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Review Article

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CHITOSAN: UNCOVERING THE TRUE POTENTIAL OF A VERSATILE POLYMER

***Salman Shaikh and Dr. Geeta Bhagwat**

Department of Pharmaceutics, H.K. College of Pharmacy, Mumbai-400092, India.

***Corresponding Author: Salman Shaikh**

Department of Pharmaceutics, H.K. College of Pharmacy, Mumbai-400092, India.

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ABSTRACT

Chitin and its deacetylated derivative chitosan are natural polymers made up of N-acetyl-D-glucosamine and randomly dispersed -(1-4)-linked D-glucosamine (deacetylated unit) (acetylated unit). Biopolymers like chitin and chitosan have a variety of characteristics that allow them to be used in a variety of fields, including biomedical science. The most recent breakthroughs in biomedical research include major new trends in mucoadhesive, anti-inflammatory, antioxidant, antimicrobial, antifungal, antihyperglycemic, antitumor, and wound-healing products. Chitin and chitosan are biocompatible materials that can be utilised in medical devices to cure, augment, or replace any bodily tissue, organ, or function. A rising number of work suggests that chitosan and its derivatives are attractive possibilities for tissue engineering support materials. This review article focuses on current research on chitin and chitosan in relation to its use in a variety of domains, including mucoadhesive, anti-inflammatory, antioxidant, antimicrobial, antifungal, antihyperglycemic, antitumor, and wound-healing properties.

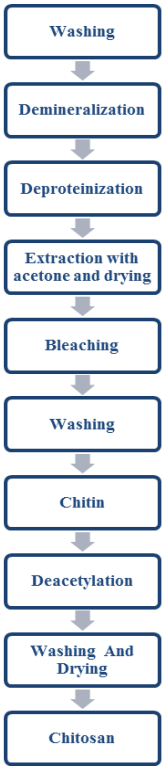
1 INTRODUCTION

Chitin and its deacetylated derivative, chitosan, are linear polysaccharides made up of N-acetyl-2 amino-2-deoxyD-glucose (glucosamine, GlcN) and 2-amino-2-deoxyD-glucose (N-acetyl-glucosamine, GlcNAc) residues in various proportions. Primary amine protonation makes chitosan soluble in aqueous acidic environments. The quantity of acetylated residues in chitin, on the other hand, is sufficient to prevent the polymer from dissolving in aqueous acidic environments. Chitin is a common biopolymer found in crustacean exoskeletons, insect cuticles, algae, and fungal cell walls. Chitosan is found in a limited number of fungus in nature (Mucoraceae). Chemical deacetylation of chitin from crustacean sources was traditionally used to make commercial chitosan samples. Due to vegan demands, chitosan from fungi has recently gained popularity in the market. Furthermore, these samples have a higher deacetylation degree and are better controlled in terms of low viscosity.[1] The rising interest in protein production from various sources has sparked research in insect cuticle production. The fascination with chitin and chitosan stems from their diverse biological and technological features. These qualities, however, are closely linked to the polymers' physical properties (namely molecular weight and acetylation degree).[2] When working with chitin and chitosan, a thorough and thorough polymer characterisation is required. Several methodologies have been described to characterize chitin, chitosan and chitooligosaccharides,[3,4]

Chitosan is the only polycation found in nature, and its charge density is affected by the degree of acetylation and media pH. The degree of acetylation and the molecular weight of the polymer determine its solubility. Chitosan oligomers are soluble at a variety of pH levels, from acidic to basic (i.e., physiological pH 7.4). On the other hand, even at high deacetylation levels, chitosan samples with larger Mw are only soluble in acidic aqueous conditions. Because of its insolubility at neutral and basic pH, chitosan has been used in various applications that need neutral physiological conditions (i.e., pH 7.4). This is why a large variety of chitosan derivatives with improved solubility have been developed. The global chitosan market was worth USD 6.8 billion in 2019, and it is predicted to grow at a CAGR of 24.7 percent between 2020 and 2027, based on revenue. The increasing use of the polymer in water treatment, as well as various high-value industries such as pharmaceutical, biomedical, cosmetics, and food, are driving market expansion.[5] Modification of polymers to increase their application; understanding of the mechanisms involved in the biological activity of chitosan, chitosan derivatives, and chitooligosaccharides; and a comprehensive investigation of chitosanolytic and chitinolytic enzymes found in various microorganisms.[6] This review seeks to give readers a broad understanding of the current status of chitosan science, including polymer chemistry, biological and technical features, and applications in drug delivery and as a biocatalyst.

