Chapter

# Chitosan-Based Nanocomposites for Biological Applications

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## Abstract

Chitosan is an important natural cationic polymer. Chitosan is produced as a deacetylated form of chitin, and its excellent biocompatible, biodegradable, nontoxic, natural chemical, and thermal stability properties have led to its common use in especially biomedical applications. The combination of nanomaterials and chitosan has been considered an excellent approach to overcoming the handicaps associated with biopolymer. The chitosan-based nanocomposites are potentially efficient in a number of areas including medical fields. Chitosan is biodegradable, biocompatible, basic, nontoxic, and also approved by GRAS (Generally recognized as safe by the United States Food and Drug Administration [US FDA]). Chitosan-based nanocomposites have different applications in drug delivery including ocular, per-oral, pulmonary, nasal mucosal, gene, buccal drug, vaccine, vaginal, and cancer therapy. Chitosan has low toxicity in both in vitro and in vivo models. *In this* chapter, we discussed the preparation techniques and various forms of chitosan materials in biomedical applications. In addition, this chapter explores recent research on chitosan-based nanocomposites for medical studies.

**Keywords:** chitosan, chitosan-based nanocomposites, clay, biomedical applications



## 1. Introduction

Chitin is the most abundant polysaccharide in nature after cellulose and is commercially produced from the waste shells of lobster, shrimp, crabs, etc. obtained. Chitosan is obtained by removing the acetyl group of chitin from the structure in a basic medium. Chitosan is a copolymer formed by connecting N-acetyl-D-glucose amine and D-glucose amine with β-1,4 glycosides bonds (**Figure 1**). Since chitosan contains free amino groups, it is a neutral polysaccharide at neutral and basic pH and is insoluble in water. However, at acidic pH, amino groups are soluble because they are protonated. Its solubility depends on the distribution of free amino and N-acetyl groups and is readily soluble in dilute 1–3% acetic acid [1]. Chitosan does not cause allergic reactions and is biocompatible with living tissues. Amino sugars are gradually broken down into harmless products in the body and are completely absorbed by the human body, and they can be easily removed from the organism without causing local side effects in the body [2]. Chitosan is a bioactive ingredient with numerous properties such as antitumor, immune enhancer, antifungal, antimicrobial, antioxidant, and

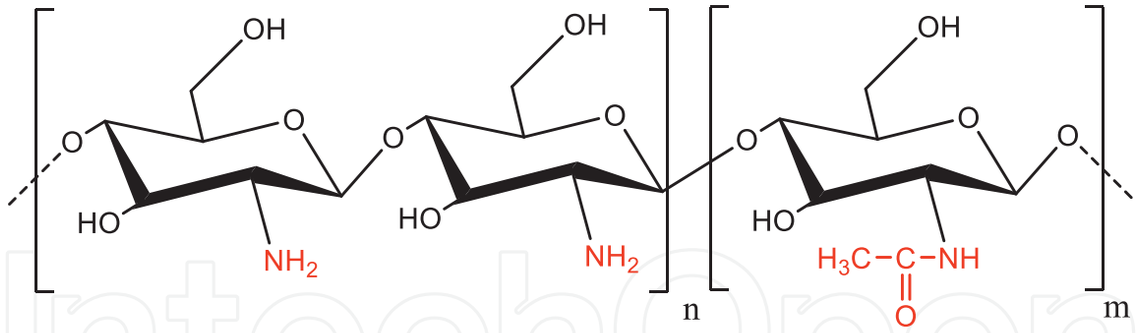


**Figure 1.**
*Chemical structure of chitosan.*

wound healing. In addition to these features, it also has features such as being biodegradable, biocompatible, low cost, and non-antigenic [3].

Chitosan also has good adhesion and coagulation properties. The presence of primary amine groups and primary and secondary hydroxyl groups in chitosan makes it very useful in biological applications. Compared to other biopolymers, chitosan is positively charged and has a structure that can adhere to the mucosa [4]. Chitosan is widely used in many industrial applications due to the following properties [1, 5].

- It is renewable and abundant.
- It has Nontoxic, biocompatible, and biodegradable properties.
- It has bioeffects such as antacid, antitumor, antioxidant, and antibacterial.
- No harmful in/organic solvents are required for dissolution.
- It is a cationic biopolymer and interacts easily with negatively charged surfaces.
- It has strong electrostatic properties and shows suitable bioadhesive properties.
- Electrostatically strong and exhibits favorable bioadhesive properties.

## 2. Properties of chitosan

The molecular structure of chitosan, which is obtained by partial deacetylation of chitin and contains β-(1,4)-D-glucosamine and N-acetyl-D-glucosamine units, is shown in **Figure 1** [5].

### 2.1 Physical and chemical properties of chitosan

There are some parameters that affect the characteristic properties of chitosan, such as molecular weight, degree of deacetylation, and solubility. These parameters vary according to the conditions applied during the production of chitosan [6]. The process of removing acetyl groups in the chitin polysaccharide chain is called deacetylation. Chitin, which usually has a degree of deacetylation greater than 50%,

is considered to be chitosan [7, 8]. The molecular weight of chitosan varies depending on the conditions applied during the deacetylation process and the source from which it is obtained. For example, exposure of chitosan to oxygen and high temperature causes its molecular weight to decrease [9].

Some parameters affecting the physical and chemical properties of chitosan are as follows:

### *2.1.1 Resolution*

Thanks to its cationic structure, chitosan can be easily dissolved in some solutions in pH <6 environments. Organic acids such as acetic acid, formic acid, and lactic acid are generally used to dissolve chitosan. On the other hand, the solubility of chitosan in inorganic acids is quite low [9]. Some mineral acids such as hydrochloric acid and nitric acid can also be dissolved, but it is not suitable to use phosphoric acid and sulfuric acid as solvents [10, 11]. Among them, the most widely used is acetic acid. The solubility of chitosan mostly depends on the degree of N-acetylation (or degree of deacetylation), distribution of acetyl groups, deacetylation time, pH, ionic strength [12], temperature, particle size, pre-treatments before isolation, base concentration, and chitin/base solution ratio [13].

The solubility problem of chitosan is a disadvantage and must be used by dissolving in acid. In order to improve this, water-soluble chitosan derivatives can be synthesized, thanks to the reactivity of the primary amine and primary and secondary hydroxyl groups in its structure. Modification of chitosan increases its solubility and stability, making it versatile as a biopolymer [14].

### *2.1.2 Deacetylation degree (DD)*

The degree of deacetylation of chitosan plays an important role in its solubility and solution properties [15]. DD and molecular weight have the effect of changing the physicochemical and biological properties of chitosan. Studies have been carried out with different chitosan samples with similar molecular weights but varying DD between 70% and 95%, and these parameters were found to be related to physicochemical properties. Chitosan samples with high DD have more crystalline regions than those with low DD. As the DD increases, the elasticity and tensile strength also increase [16]. In terms of biological properties, chitosan samples with DD exceeding 90% were examined. It has been observed that chitosan samples with high DD play a role in the regeneration of nerve cells and resemble cells in the peripheral nervous system. As a result, the DD of chitosan is a very important parameter in terms of physicochemical and biological properties [17].

