Chapter 14
Conclusion and Future Prospects of Chitosan-Based Nanocomposites

Sanjay Kumar, Abhigyan Sarmah Gogoi, Shefali Shukla, Manoj Trivedi, and Shikha Gulati

Abstract Chitosan, as a bionanocomposite has been garnering immense attention and become a center of comprehensive research, owing to its vast structural possibilities for physiochemical alterations to produce novel characteristics, functions, and utilizations, especially in the biomedical field. Chitosan is endowed with numerous properties such as biocompatibility, biodegradability, non-toxicity, antimicrobial activity, low immunogenicity, stimuli sensitivity, adjustable physical strength, and water solubility. This ideal biopolymer can be transformed into nanoparticles, nanocapsules, nano-vehicles, fiber meshes, scaffolds, and 3D printed scaffolds. The last decade is a testimony to the enormous potential of chitosan and chitosan-based nanocomposites, as showcased by the legion of research reports displaying many new applications in the field of targeted drug delivery, modern biomedical instruments, and bioimaging sensors. This chapter unfolds different aspects of chitosan, including its properties and mutations, and focuses on chitosan-based nanocomposites. Emphasis has been laid on the salient biomedical applications of chitosan-based nanocomposites including drug delivery, gene therapy, tissue engineering and regeneration, cancer diagnosis and treatment, and bioimaging among various others.

Keywords Chitosan · Bionanocomposite · Properties · Modifications · Biomedical applications

Abbreviations

AAS	Atomic absorption spectroscopy
BGC	Bioactive glass–ceramic
CG	Gelatin

S. Kumar · S. Shukla · M. Trivedi · S. Gulati (✉)
Sri Venkateswara College, Department of Chemistry, University of Delhi, Delhi 110021, India
e-mail: shikha2gulati@gmail.com

A. S. Gogoi · S. Shukla · M. Trivedi
Department of Chemistry, University of Delhi, Delhi 110007, India

S. Gulati (ed.), *Chitosan-Based Nanocomposite Materials*,
https://doi.org/10.1007/978-981-19-5338-5_14

CHO	Chinese hamster ovary
CMC	Carboxymethyl chitin
CNT	Carbon nanotubes
Cryo-SEM/TEM	Cryogenic-scanning electron microscopy and transmission electron microscopy
CS	Chitosan
CTAB	Cetrimonium bromide
DC	Direct current
DDA	Degree of deacetylation
DLS	Dynamic light scattering
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
EVA	Ethylene–vinyl acetate
FDA	Food and Drug Administration
FTIR	Fourier-transform infrared spectroscopy
GO	Graphene oxide
HAp	Hydroxyapatite
HEK	Human embryonic kidney
HIV	Human immunodeficiency virus
mRNA	Messenger ribonucleic acid
MTT	Microculture tetrazolium
NPs	Nanoparticles
NMs	Nanomaterials
PCL	Polycaprolactone
PEG	Polyethylene glycol
PVA	Polyvinyl alcohol
QDs	Quantum dots
RNAi	Ribonucleic acid interference
RT	Room temperature
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
UV	Ultraviolet
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction

1 Introduction

Natural materials outperform artificial materials in a variety of industrial applications, from cosmetics to aerospace, due to their diverse qualities and properties. When it comes to biomedical applications, natural materials are frequently found

to be preferable to synthetic ones [1]. The development of new standards to overcome technical difficulties and effectively resolve social and ecological challenges is critical for the advancement of sustainable biomedical innovation, particularly in the large-scale production of renewable materials. As a result, it is critical to reduce the use of harmful compounds and instead focus on the creation of environmentally safe nanoscale-based composite materials. In addition, the introduction of nanotechnology opens up more creative and superior options for many pharmaceutical applications, such as vaccination, tissue engineering, diagnostic imaging methods, cancer therapy, and other medicinal, and sustainable drug delivery, among others [2]. In recent years, special attention has been given to biopolymers, such as biodegradable polyesters, polysaccharides, or polypeptides. Bionanocomposites are a type of hybrid materials consisting of biopolymers and inorganic solids, having at least one dimension on the nanometer scale. The inclusion of nano-sized inorganic fillers causes biopolymer characteristics to alter and improve. The increasing interest in bionanocomposites is a global phenomenon now because of their cost-effectiveness, biodegradability, environmental friendliness, and ease of preparation. Also, polysaccharides happen to be the most popular biopolymers due to their sustainable and recyclable nature, as well as their biodegradability. They comprise a bountiful part of the biomass [3].

Chitosan is a linear polysaccharide and is acquired from nature itself. It can be made from chitin by deacetylation in the solid form under alkaline circumstances or through enzymatic hydrolysis by chitin deacetylase [4] (Fig. 1). In 1811, a French scientist named Henni Braconnot was the first to obtain chitin from mushrooms. Crab and shrimp shells are the most abundant hotspots for chitin. Chitin is a polymer made of the 2-acetamido-2-deoxy-D-glucose disaccharide that is joined together by a β bond. Chitosan was discovered in the nineteenth century, after the deacetylation of chitin having numerous acetyl glucosamine units. As a result of the partial deacetylation, chitosan is a heteropolymer with both glucosamine and acetyl glucosamine groups. Since amino groups ($-NH_2$) allow copolymerization with acrylic, styrene, urethane, and vinyl ester-based monomers to acquire auxiliary features, adjusting the attributes of this polymer is easier [1].

Non-harmfulness, biodegradability, biocompatibility, immune-enhancing, antitumoral, anti-viral, and antimicrobial action are some of the most well-known features of chitosan in the medical context. Chitosan's biodegradability was established in vitro and in vivo, where polymeric chains were disintegrated into sundry tinier segments of monomers. Chitosan and its enzymatic derivatives could harmlessly associate with living cells without any unfavorable impact on the body. Chitosan can diminish cholesterol retention, filter-free radicals from the chain oxidation process, and carry out its antimicrobial and anti-viral action against numerous yeasts and microbial organisms. Chitosan-based NPs can likewise hinder cancer cell development by instigating apoptosis. Chitosan, either singly or blended in with different polymers, active agents, and metallic nanostructures, has been widely utilized in numerous biomedical utilizations, that include wound scaffolding as an antimicrobial specialist, in drug conveyance as a nanoscale transporter to single out tumoral growths while negligibly influencing sites of unaffected tissue, for gene delivery, and

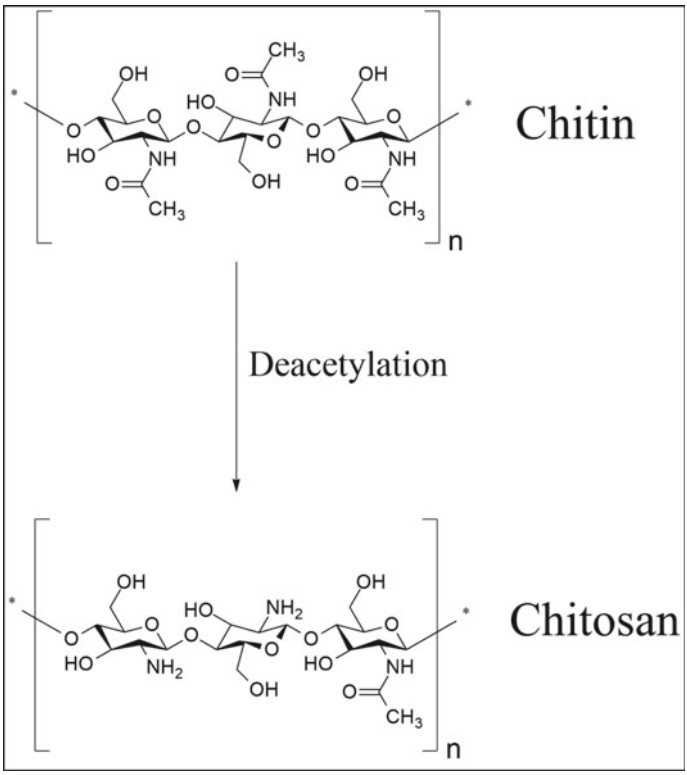

Fig. 1 Preparation of chitosan from chitin by deacetylation

dentistry and also as absorbable structure for immobilizing enzymes [4]. Since the future opportunities of chitosan-based bionanocomposite in different sectors provide us with ample room to maneuver, this chapter presents an itemized examination in regards to these issues. It is coordinated pleasantly by talking about chitosan-based bionanocomposite and their structure. From that point onward, it uncovers crucial data about the origin and attributes, and alteration techniques of chitosan, along with the essential diagrams. It likewise presents the most suitable strategies and methods for creating chitosan-based bionanocomposites. Moreover, the originality of this article is ameliorated by collecting significant data about the various applications along with the advantages and downsides intricately. Prior to closing this article, the future exploration heading is additionally given so as to progress and improve its application for different purposes soon [3].

2 Properties of Chitosan

Chitosan is made up of deacetylated D-glucosamine and N-acetyl-D-glucosamine units. It has a lesser molecular weight and a lesser number of crystalline domains than chitin (whose molecular weight is more than 100 kDa). This incredible biopolymer has also been approved by the FDA. Its design is like cellulose with the exception of the hydroxyl group at the C-2 site in cellulose is supplanted by the amino group in chitosan [6, 7].

2.1 Physiochemical Properties

Chitosan is characterized by a high extent of deacetylation and low crystallinity. This property makes it a compelling possibility for a variety of beneficial applications, such as drugs and biotechnology. Chelation, viscosity, dissolvability in various media, mucoadhesivity, polyelectrolytic behavior, and polyoxysalt formation are only a few of the physical features of chitosan [3]. Each glycosidic residue in chitosan has three receptive positions: one amino group and two hydroxyl groups. The amino group is of incredible importance because it is pH sensitive and answerable for chitosan's cationic nature. The position of the acetyl groups along the chain, deacetylation techniques, and ionic strength all play a role in its solubility. Chitosan has chelating capabilities for a few metal cations at an acidic pH [8].

2.2 Biological Properties

Chitosan has been found to be a non-toxic, biocompatible, and biodegradable polymer in several investigations [9]. Anti-inflammatory, antibacterial, antitumor, anti-fungal, hemostatic, and analgesic characteristics are all present in chitosan and its derivatives [3, 10, 11] (Fig. 2).

3 Fabrication and Chemical Alterations of Chitosan

3.1 Extraction/ Synthesis of Chitosan and Its Based Nanocomposites

The degree of deacetylation of chitin determines the molecular weight of chitosan. Deacetylation is generally done in the presence of an atmosphere of nitrogen gas or by adding sodium borohydride to the sodium hydroxide solution so as to kill any adverse side reactions. Many additional methods for making chitosan, using the exoskeleton

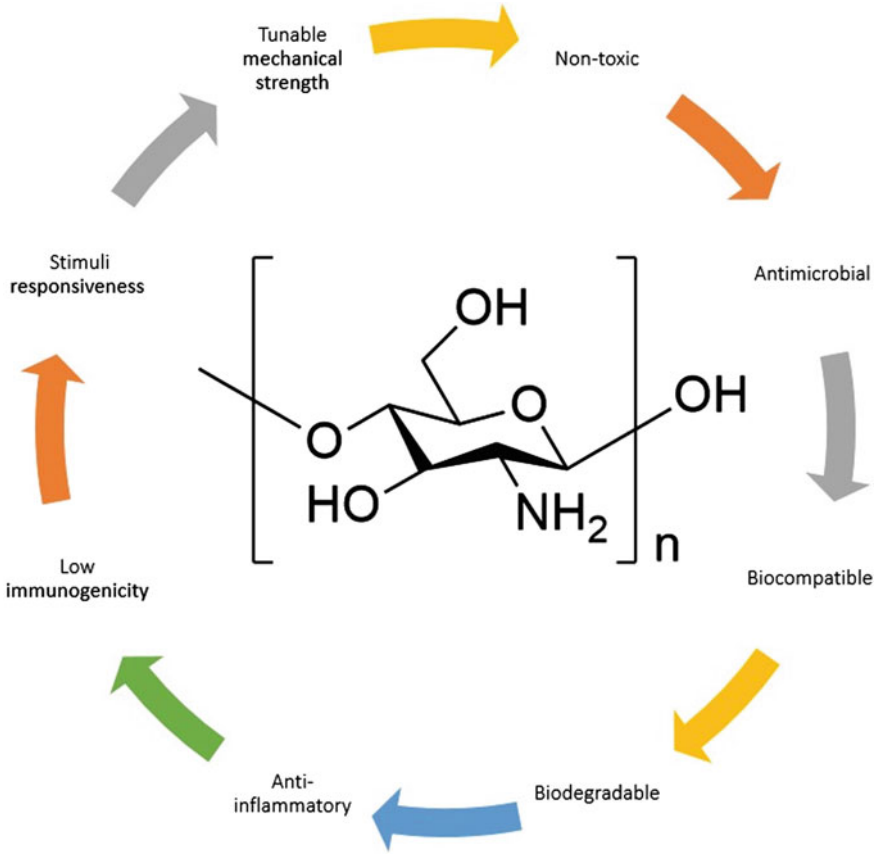

Fig. 2 Exceptional biotic properties permit the utilization of chitosan in numerous biomedical fields

of different crustaceans, fungi, cuttlefish, squid, and insects have been invented, in addition to alkali treatment [12]. To generate chitosan with variable degrees of acetylation from chitin, two techniques are now most widely used. The first approach is the solid phase heterogeneous deacetylation (Fig. 3), whereas the second involves pre-swollen aqueous phase homogeneous deacetylation under vacuum conditions [13]. These procedures employ thermo-mechanical techniques such as a cascade reactor operated at low concentrations of alkali [14]; sequential alkali analyzes with thiophenol in dimethyl sulfoxide [15]; and flash treatment with saturated steam and repeated washing with distilled water [2, 17]. The abstraction of chitosan using irradiation by microwaves has also been proposed as a more advanced approach [16]. Treatment with enzymes, rather than alkaline treatment at high temperatures, is another way to generate chitosan [18]. Following the biological methodology, chitosan is enzymatically created with chitin deacetylase. In the enzymatical fabrication strategy, the N-acetamide bond found in the basic framework of chitin gets

