, and alginate with excellent swelling ability, high porosity and great capacity to adsorb wound exudate that also maintained a comparatively moist environment of wound site have been prepared in a study. This dressing facilitated the relocation of fibroblasts cells to the wounded region thereby initiating the aggregation of platelets leading to the formation of fibrin clot. The dressing made wounded microenvironment moist and the dressing change became painless. While dressing the newly formed tissue, it was not damaged and was protected against any bacterial attack. This satisfies the requirements of people working on sea and suggested that the dressing protected against seawater immersion (Xie *et al*. 2018).

A damage in the skin, would lose its utilities and have serious health effect. Recent researches in wound healing area aims at circumventing wound infection and stimulating tissue remoulding by the development of a novel patch. As chitosan possess natural antibacterial activity, it has been exploited for wound healing and this property was utilized in the preparation of chitosan-based microneedle array patch which integrated with drug delivery for stimulating wound healing. This patch stimulated inhibition of inflammation, angiogenesis, deposition of collagen and tissue regeneration (Chi *et al*. 2020). A wound dressing material developed with superior healing properties and the ability to supress the hypertrophic scar has a good impact clinically. The cationic nature of chitosan can reduce the effects of hypertrophic scar when carboxymethyl chitosan hydrogels incorporated with different genipin concentrations was applied to the wounded area. The wound became small thin and smooth and an additional incorporation of aloevera gel showed inhibition in scar formation (Zhang *et al*. 2020).

Anticancer property

The anticancer property of chitosan makes it a versatile biomaterial which exerts different type of effects against several types of malignancies. In cancer of oral cells, chitosan exerted the anticancer activity by induction of apoptosis (Wimardhani *et al.* 2014). In human hepatocellular carcinoma HepG2 cells, chitosan unsheathed from shells of shrimp has been used to manufacture nanoparticles of biogenic silver that was successful in activating *in vitro* apoptotic activity (Priya *et al*. 2020). Another chitosan β ketosulfone derivative pose anticancer activity against different kinds of cancer cell lines, which includes the cell lines of hepatocellular, colon and breast carcinoma (Alamry *et al*. 2018).

Antimicrobial property

Low molecular weight water soluble chitosan possesses antibiotic activities and can be used to develop new anti-infective agents. It shows antifungal activities against some hyphae forming fungi and pathogenic yeasts (Park *et al*. 2008). Many factors are responsible for the antibacterial activity of chitosan in vitro against the intestinal bacteria such as deacetylation degree of chitosan and the characteristics of the bacteria such as strain and species. The pathogenic *C. perfringens* is more prone to bactericidal activity of chitosan than the resistant strains such as *Lactobacillus* and *Bifidobacterium* (Tsai *et al.* 2004). Chitosan can act against a widespread microorganism because of the occurrence of charged groups within the polymer which interact with the ionic charges present within the constituents of bacterial cell wall. Peptidoglycan hydrolysis aggravated by the leakage of electrolytes present intracellular is the major reason for the death of microorganisms. The antimicrobial activity against Gram positive and Gram-negative bacterium strains by chitosan and its derivative N, N, N-trimethylchitosan is steadily more active against the Gram-positive bacterium *S. aureus* than Gram negative *E. coli.* (Goy *et al*. 2016). Chitosan possess antibacterial activity against strains such as *Enterobacter*

cloacae, *Bacillus subtilis* and *Stenotrophomonas maltophilia* (Vilar Junior *et al*. 2016). Chitosan nanoparticles displayed higher antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* when compared to chitosan and chitin (Divya *et al*. 2017). Chitosan oligomer prepared enzymatically with chitosanase, produced by bacteria, exhibited antifungal activity by impeding the growth of *Aspergillus flavus*, *Aspergillus niger*, *Emericella nidulans* and *Eurotium amstelodami* (Fawzya *et al*. 2019).

Antioxidant property

Chitin and chitosan finds broad range of applications in various fields due to its antioxidant activity. The chitosan isolated from shrimp exoskeleton has antioxidant and cytotoxic potentials when studied by employing various analysis such as ion chelating, 1, 1-diphenyl-2-picrylhydrazyl, superoxide, hydroxyl radicals scavenging and total antioxidant activity (Rajalakshmi *et al*. 2013). The antioxidant activity of chitosan acetate, chitosan thiamine pyrophosphate, chitosan hydroxyl benzotriazole, and chitosan ethylene diamine tetra acetic acid solution were evaluated using hydroxyl and superoxide radicals scavenging, metal ion chelating and reducing power assays which revealed that these salts may be used as antioxidants for pharmaceutical uses (Charernsriwilaiwat *et al*. 2012). The development of oxidative stress in the systemic circulation has been hindered when chitosan was ingested. As a result, the levels of pro oxidants like uremic toxins, in the gastrointestinal tract declined and reduced the incidence of renal failure (Anraku *et al*. 20012). The effects of dietary supplementation of chitosan on lipid peroxidation in animal model with isoprenaline induced myocardial infarction revealed that the generation of free radicals were greatly reduced due to the action of free radical scavenging enzymes which protected myocardial membrane (Anandan *et al*. 2012). The antiulcer properties of chitin and chitosan are associated to a counteraction of free radicals through the antioxidant property; or to a neutralization of gastric juice by the release of glucosamine; and/or to the capability to maintain near to normal status activity of free-radical enzymes and the level of GSH that shields mucosa against oxidative damage by diminishing lipid peroxidation and reinforcing the mucosal barrier (Anandan *et al*. 2004).