### *2.1.3 Molecular weight (Mw)*

The Mw of chitosan varies depending on its source and deacetylation conditions (temperature, time, and base concentration). The dissolved oxygen in the solution medium reduces the stability of chitosan, causing it to decompose, and the Mw of chitosan decreases. Also, high temperature ($\geq 280^{\circ}C$) breaks the polymer chains of chitosan and lowers its Mw [9]. In the literature, it is stated that high molecular weight polymers slow down the release rate of drugs [18, 19]. The Mw, viscosity, polarity, solubility, and thermal properties of the drug carrier material matrix significantly affect the release mechanism [19].

### *2.1.4 Viscosity*

Many factors affect the viscosity of chitosan, such as the degree of deacetylation, molecular weight, ionic strength, pH, and temperature [9]. Viscosity increases with an increasing degree of deacetylation. Chitosan with a high and low degree of deacetylation has different conformations in an aqueous solution. Chitosan with a high degree of deacetylation has an expanded conformation with more flexible chains due to charge repulsion in the molecule. However, chitosan with low DD is rod-like or coil-shaped due to the low charge density in the polymer chains. The viscosity of chitosan is also affected by concentration and temperature. If the concentration of the medium increases or the temperature decreases, the viscosity of chitosan also increases [13].

The viscosity of chitosan is also highly related to its Mw. The viscosity of chitosan with a high Mw is higher than that of a low Mw one. Many studies show that physical and chemical processes affect viscosity. Processes such as increasing the grinding time, heating, autoclaving, ultrasonic, and ozonation reduce the viscosity [20]. It was stated that the viscosity decreased with increasing deproteinization and demineralization time [9, 20].

### *2.1.5 Color*

The pigment in the shellfish structure forms a complex with chitin. The color of powdered chitosan can vary from light yellow to white. When obtaining chitosan from chitin, decolorization can be done by extraction with acetone followed by interaction with 0.3% NaOCl. Chemicals such as $KMnO_4$, $NaHSO_3$, $Na_2S_2O_4$, and $H_2O_2$ are also used for color removal [9].

The amino and hydroxyl functional groups in chitosan allow it to form stable covalent bonds with other materials. It can carry out esterification and etherification reactions with alcohol groups, while amino groups on D-glucosamine can be quaternized or react with aldehyde functions [21]. Thanks to its amino groups, chitosan can form complexes with metal ions, and thus it can be used in the treatment of wastewater and recovery of heavy metals [22, 23]. The complexing feature of chitosan also enables it to be used in the purification of beverages (juices, wine, etc.) [22].

Chitin has a stable structure and is insoluble in water, alcohol, dilute acid, and base solutions, and its chemical reactivity is quite low. Due to these features, it is not widely used in industrial applications [23, 24]. Today, chitosan is used in numerous fields such as food, agriculture, cosmetics, textile, medicine, and pharmacy sectors. Thanks to the mentioned properties of chitosan, its field of study can be further increased by its modifications with various functional groups in the future.

## 3. Biomedical applications chitosan-based nanocomposites

### 3.1 Controlled drug delivery systems

Before controlled release systems were developed, there were many systems with long-acting, different drug releases, and different names. Modified release systems, which differ from basic drug delivery methods, fall into two groups:

1. Delayed-release systems

2. Extended-release systems:

- Controlled release systems
- Sustained-release/prolonged systems

The main systems defined in the modified release systems classification and differing from each other in active substance releases are as follows [25, 26]:

*Repeat Action Systems:* There is more than one dose of the active substance in the applied dosage, and these doses are released at certain time intervals.

*Delayed-Release Systems:* The release of the active substance from the system takes place in a certain region. It is used for enteric-coated tablets, generally.

*Sustained Release Systems:* These systems can maintain the plasma and tissue levels of the active substance for a longer period of time than conventional release systems. However, since the system is affected by environmental conditions, it is difficult to determine the release mechanism in advance. In general, its velocity is compatible with first-order kinetics.

*Controlled Release Systems:* These systems exhibit different behavior from sustained-release systems in that release rates can be planned in advance and can realize drug release with zero-order kinetics [25, 27].

Controlled release is a constantly evolving topic with applications in many different fields such as medicine, pharmacy [28], chemistry [29], environment, agriculture [30], and veterinary medicine [31]. Thanks to the controlled release systems, the pollution caused by the traditional application methods in the soil can be prevented by using low amounts in agriculture (agricultural pesticides such as fertilizers and insecticides) and applications related to environmental protection [30]. It is used in controlled release applications of parasitic drugs, hormones, vaccines, antibiotics, substances that increase milk yield, and birth control drugs in veterinary medicine [31]. In chemical applications, it is also used for the controlled release of expensive and waste-producing materials, thus ensuring economic and continuous production. Controlled release practices in medicine and pharmacy have emerged in order to better control drug administration, facilitate the treatment of the patient, and increase the quality of life of the patient [32].

Controlled drug release is a method in which the active substance is designed to be released from a system at the desired time, at a determined rate, and in the required quantities [33]. The interest in controlled drug delivery systems is increasing day by day because developing a new drug takes a long time and is economically burdensome. Thanks to controlled drug release systems, the drug dose used decreases, the dosage range increases, and the side effects of the drug are largely eliminated [32]. After the active substance mixes into the blood, its level blood should remain within the plasma range where it is effective. In classical release systems, the drug level fluctuates between the maximum and minimum values, and when the drug life is over, it is necessary to take the drug in high doses again. In controlled drug release systems, the drug can be released at the optimum plasma concentration, in a stable manner, and over a long period of time [25, 34].

In order for the drug to be effective, it must first form the dosage form that carries the active substance, then mix it into the blood safely and effectively, disperse into the tissues, and show its effect in the target area. The dosage should be maintained in a range above the effective amount and below the toxic amount after mixing with the blood. Each dose of the drug taken reaches a peak according to its specific half-life in the blood, then decreases below the effective amount, and is finally eliminated from the body. In conventional drug systems, it is not possible for the drug to select its place of

action or to mix into the blood in a controlled manner [25, 35]. Controlled drug release application, it is aimed to show the effectiveness of the drug according to a pre-planned process in the body and to perform treatment at longer intervals, with low doses, without side effects. Thus, the life of the drug in its circulation in the body is prolonged, the absorption is accelerated and its target ability to the effect site is ensured [36].

The conventional and controlled exchange of the drug in the blood is shown schematically in **Figure 2**.

Drug delivery systems provide predetermined and reproducible controlled drug release for long-term treatment locally or systemically at specified time intervals. In the traditional method, the drug is given at once and in high doses, and the dose is repeated after a few hours or a day. This method is not economical and has side effects [37, 38]. The purpose of controlled drug release systems is to improve the performance of drug therapy. This mechanism enhances the therapeutic activity and reduces side effects by reducing the toxicity caused by overdose during treatment. If the drug concentration level is not stable, the drug falls below the normal level or rises above the toxic level. This may cause undesirable side effects in the patient. The controlled drug release system maintains a constant level of drug concentration in the blood plasma [39, 40].