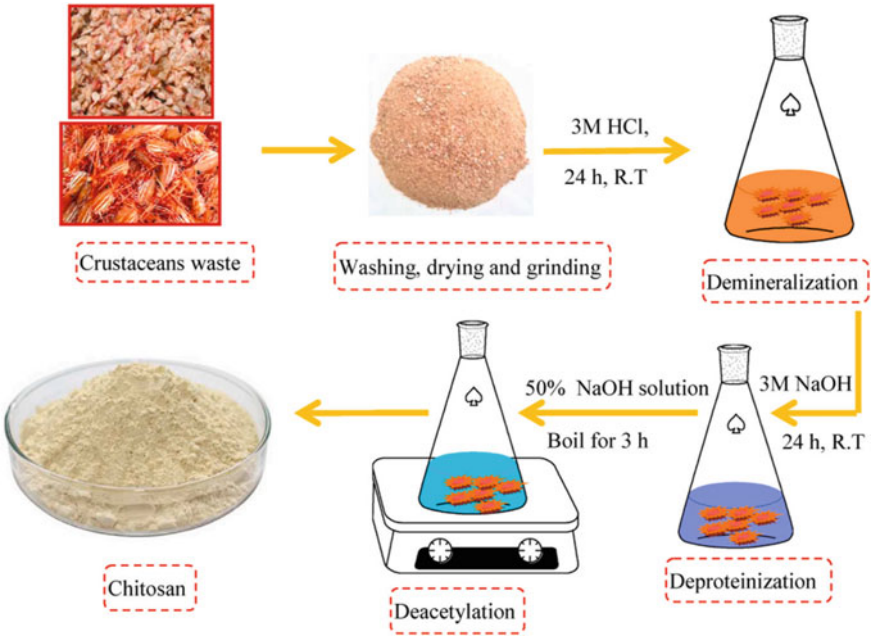

Fig. 3 Traditional method for the extraction of chitosan using the extracellular matrix of crustaceans. Reproduced from Ajahar et al. (2021) with permission from Elsevier

hydrolyzed by the chitin deacetylase enzyme. Prior to hydrolysis, chitin is artificially changed into unrefined chitin, as unrefined chitin is a poor enzyme substrate. As a result, chitin undergoes reprecipitation, glycolation, and depolymerization before being hydrolyzed [2, 19, 20].

Also, typical techniques generally used for the synthesis of chitosan-based bionanocomposites are listed below [3]:

i. Solution-casting method
ii. In-situ technique
iii. Electrospinning technique
iv. Freeze-drying technique

3.2 Modification of Chitosan

Natural chitosan has lower transfection effectiveness than synthetic chitosan, and it lacks several characteristics that are essential for some applications [2]. In addition to this, their inferior solubility in many polar solvents and water limits their applicability. To overcome this problem, chemical/enzymatic methods (Fig. 4) can be used to modify the chitosan molecule, resulting in new derivatives that not only improve its solvability for prudent use in various biomedical utilizations but also

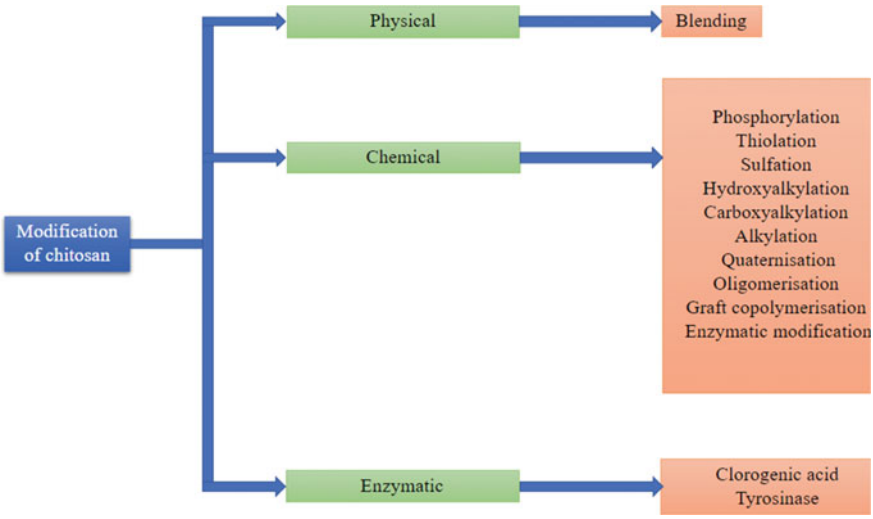

Fig. 4 Techniques to modify chitosan

serve as a powerful resource to promote novel biological actions and change its physical attributes [3]. Surprisingly, structural alterations to chitosan do not modify the molecule's basic structure, but they do give it new characteristics. Chitosan's structural morphology allows it to undergo a wide variety of reactions (like reduction, oxidation, acylation, cross-linking, complexation, halogenation, and phosphorylation) that result in novel derivatives [21]. Chemical or physical processes such as compositing with appropriate fillers, substituent inclusion, cross-linking, blending, curing, graft polymerization, and so on can be used to modify the material. Chemically modified chitosan and its derivatives as biomaterials for antimicrobial action, drug transport, and other biomedical utilizations have been examined extensively by Sashiwa and Alves et al. [22, 23]. Also, Mourya et al. have outlined the various approaches for modifying chitosan in great detail [24].

3.2.1 Physical Modification

Physical modification is commonly accomplished by combining two polymers to create a new material with distinct physical properties. Polymer blending is employed for the creation of materials with the perfect mechanical, chemical, biological, morphological, and structural attributes. When compared to other readily available tactics, this method is considered practicable and judicious, as well as less tedious [25, 26]. Hydrophilic polymers like polyethyl oxide (PEO), polyvinyl alcohol (PVA), and polyvinyl pyrrolidone (PVP) are all known to mix nicely with chitosan [27, 28]. The qualities of the component polymers and the chitosan/PVA blend, which is widely utilized in food applications, are improved through molecular interaction [29].

Furthermore, chitosan combined with PVA has demonstrated excellent mechanical properties for pharmaceutical items and controlled drug conveyance systems.

3.2.2 Chemical Modification

The polymer chain of chitosan possesses reactive amino (-NH_2) and hydroxyl (-OH) groups that aid the favorable chemical alteration of the molecule, without amending its fundamental characteristics. The modification of chitosan can be performed by various chemical reactions as listed below [3, 30]:

i. Phosphorylation
ii. Thiolation
iii. Sulfation
iv. Hydroxyalkylation
v. Carboxyalkylation
vi. Alkylation
vii. Quaternization
viii. Oligomerization
ix. Graft copolymerization
x. Enzymatic modification

4 Characterization Techniques for Chitosan and Chitosan-Based Nanocomposites

Characterization techniques such as FTIR, DLS, XPS, XRD, TEM, AAS, and SEM give reliable, consistent, and accurate results for improving the chemical and physical properties of nanomaterials. Nanomaterials are characterized in order to gain a better understanding of them, improve already-existing methods, and their applications sustainably.

4.1 Dynamic Light Scattering

The features of chitosan-based nanomaterials, such as particle size, surface charge, and size distribution, can be determined using the dynamic light scattering (DLS) approach. This is the most reliable and profoundly effective method when contrasted with others. DLS is a non-invasive method for estimating the size, size distribution, and particle charge of nanomaterials, especially in the submicron and <1 nm ranges, using cutting-edge technology. Brownian motion of particles (present in suspension) having varied scattering angles (θ) is used to determine particle size in DLS. This

technique is primarily focused on the measurement of particles suspended in liquid media. Brownian motion is slowed as the particle size increases. The Stokes–Einstein equation is applied to find the hydrodynamic size by analyzing intensity fluctuations and Brownian motion velocity. The probable size of the dynamic hydrated/solvated particle is determined by the diameter derived from the particle's diffusional characteristics. Using a combo of light scattering and microscopy techniques, a more thorough examination of the size and distribution of NPs can be performed. Chitosan nanoparticles in water have a size range of 40–374 nm, with an average size of ~ 250 nm, as observed by DLS. Furthermore, it was revealed by the study of the number distribution data that 91.6% of chitosan nanoparticles had diameters less than 100 nm [91.28 nm (1.6%), 78.82 nm (6.3%), 68.06 nm (17.6%), 58.77 nm (30.2%), 50.72 (26.8%), and 43.82 (9.1%)] [31–33] .

4.2 Interaction Analysis of Nanomaterials by FTIR Spectroscopy

Interaction evaluation, functional group evaluation, and the types of particular bonds/interactions occurring between various groups are all used to investigate the chemical characteristics of chitosan-based nanomaterials, for which Fourier-transform infrared spectroscopy is employed. Their calculations are crucial when it comes to changing or adjusting the attributes of nanomaterials. The paired peaks of the amino and hydroxyl groups stretching vibration in chitosan are responsible for a band at 3420 cm^{-1}. Furthermore, the C–N, $-CONH_2$ stretching vibration of primary amine, and anhydrous glycosidic bond are indicated by bands at 1385, 1649, and 892 cm^{-1}, respectively. These peaks are sharper and reposition toward 1315, 1640, and 894 cm^{-1} in Cu-chitosan nanoparticles, indicating boosted interactions. As a result, the synthesis of chitosan NPs is revealed by the relocation of vibrations from greater to smaller wavenumbers [33].

4.3 Elemental Analysis of Nanomaterials

4.3.1 X-Ray Photoelectron Spectroscopy (XPS)

Nanomaterials' surfaces provide sites of interface with other materials and the environment. As a result, a proper understanding of the chemical and physical properties of these nanostructures is a mammoth errand that can be accomplished with the help of XPS. It is a statistical spectroscopic tool for delving into data regarding the chemical and elemental composition of substances found inside nanomaterials [34]. Cu, C, O, N, and P elements are found in Cu-chitosan nanoparticles, according to

quintessential elemental research findings. C and O were the most plentiful components found in nanomaterials, while N and P were detected in limited proportions [35].

4.3.2 Atomic Absorption Spectroscopy

AAS is used to evaluate the concentration of elements present in nanomaterials. Photons are absorbed by atoms, which allows them to get excited from lower to higher energy levels. The ionization energy required for the excitation of the electron is specific to the element. As a result, each atom has its own unique wavelength profile, allowing for a qualitative study of chitosan-based nanomaterials. Furthermore, AAS can be used to focus on the entrapped metal ion release profile in chitosan-based NMs at different pH levels, agitation intervals, and rates [33].

4.4 Solid-State Characteristics of Nanomaterials by X-ray Diffraction (XRD)

X-ray diffraction is often employed to study the solid-state characteristics of nanomaterials. The molecular design of nanomaterials can be elucidated using solid-state characteristics data. Understanding the fundamental architecture of nanomaterials can aid in the efficient and precise functionalization and modification of these materials. Furthermore, these features can be used to further create an extraction strategy for chitosan nanoparticles, resulting in a structure that is suitable for a wide range of applications [33]. XRD is a non-destructive technology that studies the structure of materials on an atomic or molecular scale. XRD analysis of chitosan has been reported in a number of research studies. The crystalline peak intensity in the XRD diffractogram was found to be low intensity ($2\theta = 19.5$ and 21.0), which revealed the presence of Cu-chitosan NPs, allowing solid-state characteristics to be assessed [36, 37].

4.5 Internal and Surface Characteristics of Nanomaterials

The properties of the superficial and internal environment of chitosan-based nanomaterials, such as topology, inner architecture, biochemical functionalization, characterization, purity assessment, degree of agglomeration, and dimensions, are studied using electron microscopy and cryogenic electron microscopy [38].

4.5.1 Scanning Electron Microscopy (SEM)

SEM can be considered to be a crucial method for studying geometry, external morphology, and functionalization of diffused and aggregated chitosan-based nanostructures from top to bottom. The exterior of the nanomaterials is examined using a high-energy beam of electrons. Under optimal conditions, many of the state-of-the-art SEM machines can unveil data even in the 2 nm range. Chemical data on various nanomaterials can be obtained using a combination of SEM and energy-dispersive X-ray spectroscopy (SEM–EDX). In the various research publications, SEM data of chitosan-based nanomaterials has been frequently described [31, 39, 40].

4.5.2 Transmission Electron Microscopy (TEM)

Because of its higher spatial resolution relative to SEM, the most popular electron microscopy tool for the evaluation of chitosan-based nanoparticles is TEM. It is a key technique for the characterization process, which includes analysis of size, internal structure, and interactions between nanomaterials [33]. TEM can provide information in the 0.2 nm range under optimum circumstances. TEM images of spherical-shaped chitosan-based nanomaterials have been reported in several publications [39, 41].