Gut microbiota

Any alteration in the gut microbiota is known as dysbiosis and thus a change in the activity of gut bacteria disturbs energy storage and host metabolism leading to the development of conditions like obesity. Recent studies show that chronic ingestion of chitin–glucan has many favourable effects. It can reduce the incidence of obesity and other metabolic disorders such as diabetes, hepatic steatosis etc. The microorganism linked to the renewal of the composition and action of gut bacteria, is clostridial cluster XIVa (Neyrinck *et al.* 2012). Another experiment conducted to study the arrangement of the intestinal microflora in mice revealed that chitosan addition to the diet significantly changed the intestinal microflora and also provided resistance to infection by *Citrobacter rodentium*. It was found that there is a repression in the activation of NF-κB and reduction in TNF-α and IL-6 production led to control of infection with *C. rodentium* (Guan *et al*. 2016).

When sugar rich diet is consumed, there is significant alteration in the gut microbiota and it induces inflammation and insulin resistance. Dysbiosis along with elevated lipopolysaccharide levels in plasma and reduced short chain fatty acids was observed. This significantly impaired insulin signaling pathway by diverse molecular mechanisms that ultimately lead to the development of diabetes. Chitosan has an inherent capacity to safeguard the degradation of insulin enzymatically and its well-ordered inter-epithelial transport and hence chitosan-based formulations played key role in insulin delivery system. Also, prebiotic character of chitosan had been reported to increase the growth of *Lactobacillus* and *Bifidobacteria*, which lead to the reduction in metabolic endotoxemia and inflammation. Administration of chitosan has diminished the expression of innate immune receptors i.e. TLRs and NLRs, which decreased activation of NF-kB to reduce the inflammation which ultimately can prevent type II diabetes mellitus (Prajapati *et al*. 2016). The effect of chitosan oligosaccharides on type II diabetes mellitus revealed that chitosan oligosaccharides may control the metabolic pathways of gut microbiota. The usage extraordinarily enriched glucose metabolism and remade the unbalanced gut microbiota in diabetic mice (Zheng *et al*. 2018).

Drug delivery

The abundance of amino groups in chitosan increased the properties like *in situ* gelation, mucoadhesion, permeation enhancement, transfection, efflux pump inhibitory properties and controlled drug release which can be improved by derivatization of chitosan and this resulted in more potent functions of chitosan (Bernkop-Schnürch and Dünnhaupt 2012).

Familial adenomatous polyposis, a precancerous condition of the colon is usually treated using Celecoxib, a generalized drug for cancer therapy and due to its side effects, the usage was restricted. When chitosan derived hydroxyapatite nanocarriers-mediated celecoxib was developed, *in vitro* studies were carried in colon cancer cells. The results showed significant anti-proliferation, apoptosis and time-dependent cytoplasmic uptake of the nanoparticle. Additionally, studies conducted *in vivo* established that the treatment with the nanoparticle showed inhibition of the tumor growth (Venkatesan *et al*. 2011). Another main challenge in cancer treatment is the selective delivery of nanoparticles to the target site. A novel nanoparticle developed using chitosan and chondroitin sulphate, named as folate-targeting self-assembled nanoparticles with a hydrophobic drug, bortezomib has been used for colorectal carcinoma therapy (Soe *et al*. 2019).

Most chemotherapeutic drugs have reduced solubility and permeability which lead to insufficient availability of the drug. To enhance tumor penetration, positively charged nanoparticles reinforced with chitosan synthesized of 10-Hydroxycamptothecin augmented the chemotherapeutic

effect on melanoma and the investigations implied that the nanoparticle could be used as an efficient drug delivery nanosystem (Guo *et al*. 2020). An improved drug delivery carrier was established using biopolymeric materials for applications on oral mucosa capable of *in situ* release. Chitosan have gained considerable attention among these biocompatible carriers which are able to enhance the delivery of chitosan-based formulations. It may be explored for therapy purposes or to develop *in vivo* dental care such as the preparation of toothpastes or cosmetics for daily oral care (Chronopoulou *et al*. 2016).

Cosmetics

Chitin and chitosan are of significant importance in the field of cosmeceutical industries due to their unique biochemical nature. Chitosan has many functional roles in cosmetic applications such as oral hygiene agent, skin and hair care ingredients, and carrier of bioactive compounds (Aranaz *et al.* 2013). In hair care cosmetics, the mechanical property of chitosan- collagen-hyaluronic acid based triple component blend films is used to cover the hair surface and to increase the thickness of hair (Sionkowska *et al*. 2017).