Controlled drug delivery systems are produced by combining a polymer with a drug or other active agent. Polymers used in drug delivery systems should be nontoxic and non-allergenic, high purity and reusable, biodegradable in vivo, and structures formed after the degradation process should be usable in metabolism [38]. These polymers used as biomaterials can be natural or synthetic. Despite the advantage that synthetic polymers can be synthesized at any time, biopolymers have advantages such as being easily obtained from nature, cheap, and chemically modified [1, 5]. Chitosan has a very important role in medical applications due to its nontoxicity, biocompatibility, biodegradability, mucoadhesion, and low allergenic. Its high biocompatibility and biodegradable properties are its most important advantages for drug delivery systems [38, 39].

### 3.2 Advantages and disadvantages of controlled drug delivery systems

Controlled release systems are used successfully in the treatment of many diseases today. In the coming years, with the use of controlled-release drugs (especially tablets and capsules), the effectiveness and safety of the treatment will increase. The

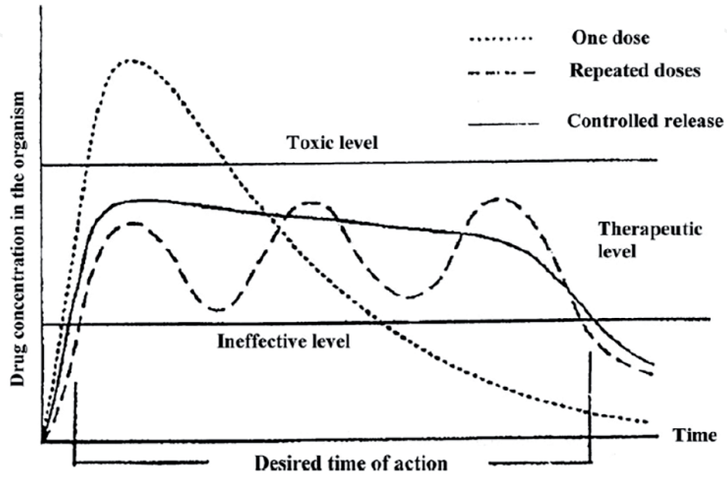


**Figure 2.**
*A schematic drawing of drug release [12].*

advantages and disadvantages of controlled release systems can be summarized as follows [25, 32–42]. Advantages of controlled-release systems:

- The plasma level of the drug can be kept at the desired value for the specified time.
- Thanks to infrequent drug intake, drug compliance increases.
- The total drug dose loaded into the system is less than conventional dosage forms.
- Eliminating or reducing the toxic and side effects of the drug.
- Preventing the degradation of drugs with short in vivo half-lives and prolonging their half-life.
- Controlled release systems can be targeted to the desired region, organ, and tissue.
- It provides convenience to patients, their relatives, and caregivers, and drug use can be controlled, especially in underdeveloped regions where medical control cannot be fully achieved.

Despite all these benefits, there are still unsolved problems with controlled release systems. Main disadvantages:

- The polymer itself or the decomposed products may exhibit toxic effects or biocompatibility.
- After the system is used, the drug release cannot be stopped at any time.
- Reliability of the system is weak in case of physical changes that may occur during production, storage, and distribution (capillary cracks, etc.).
- It may not be possible to prepare every drug with this system, and there is no single technology that can be applied to drugs.
- This system may increase the cost of drugs.

In some of these systems, the cost may be greater than in conventional dosage forms. However, well-designed controlled release systems are preferred because they reduce overall health expenditures when evaluated in terms of cost-effectiveness. Controlled release systems of cancer, hormone, enzyme, antibiotic, antirheumatic drugs, and some existing drugs produced in biotechnology or classical technologies have been prepared. Thanks to the developments in molecular medicine in recent years, controlled release systems have also given successful results in the use of protein and peptide drugs.

### 3.3 Chitosan-based clay-containing material applications in drug delivery systems

For controlled drug release systems, some carrier materials such as natural or synthetic polymers, metals, ceramics, oils, antibodies, magnetic components, and

carbon are used in the world today. The most widely used of these materials are polymers, and some of these studies are in the stage of animal trials. Biocompatibility and biodegradability properties are sought in polymers used in drug delivery systems. Biodegradable polymers are polymers that can be enzymatically, microbiologically, or hydrolytically degraded in a physiological environment. The degradation products of biodegradable polymers must also be nontoxic. Biopolymers, modified natural polymers, and synthetic polymers are frequently used in the preparation of drug delivery systems. Along with this polymer, chemicals such as calcium hydroxyapatite, clay, organic-containing clay derivatives, magnetite and maghemite nanoparticles (for magnetic control), and graphene oxide are also used. In the following paragraphs, these subjects will be explained and studies on chitosan will be emphasized.

In recent years, studies have been carried out on the preparation and application of biodegradable polymer nanocomposites as controlled drug delivery systems [38–40]. Clay types such as montmorillonite (MMT), halloysite, organoclay, etc. are used in the preparation of these nanocomposites. MMT is a widely used material for controlled drug release as it retains the drug, thanks to its high cation exchange capacity. Clay minerals are natural cation exchangers and bind to the drug in solution by electrostatic attraction. Depending on the cation exchange capacity of the clay, cationic of the drug, release medium, and pH decide the release kinetics of the drug. It is possible to have different interactions such as hydrophobic, hydrogen bonding, ligand exchange, and water bridge, independent of electrostatic forces. These properties of clay encourage the use of clay for sustainable drug release [43]. However, the ability of clay particles to adsorb negative charged or neutral drugs is low. This limits the applications of negatively charged or neutral drugs. When preparing drug delivery systems, this disadvantage of clay should be taken into account [43].

Yuan et al. observed that the swelling rates of chitosan-clay biocomposites that they synthesized for drug release were lower than clay alone and chitosan alone. However, they stated that the drug release from the chitosan-clay biocomposite was more than that of clay and less than chitosan. They stated that with the addition of clay to chitosan, the interaction between the negatively charged groups in the clay and the positively charged $NH_3^+$ groups in the chitosan creates strong crosslinks. They stated that this situation affects the swelling behavior of the composite and, therefore, affects the diffusion of the drug in the structure [43]. Hua et al. studied the release of the drug ofloxacin from chitosan-MMT and chitosan-only hydrogel. They prepared different ratios of drug and clay with the same amount of chitosan, and they observed that the hydrogel containing the highest clay had the highest drug retention efficiency. With its high surface area, MMT adsorbed the drug not only on the inner and outer surfaces but also between the clay layers. With the addition of MMT, the drug release decreased and the dispersion viscosity of the drug increased. In the swelling test, the least swelling value was observed in chitosan, and the maximum swelling value was observed in the hydrogel with the highest MMT content. The amount and rate of drug release decreased as the amount of MMT increased. In the XRD analysis, the presence of the drug was observed in the medicated composite and chitosan, while the drug was lost in the hydrogel with high clay content. This indicates that drug molecules are lost at the polymeric level or get into the clay layers [44]. Cheikh et al. synthesized nanocomposite by intercalation method using chitosan and purified beidellite. In this study, they selected diclofenac sodium as a model drug and examined its release. According to the results of the analysis, the drug was both distributed between the layers of the clay and detected on the surface of the nanocomposite. They reported that with the nanocomposites they obtained, they reached 60% cumulative