4.5.3 Cryogenic-Scanning Electron Microscopy and Transmission Electron Microscopy

Cryo-SEM/TEM has recently emerged as a fundamental method for characterizing nanomaterials. This technique allows researchers to examine nanomaterials in a chemically unaffected and fully hydrated form at cryogenic temperatures (usually −100 to −175 °C) [42, 43]. It can be used to investigate the surface profile, geometry, dimension, and inner structural properties of chitosan-based nanomaterials in the same way that SEM and TEM can. The increased adoption of these approaches is due to the speedy pace of specimen processing, efficiency, the convenience of use, and imaging quality. Cryo-SEM/TEM edges out conventional SEM or TEM due to their numerous advantages like excellent resolution, swiftness, and examination in a completely hydrated condition, less dispersed material translocation, and suitability for liquid/semiliquid substances [43].

5 Applications of Chitosan

The creation of efficient and safe chitosan-based biomaterials for diverse biomedical utilizations (Fig. 5) like drug delivery, biosensors, tissue engineering, and wound healing has been the focus of extensive research and development during the last several years. The central emphasis was on prospective biomedical uses

based on chitosan nanocomposite, with a specific focus on its upgraded physico-chemical and biological attributes. The customary biological uses of chitosan-based nanocomposites are depicted in the diagram below.

As per the requirement of the desired application, chitosan can be rendered into numerous reactive formulations as shown in Fig. 6.

The next sections focus on the various biomedical application in a summarized manner.

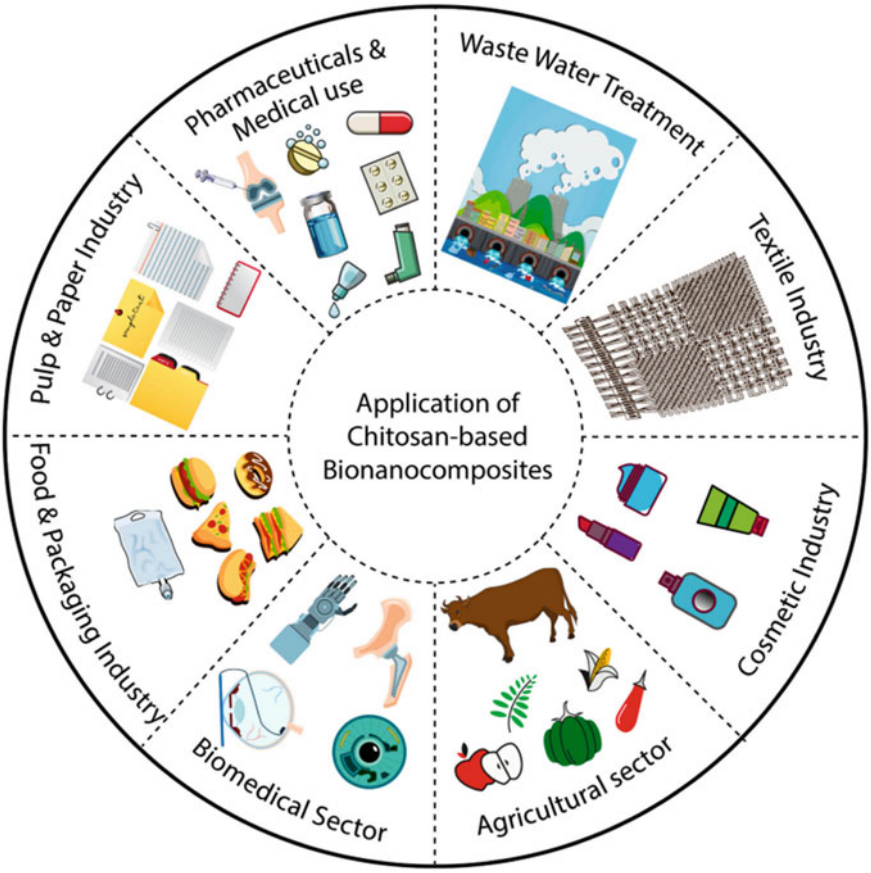

Fig. 5 Broadened application of chitosan-based bionanocomposites. Reproduced from Motia et al. (2021) with permission from Elsevier

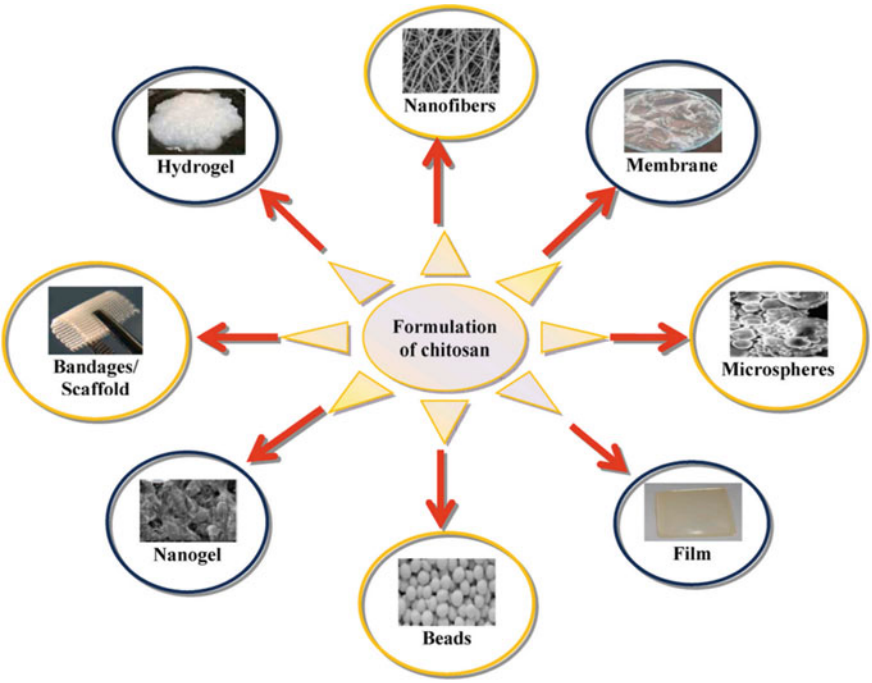

Fig. 6 Different formulations of chitosan. Reproduced from Ajahar et al. (2021) with permission from Elsevier

5.1 Drug Delivery

The use of nanomaterials in pharmaceuticals is becoming increasingly popular. Because of their tiny dimensions, nanoparticles can permeate through various biological membranes and convey drugs to specific areas with greater effectiveness [44]. Chitosan is perhaps the most essential polysaccharide for drug delivery due to its cationic characteristics and the existence of primary amino groups. These are in charge of many of their properties, including permeation augmentation, in-situ gelations, mucoadhesion, and calculated release of drugs [45]. Premeditated chitosan bio-decomposition allows for the controlled and steady discharge of loaded moieties while decreasing dosing frequency, which is beneficial for improving patient medication adherence [46].

Numerous research publications have identified chitosan-based nanoparticles to be enticing candidates for oral drug conveyance, owing to their unique perks like enhanced solvation of loaded hydrophobic medications, regulated drug delivery, minimal cytotoxicity, and improved therapeutic performance. Furthermore, nanoparticles in the intestinal system prevent unstable medications from deteriorating enzymatically. Nanoclays, reduced graphene oxide, gold NPs, layered double hydroxide, hydroxyapatite, mesoporous zeolites, and Fe and SiO_2 NPs are among the various

nanoparticles employed in the fabrication of chitosan-based drug carrier systems, according to the literature [1, 47–49]. Also, because of the creation of grueling routes for clays, 2D layered nanosheets such as carbon nanotubes (CNT), MXenes (Ti_3C_2, Nb_2C) nanoclay, or graphene exhibit better drug release profiles [50]. Moreover, other researchers have sought to incorporate mesoporous silica and other nanoparticles, such as hydroxyapatite, into chitosan frameworks in order to fine-tune drug-loading efficiency and release properties [51, 52].

Dev et al. [53] adopted an emulsion approach to produce poly(lactic acid) (PLA)/CS nanoparticles for anti-HIV medication delivery. Lamivudine, a hydrophilic antiretroviral, was incorporated into PLA/CS nanoparticles. In vitro, drug release tests revealed that when the medium's pH changed from basic to acidic to neutral, the rate of drug release from PLA/CS nanoparticles reduced. When comparing acidic and basic pH, the rate of drug release decreased in acidic pH. This could be owing to the fact that H^+ ions repel cationic groups found in polymeric NPs. These findings suggest that PLA/CS NPs are a potential delivery system for controlled anti-HIV and cancer medication delivery.

Sandhyarani and Chandran [54] used the solution-casting method to synthesize electric field-sensitive nanocomposite thin films comprised of chitosan/Au NPs and incorporated 5-Fluorouracil. In an electrolyte solution, the produced nanocomposites had a greater drug delivery efficacy (63%) and consistent-release regulated by an externally manifested electric field (DC). Shah et al. [55] used in-situ co-precipitation to create chitosan/Ag NP composite films with integrated moxifloxacin medications. The drug-encased nanocomposite films have superior mechanical qualities and antibacterial activity against a variety of microorganisms. These achievements in the realm of drug delivery are piquing attention and sending a clear message to produce sophisticated and upgraded nanomaterials/nanodevices for enhancing drug delivery efficiency by employing chitosan-based bionanocomposites (Table 1).

5.2 Gene Therapy

Chitosan-based bionanopolymers are strongly cationic, making them ideal choices for intracellular transport of nucleic acid biomolecules. Due to the cationic property of chitosan, it produces a complex of polyelectrolytes with oppositely charged nucleic acid moieties, which shields nucleic acids from nuclease disintegration [56].

Multiple illnesses such as cystic fibrosis and Parkinson's can be treated employing these genetic components (ribonucleic acid and deoxyribonucleic acid). The advantage of chitosan carriers is that they minimize the cytotoxicity problem that plagues the plurality of the artificial polymeric devices, in addition to having the unique capability of transcellular movement. Furthermore, because the amine groups are positively charged, they can transfer plasmid DNA (pDNA) into cells via membrane invagination and endocytosis [57].

Different techniques for constructing chitosan-based protein-embedded nanoparticles have been documented. Emulsification along with cross-linking is the most

Table 1 Some examples of chitosan and its derivatives-based nanocomposites in diversified functional formulations for drug delivery [2]

Serial no	Nanocomposites	Formulation	Receiver	Characteristics
1	Glycol chitosan-o-nitrobenzyl succinate	Nano-micelles	Mouse	Biologically compatible and superior drug carrier system for antitumor treatment
2	Chitosan-Mg–Al-PO_4-nanoclay	Nanohybrid hydrogel, scaffold	Mouse	Benign, more biologically compatible, and protracted distribution with site-specific cellular proliferation
3	Chitosan cross-linked-6-phosphogluconic trisodium	Hydrogel (ionic)	Swine	Benign, causes no skin itching, may be used as a medicine carrier and also for the dressing of wounds
4	Interferon-alpha embedded chitosan	NPs	Mouse	Oral delivery, harmless, intrusive, and more patient-friendly organic medication can be rendered as cytokines and protein therapeutics

popular method of preparation, although the use of organic solvents and cross-linkers can have a deleterious impact on protein expression [58]. To improve chitosan's effectiveness for gene delivery, researchers tried grafting polyethyleneimine into 1-butyl-imidazolium acetate [59] or fabricating chitosan derivatives in 1-ethyl-3-methylimidazolium chloride [57]. Consolidation of nucleic acids, shielding against breakdown, preservation in physiological settings, cellular incorporation, endolysosomal discharge, unloading, and transportation of the genetic material to the nucleus are all essential steps in the gene delivery process [60]. A range of methodologies has been attempted to increase chitosan's protein-sponging capacity, including the insertion of various functionalities (imidazole, histidine, etc.). In vitro and in vivo, chitosan has been exploited to transfect an array of cell varieties. A549, HeLa, HEK293, and COS-1 seem to be the most widely transfected cell varieties with chitosan-pDNA complexes under in-vitro conditions [61]. RNAi was also effectively implanted into HEK293, H1299, CHO-K1, and HepG2 cells using chitosan-RNAi complexes [61]. For pharmaceutical application, gene medications can be administered in a multitude

of ways, but one of the more inventive strategies involved incorporating complexes into a scaffold and propagating them to the problematic location [4].

In an exclusively built-in-situ reactor, Chen et al. [57] detailed the production of O-alkylated chitosan derivatives in 1-ethyl-3-methylimidazolium chloride solvent with N, N′ -carbonyldiimidazole as a binder. It is claimed that the ionic liquid solvent's unique properties are responsible for the selective alkylation of hydroxyl groups without protecting the amino ($–NH_2$) groups in chitosan. Furthermore, the chitosan derivatives' increased solubility in the presence of organic solvent may facilitate their future use in gene delivery studies [2].

Bionanocomposites based on chitosan offer hope for genome treatment and nucleic acid transport so as to cure genetic diseases. Future studies on improving the formulation of nucleic acid and gene delivery to targeted cells will hopefully upgrade the features and enhance the conveyance of nucleic acid and gene therapy on target sites.