2 Preparation of Chitosan

Shellfish waste from food processing (Shrimp, Squid, Crab).



3 Chemistry of Chitosan

A primary amino group (C2), as well as primary and secondary hydroxyl groups, are contained in chitosan, as shown in Figure 1. (C6, C3). Also considered functional groups include glycosidic linkages and the acetamide group. These functional groups enable a wide range of changes, resulting in polymers with novel characteristics and behaviours.

Chitosan derivatives have been created with the goal of improving the qualities of chitosan, such as solubility or biodegradability, or adding new functions or properties. Deacetylation, depolymerization, and quaternization, among other procedures, have enhanced solubility in water aqueous conditions.[7] After modification, new chitosan actions have been discovered, such as 6-O-sulphated chitosan promoting neuronal development and phosphorylated chitosan inhibiting corrosion.[8,9]

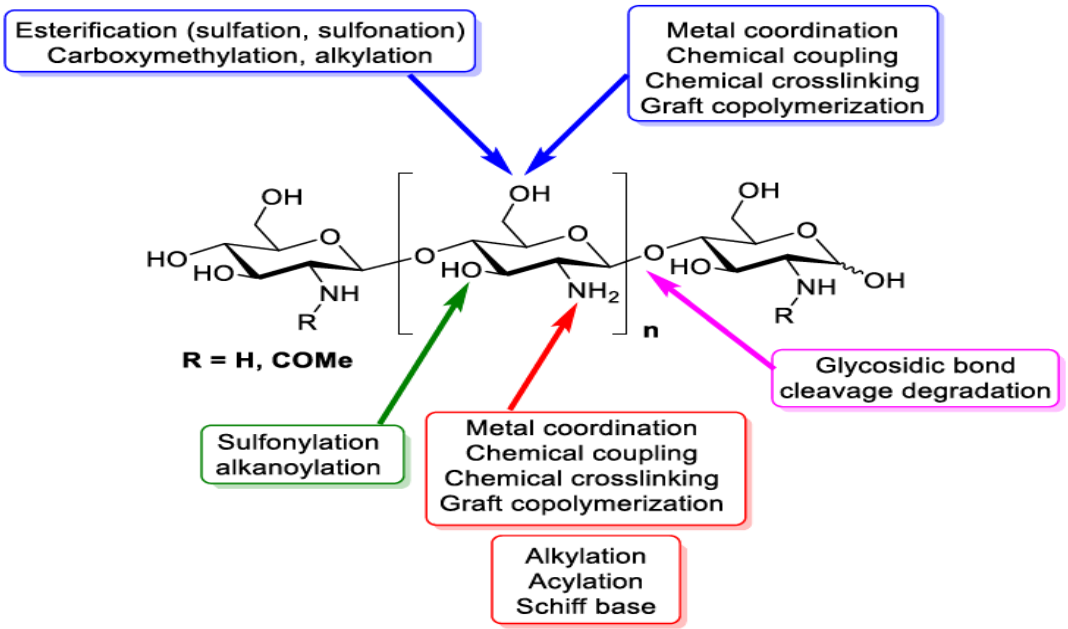


Figure 1: Functional groups in chitosan's structure that are able to be chemically modified

4 Properties of Chitosan

Table 1: Properties of Chitosan.

Property Activity	Reference
Mucoadhesive	[10,11]
Anti-inflammatory	[12]
Antioxidant	[13]
Antimicrobial	[14]
Antifungal	[15]
Antihyperglycemic	[16]
Antitumor	[12-21]
Wound healing	[18]

4.1 Mucoadhesive Property

Because of its low toxicity, biocompatibility, antibacterial activity, mucoadhesive qualities, and permeation boosting effects, chitosan has been widely used in different biomedical and drug delivery fields.[19,20,21,22,23,24,25,26] It has been thoroughly investigated as a possible excipient for peptide delivery by mouth.[27] Because of their mucoadhesive qualities and strong interactions with the intestinal barrier, Alonso and colleagues discovered that chitosan nanocapsules increased and prolonged intestinal absorption of salmon calcitonin.[28]