drug release in 8 hours at pH 6.8 [45]. In another study, Sharma et al. synthesized silymarin-loaded chitosan-MMT microbeads by ionotropic gelation method for the potential treatment of gastric ulcer [46]. Depan et al. grafted lactic acid onto chitosan and obtained the chitosan-g-lactic acid/$Na^{+}$MMT bionanocomposite. They characterized the structure of the composite by FTIR, XRD, SEM, and [1]H-NMR, and thought that this bionanocomposite could be used in controlled drug release and tissue engineering. For this purpose, they used the synthesized bionanocomposite for the transport of the drug sodium ibuprofen [47]. Sahoo et al. synthesized Chitosan/polycaprolactone/Cloisite30B bionanocomposites using an 80:20 ratio of chitosan: polycaprolactone and Cloisite30B (organoMMT) at 1, 2.5 and 5% by mass and characterized their structures by FT-IR, XRD, and SEM. They reported that the synthesized biocomposite could be used in the controlled release of the drug doxycycline [48]. Parida et al. synthesized the Chitosan/Polyvinyl Alcohol/Cloisite30B bionanocomposite using Cloisite30B at different rates and reported that the composite could be used in controlled drug release [49]. In another study, Nanda et al. synthesized (by solvent removal method) Chitosan/Polylactic Acid/Cloisite30B bionanocomposites using different ratios of chitosan, polylactic acid, and Cloisite30B and characterized their structures with FT-IR, SEM, and XRD. They stated that the drug release properties of these biocomposites, which they used in the controlled release of paclitaxel anticancer drugs, were sensitive to pH and the amount of drug loaded [50]. Cojocariu et al. synthesized the Chitosan/Cloisite15A (organoMMT) bionanocomposite using different amounts of clay. They stated that the bionanocomposite has an intercalated structure by XRD and SEM analysis. They reported that the bionanocomposite containing 9% by mass of Cloisite15A delayed the controlled drug release [51].

It is known that hydrogels as new drug delivery systems have been used extensively in controlled drug release in recent years. Hydrogels provide control of drug release by showing swelling-shrinking behavior at different rates in different environments (temperature, pH, humidity, electric current, magnetic field, light, etc.) according to the cross-linker ratios in their content. Similarly, in nanoparticle synthesis, hydrogels are used for controlled size adjustment and stabilization of the produced nanometal particles [52]. Wang et al. synthesized a series of polymeric materials containing different amounts of attapulgite clay from the material they prepared with pH-sensitive chitosan, acrylic acid, attapulgite, and sodium alginate. Diclofenac sodium active drug substance was used to examine the controlled drug loading and release kinetics of the prepared materials. They reported that the material released 100% drug in basic medium and 3.76% in acidic medium [53]. Dinu et al. prepared chitosan/clinoptilolite biocomposite cryogels containing clinoptilolite clay in different proportions and investigated their drug release properties. SEM photographs revealed that the pores became smaller and the pore walls thickened as the clay content in the biocomposite increased. They reported that the swelling behavior of the cryogels they obtained and the drug release properties showed parallelism [54]. Luo and Mills used halloysite clay to strengthen chitosan hydrogels and prepared a biocompatible and biodegradable drug delivery system. They loaded gentamicin on both halloysite clay and chitosan-halloysite hydrogel. They observed that drug-loaded chitosan-halloysite hydrogels released drugs more slowly than drug-loaded halloysite hydrogels. They stated that as the rate of chitosan increased, long and effective drug release occurred over time [55]. Hua et al. synthesized chitosan/ofloxacin/MMT nanocomposite hydrogels using ofloxacin, a synthetic antibiotic, using sodium tripolyphosphate as the anionic cross-linker. In the drug delivery system, while the chitosan beads deteriorated within 3 hours at pH=1.2, it was determined as a result of the analysis that the synthesized

nanocomposite hydrogel degraded in 12 hours [55]. Ma et al. synthesized pH and temperature-sensitive carboxymethyl chitosan-g-poly(N, N-dimethyl acrylamide) polymers. They used vitamin B2 as a model drug and aimed to achieve intestinal-targeted controlled release of the developed drug-loaded hydrogels. Hydrogel beads were prepared by $Ca^{2+}$ ionic crosslinking in an acidic solution and a double crosslinked network structure was obtained. The morphological features of the obtained products were also characterized. It was determined that the synthesized hydrogels performed an effective controlled release under gastrointestinal system conditions [56]. Aycan and Alemdar used hydroxyapatite-natural bone powder to increase the thermal and mechanical strength of chitosan hydrogels. They investigated the controlled and effective release of the drug under different physical conditions of the stomach and intestinal environment by loading the active ingredient of amoxicillin, which is used in the treatment of gastric ulcers, into hydrogels [57]. Yücel et al. prepared chitosan nanoparticles with quercetin, which is one of the polyphenolic compounds with antioxidant properties, and conducted in vitro and release studies. They observed that the quercetin release of nanoparticles could be sustained for 24 hours [58]. İnal investigated the release of indomethacin drug by synthesizing chitosan/κ-carrageenan/chitosan trilayer microspheres. Controlled release studies were performed in pH 1.2 and 7.4 buffers and characterized by FTIR, SEM, and DSC. The drug entrapment efficiency of microspheres and the equilibrium swelling degree were determined by the particle size and controlled release data. He stated that the newly obtained system is a suitable controlled release system for drugs that cause stomach irritation [4]. Ulu A. synthesized allantoin-loaded chitosan nanoparticles and investigated the effect of chitosan molecular weights (low, medium, and high) on drug release. He proved that allantoin-loaded nanoparticles are affected by the molecular weight of chitosan by morphology, size, zeta potential, and loading efficiency methods. From the in vitro release results, it was observed that nanoparticles synthesized with chitosan with the lowest molecular weight were more effective in drug release [59].

Hospital infections significantly affect the success of implant materials placed in the body. Önder S. conducted a controlled release study of gentamicin, an antibiotic type, on a chitosan/titanium system that can be used to prevent infections. He first immobilized the antibiotic-laden chitosan onto titanium surfaces, and then exposed it to drying conditions (air and freeze-drying). It was observed that the release of antibiotics was higher on the freeze-drying surfaces. From the cell proliferation tests, it was observed that bone cells proliferated more in the chitosan/titanium system compared to the flat chitosan-coated surfaces [60]. In order to prevent infections related to implants and increase tissue interaction, studies are also carried out on the functionalization of implant surfaces and their drug release. Erşan and Önder S., in another of their studies, synthesized and characterized chitosan microspheres that can be used as bone filling material and drug delivery system, and determined the performance of microspheres in vitro. Microspheres were produced by the emulsion cross-linking method and used antibiotic ciprofloxacin as a drug. They tested the bioactivity of the microspheres in artificial body fluid and found that chitosan microspheres from the bioactivity tests had the potential to increase osteointegration. They stated that these spheres could be used as a filling material that can release biomolecules locally in bone tissue damage and can be used in surface modification of implant materials [38].