5.3 Tissue Engineering and Regenerative Medicine

Tissue engineering is the process of leveraging live cells to create biological replacements for insertion into the body and/or to encourage active tissue reformation by manipulation of their extracellular space or genetics. It is an effective technique for repairing, replacing, preserving, or augmenting the performance of a particular organ/tissue [62].

A detailed grasp of bone architecture and the process of healing is required to select suitable biological materials for tissue engineering. In terms of selecting appropriate biomaterials, chitosan nanocomposites containing nanofillers such as bioactive glass, zeolite, hydroxyapatite, Cu NPs, carbon filler, etc., are widely used in tissue engineering applications in the form of fiber meshes, scaffolds, thin films, and hydrogels [1]. Chitosan possesses a hydrophilic surface that stimulates cell adhesion and growth, unlike many artificial polymeric materials. Chitosan molecules are very adaptable, allowing them to be easily changed into thin films and scaffolds, with a wide range of uses in tissue regeneration and cell transplantation. Chitosan may be treated in a variety of ways to create 3D scaffolds with a range of porous structures for bone-tissue regeneration. These functionalized scaffolds induce bone-forming osteoblast cell growth as well as the generation of a mineralized bone framework [3].

Multiple physiologically functional moieties, extracellular matrix (ECM) constituents, and cells are now well understood to coordinate at the nanometer scale. Electrospun nanofiber mat has a structure that is highly comparable to human native ECM, making it suitable scaffolding equipment for tissue engineering and cell culture [63]. Water-soluble carboxymethyl chitin (CMC)/PVA blend, electrospun for tissue engineering applications was described by Shalumon and colleagues [64]. To produce nanofibers, the proportion of PVA (8%) and CMC (7%) was tuned, then combined

in various ratios (0–100%) and electrospun. By cross-linking fibers with glutaraldehyde fumes and then heating them, the fibers became insoluble in water. This led to the creation of bioactive and biocompatible nanofibers. Cells were able to adhere and proliferate in nanofibrous scaffolds, according to cell attachment experiments. These findings show that the nanofibrous CMC/PVA scaffold promotes cell proliferation and adherence, indicating that it could be a great pick for the purpose of tissue engineering.

Nanosurfaces have a significant impact on cell behavior. Nanophase ceramics, compared to microphase ceramics, are known to have better cell–material interconnections [65]. The development of chitosan/nBGC and chitin/nBGC composite scaffolds for the purpose of tissue engineering is a good example of this [62]. The lyophilization procedure was used to create chitosan or chitin /nBGC composite scaffolds. When the nBGC were evenly distributed on the pore walls, the composite scaffolds displayed satisfactory porosity. Apart from their potential to become bioactive, the generated nanocomposite scaffolds exhibited acceptable swelling and disintegration characteristics. Direct contact test, MTT assay, and cell attachment tests were used to analyze the chitin/nBGC and chitosan/nBGC scaffolds' cytocompatibility. There was no evidence of toxic effects, and the cells were discovered to adhere to the scaffolds' pore walls. These findings indicated that the composite scaffolds generated may be employed in the field of tissue engineering [62].

5.3.1 Cartilage

Because of its similarities to glycosaminoglycans found in the extracellular matrix, chitosan is the most commonly employed biopolymer for cartilage tissue engineering [66]. Due to the biocompatible and non-poisonous characteristics of biopolymers like gelatin, silk fibroin, alginate, and collagen, their chitosan-based nanocomposites have garnered significant research in the area of cartilage tissue engineering [67]. By using an in-situ precipitation approach, Zhi-Sen et al. [68] were able to create a robust, porous, and resistant chitosan–gelatin-based hydrogel with Young's modulus of 3.25 MPa and a tensile rigidity of 2.15 MPa. The hydrogel was able to demonstrate multiplication and wonderful adhesion of human thyroid cartilage cells due to its incredibly porous architecture, which aided the cells' growth and transit of nutrients. Furthermore, it was observed that 65.9% of the produced hydrogel was decomposed after a period of 70 days.

5.3.2 Bone

Chitin and chitosan have minimal mechanical capabilities by nature. As a result, chitin can be employed as a bone-substituent material for bone healing and rebuilding, only if the mechanical characteristics can be enhanced by adding biomaterials such as bioactive glass–ceramic (BGC), hydroxyapatite (HAp), or other biomaterials. BGCs are a class of bone-healing materials consisting of osteoconductive silicates. Chitosan

(CS)-gelatin (CG) composite scaffolds with nBGC were developed by integrating gelatin and chitosan with nBGC [69]. According to the findings, the scaffold has a macroporous internal structure with pore sizes varying from 150 to 300 μm. With the addition of nBGC, protein adsorption improved and the nanocomposite scaffolds' disintegration and swelling tendency decreased. As incubation time rose, a higher degree of mineral deposition on the nanocomposite scaffold was revealed by biomineralization investigations. The performance of these nanocomposite scaffolds in the direct contact test, MTT assay, and cell attachment experiments showed that they are more favorable choices for cell spreading and attachment. Hence, these scaffolds can be employed to regenerate alveolar bone successfully [69] (Table 2).

5.4 Cancer Diagnosis and Treatment

Cancer is an exceptional disease portrayed by abnormal cell multiplication, which in the long run prompts the development of a cluster of cells called malignant cancer. Chemotherapy is usually utilized for the therapy of various diseases like lung cancer, breast cancer, prostate cancer, and so on. Despite the fact that chemotherapy showed strong anti-cancer action, it has numerous limits which at times hinder its application in medical settings. Chemotherapy is limited by three factors: (1) brief half-life, (2) non-specific anti-cancer activity, and (3) extreme aftereffects. To defeat these impediments, a targeted drug transportation system has emerged as a promising option for traditional chemotherapy. Deliberate preying on tumor cells, extending medication half-life, and regulating the release of the drug have all been demonstrated with the designated drug carrier framework. Due to the sheer diminutive dimensions of nano-transporters and their ability to preserve the drug from enzymatic defilement along with renal sieving, target-tailored drug carrier devices prolong the half-life of chemotherapeutic circulation. Even though these drug-loaded nanocarrier frameworks can evade enzymatic degradation and renal sieving, they were discovered to be eliminated through absorption of the reticuloendothelial framework. PEG-covered nanocarriers and PEGylated nanocarriers [70, 71] have been developed in the recent years and are regarded as remarkable enhancements due to their cloak and dagger effect, which allows drug-loaded nanocarriers to avoid renal sieving and enzymatic degradation and thus disseminate freely in the bloodstream, resulting in increased bioavailability of the drug at the assigned location and broadening its restorative competency.

The pharmacodynamics and pharmacokinetics of the drug-encased chemotherapeutic medications are substantially influenced by the nanocarriers used. Several chitosan nanoparticle-based chemotherapeutic formulations have been designed and tested in vivo and in vitro on various tumors in an attempt to identify a superior tumor treatment [70]. Bionanocomposites based on chitosan can preferentially permeate the membranes of cancerous cells and provide an antitumor effect through a variety of antiangiogenic, enzymatic, apoptotic, and immune-enhancing mechanisms. They are hidden from non-tumor cells and attack cancerous cells with increased bioavailability

Table 2 Summarizes a few popular chitosan nanocomposites for the purpose of bone engineering [1]

Nanocomposites additives	Chitosan information/synthesis technique	Attributes compared to original chitosan materials	Study of cell lineage and cytotoxicity when compared to original chitosan materials
Tobermorite type nanoclay (chitosan film)	Molecular mass low/solvent casting	Cell compatible; bioactive and biodegradable	MG-63 human osteosarcoma cells; viability of cells improved up to 30%
Hydroxyapatite (chitosan film)	DDA of chitosan >95%/solution casting	Alkaline phosphatase level rose by 377%, collagen I grew by 479%, and osteopontin climbed by 597%: all outstanding osteodifferentiation features	Mesenchymal stem cells; viability of cells enhanced by 52%
Silicon dioxide and zirconia NPs (chitosan scaffold)	75–85% DDA and low molecular mass/freeze-drying technique	Protein adsorption, deswelling, and biodegradation rates, and accelerated biomineralization abilities are significantly improved	Osteoprogenitor cells; zero toxicity shown by nanocomposites scaffold at fewer proportions of SiO_2/Zirconia NPs encapsulation
Nano-crystalline calcium phosphate (chitosan scaffold)	DDA = 92.3%/solution approach	Enhanced proliferation, mechanical characteristics, fibronectin, and cell adhesion	Human embryonic palatal mesenchymal cells; viability of cells > 65%
Bioglass (chitosan scaffold)	Needle punching technique	Porosity was raised by around 86% without impacting mechanical integrity; cell adherence and multiplication were enhanced	Human bone marrow stromal cells; viability of cells raised by four times after seven days of culture

over time. The magnetic sensitivity of bionanocomposites speeds up the antitumor agent's intravenous dispersion while reducing cytotoxicity [72]. Chitosan coating significantly enhanced the selectivity of magnetic NPs under conditions of hyperthermia. Thus, magnetic hyperthermia is indeed a potential tumor treatment method. The use of chitosan coating increased the targeting of magnetic nanoparticles in hyperthermia. Anti-cancer action of the complexes of chitosan/metal is owing to their interactions with cellular DNA and antioxidative capacity. The multiplication of cancerous cells has been reported to be suppressed by the more dissoluble variants with lower molecular weight chitosan oligosaccharides [73]. Thus, drug transport

devices combined with the bioavailability, and antioxidative attributes of chitosan-based nanocomposites can create a new vista for creating novel medications and techniques for the treatment of cancer.

For the treatment of bladder tumors, chitosan hydrogels embedded with β-glycerophosphate and magnetic NPs (for the steady and long-term administration of bacillus Calmette Guérin) were synthesized by Zhang and colleagues [74]. The apoptosis of cancerous cell clusters was studied by Bae and team [75] using chitosan-g-PEG/heparin bio-nanocomplexes. These complexes showed superior internalization of cells in comparison with unbound heparin exclusively. Furthermore, after the internalization of cells, heparin discharges induced apoptosis of the tumorous growth via activation of caspase. Sasidharan and colleagues [62] applied an aqueous chemistry approach at RT to synthesize a breakthrough nanomaterial structure centered on mannosylated ZnS with robust fluorescence intensity and extensive durability. Under this investigation, d-Mannose was utilized to effectively functionalize chitosan entrapped zinc sulfide NPs, thereby generating mannosylated ZnS with a dimension of approximately 120 nm. According to in-vitro cytotoxicity assessment using MTT assay, the mannosylated zinc sulfide NPs had negligible toxicity for normal and tumor cells alike. The mannosylated nanostructures were used to specifically attack tumor cells. The target specificity of mannosylated zinc sulfide nanostructures toward mannose-containing KB cells was revealed by fluorescence microscopic investigations, with no particular adhesion on normal cells. These studies greatly demonstrate the importance of chitosan-centric nanomedicine in cancer treatment (Table 3).

Table 3 Summary of a few chitosan-based nanocomposites in diversified formulations having anti-cancer action [2]

Bionanocomposites	Formulations	Receiver	Characteristics
Glycol chitosan-o-nitrobenzyl succinate	Nano micelles	Mouse	Biocompatibility; superior drug carrier for tumor treatment
GO/dimethylmaleic anhydride-altered chitosan	NPs	HepG2 cells	Smart antitumor nanocomposite having enhanced curative activity
Chitosan/dextran sulfate/chitosan	NPs	HepG2 cell	Nanoscale drug carrier with enhanced impediment toward cancerous clusters
Disulfide-bridged chitosan-Eudragit S-100	NPs	Mouse	Steady release of drugs at the specified site; tumors in the colorectal area are affected
GO-functionalized chitosan polyelectrolyte	Nanocomposite	MCF7, HeLa and L929 cells	Antitumor medication delivered in a regulated manner to the desired site

5.5 Bioimaging

Bioimaging has been widely utilized for both scientific surveys and primary diagnostic trials [76, 77]. This approach aids in the investigation of physiological processes spanning from cells at the nanoscopic level to the mammalian degree. Because of its ability to automatically measure, recognize, and profile phenotypic variations, this method is regarded as a viable technique for learning more about complex physiological phenomena [78, 79]. Altered chitosan and its hybrid nano polymers have evolved as interesting bioimaging materials of late. Chitosan's photoluminescent property was studied for bioimaging, and it showed photoluminescence in the aqueous phase.

Salehizadeh et al. [80] created a magnetically susceptible core–shell nano polymer for bioimaging by mixing Fe_3O_4-AuNPs with chitosan as the stabilizer. The superior magnetic character of magnetite ensured magnetic resonance for imaging, while gold NPs served the purpose of photothermal conversion, and stimulate optical qualities. So as to create exceptionally robust covalently cross-polymerized fusion nanogels, Wu's research group [81] demonstrated in-situ confinement of cadmium selenide QDs in chitosan–poly (methacrylic acid) nanogels. They came to the conclusion that the synthesized bionanocomposite can be used for efficient bioimaging and biosensing. The nanogels created established the likelihood of concurrent surveillance and assessment responsiveness during therapies. Lin's team [82] used an ethyl alcohol-mediated counter-ion complex formation technique in an aqueous medium to fabricate CdSe/ZnS quantum dot-embedded chitosan fusion nanospheres. It was discovered that produced fusion nanostructures may well be absorbed by cancer cells and hence act as cell imaging tagging tools. It might be used to image malignancies in rodents having tumors, utilizing intra-tumoral injection of a substance that would concentrate at the cancer site through the circulatory system.