A film prepared with neutralized chitosan citrate exhibited better swelling capacities, greater thicknesses owing to superior physical integrity, lower moisture absorbance and reduced solubility in the acid medium. For cosmetic application, when thermal analyses were performed, the films showed better interaction with water. Skins treated with the films have shown to hydrate desquamation of stratum corneum within 10 min with enhanced skin exfoliation (Libio *et al*. 2016). The anti-aging skin masks developed using chitosan matrix entrapping antioxidants such as vitamin C and *Bixa orellana* L., commonly called as annatto are found to be more flexible, selective permeable and non-cytotoxic (Afonso *et al*. 2019).

Ophthalmology

In the field of ophthalmology, chitin and chitosan have gained attention of many researchers due to the peculiar features that have replaced the usage of polymers of synthetic origin. The administration of chitosan-N-acetylcysteine containing eye drops has hastened wound healing process in corneal region of rabbits (Fischak *et al.* 2017). Chitosan and its thiolated derivative is recognized as potential anti-angiogenic and anti-fibrotic agents for treating corneal injuries. Increased mucoadhesion of the chitosan derivatives at the corneal surface has been effective in inhibiting corneal haze after chemical injuries (Zahir-Jouzdani *et al*. 2018). Recent studies also have shown that chitosan-N-acetylcysteine eye drops is successful in treating dry eye disorder, a pathophysiological condition that causes tear-film instability, ocular discomfort, and visual disturbance and also possessed regenerative effect (Nepp *et al*. 2020).

Antilipidemic effect

In the recent years, the number of individuals at a risk of myocardial infarction has greatly increased due to many reasons like physical inactivity, abnormal nutritional habits and family history. In all these cases, there is disarrangement in lipid profile parameters like an elevated triglyceride, reduced HDL levels and so on. The hypolipidemic character of chitosan and its oligosaccaharides has been well established in high fat diet experimental models. The cardiac indices, body fat ratio, serum and liver antioxidant status were significantly modified after the intake of chitosan and effectively improved the metabolism of lipid in liver by stabilizing the expressions of peroxisome proliferator-activated receptor-α and its target regulatory protein hepatic lipase. The liver can be protected from the oxidized trauma by enhancing hepatic function, which could be used to treat hyperlipidemia (Pan *et al*. 2016). The antilipedimic effect of chitosan when ingested orally has been studied in isoprenaline induced myocardial infarction in animal models. It exerted significant anitlipidemic effect by maintaining the cholesterol levels, free fatty acids, triglycerides and phospholipids levels in plasma and heart tissue. Previous reports by Sivakumar *et al.* (2007), have indicated that the dietary chitosan supplementation is capable of ameliorating isoprenaline induced oxidative stress and hyperlipidaemia in myocardium of rats by its antioxidant activity and hypolipidemic property.

Hepatoprotection

In the human body, liver has several important functions which include most of the synthetic processes of metabolism and detoxification of all major toxins and drugs that can cause severe damage to hepatocellular homeostasis, leading to death and progression to hepatitis, fibrosis, and cirrhosis and hepatocellular carcinoma. Also, the reactive oxygen species and free radicals play deleterious functions in the pathogenesis of various liver diseases and disorders. Chitosan is being considered as one of the most preferred candidates in the formulation of human healthcare nutraceuticals in the field of nutritional medicine due its ability to prevent the hepatocellular necrotic damage by maintaining cellular enzymatic and non-enzymatic antioxidant defense system at higher levels. Experimental investigations on the antioxidative and hepatoprotective actions of chitosan derived from *Sepia kobiensis* against carbon tetrachloride induced liver toxicity has revealed that there is significant reduction in the levels of diagnostic markers enzymes, free fatty acids, total cholesterol and triglycerides in plasma and tissue with concomitant elevation in hepatic and circulatory antioxidant enzyme levels. The hepatoprotective effect of chitosan is probably related to its antioxidant and antilipidemic properties (Ramasamy *et al*. 2014). Studies by Santhosh *et al.* (2007), have shown that the dietary intake of chitosan is capable of attenuating antitubercular drugs mediated aberrations in the levels of bilirubin, albumin/globulin ratio, hepatic diagnostic markers and lipogenesis in experimental animals.

CONCLUSION

From the aforementioned review it could be revealed that Chitosan on account of its cationic nature contributes to the likelihood to form electrostatic materials and multilayer

moieties which might be efficiently used for the preparation of sponge, film, fibre, bead, gel or solution. The occurrence of free -NH_2 groups in the structural moiety of chitin and chitosan permits it to mediate significant changes under mild conditions even in aqueous conditions. It very well may be mixed or engineered with polymers proteins, DNA, alginate, hyaluronan, and so forth. Aside from this, anticancer, antimicrobial and cancer prevention properties makes it a remarkable nutraceutical. Chitosan has likewise various applications particularly in hair and healthy skin management. All these characters make it an incredible up-and-comer with numerous biomedical applications.

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