In drug delivery systems, functionalized, superparamagnetic magnetite ($Fe_3O_4$), and maghemite ($\gamma$-$Fe_2O_3$) nanoparticles enable the drug to reach the desired target cells with the effect of an externally applied magnetic field. Thus, the side effects

of the drug are minimized. The surfaces of these nanoparticles, which are used as carrier systems, are usually functionalized with drugs, proteins, and genetic materials; and with these particles, the therapeutic agent is released at the targeted site [61]. Magnetite and maghemite nanoparticles are of great importance for biomedical applications because they are biocompatible and show low levels of toxicity [62]. Most of the applications in this field are biological investigations used to the orientation of the nanoparticle with the help of an externally applied magnetic field. Such materials, prepared by embedding ferromagnetic particles into the gel, are placed inside the body, the magnetic field is activated by a device used to provide a magnetic field, and the drug in the gel begins to be released [63]. Mahdavinia et al. synthesized magnetic and pH-sensitive hydrogel spheres obtained from carrageenan and carboxymethyl chitosan for a drug delivery system. Magnetic $Fe_3O_4$ nanoparticles were added to the biopolymer mixture by in situ polymerization method. The structural properties of the hydrogel spheres were characterized by TEM, SEM, XRD, and VSM techniques. The pH-dependent swelling behavior of hydrogels was investigated in various buffer solutions, and the swelling capacity of magnetic hydrogels was affected by the incorporation of magnetite nanoparticles into the carrageenan/chitosan complexes. The water absorption capacity of hydrogels decreased with increasing magnetite content. In the study, methotrexate, an anticancer drug was loaded into hydrogels as a model drug, and its release profiles were investigated at pH 7.4 and 5.3. Methotrexate encapsulation efficiency increased with increasing magnetite and chitosan content [64]. Long et al. synthesized chitosan/carrageenan/$Fe_3O_4$ nanoparticles, adsorbed bovine serum albumin into them, and studied the release behavior of protein from the nanoparticles [65]. Wang et al. prepared magnetic chitosan-5-fluorouracil nanoparticles for the target drug delivery system. The results showed that the loading capacity was 13.4%, and the percentage of release in phosphate buffer solution (pH=7.2) was 68% at 30 hours [66].

## 4. Other applications of Chitosan-based nanocomposites

Significant attention has been paid to many kinds of biomedical fields because of Chitosan-based nanomaterials due to their special chemical properties, including desired biodegradability, compatibility, and nontoxicity. Chitosan is a convenient biomaterial to construct extracellular tissue matrixes in tissue engineering [67]. Chitosan can be used as a carrier for drug delivery and also for different types of therapeutic molecules such as genes, drugs, and proteins [68]. It is greatly used as a carrier in delivering active agents and drugs [69], in gene and cancer therapy [70], and also in biosensor monitoring and bioimaging [71, 72]. Chitosan behaves like an anti-plaque agent and can intervene with all microorganisms while performing antibacterial activities in dentistry [73]. Chitosan is more generally used in wound dressing as an antimicrobial and antifungal agent because of its perfect tissue adhesive features (**Figure 3**).

### 4.1 Wound healing applications of Chitosan-based nanocomposites

Polymer nanocomposites are described as sophisticated materials, which carry nanoparticles. They can also be presented as core-shell nanoparticles. Chitosan produced an amino group that can be operationalized further to be reconciled for a great variety of applications. Antimicrobial chitosan nanocomposites are also attractive in food preservation as well as in medical fields. The downside of chitosan

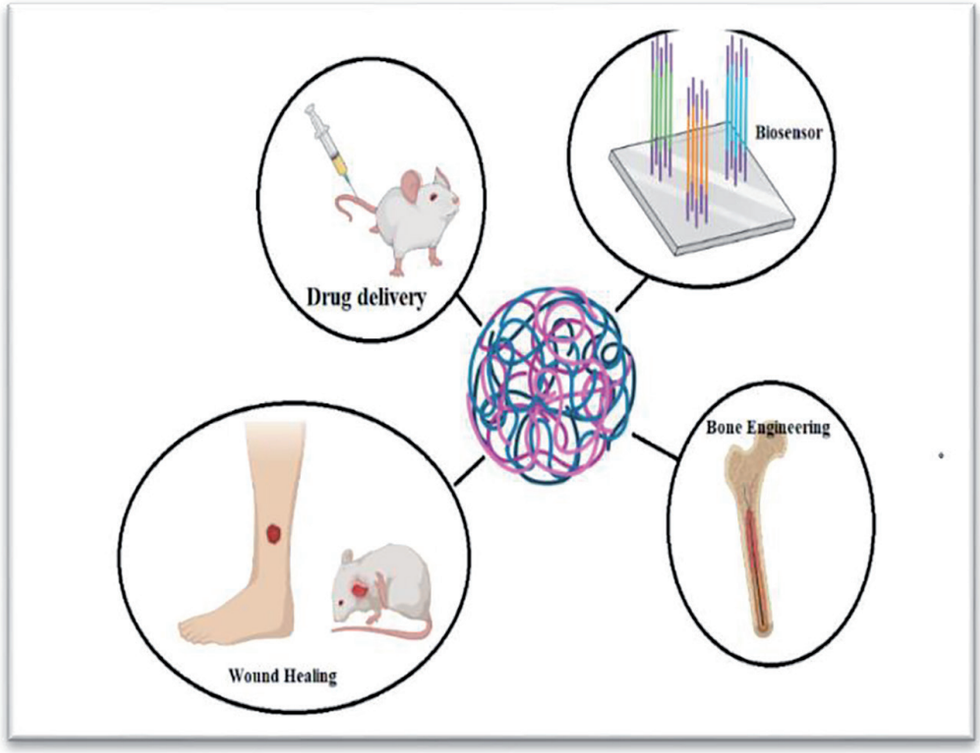


**Figure 3.**
*Schematic Representation of Chitosan-based nanocomposites in biomedical applications (https://biorender.com/).*

is its solubility being in only an acidic solution. This is solved by using chitosan as a derivative of lactose to form chitilac [74, 75]. Burst release is less effective than the controlled release of drugs through nano carrier matrix-like nanofibers, membranes, spheres, capsules, tubes, etc.

The antimicrobial activity of chitosan nanocomposite [76] was utilized by Youssef. The effect of Montmorillonite-chitosan-silver sulfadiazine nanocomposites on skin lesions was also prepared and utilized by Sandri. Montmorillonite has hemostatic and absorbent features and it is used in the healing of lesions and ulcers [77, 78].