Due to the diverse functions of chitosan-centric biomaterials, the bioimaging domain appears to be a viable arena for assisting and speeding up the curative procedure. In any event, a slew of testing difficulties persists to this day. These can be answered by large-scale medical tests and further analysis of the reconfigured nanocomposites.

5.6 Wound Healing

Healing of wound progressive mechanism that involves the controlled synthesis of inflammatory, vascular, and connective tissues. The injury dressing biomaterials must create a moist condition surrounding the wound, soak secretions, mechanically maintain the tissue, prevent drying of the wound, stimulate cellular proliferation, and function as a barrier against microbial pathogens while allowing for the exchange of gases. To accelerate wound regeneration, the perfect wound patch should not be allergenic or virulent and have a high antibacterial and cytocompatibility rating [83,

84]. A variety of biomaterials have been evaluated as wound coverings for both acute and persistent wounds. Inflammation, hemostasis, motility, proliferation, and restructuring are all coordinated activities in the wound repair mechanism, and the tempo of regeneration fluctuates according to the biopolymers utilized.

Even though numerous artificial or organic materials are being used, chitosan-centric nanopolymers (Fig. 7) have received much interest for a variety of reasons, including superior microbiological resistance, oxygen penetration, photothermal consequences, stimuli sensitivity, and simplicity of usage. Antibiotics like sulfadiazine, ciprofloxacin, or tetracycline, metallic antibacterial particulates (nCu, nAg, or nZnO), and natural substances/extracts (Salix alba leaves, Juglena regia, aloe vera, and so on) can all be incorporated into chitosan for the production of enhanced antimicrobial wound dressing [85–88]. Nanofibers made of chitosan trigger macrophages and speed up tissue repair. Furthermore, chitosan-induced re-epithelialization and restoration of the granular surface of the skin have also been documented [89].

Via electrospinning followed by photo-polymerization, Zhou's research team [90] effectively created a bio-nanofibrous scaffold exhibiting increased stability in water. They used photo-cross-linked maleilated chitosan/methacrylated poly(vinyl alcohol) in the aqueous phase. The developed product has great biocompatible features, according to in-vitro cytotoxicity evaluation and could be used as a potential bandaging material for wounds. A micro-porous, elastic, and chitosan hydrogel/nano ZnO-based bandage for wound treatment was created by Sudheesh Kumar and colleagues [91]. It works as a barrier against microbes and offers a moist atmosphere and cooling effect. These synthesized chitosan-based bandages improved edema, blood coagulation, and antimicrobial properties. Bionanocomposite scaffolds are cytocompatible with the dermal fibroblast cells of human beings, which promotes their usage for diabetic foot ulcers, burn injuries, and chronic injuries.

With the addition of bio-nanofillers, it is anticipated that researchers will soon create dramatically improved chitosan-centric bionanocomposites that will accelerate re-epithelialization and optimize the efficiency to eliminate microbes from the injured area.

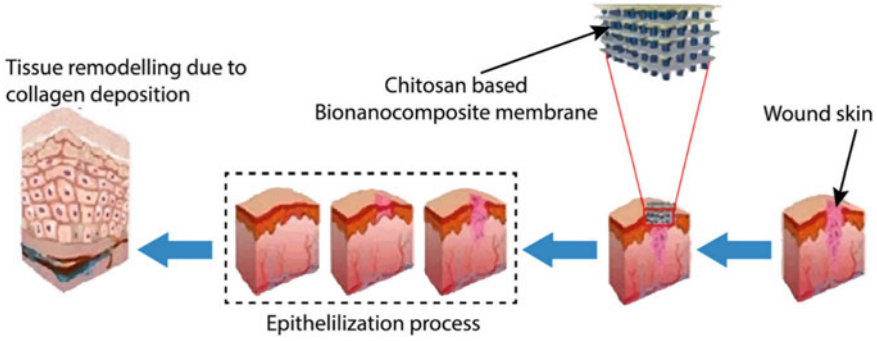

Fig. 7 Mechanism of wound healing with bionanocomposites based on chitosan. Reproduced from Motia et al. (2021) with permission from Elsevier

5.7 Antibacterial and Anti-fungal Action

Chitosan provides a variety of antagonistic efficacies against various fungi and bacteria. Attributed to variations in cellular framework, the mechanism of antibacterial action differs for both Gram-positive and Gram-negative bacteria. Chitosan is actually believed to be fungistatic instead of fungicidal, similar to bacterial activity [4]. Chitosan does not require chemical manipulation to trigger its antimicrobial effects. Nevertheless, chitosan NPs considerably boost their anti-fungal action.

Multiple concepts on the mechanism of antimicrobial activity, including the following, have lately been suggested and endorsed, despite the fact that the precise mechanism for the antibacterial and anti-fungal activity of chitosan and its derivatives has not yet been adequately elucidated.

- Polycationic nature of chitosan [92, 93]
- Interaction with bacterial DNA (mRNA suppression) [4, 94]
- Chelation agent (nutrients and essential metals) [95, 96]
- Chitosan has the ability to envelop the bacterial cell surface which blocks the uptake of nutrients. Additionally, it obstructs the O_2 pathway and prevents the development of aerobic microorganisms [97, 98].

For the enhancement and modification of the antibacterial action of both artificial and organic compounds, chitosan was used as a nanodevice for their transportation. Antibiotics, natural chemicals, antimicrobial peptides, and proteins are some of these entities. To boost the efficiency of the medication against bacterial development, various kinds of antibiotics including penicillins, tetracycline, vancomycin, cephalosporins, and aminoglycosides were encased in chitosan nanocarriers [99]. These nanocarriers were used, especially to enhance the administration of antibiotics into cells infiltrated by intracellular bacteria or to strengthen their potency against pathogens with multiple resistances.

The most popular chitosan-metal complexes for research studies are Ag-based nanostructures. According to studies done by Du's team [100], chitosan solution and nanoparticles comprising of metals like Cu(II), Mn(II), Zn(II), and Fe(II), all had reduced antibacterial effects against Gram-negative and Gram-positive bacteria (MIC varying from 3.0 to 6.0 g mL^{-1}) in comparison with Ag^{+}/chitosan complex NPs. The effectiveness of cefazolin-embedded chitosan NPs against multi-resistant Gram-negative bacteria like P. aeruginosa, E. coli and K. pneumonia was assessed by Jamil and colleagues [101]. Data obtained from the agar well diffusion assay and broth microdilution method showed that the drug-embedded chitosan NPs boosted antibacterial activity against the three pathogens, relative to simple cefazolin solution. Furthermore, Vancomycin's effectiveness against drug-resistive S. aureus, as proven by Chakraborty and others [102], firmly established the potency of drug-embedded chitosan NPs against antibiotic-resistive strains of bacteria. In a different investigation by Zhang's team [103], catechin and catechin/zinc complex were incorporated into chitosan NPs to increase the bioavailability and antibacterial impact of catechin. *L. innocua* & *E. coli* were used to test the antibacterial effect, and

the catechin-zinc complex encased chitosan NPs showed a greater impact than the normal catechin-encased kind. Additionally, all specimens, along with the unloaded chitosan NPs, showed greater antibacterial action against *L. innocua.* A study done by Panwar's group [104] showed that the surface of chitosan NPs has a positive charge which was critical in promoting their engagement with the fungal cells' oppositely charged plasma membrane. This prevented Candida albicans from forming a biofilm by compromising its physical stability. These findings were consistent with research done by Ing and colleagues [105], which revealed that Candida albicans were successfully eliminated by chitosan NPs at a dosage of 1 mg/mL. For enhanced anti-fungal action, Fawzya et al. [3, 106] created chitosan oligosaccharide polymers by hydrolyzing chitosan with an enzyme from Aeromonas media KLU 11.16. In another study [107], EVA/chitosan-based PEG-PCL micelles nanocomposite films were produced by solution-casting technique. These nanocomposites possessed improved anti-fungal efficacy and temperature-responsive drug deliverance. Through pre-harvest fertilization, they led to the production of grape-fruit of superior standards.

5.8 Anti-viral Action

Living beings frequently suffer from a variety of viral diseases, some of which can be fatal. Although standard anti-viral medications are effective in curing viral infections, they can have negative consequences or even be toxic to the body. Several methods emphasizing the physical methodology have been used to create Ag nanoparticle/chitosan blends [108]. In order to create Ag/chitosan bionanocomposites with a range of reducing and/or stabilizing components, thermal techniques like the freeze-drying process and also specialized materials like polyvinylpyrrolidone and carboxymethyl chitosan have been used [108]. Ghosh and his team [109] applied a layer-by-layer synthesizing approach, which involved a thin layer of Ag-chitosan composite being poured onto a quartz and stainless-steel strip. More recently, applying the principles of green chemistry, chitosan-based bionanocomposites with integrated silver nanoparticles have been created using a natural polymer that performs double duty as a stabilizing agent and a reductant [110].

Silver nanoparticles with chitosan coating were developed by Mori and colleagues, and their efficacy against viruses like H1N1 was examined. The findings showed that the composites with finer silver nanoparticles had a considerable inhibitory action, whereas chitosan in the pure form alone showed little anti-viral action [111]. Additionally, a new study by Loutfy et al. demonstrated a chitosan-curcumin nanohybrid that can cure human hepatoma cells of the genotype 4a of hepatitis C virus [112]. In a different research, antimicrobial and anti-viral films for the safe packaging of foods were created by integrating green tea extracts with eatable coatings of chitosan. The nano-polymeric film synthesized successfully suppressed and neutralized the murine norovirus and Escherichia coli bacterial colonies [113].

5.9 Orthopedics and Dentistry

The most significant and common dental remedy for preventing oral caries is fluoride. However, the substantial prevalence of dental fluorosis raises alarm [56].

Chitosan and bioactive glass–ceramic (BGC) blended nano scaffold has been created and is being researched for a variety of orthopedic applications, including bone regeneration and engineering [70]. BGC is an assortment of osteoconductive bone-repairing agents which are based on silicates. Hench synthesized BGC for the first time in 1991, and it was employed as a biocompatible substance for bone healing. Owing to its prospective capacity to bind to hard as well as soft tissues, BGC is now an important material in the world of dentistry and orthopedics. A nanophase version of bioactive glass–ceramic has recently been created using the sol–gel method. When compared to micro-BGC, it has demonstrated improved interactions between the material and the cells [62]. A chitosan/macroporous nano-BGC scaffolding frame with dimensions of pores spanning from 150 to 500 μm was created by Peter's team [69]. This bandaging nanostructure enhances adherence, dispersion, and proliferation of cells, according to in-vitro research on osteoblast-like cells (MG-63) [114]. A biofilm for laminating orthopedic implants, consisting of chitosan and multi-walled carbon nanotubes, was developed by Ahmed and colleagues [56]. Titania and zirconia are two of the most used materials for orthopedic laminations and bone implantations. For laminating 316L stainless-steel substratum, Clavijo et al. [115]. examined the bioglass/chitosan/TiO_2 matrix and achieved superior biomechanical characteristics for implantation purposes. In addition, Bartma'nski and his team achieved excellent biocompatibility in a Ti13Zr13Nb alloy nanocomposite wrapped with chitosan/nAg [116].

Chitosan and propolis together showed a synergistic relationship for enhancing antibacterial action. Chitosan incorporated with dentifrice significantly inhibited the demineralization of enamel around the dental braces during orthodontic therapy. Both gypsum-based chitosan and calcium phosphate carboxymethyl-chitosan bionanocomposites can be used as pulp capping agents to enhance cytocompatibility and proliferation of human dental pulp stem cells. The calcium phosphate carboxymethyl-chitosan composite has the odontogenic capability, enhanced physical qualities, accelerated curing, is biocompatible, and solidifies quickly upon freezing, thereby satisfying the fundamental requirements of a prospective agent for pulp capping and regeneration [117, 118]. Using the freeze-drying technique, a porous chitosan-based scaffolding nanostructure was produced. The surface of the fabricated scaffold was then loaded with human dental pulp stem cells. The observations indicated improved gene delivery and that the dental pulp stem cells developed into an odontoblast-like phenotype [56]. Moreover, Costa's team suggested that chitosan NPs can be used to fight plaque on the enamel surface, which is linked to gingivitis, periodontitis, and cavities in human beings [99]. The antibacterial effects of chitosan NPs with varying degrees of acetylation and molecular weight on Streptococcus mutans biofilm were studied by Chavez de Paz and colleagues [119]. The

chitosan nanoparticle formulations with low molecular weights (up to 150 kDa) effectively engaged with bacterial cells (more than 95% of afflicted cells) and disrupted the cell membrane stability in Streptococcus mutans throughout the whole region affected by plaque formation. The bactericidal efficacy of chitosan NPs against four distinct species of cariogenic streptococci (Streptococcus sanguis, Streptococcus mutans, Streptococcus sobrinus, and Streptococcus salivarius) was assessed in the research paper published by Aliasghari's team [120]. According to the findings, these compounds can inhibit the formation of biofilms in vitro, in addition to having antibacterial and anti-adhesion properties.