Researchers have evaluated the mucoadhesiveness of chitosan using a variety of approaches, including mucin-particle interaction,[20] tensile strength,[29] and, most recently, flow-through technique combined with fluorescence microscopy.[30,31] Fluorescein isothiocyanate-chitosan (FITC-chitosan) was utilised as a positive control in the most recent study, and it was compared to other materials as well as FITC-dextran (non-mucoadhesive or negative control). Ex vivo mucosal tissues (e.g., pig urine bladder or bovine eyes) were coated with fluorescent materials and rinsed with bio-relevant fluids. After many wash cycles, fluorescence images were acquired, and the intensity of the fluorescence was utilised to compare the retention of each substance on the mucosal tissues. Although there were some variances in the extent of chitosan's mucoadhesive potential in different mucosal tissues, it was found that it had excellent mucoadhesive characteristics in all cases.[31,32,33] Figure 2 depicts the results of an ex vivo mucoadhesion study of several silica nanoparticles in the pig urinary bladder. After washing, the fluorescence signal of chitosan was stronger than that of other materials, indicating that it has superior mucoadhesive qualities. The following was the order of material retention: FITC-chitosan > thiolated silica nanoparticles > PEGylated (polyethylene glycol, 750 Da) silica nanoparticles > PEGylated (5000 Da) silica nanoparticles > FITC-dextran.[31]

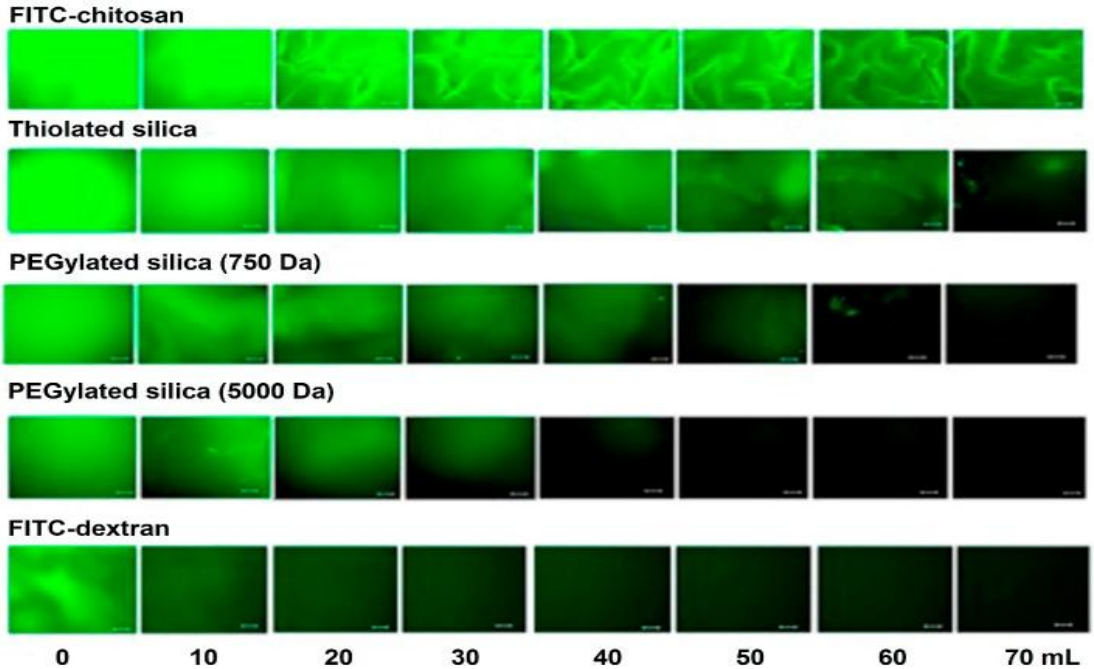


Figure 2: Representative microscopic fluorescence images of ex vivo porcine urinary bladder mucosa incubated with FITC-chitosan, thiolated silica, PEGylated silica (750 Da), PEGylated silica (5000 Da) and FITC-dextran and washed with different volumes of artificial urine solution. Scale bar = 200 µm.[31]