Wound healing is not the simple programmed structure of cellular and molecular developments including swelling, cell immigration, angiogenesis, temporal matrix syntheses, collagen deposition, and re-epithelization. Mostly, an effective wound dressing has the subsequent properties: (1) an appropriate water vapor transmission rate (WVTR), which produces a humid environment on the wound beds, without risking dehydration or exudates accumulation; (2) adequate gas penetrability for oxygen-requiring reparative methods; (3) a great level of fluid absorption ability to get rid of too many exudates, which cover nutrients for bacteria from the wound beds; (4) a good blockade trans the distribution of contagion producing microorganisms; (5) activity of antibacterial to overwhelm bacteria growth lower the dressing; and (6) the lack of any cytotoxic effects in the event of side damage to the neonatal tissues. Thus far numerous wound dressing materials types have been stated; however, they have some severe faults such as low water vapor/gas transmission rate, the capability of poor fluid absorption, and low flexible strength. So, we choose chitosan as a dressing material because of its biocompatibility [79–81] biodegradability [82], hemostatic activity [83, 84], the activity of anti-inflectional [85, 86], and property to accelerate wound healing [87–89]. The N-acetyl glucosamine (NAG) current in chitin and chitosan is a chief element of dermal tissue that is vital for the repair of scar tissue

[90, 91]. Its positive surface charge permits it to effectively support cell growth [92] and helps surface stimulated blood clotting and blood coagulation [93].

### 4.2 Tissue engineering applications of chitosan-based nanocomposites

3-D porous scaffolds for bone tissue studies should be biocompatible and let osteogenesis. Various techniques have been enlarged to imitate natural mineralized materials' properties and microstructure. Yet, large-size bulk materials' simple and fast fabrication with the content of high calcium under environmental aspects remains a big difficulty. Throughout gelation, a manageable inorganic gradient supply formula, accompanied by mineralization, where urea was used, and naturally hierarchically well-ordered hydrogel microstructures were shaped. This construction route takes a couple of hours to complete the gelation and processes of mineralization [94].

Composite scaffolds have been fabricated which use a coaxial electrospinning method to prepare gelatin-chitosan core-shell structured nanofibers. An arginine-glycine-aspartic acid (RGD)-like structure was shaped to imitate the structural component of the extracellular matrix of natural bone. After, by a wet chemical technique, hydroxyapatite was deposited on the prepared-shell structured nanofibers surface. Hydroxyapatite is the key mineral component of natural bone. Gelatin-chitosan core-shell structured nanofibers enhanced the mineralization productivity of hydroxyapatite compared to chitosan nanofibers [95]. Biologically-active scaffolds design is focused on cell-adhesive protein applications or bioceramic nanoparticles to produce a cell-sensitive surface. Hence, trace metals found in the alive organisms were used to prepare hydrogels of biocompatible chitosan. These were modified by copper (II) ions through complexation connections and generated a fewer constant cytocompatible to more constant cytotoxic structure for a copper-chitosan system [96, 97].

### 4.3 Cancer therapy applications of chitosan-based nanocomposites

Carcinoma involves the cell's uncontrolled proliferation. Operation and chemotherapy frequently are combined as a full strategy to defeat the tumor [98] Chemotherapy is a systemic treatment with the benefit of reducing lasting potential metastatic lesions after the operation. It has the benefit to treat numerous wounds contemporaneously, on the other hand, it is also apparent because of the systemic side effects, which may affect healthy tissue [99]. Numerous optimized chemotherapy strategies have been developed to solve the above-referred problems. Nanomaterials have been designed to target particular tissues or react to specific environmental conditions. CS is mostly developed to antitumor nanovesicles for the carcinoma behavior due to its matchless properties such as mucoadhesiveness and structural changefulness [100].

There are various articles about chitosan-based systems for antitumor drug delivery in advanced methods [101, 102]. Effective drug delivery systems are enhanced for therapy of anti-cancer based on environmental responsibility and directing principles to convey drugs, vaccines, etc. Furthermore, the drug delivery vectors were also designed with a mixture of photodynamic and hyperthermia cure (**Figure 4**) [103, 104].

Numerous healing anticancer drugs are limited in their scientific applications due to their toxicities and are not high solubility in aqueous media [105–107]. For example, doxorubicin (DOX) is one of the greatest commonly used drugs in cancer therapy. Yet, it can cause side reactions such as cardiotoxicity and drug resistance. Also, it is hard to manage intravenously due to its not high solubility in aqueous media.

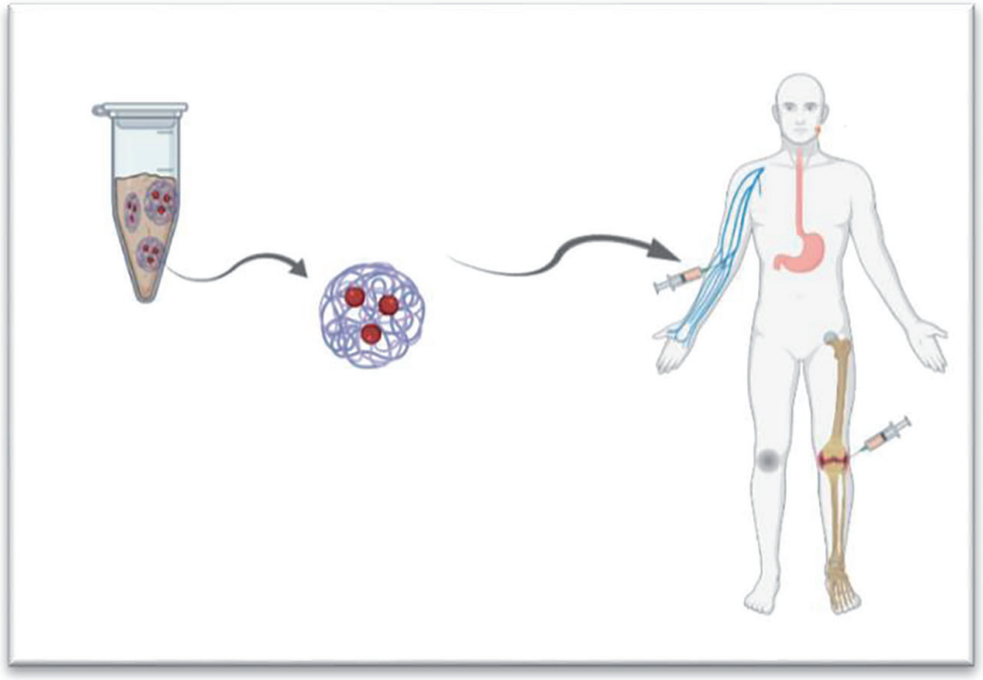

**Figure 4.**
*Drug delivery (https://biorender.com/).*

Nanomaterial-based drug delivery systems have received attention in overwhelming this downside. These systems can be made from several organic and inorganic materials including nondegradable and biodegradable polymers and inorganic nanocrystals. Polymeric micelles based on amphiphilic block copolymers have the benefits of great biocompatibility and capacity of drug-loading with less toxicity since they can self-assemble into polymeric micelles in aqueous media [108–110]. The mass in tumors through an improved penetration and retention (EPR) effect compared to single minor molecules, principal to special spatial distribution in the tumor. Nevertheless, the drug release performance of polymeric micelles is hard to control; they spontaneously release the drug before reaching tumors, which could give increased undesirable side effects and less therapeutic efficacy [111]. Well-designed drug delivery systems need to be advanced to enable cancer chemotherapy, which basically improves therapeutic efficacy by reducing drug release in unwanted sites. With these systems, a particular drug concentration can be delivered to tumors to diminish side effects. Drug delivery systems can be designed to release drugs stimulated by environmental parameters such as pH, enzymes, and temperature [112–114].