6 Drawbacks and Challenges

Deliberations about the drawbacks enable us to improvise material-based investigations and explore novel utilizations. Chitosan has promising prospects for various applications in the biomedical field, as evidenced by the rising number of research reports on chitosan-based nanocomposites. But despite having unprecedented biological and physiochemical attributes, chitosan suffers from certain pitfalls that pose significant hurdles and restricts its utilization in some foremost sectors. In order to fully exploit the benefits of this promising biopolymer and guarantee its availability for any application in distinctive sectors, the following noteworthy limitations must be addressed imperatively:

- Inferior solubility in physiological pH is a constraint in the biomedical sector [121].
- The Food and Drug Administration (FDA) has deemed it as food-contact material, but the European Food Safety Authority says otherwise. So, the safety of this material is still questionable [122].
- Low colloidal stability inhibits drug delivery [123].
- Economic practicality of eco-friendly bio-nanopolymer in the market of the real world [3].
- Efficacy of drug discharge, drug-loading capability, rate of decomposition of used nanocomposites, and delivery time period are other challenges faced by researchers [2, 124].

7 Future Prospects

The future holds great prospects for the innovation of chitosan and chitosan-based nanocomposites.

- *Development in agriculture*

Recent in-vivo and in-vitro studies showcase chitosan-based nanocomposites as a game-changer in agri-economics to lessen the negative environmental consequences

of traditional chemical fertilizers and deliver the upcoming generation of sustainable, compostable, and environment-friendly fertilizers. Primary usages include pisciculture improvement, pest control, meat production, seed preservation, and enhancement of plant immune by gradual, regulated, and selective nutrient conveyance to plants. Chitosan-based nanomaterials serve as a rich supply of nutrients for plants, providing them with C (~54–48 wt%), O (~42–30 wt%), N (~8–6 wt%), and P (~6–3 wt%) [125]. Additionally, via its functional groups, chitosan nanomaterials can be further modified to provide additional nutrient content. Thus, extensive research can be committed to developing this efficient and realistic approach to safeguard farming operations and also our ecosystem.

- *Development of chitosan carrier for brain drug delivery*

Chitosan-based nanocarriers have received a lot of attention for the delivery of drugs to the brain, and extensive research has been done on them with respectable outcomes. But to dismay, it was discovered that the chitosan-based nano delivery device interacted with the antibodies. Following this, in-vivo studies were conducted which showed their nanocarriers conjugating with antibodies, and their cytotoxicity, in the long run, was also assessed [126]. Therefore, the scholar can focus on these topics in their subsequent research investigation.

- *Improving the properties for medical application*

Studies have shown that the tissue sensitivity for chitosan-based biopolymers is discernably different when contrasted with lone materials. Cellular feedback, adherence, differentiation, and proliferation can be modulated through surface functionalization of nanoparticles and modifications of chitosan by moieties such as peptides, proteins, small biomolecules, and polymers, which in turn provides for improved bio-sensitivity, thereby facilitating enhanced cell-substrate reciprocation, and ameliorate physical properties of the substrate. Chitosan-based nanomaterials with bespoke characteristics show encouraging end results, such as selective drug/gene conveyance, faster bone rebuilding, superior wound regeneration, boosted osteogenesis, and angiogenesis. Nonetheless, it is of the utmost importance to perform research and surveys before starting clinical trials, since chitosan-based nanomaterials may elute as time goes by and also, the study of biodistribution in long-term animal trials should be done exhaustively. To be more precise, cells may absorb the nanoparticles released from the nanocomposites through endocytotic pathways, as exhibited by nanographene sheets that have been proven to infiltrate cells through phagocytotic uptake or clathrin-mediated endocytosis. The molecular mass and degree of deacetylation of chitosan are some additional disadvantages that severely affect its use. One recent study found that chitosan promotes the rupture of lysosomes that causes macrophage cytokines to be produced when glucosamine concentrations are more than 30,000 g/mol [127]. Moreover, the nanoparticulate concentration (such as CNTs, silver sulfadiazine, and bioactive glass), employed as fillers beyond a particular wt% leads to an appreciable decline in cell viability which hampers tissue engineering applications. Other nanoparticles did not enhance any

properties. However, regardless of these blemishes of chitosan-based nanomaterials, they have a lot of potential for further study in the biomedical field.

- *Wound healing*

UV irradiated chitosan/ZnO nanocomposite is a superb research topic in the near future, owing to the distinctive optical and semiconducting attributes of ZnO combined with UV's ability to promote photocatalysis. All these factors improve the bacteriostatic characteristics of the synthesized nanocomposite [128].

- *Heterogeneous nanocatalysis*

Inefficiency, impotent and low yield of conventional catalysts like iBr, CTAB, CuBr, salicylic acid, and L-proline are huge barriers in the synthesis of essential derivatives having antidiabetic, antitumor, geroprotective, antitubercular, and pain-killing activities. This is where chitosan and chitosan-based bionanocomposites come in as excellent future research options for heterogeneous bio-nanocatalysis because lately, Asgharnasl and his team achieved encouraging results from magnetically susceptible chitosan-terephthaloyl-creatine bionanocomposite [129].

- *Discovering substitute sources*

The exoskeleton of crustaceans is primarily made of chitin, which is also present in several other species, including fungi, mollusks, and insects. The a-chitosan from crustacean chitin is produced from recycled shrimp and crab shells and is the most widely exploited variant of chitosan. An enzyme known as chitosanases that is found in the species itself can also be used to extract chitosan from bacteria and fungus. But with rising popularity comes the fear that these natural resources will disappear. To restore the ecological balance, researchers must, therefore, hunt for alternate supplies.

- *Eco-friendly and cost-efficient production*

The traditional chemical technique of obtaining chitin poses several environmental concerns. Ionic liquids, microbial fermentation, deep eutectic solvents, microwave-mediated extraction, enzyme-mediated extraction, ultrasonic-mediated extraction, subcritical water extraction, and electrochemical extraction are a few methods that have been fruitfully established recently, using green harvesting methods for retrieving chitosan and chitin from different sources.

8 Conclusion

The indiscriminate exploitation of non-renewable resources and the harmful effects of non-biodegradable materials have led to the rise of a new era in the study and development of environmentally safe materials. Bionanocomposites are the leading contenders among the possible candidates with the intrinsic qualities of biocompatibility, biodegradability, and non-toxicity, along with their improved structural and

functional features. They possess numerous extraordinary properties which can be considered to be a boon to the human race.

This chapter has expounded on the chitosan-based bionanocomposites and the apprehension of their exceptional qualities. Different sources of chitosan, alteration techniques, and manufacturing methods have also been discussed. Global applications, advantages, and limitations of the chitosan-based bionanocomposites are described in depth. This chapter also draws attention to certain areas that have scope for further scientific study and investigation and provides insights to the scientists to work on ways to augment the physiochemical properties of this biopolymer. This chapter eventually concludes that even though multiple chitosan bionanocomposite-based utilizations are still in infancy, a booming future lies ahead of it with enhanced distinctive attributes like thermal, mechanical, protective properties, crystallization, and decomposition rate, which reflects their métier in the biomedical field, industrial field, agronomic field, and so on.

References

1. Murugesan S, Scheibel T (2021) Chitosan-based nanocomposites for medical applications. J Polym Sci 59(15):1610–1642, Wiley. https://doi.org/10.1002/pol.20210251
2. Khan A, Alamry KA (2021) Recent advances of emerging green chitosan-based biomaterials with potential biomedical applications: a review. Carbohydrate Res 506. Elsevier Ltd. https://doi.org/10.1016/j.carres.2021.108368
3. Azmana M, Mahmood S, Hilles AR, Rahman A, Bin Arifin MA, Ahmed S (2021) A review on chitosan and chitosan-based bionanocomposites: promising material for combatting global issues and its applications. Int J Biol Macromolecules 185:832–848, Elsevier B.V. https://doi.org/10.1016/j.ijbiomac.2021.07.023
4. Kravanja G, Primožič M, Knez Ž, Leitgeb M (2019) Chitosan-based (Nano)materials for novel biomedical applications. Molecules 24(10). MDPI AG. https://doi.org/10.3390/molecules24101960
5. Bagheri-Khoulenjani S, Taghizadeh SM, Mirzadeh H (2009) An investigation on the short-term biodegradability of chitosan with various molecular weights and degrees of deacetylation. Carbohydr Polym 78(4):773–778. https://doi.org/10.1016/j.carbpol.2009.06.020
6. Moreno JAS et al (2018) Development of electrosprayed mucoadhesive chitosan microparticles. Carbohydr Polym 190:240–247. https://doi.org/10.1016/j.carbpol.2018.02.062
7. Husain S et al (2017) Chitosan biomaterials for current and potential dental applications. Materials 10(6). MDPI AG. https://doi.org/10.3390/ma10060602
8. Kumirska J, Weinhold MX, Thöming J, Stepnowski P (2011) Biomedical activity of chitin/chitosan based materials-influence of physicochemical properties apart from molecular weight and degree of N-Acetylation. Polymers 3(4):1875–1901. https://doi.org/10.3390/polym3041875
9. Ghanbari B, Shahhoseini L, Hosseini H, Bagherzadeh M, Owczarzak A, Kubicki M (2018) Synthesis of Pd(II) large dinuclear macrocyclic complex tethered through two dipyridine-bridged aza-crowns as an efficient copper-and phosphine-free Sonogashira catalytic reaction. J Organomet Chem 866(Ii):72–78. https://doi.org/10.1016/j.jorganchem.2018.04.009
10. Kim S (2018) Competitive biological activities of Chitosan and its derivatives: antimicrobial, antioxidant, anticancer, and anti-inflammatory activities. Int J Polym Sci. https://doi.org/10.1155/2018/1708172

11. Ardila N, Daigle F, Heuzey MC, Ajji A (2017) Effect of Chitosan physical form on its antibacterial activity against pathogenic bacteria. J Food Sci 82(3):679–686. https://doi.org/10.1111/1750-3841.13635
12. Rashid TU, Rahman MM, Kabir S, Shamsuddin SM, Khan MA (2012) A new approach for the preparation of chitosan from γ-irradiation of prawn shell: effects of radiation on the characteristics of chitosan. Polym Int 61(8):1302–1308. https://doi.org/10.1002/pi.4207
13. Methacanon P, Prasitsilp M, Pothsree T, Pattaraarchachai J (2022) Heterogeneous N-deacetylation of squid chitin in alkaline solution. [Online]. Available: www.elsevier.com/locate/carbpol
14. Focher B, Beltrame PL, Naggi A, Torri G (1990) Alkaline N-Deacetylation of Chitin enhanced by flash treatments. Reaction Kinetics and Struct Modif
15. Pelletier A, Sygusch J, Chornet E, Overend RP (1990) Chitin/Chitosan transformation by thermo-mechano-chemical treatment including characterization by enzymatic depolymerization
16. Lertwattanaseri T, Ichikawa N, Mizoguchi T, Tanaka Y, Chirachanchai S (2009) Microwave technique for efficient deacetylation of chitin nanowhiskers to a chitosan nanoscaffold. Carbohydr Res 344(3):331–335. https://doi.org/10.1016/j.carres.2008.10.018
17. Aranaz I et al (2009) Functional characterization of Chitin and Chitosan
18. Cai J et al (2006) Enzymatic preparation of chitosan from the waste Aspergillus niger mycelium of citric acid production plant. Carbohydr Polym 64(2):151–157. https://doi.org/10.1016/J.CARBPOL.2005.11.004
19. Zhao Y, Park R-D, Muzzarelli RAA (2010) Chitin deacetylases: properties and applications. Mar Drugs 8:24–46. https://doi.org/10.3390/md8010024
20. Yamada M, Kurano M, Inatomi S, Taguchi G, Okazaki M, Shimosaka M (2008) Isolation and characterization of a gene coding for chitin deacetylase specifically expressed during fruiting body development in the basidiomycete Flammulina velutipes and its expression in the yeast Pichia pastoris. https://doi.org/10.1111/j.1574-6968.2008.01361.x
21. Qin Y, Li P, Guo Z (2020) Cationic chitosan derivatives as potential antifungals: a review of structural optimization and applications. Carbohydrate Polym 236, Elsevier Ltd https://doi.org/10.1016/j.carbpol.2020.116002
22. Sashiwa H, Aiba SI (2004) Chemically modified chitin and chitosan as biomaterials. Prog Polym Sci 29(9):887–908. https://doi.org/10.1016/J.PROGPOLYMSCI.2004.04.001
23. Alves NM, Mano JF (2008) Chitosan derivatives obtained by chemical modifications for biomedical and environmental applications. Int J Biol Macromol 43(5):401–414. https://doi.org/10.1016/J.IJBIOMAC.2008.09.007
24. Mourya VK, Inamdar NN (2008) Chitosan-modifications and applications: opportunities galore. React Funct Polym 68(6):1013–1051. https://doi.org/10.1016/J.REACTFUNCTPOLYM.2008.03.002
25. Meredith JC et al (2003) Combinatorial characterization of cell interactions with polymer surfaces
26. Abolhasani MM, Arefazar A, Mozdianfard M (2010) Effect of dispersed phase composition on morphological and mechanical properties of PET/EVA/PP ternary blends. J Polym Sci Part B Polym Phys 48(3):251–259. https://doi.org/10.1002/polb.21857
27. Khor E, Lim LY (2003) Implantable applications of chitin and chitosan. Biomaterials 24(13):2339–2349. https://doi.org/10.1016/S0142-9612(03)00026-7
28. Khoo CGL, Frantzich S, Rosinski A, Sjöström M, Hoogstraate J (2003) Oral gingival delivery systems from chitosan blends with hydrophilic polymers. Eur J Pharm Biopharm 55(1):47–56. https://doi.org/10.1016/S0939-6411(02)00155-8
29. Park SY, Jun ST, Marsh KS (2001) Physical properties of PVOH/chitosan-blended films cast from different solvents. Food Hydrocoll 15(4–6):499–502. https://doi.org/10.1016/S0268-005X(01)00055-8
30. Kumari S, Singh RP (2012) Glycolic acid-g-chitosan-gold nanoflower nanocomposite scaffolds for drug delivery and tissue engineering. Int J Biol Macromol 50(3):878–883. https://doi.org/10.1016/J.IJBIOMAC.2011.10.014