4.2 Anti-Inflammatory Properties

The inflammatory response is the body's natural physiological response to tissue injury. The primary purpose of the inflammatory response is to direct circulating leukocytes and plasma proteins to the site of infection or tissue injury, eradicate the causal culprit if feasible, and begin the healing process. Although inflammation is important for survival, it can cause harm if it is excessive, unable to eliminate the causative agent, or directed against the host. The production of free radicals is intimately linked to the inflammatory process. When the molecular weight of the chitosan is reduced and the activity of chitooligosaccharides is increased, this activity appears to be even more impressive. In a model of lipopolysaccharide (LPS)-induced murine RAW 264.7 macrophages, the activity of degraded polymers with medium molecular weight, low molecular weight, and chitooligosaccharides(156, 72, 7, and 3.3 kDa) was tested in terms of NO secretion, cytokine production, and mitogen-activated protein kinase pathways. NO generation was considerably decreased by chitosan samples (parent, medium, and low). The COS, on the other hand, had the exact opposite impact. The medium and low Mw chitosan used different mechanisms to suppress NF- B activation and iNOS expression. The binding to CR3 receptor happened for medium chitosan (156 kDa), while the binding to CR3 and TLR4 receptors occurred for low molecular weight chitosan. By binding to CD14, TLR4, and CR3 receptors to activate JNK signalling pathways, lower molecular weight chitosans activated NF- B and increased iNOS expression.[34] Chitooligosaccharides have been researched in greater depth for this application than chitosan, owing to its superior solubility in aqueous media and performance.

The impact of acetylation on COS' anti-inflammatory properties has also been investigated. Chitooligosaccharides with MWs ranging from 0.2 to 1.2 kDa were depolymerized and, depending on the enzyme, fully deacetylated (fdCOS, primarily GlcN).

(GlcN)2, (GlcN)3, and (GlcN)4), partially acetylated (paCOS: at least 11 Cos) with varying GlcNAc/GlcN ratios), and fully acetylated (faCOS, mostly).

GlcNAc, (GlcNAc)2, and (GlcNAc)3 were synthesised. The anti-inflammatory properties of The ability of three COS mixes to lower TNF-_ in the blood was investigated.

Mice with LPS-stimulated macrophages (RAW264.7). Only fdCOS and faCOS were able to diminish this factor significantly.[35,36] The reduction of NO secretion by COSs demonstrated that COSs that were 10% acetylated decreased NO secretion much more than COSs that were 50% acetylated.[37] Citronellol grafted chitosan oligosaccharide derivatives with degrees of substitution of 0.165, 0.199, and 0.182 were created to improve the anti-inflammatory action of the oligosaccharides. The derivatives consistently outperformed the parent COS in every situation. TNF-_ expression was lowered by increasing the production of IL-4 and IL-10, and the NF-B signalling pathway was inactivated by blocking the phosphorylation of p65, IKB_, and IKK.[38]

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4.3 Antioxidant Acvities

Because of the link between oxidative stress and diseases like Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and cancer, antioxidants are gaining popularity. It's also linked to issues in other disorders like diabetes.[40–42] Chitosan includes an amino acid and a number of hydroxyl groups that can react with free radicals to provide scavenging activity. The antioxidant activity of several chitosan derivatives, such as chitosansulphates or N-2 carboxyethyl chitosan, was improved.[43–45]

Chitooligosaccharides have also been chemically changed to increase their antioxidant activity, for as by treating the polymers with gallic acid or phenolic chemicals.[46,47,48] DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid), and FRAP (ferric antioxidant power) assays, peroxide and hydroxyl radical scavenging assays, and macrophage models have all been used to determine chitosan and its derivatives' antioxidant assays. The DPPH and ABTS assays use electron and H atom transfer, respectively, while the FRAP assay uses an electron transfer reaction. Antioxidant activity is also measured using the ORAC (oxygen radical absorbance capacity assay). Because of the variation between the polymers examined and the procedures used to test the activity, polymer concentrations varied from 50 g/mL to 400 mg/Ml.[45] Antioxidant activity is higher in low molecular weight samples than in high molecular weight samples because shorter chains generate fewer intramolecular hydrogen bonds, making reactive groups more accessible and contributing to radical scavenging activity.[49,50] In terms of the influence of acetylation degree, it appears that as this parameter increases, antioxidant activity decreases.[50]

4.4 Anti Microbial Activity

In investigations including in vivo and in vitro interactions with chitosan in various forms, chitin and chitosan have been examined as an antibacterial substance against a wide range of target organisms such as algae, bacteria, yeasts, and fungi (solutions, films and composites). The antibacterial potential of chitin, chitosan, and its derivatives was first described in the 1980s and 1990s.[51-56] Generally, chitosan is classified as either bacteriocidal (kills live bacteria or a fraction thereof) or bacteriostatic (hinders the growth of bacteria but does not indicate that bacteria are killed) in these research, with little differentiation between the two actions. Chitin and chitosan have been studied as antibacterial substances against a wide range of target organisms, including algae, bacteria, yeasts, and fungi, in vivo and in vitro interactions with chitosan in various forms (solutions, films and composites). Chitin, chitosan, and its derivatives were originally discovered to have antibacterial properties in the 1980s and 1990s.[51-56] In these studies, chitosan was classed as either bacteriocidal (kills live bacteria or a fraction thereof) or bacteriostatic (hinders bacterial growth but does not suggest that bacteria are destroyed), with little distinction between the two effects.