### 4.4 Gene delivery applications of chitosan-based nanocomposites

Gene therapy holds promise for challenging disease therapy such as innate genetic diseases [115], infections [116], and cancer [117]. A serious step for gene therapy is the gene's successful delivery. Naked nucleic acids cannot cross the cell membrane and are simply reduced by serum nucleases. Therefore, safe and efficient gene delivery systems are crucial for the accomplished application of gene therapy. Safety concerns about immunogenicity and toxicity are the key difficulties for the common use of viral systems [118]. Amongst virus-related vectors, adeno-associated virus (AAV) is now the most common gene delivery vector since it seldom includes the host genome, however, aggregate can be sensed [119, 120]. No viral vectors are good options for gene delivery

because of their no genotoxicity, scalable manufacture, and structural elasticity [121]. They have been used in studies related to RNAi [122], gene editing [123], and CAR-T cell treatment [124]. No viral vectors are used more regularly in cancer treatment than in other uses for cancer and are a key worldwide public health matter [125]. Chitosan and its derivatives are commonly used to formulate gene delivery vectors. Various studies have been conducted to theory gene vectors based on chitosan. This assessment will argue the present development of policies encouraging the restrictions and chitosan applications in gene delivery systems and upcoming forecasts [126].

Gene therapy has developed very promptly due to its incredible healing possible to treat various genetic diseases by the inclusion of new genes (DNA and RNA) into target cells with transgene expression. Yet, the in vivo delivery of naked healing genes is unrealistic and tense with difficulties, including the gene susceptibility degradation by nucleases in the plasma, non-specificity to directed cells, in addition to the incapability of the negative genes from incoming negative cellular membranes. Certainly, with nearly 2600 gene therapeutics having finalized or ongoing medical trials, about six genes healing has received confirmation in the west [127, 128]. It is approved that the administration of the naked gene either systemically or locally is not effective, because of the concerns mentioned above. Consequently, the simple difficulties for gene treatment is the safe development and efficient gene transfection vector (**Figure 5**) [129].

### 4.5 Bio-imaging applications

Chitosan is a naturally happening amino-polysaccharide, with tempting physiochemical and biological features, gotten from the deacetylation of chitin, the second-highest biopolymer contemporary in the world afterward cellulose. The amino and hydroxyl groups current on the skeleton of chitosan deliver a route for reaction with

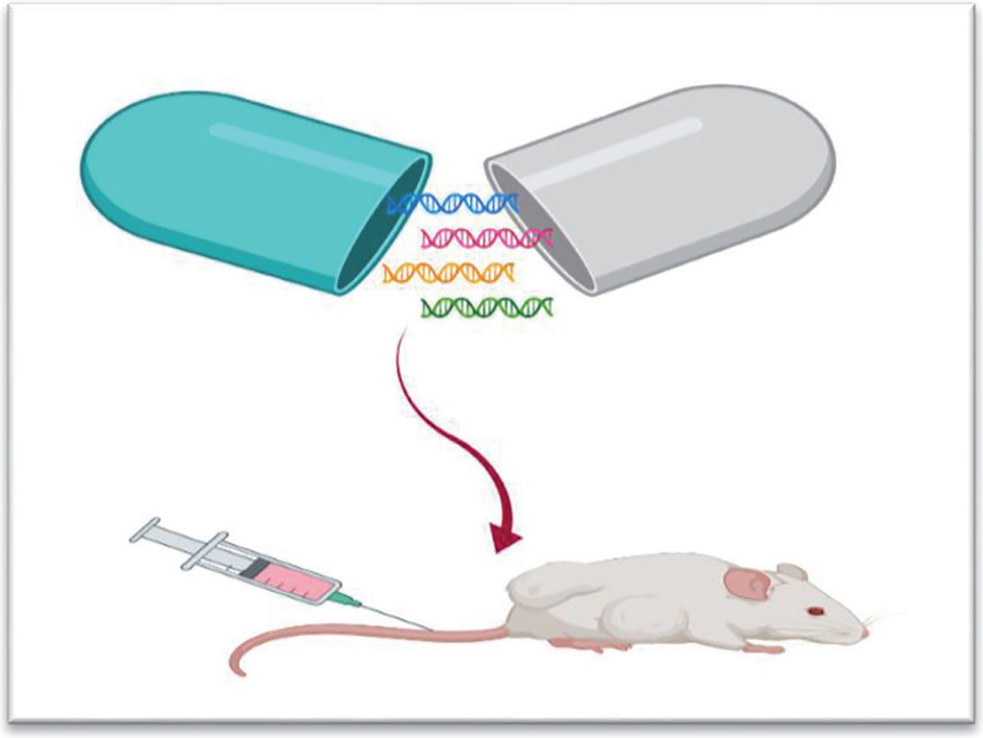

**Figure 5.**
*Gene delivery in the cancer therapy (https://biorender.com/).*

organic practical molecules [130]. Moreover, chitosan is used as a biomaterial due to its outstanding biodegradability and low toxicity. Also used as bio imaging agent [131]. The introduction of imaging agents in chitosan permitted its use for bioimaging. Such as the integration of imaging agents, for example, $Fe_3O_4$ figure magnetic resonance imaging; the self-assembled nanoparticles improve the perception of hepatocyte targeted imaging [132]. Chitosan decreased gold nanoparticles have performed as photo thermal-converter, photodynamic-treatment besides photodynamics carrier and thus figure in the bio-imaging implementation plus used to annihilate the breast cancer cells (MCF-7) [133]. Gold-covered $Fe_3O_4$ nanoparticles were made by chemical co-precipitation technique with an average size of 9.8 nm in diameter by the decrease of glucose and stabilized with chitosan in the face of formaldehyde as a crosslinking agent. The prepared nanomaterial performed as well substantial for bio establisher applications [134, 135].

## 4.6 Applications of biosensor

Biosensors are equipment to transform biological, physical, and chemical indications of biological schemes into electrical by classifying particular responses to aim analyses [136]. A blood glucose biosensor is a typical sample of a characteristic biosensor that uses the enzyme Glucose oxidase. Electrochemical biosensors particularly react with target moieties and produce an electrical signal linked to particular analyze concentrations, pH, and temperature [137]. Biosensors are a vital role in tissue engineering, and also chitosan nanocomposites are well worn in these kinds of applications. Generally, metal nanoparticles display higher conductivity and electronic features than transmission polymer, however, the flexibility of the polymer creates them matchless for numerous applications. The addition of transmission