31. Choudhary RC et al (2017) Cu-chitosan nanoparticle boost defense responses and plant growth in maize (Zea mays L.) OPEN. https://doi.org/10.1038/s41598-017-08571-0
32. Saharan V, Pal A (2016) Properties and types of Chitosan-based nanomaterials. pp 23–32. https://doi.org/10.1007/978-81-322-3601-6_3
33. Choudhary RC et al (2019) Characterization methods for Chitosan-based nanomaterials. In: Nanotechnology in the life sciences, Springer Science and Business Media B.V., pp 103–116. https://doi.org/10.1007/978-3-030-12496-0_5
34. Korin E, Froumin N, Cohen S (2017) Surface analysis of nanocomplexes by X-ray photoelectron spectroscopy (XPS). ACS Biomater Sci Eng 3(6):882–889. https://doi.org/10.1021/acsbiomaterials.7b00040
35. Matienzo LJ, Winnacker SK (2002) Dry processes for surface modification of a biopolymer: Chitosan
36. Ieva E, Trapani A, Cioffi N, Ditaranto N, Monopoli A, Sabbatini L (2009) Analytical characterization of chitosan nanoparticles for peptide drug delivery applications. Anal Bioanal Chem 393(1):207–215. https://doi.org/10.1007/s00216-008-2463-4
37. Li LH, Deng JC, Deng HR, Liu ZL, Xin L (2010) Synthesis and characterization of chitosan/ZnO nanoparticle composite membranes. Carbohydr Res 345(8):994–998. https://doi.org/10.1016/j.carres.2010.03.019
38. Han J, Zhou C, Wu Y, Liu F, Wu Q (2013) Self-assembling behavior of cellulose nanoparticles during freeze-drying: Effect of suspension concentration, particle size, crystal structure, and surface charge. Biomacromol 14(5):1529–1540. https://doi.org/10.1021/bm4001734
39. Saharan V, Mehrotra A, Khatik R, Rawal P, Sharma SS, Pal A (2013) Synthesis of chitosan based nanoparticles and their in vitro evaluation against phytopathogenic fungi. Int J Biol Macromol 62:677–683. https://doi.org/10.1016/J.IJBIOMAC.2013.10.012
40. Nguyen Van S, Dinh Minh H, Nguyen Anh D (2013) Study on chitosan nanoparticles on biophysical characteristics and growth of Robusta coffee in green house. Biocatal Agric Biotechnol 2(4):289–294. https://doi.org/10.1016/j.bcab.2013.06.001
41. Liu H, Gao C (2009) Preparation and properties of ionically cross-linked chitosan nanoparticles. Polym Adv Technol 20(7):613–619. https://doi.org/10.1002/pat.1306
42. Sriamornsak P, Thirawong N, Cheewatanakornkool K, Burapapadh K, Sae-Ngow W (2008) Cryo-scanning electron microscopy (cryo-SEM) as a tool for studying the ultrastructure during bead formation by ionotropic gelation of calcium pectinate. Int J Pharm 352(1–2):115–122. https://doi.org/10.1016/j.ijpharm.2007.10.038
43. Kuntsche J, Horst JC, Bunjes H (2011) Cryogenic transmission electron microscopy (cryo-TEM) for studying the morphology of colloidal drug delivery systems. Int J Pharmaceutics 417(1–2):120–137, Elsevier B.V. https://doi.org/10.1016/j.ijpharm.2011.02.001
44. Wang JJ et al (2011) Recent advances of chitosan nanoparticles as drug carriers. Int J Nanomed 6:765–774. https://doi.org/10.2147/ijn.s17296
45. Barman M, Mahmood S, Augustine R, Hasan A, Thomas S, Ghosal K (2020) Natural halloysite nanotubes /chitosan based bio-nanocomposite for delivering norfloxacin, an anti-microbial agent in sustained release manner. Int J Biol Macromol 162:1849–1861. https://doi.org/10.1016/J.IJBIOMAC.2020.08.060
46. Rajitha P, Gopinath D, Biswas R, Sabitha M, Jayakumar R (2016) Chitosan nanoparticles in drug therapy of infectious and inflammatory diseases. Expert Opinion on Drug Delivery 13(8):177–1194, Taylor and Francis Ltd. https://doi.org/10.1080/17425247.2016.1178232
47. Liu W et al (2018) Molecules Galactosylated Chitosan-functionalized mesoporous silica nanoparticle loading by Calcium Leucovorin for colon cancer cell-targeted drug delivery. https://doi.org/10.3390/molecules23123082
48. Baktash MS, Zarrabi A, Avazverdi E, Reis NM (2021) Development and optimization of a new hybrid chitosan-grafted graphene oxide/magnetic nanoparticle system for theranostic applications. J Mol Liq 322:114515. https://doi.org/10.1016/J.MOLLIQ.2020.114515
49. Gowda BHJ et al (2022) Current trends in bio-waste mediated metal/metal oxide nanoparticles for drug delivery. J Drug Deliv Sci Technol 71:103305. https://doi.org/10.1016/J.JDDST.2022.103305

50. Murugesan S, Scheibel T (2020) Copolymer/clay nanocomposites for biomedical applications. https://doi.org/10.1002/adfm.201908101
51. Ahmed A, Hearn J, Abdelmagid W, Zhang H (2012) Dual-tuned drug release by nanofibrous scaffolds of chitosan and mesoporous silica microspheres. J Mater Chem 22(48):25027–25035. https://doi.org/10.1039/c2jm35569h
52. Pourshahrestani S et al (2017) Potency and cytotoxicity of a novel gallium-containing mesoporous bioactive glass/chitosan composite scaffold as hemostatic agents. ACS Appl Mater Interf 9(37):31381–31392. https://doi.org/10.1021/acsami.7b07769
53. Dev A et al (2010) Preparation of poly(lactic acid)/chitosan nanoparticles for anti-HIV drug delivery applications. Carbohydr Polym 80(3):833–838. https://doi.org/10.1016/J.CARBPOL.2009.12.040
54. Chandran PR, Sandhyarani N (2014) An electric field responsive drug delivery system based on chitosan-gold nanocomposites for site specific and controlled delivery of 5-fluorouracil. RSC Adv 4(85):44922–44929. https://doi.org/10.1039/c4ra07551j
55. Shah A, Ali Buabeid M, Arafa ESA, Hussain I, Li L, Murtaza G, (2019) The wound healing and antibacterial potential of triple-component nanocomposite (chitosan-silver-sericin) films loaded with moxifloxacin. Int J Pharm 564:22–38. https://doi.org/10.1016/j.ijpharm.2019.04.046
56. Sharifianjazi F et al (2022) Advancements in fabrication and application of Chitosan composites in implants and dentistry: a review. Biomolecule 12(2). MDPI. https://doi.org/10.3390/biom12020155
57. Chen H et al (2012) O-Alkylation of Chitosan for gene delivery by using ionic liquid in an in-situ reactor. Engineering 04(10):114–117. https://doi.org/10.4236/eng.2012.410b029
58. Amidi M, Mastrobattista E, Jiskoot W, Hennink WE (2010) Chitosan-based delivery systems for protein therapeutics and antigens. Adv Drug Deliv Rev 62(1):59–82. https://doi.org/10.1016/J.ADDR.2009.11.009
59. Chen H, Cui S, Zhao Y, Zhang C, Zhang S, Peng X (2015) Grafting Chitosan with polyethylenimine in an ionic liquid for efficient gene delivery. https://doi.org/10.1371/journal.pone.0121817
60. Ahsan SM, Thomas M, Reddy KK, Sooraparaju SG, Asthana A, Bhatnagar I (2018) Chitosan as biomaterial in drug delivery and tissue engineering. Int J Biol Macromol 110:97–109. https://doi.org/10.1016/J.IJBIOMAC.2017.08.140
61. Raftery R, O'brien FJ, Cryan S-A (2013) Molecules Chitosan for gene delivery and orthopedic tissue engineering applications. Molecules 18:5611–5647. https://doi.org/10.3390/molecules18055611
62. Jayakumar R, Menon D, Manzoor K, Nair SV, Tamura H (2010) Biomedical applications of chitin and chitosan based nanomaterials—a short review. Carbohydrate Polym 82(2):227–232. https://doi.org/10.1016/j.carbpol.2010.04.074
63. Jayakumar R, Prabaharan M, Nair SV, Tokura S, Tamura H, Selvamurugan N (2010) Novel carboxymethyl derivatives of chitin and chitosan materials and their biomedical applications. Prog Mater Sci 55(7):675–709. https://doi.org/10.1016/J.PMATSCI.2010.03.001
64. Shalumon KT et al (2009) Electrospinning of carboxymethyl chitin/poly(vinyl alcohol) nanofibrous scaffolds for tissue engineering applications. Carbohydr Polym 77(4):863–869. https://doi.org/10.1016/j.carbpol.2009.03.009
65. Webster TJ, Ergun C, Doremus RH, Siegel RW, Bizios R (2000) Enhanced functions of osteoblasts on nanophase ceramics. Biomaterials 21(17):1803–1810. https://doi.org/10.1016/S0142-9612(00)00075-2
66. Francis Suh JK, Matthew HWT (2000) Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review. Biomaterials 21(24):2589–2598. https://doi.org/10.1016/S0142-9612(00)00126-5
67. Kuo CY, Chen CH, Hsiao CY, Chen JP (2015) Incorporation of chitosan in biomimetic gelatin/chondroitin-6-sulfate/hyaluronan cryogel for cartilage tissue engineering. Carbohydr Polym 117:722–730. https://doi.org/10.1016/J.CARBPOL.2014.10.056

68. Sen Shen Z, Cui X, Hou RX, Li Q, Deng HX, Fu J (2015) Tough biodegradable chitosan-gelatin hydrogels via in situ precipitation for potential cartilage tissue engineering. RSC Adv 5(69):55640–55647. https://doi.org/10.1039/c5ra06835e
69. Peter M, Binulal NS, Nair SV, Selvamurugan N, Tamura H, Jayakumar R (2010) Novel biodegradable chitosan-gelatin/nano-bioactive glass ceramic composite scaffolds for alveolar bone tissue engineering. Chem Eng J 158(2):353–361. https://doi.org/10.1016/j.cej.2010.02.003
70. El-Sherbiny IM, El-Baz NM (2015) A review on bionanocomposites based on chitosan and its derivatives for biomedical applications. Adv Struct Mater 74:173–208. https://doi.org/10.1007/978-81-322-2473-0_6
71. Hu C-MJ, Zhang L (2009) Therapeutic nanoparticles to combat cancer drug resistance
72. Arias JL, Reddy LH, Couvreur P (2012) Fe_3O_4/chitosan nanocomposite for magnetic drug targeting to cancer. J Mater Chem 22(15):7622–7632. https://doi.org/10.1039/c2jm15339d
73. Sharan Adhikari H, Nath Yadav P (2018) Anticancer activity of Chitosan, Chitosan derivatives, and their mechanism of action. https://doi.org/10.1155/2018/2952085
74. Zhang D et al (2013) A magnetic chitosan hydrogel for sustained and prolonged delivery of Bacillus Calmette-Guérin in the treatment of bladder cancer. Biomaterials 34(38):10258–10266. https://doi.org/10.1016/J.BIOMATERIALS.2013.09.027
75. Bae KH, Moon CW, Lee Y, Park TG (2009) Intracellular delivery of heparin complexed with chitosan-g-poly(ethylene glycol) for inducing apoptosis. Pharm Res 26(1):93–100. https://doi.org/10.1007/s11095-008-9713-1
76. Lin J, Chen X, Huang P (2016) Graphene-based nanomaterials for bioimaging. Adv Drug Deliv Rev 105:242–254. https://doi.org/10.1016/J.ADDR.2016.05.013
77. Chen M, Yin M (2014) Design and development of fluorescent nanostructures for bioimaging. Prog Polym Sci 39(2):365–395. https://doi.org/10.1016/J.PROGPOLYMSCI.2013.11.001
78. Chen W, Xia X, Huang Y, Chen X, Han JDJ (2016) Bioimaging for quantitative phenotype analysis. Methods 102:20–25. https://doi.org/10.1016/J.YMETH.2016.01.017
79. Yang J et al (2014) Multifunctional quantum dot-polypeptide hybrid nanogel for targeted imaging and drug delivery. Nanoscale 6(19):11282–11292. https://doi.org/10.1039/c4nr03058c
80. Salehizadeh H, Hekmatian E, Sadeghi M, Kennedy K, (2012) Synthesis and characterization of core-shell Fe_3O_4-gold-chitosan nanostructure. J Nanobiotechnol 10. https://doi.org/10.1186/1477-3155-10-3
81. Wu W, Shen J, Banerjee P, Zhou S (2010) Chitosan-based responsive hybrid nanogels for integration of optical pH-sensing, tumor cell imaging and controlled drug delivery. Biomaterials 31(32):8371–8381. https://doi.org/10.1016/J.BIOMATERIALS.2010.07.061
82. Lin Y et al (2011) Water-soluble chitosan-quantum dot hybrid nanospheres toward bioimaging and biolabeling. ACS Appl Mater Interfaces 3(4):995–1002. https://doi.org/10.1021/am100982p
83. Gomes SR et al (2015) In vitro and in vivo evaluation of electrospun nanofibers of PCL, chitosan and gelatin: a comparative study. Mater Sci Eng C 46:348–358. https://doi.org/10.1016/J.MSEC.2014.10.051
84. Khorasani MT, Joorabloo A, Moghaddam A, Shamsi H, MansooriMoghadam Z (2018) Incorporation of ZnO nanoparticles into heparinised polyvinyl alcohol/chitosan hydrogels for wound dressing application. Int J Biol Macromol 114:1203–1215. https://doi.org/10.1016/J.IJBIOMAC.2018.04.010
85. Simões D, Miguel SP, Ribeiro MP, Coutinho P, Mendonça AG, Correia IJ (2018) Recent advances on antimicrobial wound dressing: a review. Eur J Pharm Biopharm 127:130–141. https://doi.org/10.1016/J.EJPB.2018.02.022
86. Yang L et al (2016) Fabrication of a novel chitosan scaffold with asymmetric structure for guided tissue regeneration. RSC Adv 6(75):71567–71573. https://doi.org/10.1039/c6ra12370h
87. Huang X et al (2015) Using absorbable chitosan hemostatic sponges as a promising surgical dressing. Int J Biol Macromol 75:322–329. https://doi.org/10.1016/J.IJBIOMAC.2015.01.049