The interaction between positively charged chitin/chitosan molecules and negatively charged microbial cell membranes is the most acceptable of three models suggested. Electrostatic interactions between protonated NH+3 groups and negative residues drive the interaction in this model,[58] likely by competing with Ca2+ for electronegative locations on the membrane surface.[59]

This electrostatic interaction has two effects: I it promotes changes in membrane wall permeability, which causes internal osmotic imbalances and inhibits microorganism growth[60,61], and ii) it causes the hydrolysis of peptidoglycans in the microorganism wall, which leads to the leakage of intracellular electrolytes such as potassium ions and other low molecular weight proteinaceous constituents (e.g. proteins, nucleic acids).[62]

Raafat et al.[63] studied in to model and noticed ultrastructural alterations in S. simulans 22 cells after exposure to positively charged chitosan using a transmission electron microscope. Chitosan molecules adhering to bacterium cell surfaces may be seen and identified. The cell membrane became locally separated from the cell wall at the interaction sites, resulting in "vacuole-like" formations beneath the cell wall. The separation produces ions and water outflow, causing the internal bacterium pressure to drop. On both gram-negative and gram-positive bacteria, visual confirmation of successful membrane lysis has been reported.[64-65]

Because this mechanism relies on electrostatic contact, it stands to reason that the higher the amount of cationized amines, the greater the antibacterial activity.[66,67] This shows that chitosan has higher activity than chitin, which has been empirically confirmed.[68,69] It's worth noting that when chitosan concentration rises, the amount of polycationic chitosan accessible to bind to a charged bacterial surface appears to decrease.[70,71] One explanation is that in the presence of a larger number of charged sites, the chains tend to form clusters as molecules aggregate in solution. Observations have shown that at greater concentrations, chitosan forms a covering over the bacteria, which is not always adhered to the surface and is independent of the bacteria type. Adjusting the pH in such a situation could be critical for proper solubility and keeping the chains apart.

In terms of surface polarity, gram-negative bacteria's outer membrane is primarily composed of lipopolysaccharides containing phosphate and pyrophosphate groups, which result in a higher density of negative charges on the surface than gram-positive bacteria (membrane composed of peptidoglycan associated with polysaccharides and teichoic acids).[72] This adds to the data that chitosan causes more intracellular material leakage in gram-negative bacteria than in gram-positive bacteria.[73-74]

However, the efficiency of bacteria against gram-positive and gram-negative bacteria is debatable. According to some researchers, chitosan has a stronger effect on gram-positive bacteria (e.g. Listeria monocytogenes, Bacillus megaterium, Bacillus cereus, Staphylococcus aureus, Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus bulgaris, etc.) than gram-negative bacteria (e.g. E. coli, Pseudomonas fluor In contrast, it has been shown that gram-negative bacteria have considerably higher hydrophilicity than gram-positive bacteria, making them the most sensitive to chitosan.[76] In vitro tests show that gram-negative bacteria are more sensitive to chitosan than gram-positive bacteria, with higher morphological alterations after treatment when compared to gram-positive bacteria.[77] The amount of adsorbed Chitosan is determined by the charge density on the cell surface. More adsorbed chitosan would evidently result in greater changes in the structure and in the permeability of the cell membrane. This would suggest that the antibacterial mode of action is dependent upon the host microorganism.[78]

Another mechanism hypothesised is the interaction of chitosan with microbial DNA, which results in the suppression of mRNA and protein synthesis via chitosan penetration into the nuclei of microorganisms.[79] It is hypothesised that chitosan molecules can penetrate through the bacterial cell wall, which is made up of multilayers of cross-linked murein, and reach the plasma membrane. The presence of chitosan oligomers (a chain with a small number of monomer units) inside E. coli treated to chitosan under various circumstances was confirmed using confocal laser scanning microscopy.[7] Despite being regarded as a conceivable mechanism,

Raafat et al.[80] remarked that the likelihood of it occurring is extremely low. The prevailing contention is that chitosan acts essentially as an outer membrane disruptor rather than as a penetrating material.[81]