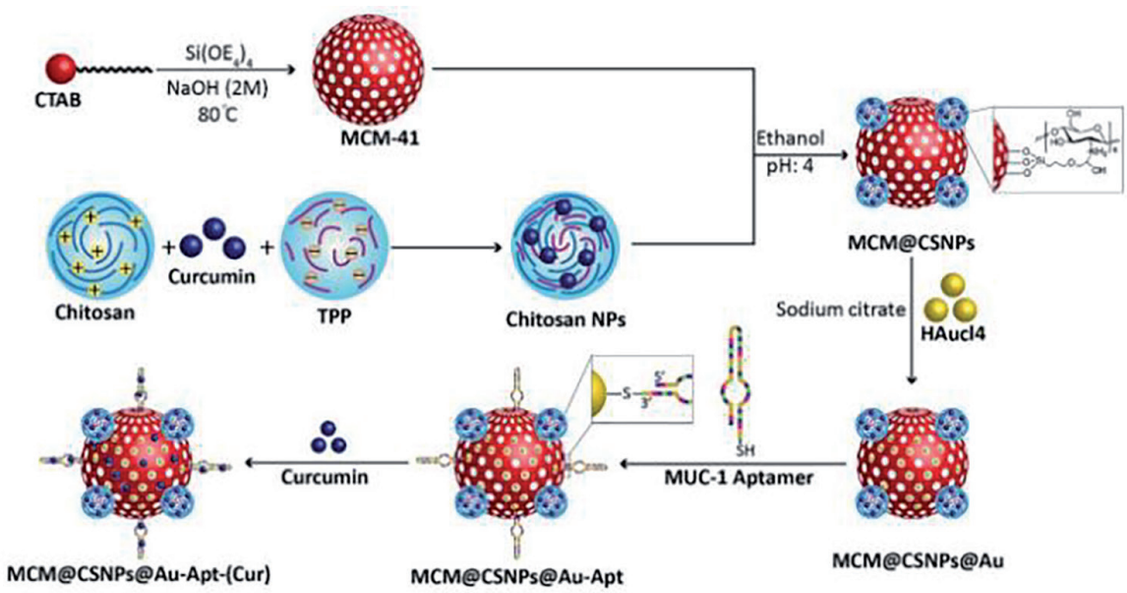


**Figure 6.**
*Stepwise schematic of the nanosystem synthesis: MCM-41 was prepared through a surfactant-assisted sol-gel method, then, the fabricated chitosan-curcumin nanoparticles (CSNPs) were crosslinked onto MCM-41 through TPP (MCM@CS). In the next step, the synthesized AuNPs were modified physically onto the MCM-41 surface as well as loaded into CSNPs networks (MCM@CS@Au), and MUC-1 aptamer as a targeted agent linked to AuNPs via sulfuric bonds (MCM@CS@Au-Apt); eventually curcumin was loaded into the final nanosystem (MCM@CS@Au-Apt (CUR)) [147] (Esmaeili Y, Khavani M, Bigham A, Sanati A, Bidram E, Shariati L, Zarrabi A, Jolfaie NA, Rafienia M. Mesoporous silica@chitosan@gold nanoparticles as "on/off" optical biosensor and pH-sensitive theranostic platform against cancer. Int J Biol Macromol. 2022 Mar 31;202:241–255).*

| Materials | References |
|---|---|
| Laccase-Based Biosensor Encapsulated in a Galactomannan-Chitosan Composite | [148] |
| Ti 3 C 2 T X MXene/Chitosan Nanocomposite-Based Amperometric Biosensor | [149] |
| Glucose oxidase-chitosan immobilized paper biosensor | [150] |
| Diffraction-based chitosan leaky waveguide (LW) biosensors | [151] |
| Carboxymethyl chitosan assembled piezoelectric biosensor | [152] |
| Au nanoparticles/MoS 2 nanosheets—Chitosan modified screen-printed electrode as chlorpyrifos biosensor | [153] |
| Temperature-sensitive poly(N-isopropylacrylamide)-chitosan hydrogel for fluorescence sensors | [154] |

**Table 1.**
*Chitosan-based biosensor applications.*

nanoparticles to chitosan materials increases the nanocomposite electrical conductivity on top of stimuli-responsive features that could be used for sensing biological types [138]. For particularly improving the sensitivity to biological moieties, biosensor faces have been changed using enzymes similar to cholesterol esterase [139] and cholesterol oxidase [140] to determine cholesterol substance in the blood or human serums. As it is seen chitosan nanocomposites-based biosensors are higher efficient, sensitive, and durable in comparison to pure chitosan [138]. A lot of nanostructured inorganic materials for instance cuprous oxide nanoparticles [141] and $Fe_3O_4$ nanoparticles [142]. $NiFe_2O_4$ nanoparticle [143], Cerium oxide nanoparticle [144], and $TiO_2$ nanoparticles [145] are often used to increase the electronic features, moreover, the chitosan electrical conductivity-based materials in nanocomposites [146].

In another study, Esmaelili *et al.* developed a multifunctional nanosystem of mesoporous silica@chitosan@gold-aptamer (MCM@CS@Au-Apt) encapsulated curcumin for MUC-1 positive tumor cells targeting and drug delivery (**Figure 6**). They found that MCM@CS@Au has "on/off" fluorescence bio-sensing ability with selective performance against MUC-1 positive tumor cells

**Table 1** showed chitosan-based biosensor applications in the recent years

## 5. Conclusion and future perspectives

Chitosan is a natural polymer and has many advantages including nontoxicity, biocompatibility, and biodegradability, and it can be applied in many fields, especially in medicine. The importance of chitosan-based bionanocomposites is skyrocketing due to their numerous advantages. Because the use and potential of chitosan-based bionanocomposite expanding in a variety of industries, this paper provides a thorough examination of these issues. The negative effects of non-biodegradable materials on humans and the environment have sparked interest in biodegradable materials among scientists and environmentalists. As a result of its intrinsic qualities such as biocompatibility, biodegradability, and nontoxicity, as well as their improved structural and functional aspects, bionanocomposites have become a focus of considerable research. Bionanocomposites based on chitosan are the most popular at the moment. They have a lot of great features and advantages. This chapter primarily focuses on the properties of chitosan, and applications of chitosan-based nanocomposites. In summary,

nanocomposites based on chitosan have great potential for research and development of new nanomaterials in the future.

## Scope of the review and its significance

Recent research on chitosan-based nanocomposites for biomedical applications is based on the field's expanding understanding of chitosan properties and chemical/physical alteration. This chapter discussed the current understanding of the effect of chitosan-based nanocomposites in medical studies.

## Translational relevance

Usage of polymer-based nanomaterials has been analyzed for their varied application and the most important of these is the use of chitosan-based polymeric nanomaterial in the medical field. Studies confirm that the use of chitosan-based materials in the clinic is increasing day by day (https://clinicaltrials.gov).

## Clinical relevance

In the chapter, the chitosan-based nanocomposites and their biomedical applications in the biomedical field involving drug delivery systems and other applications have been addressed highlighting the recent advancements.

## Author disclosure and ghostwriting

There are no competing interests to declare. No ghostwriters were used to write this article.

## Abbreviations

| | |
|---|---|
| DD | Deacetylation degree |
| Mw | Molecular Weight |
| MMT | Montmorillonite |
| organoMMT | Cloisite30B/15A |
| FTIR | Fourier-transform infrared spectroscopy |
| XRD | X-ray Diffraction Analysis |
| SEM | Scanning Electron Microscopes |
| DSC | Differential scanning calorimetry |
| WVTR | Water vapor transmission rate |
| NAG | N-acetyl glucosamine |
| DOX | Doxorubicin |
| EPR | Penetration and retention effect |
| AAV | Adeno-associated virus |

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