88. Mi FL, Shyu SS, Wu YB, Lee ST, Shyong JY, Huang RN (2001) Fabrication and characterization of a sponge-like asymmetric chitosan membrane as a wound dressing. Biomaterials 22(2):165–173. https://doi.org/10.1016/S0142-9612(00)00167-8
89. Jayakumar R, Prabaharan M, Sudheesh Kumar PT, Nair SV, Tamura H (2011) Biomaterials based on chitin and chitosan in wound dressing applications. Biotechnol Adv 29(3):322–337. https://doi.org/10.1016/J.BIOTECHADV.2011.01.005
90. Zhou Y et al (2017) Photocrosslinked maleilated chitosan/methacrylated poly (vinyl alcohol) bicomponent nanofibrous scaffolds for use as potential wound dressings. Carbohydr Polym 168:220–226. https://doi.org/10.1016/J.CARBPOL.2017.03.044
91. Sudheesh Kumar PT et al (2012) Flexible and microporous chitosan hydrogel/nano ZnO composite bandages for wound dressing: In vitro and in vivo evaluation. ACS Appl Mater Interf 4(5):2618–2629, American Chemical Society. https://doi.org/10.1021/am300292v
92. Severino R, Ferrari G, Vu KD, Donsì F, Salmieri S, Lacroix M (2015) Antimicrobial effects of modified chitosan based coating containing nanoemulsion of essential oils, modified atmosphere packaging and gamma irradiation against *Escherichia coli* O157:H7 and Salmonella Typhimurium on green beans. Food Control 50:215–222. https://doi.org/10.1016/J.FOODCONT.2014.08.029
93. Rabea EI, Badawy MET, Stevens CV, Smagghe G, Steurbaut W (2003) Chitosan as antimicrobial agent: applications and mode of action. Biomacromol 4(6):1457–1465. https://doi.org/10.1021/bm034130m
94. Sudarshan NR, Hoover DG, Knorr D (1992) Antibacterial action of Chitosan. Food Biotechnol 6(3):257–272. https://doi.org/10.1080/08905439209549838
95. Wang X, Du Y, Fan L, Liu H, Hu Y (2005) Chitosan- metal complexes as antimicrobial agent: synthesis, characterization and Structure-activity study. Polym Bull 55(1–2):105–113. https://doi.org/10.1007/s00289-005-0414-1
96. Dutta PK, Tripathi S, Mehrotra GK, Dutta J (2009) Perspectives for chitosan based antimicrobial films in food applications. Food Chem 114(4):1173–1182. https://doi.org/10.1016/J.FOODCHEM.2008.11.047
97. Devlieghere F, Vermeulen A, Debevere J (2004) Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. Food Microbiol 21(6):703–714. https://doi.org/10.1016/J.FM.2004.02.008
98. Yuan G, Lv H, Tang W, Zhang X, Sun H (2016) Effect of chitosan coating combined with pomegranate peel extract on the quality of Pacific white shrimp during iced storage. Food Control 59:818–823. https://doi.org/10.1016/J.FOODCONT.2015.07.011
99. Perinelli DR et al (2018) Chitosan-based nanosystems and their exploited antimicrobial activity. European J Pharmaceutical Sci 117:8–20. Elsevier B.V. https://doi.org/10.1016/j.ejps.2018.01.046
100. Du WL, Niu SS, Xu YL, Xu ZR, Fan CL (2009) Antibacterial activity of chitosan tripolyphosphate nanoparticles loaded with various metal ions. Carbohydr Polym 75(3):385–389. https://doi.org/10.1016/j.carbpol.2008.07.039
101. Jamil B et al (2016) Cefazolin loaded chitosan nanoparticles to cure multi drug resistant Gram-negative pathogens. Carbohydr Polym 136:682–691. https://doi.org/10.1016/J.CARBPOL.2015.09.078
102. Chakraborty SP, Sahu SK, Pramanik P, Roy S (2012) In vitro antimicrobial activity of nanoconjugated vancomycin against drug resistant Staphylococcus aureus. Int J Pharm 436(1–2):659–676. https://doi.org/10.1016/J.IJPHARM.2012.07.033
103. Zhang H, Jung J, Zhao Y (2016) Preparation, characterization and evaluation of antibacterial activity of catechins and catechins-Zn complex loaded β-chitosan nanoparticles of different particle sizes. Carbohydr Polym 137:82–91. https://doi.org/10.1016/j.carbpol.2015.10.036
104. Panwar R, Pemmaraju SC, Sharma AK, Pruthi V (2016) Efficacy of ferulic acid encapsulated chitosan nanoparticles against Candida albicans biofilm. Microb Pathog 95:21–31. https://doi.org/10.1016/J.MICPATH.2016.02.007
105. Ing LY, Zin NM, Sarwar A, Katas H (2012) Antifungal activity of Chitosan nanoparticles and correlation with their physical properties. Int J Biomater 2012. https://doi.org/10.1155/2012/632698

106. Jeon YJ, Shahidi F, Kim SK (2000) Preparation of chitin and chitosan oligomers and their applications in physiological functional foods. Food Rev Int 16(2):159–176. https://doi.org/10.1081/FRI-100100286
107. Xiao NY et al (2020) Construction of EVA/chitosan based PEG-PCL micelles nanocomposite films with controlled release of iprodione and its application in pre-harvest treatment of grapes. Food Chem 331:127277. https://doi.org/10.1016/J.FOODCHEM.2020.127277
108. Jeevanandam J et al (2022) Synthesis approach-dependent antiviral properties of silver nanoparticles and nanocomposites. J Nanostruct Chem. https://doi.org/10.1007/s40097-021-00465-y
109. Ghosh S, Ranebennur TK, Vasan HN (2011) Study of antibacterial efficacy of hybrid chitosan-silver nanoparticles for prevention of specific biofilm and water purification. Int J Carbohydr Chem 2011:1–11. https://doi.org/10.1155/2011/693759
110. Sharma S, Sanpui P, Chattopadhyay A, Ghosh SS (2012) Fabrication of antibacterial silver nanoparticle—sodium alginate-chitosan composite films. RSC Adv 2(13):5837–5843. https://doi.org/10.1039/c2ra00006g
111. Mori Y et al (2011) Simple and environmentally friendly preparation and size control of silver nanoparticles using an inhomogeneous system with silver-containing glass powder. J Nanoparticle Res 13(7):2799–2806. https://doi.org/10.1007/s11051-010-0168-z
112. Loutfy SA et al (2022) Antiviral activity of chitosan nanoparticles encapsulating curcumin against hepatitis C virus genotype 4a in human hepatoma cell lines. Int J Nanomed 2020:15–2699. https://doi.org/10.2147/IJN.S241702
113. Mallakpour S, Azadi E, Hussain CM (2021) Recent breakthroughs of antibacterial and antiviral protective polymeric materials during COVID-19 pandemic and after pandemic: coating, packaging, and textile applications. Current Opinion in Colloid and Interf Sci 55. Elsevier Ltd. https://doi.org/10.1016/j.cocis.2021.101480
114. Liu X-P et al (2010) Open access research anti-tumor activity of N-trimethyl chitosan-encapsulated camptothecin in a mouse melanoma model. https://doi.org/10.1186/1756-9966-29-76
115. Clavijo S, Membrives F, Quiroga G, Boccaccini AR, Santillán MJ (2016) Electrophoretic deposition of chitosan/Bioglass® and chitosan/Bioglass®/TiO_2 composite coatings for bioimplants. Ceram Int 42(12):14206–14213. https://doi.org/10.1016/J.CERAMINT.2016.05.178
116. Bartmá Nski M et al (2020) Electrophoretic deposition and characteristics of Chitosan-nanosilver composite coatings on a nanotubular TiO_2 layer. https://doi.org/10.3390/coatings10030245
117. Mu¸sat VM et al (2021) A Chitosan-Agarose polysaccharide-based hydrogel for biomimetic remineralization of dental enamel. https://doi.org/10.3390/biom11081137
118. Zhang C et al (2021) Preparation and application of chitosan biomaterials in dentistry. Int J Biol Macromol 167:1198–1210. https://doi.org/10.1016/J.IJBIOMAC.2020.11.073
119. de Paz LEC, Resin A, Howard KA, Sutherland DS, Wejse PL (2011) Antimicrobial effect of chitosan nanoparticles on Streptococcus mutans biofilms. Appl Environ Microbiol 77(11):3892–3895. https://doi.org/10.1128/AEM.02941-10
120. Aliasghari A, Khorasgani MR, Vaezifar S, Younesi H (2022) Evaluation of antibacterial efficiency of chitosan and chitosan nanoparticles on cariogenic streptococci: an in vitro study antimicrobial effects of *Camellia sinensis* and *Achillea millefolium* on several antibiotic resistant strains view project biodegradable PLGA implants containing anti cancer drug-loaded chitosan nanoparticles view project. [Online]. Available: https://www.researchgate.net/publication/303788651
121. Kumari S, Singh RP, Chavan NN, Annamalai PK (2016) Chitosan-based bionanocomposites for biomedical application. Bioinspired Biomim Nanobiomater 7(4):219–227. https://doi.org/10.1680/jbibn.15.00015
122. Butnaru E, Stoleru E, Brebu A, Nicoleta Darie-Nita R, Bargan A, Vasile C (2019) Materials Chitosan-based bionanocomposite films prepared by emulsion technique for food preservation. https://doi.org/10.3390/ma12030373

123. Onnainty R, Granero G, Onnainty R (2017) In: Onnainty R, Granero G (eds) Chitosan-Clays based nanocomposites: promising materials for drug delivery applications
124. Barra A et al (2020) Biocompatible chitosan-based composites with properties suitable for hyperthermia therapy. J Mater Chem B 8(6):1256–1265. https://doi.org/10.1039/c9tb02067e
125. Prajapati D et al (2022) Chitosan nanomaterials: a prelim of next-generation fertilizers; existing and future prospects. Carbohydr Polym 288:119356. https://doi.org/10.1016/J.CARBPOL.2022.119356
126. Aderibigbe BA, Naki T (2019) Chitosan-based nanocarriers for nose to brain delivery. https://doi.org/10.3390/app9112219
127. Fong D et al (2017) Lysosomal rupture induced by structurally distinct chitosans either promotes a type 1 IFN response or activates the inflammasome in macrophages. Biomaterials 129:127–138. https://doi.org/10.1016/J.BIOMATERIALS.2017.03.022
128. Wang K et al (2020) Review article recent advances in Chitosan-based metal nanocomposites. https://doi.org/10.1155/2020/3827912
129. Asgharnasl S, Eivazzadeh-Keihan R, Radinekiyan F, Maleki A (2020) Preparation of a novel magnetic bionanocomposite based on factionalized chitosan by creatine and its application in the synthesis of polyhydroquinoline, 1,4-dyhdropyridine and 1,8-dioxo-decahydroacridine derivatives. Int J Biol Macromol 144:29–46. https://doi.org/10.1016/J.IJBIOMAC.2019.12.059