Metal chelation, spore suppression, and binding to critical nutrients for microbial growth are the third mechanisms. It is well known that chitosan has good metal-binding properties, with the amine groups in the chitosan molecules being responsible for the chelation absorption of metal cations. Because the amine groups are unprotonated and the electron pair on the amine nitrogen is available for donation to metal ions at high pH, such a process is more efficient when positive ions are coupled to chitosan. The pH dependence on the proportion of accessible sites for interacting in the polysaccharide backbone is related by a model proposed based on the system chitosan-Cu.[82] At pH 6, the complexation involves only one NH2 group and three hydroxyls or H2O molecules, however at pH > 6.7, the complexation is more likely to involve two NH2. Deprotonation of hydroxyl groups is thought to occur at higher pHs, such as 7-9, and the prevailing complexation is dominated by two -NH2 and two hydroxyl groups dissociated. Wang et al.[83] proposed a model in which the metal is placed as an electron acceptor coupled to one or more chitosan chains via -NH2 and building bridges to hydroxyl groups.

4.5 WOUND HEALING

Wound healing is a biological pathway that is linked to the overall phenomenon of tissue regeneration and growth. It goes through a succession of interdependent and matching stages in which a variety of cellular and matrix components work together to restore damaged tissue's integrity and replace lost tissue.[84] Wound healing is a multi-phased regenerative process that includes haemostasis, inflammation, proliferation, and remodeling.[85] The goal has been to create bacterial-resistant prosthetic equivalents by attaching an antibiotic to the materials. In vitro and in vivo, chitosan hydrogel covered grafts that were crosslinked by UV light irradiation showed resistance to E. coli. Wound healing is a biological pathway that is linked to the overall phenomenon of tissue regeneration and growth. It goes through a succession of interdependent and matching stages in which a variety of cellular and matrix components work together to restore damaged tissue's integrity and replace lost tissue.[84] Wound healing is a biological process that is linked to tissue regeneration and growth as a whole. It passes through a series of interdependent and matched stages in which a variety of cellular and matrix components collaborate to restore the integrity of injured tissue and replace lost tissue.[84] Wound healing is a multi-phased regeneration process involving hemostasis, inflammation, proliferation, and remodeling.[85] By adding an antibiotic to the materials, the idea was to build bacterial-resistant prosthetic counterparts. Chitosan hydrogel coated grafts that were crosslinked by UV light irradiation showed resistance to E. coli in vitro and in vivo.

Infectious complications in combat wounds continue to be a burden for caretakers, and haemorrhage remains a primary cause of early mortality after trauma. Despite the fact that chitosan dressings were developed to address these issues, they are not always successful in controlling bleeding or killing microorganisms. Chitosan hydrogels immobilised on the surface of poly(N-isopropylacrylamide) gel/polypropylene nonwoven composites employing the cross-linking agent (glutaraldehyde) showed antibacterial activity against E. coli and Staphylococcus aureus. Meanwhile, the product demonstrated the ability to be easily taken off without causing damage to newly regenerated tissue when removed from the wound.[89] Curcumin/chitosan/gelatin composite sponges were made in varied ratios of chitosan and gelatin and showed increased water uptake, antimicrobial activity, and wound closure. The composite sponge with a higher gelatin content had a faster release time of up to 240 minutes. These composite sponges were also demonstrated to promote wound healing activity by enhancing collagen production and wound closure in vivo.[90]

CONCLUSION

Chitosan is a biodegradable and low-cost polymer with a wide range of uses in the biomedical and pharmaceutical industries. Chitosan and its derivatives have been the subject of extensive investigation for tissue engineering, drug delivery, wound healing, water treatment, anticancer, and antibacterial activities.

Chitosan has anticancer properties both as a drug and as a drug carrier. It has been proposed that the anticancer effect of these drugs is linked to their ability to promote cytokine synthesis through enhanced T-cell proliferation. MMP-9 suppression and powerful pro-apoptotic actions against tumour cells, according to other researchers.

Wound-care products such as hemostatic dressings are still uncommon nowadays due to their higher cost, despite the fact that they are more effective for quick recovery. However, employing these items allows for fewer dressing changes and less patient attention, reducing the resources and workload required by healthcare staff.

Chitosan appears to be safe to consume for the treatment of obesity, with no documented negative effects. However, its claim for obesity treatment appears to be unsupported by science, and additional research is needed to determine the validity of such a claim.

Quaternized chitosan improves antibacterial activity over a wide pH range by introducing permanently positively charged quaternary groups to the hydroxyl group or amino group of the polymers. In addition, quaternized chitosan can be used as an antibacterial covering for

orthopaedic and dental implants, as well as a surgical wound dressing material.

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