

# Chapter 14

## Chitin/Chitosan: Versatile Ecological, Industrial, and Biomedical Applications



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**Abstract** Chitin is a linear polysaccharide of N-acetylglucosamine, which is highly abundant in nature and mainly produced by marine crustaceans. Chitosan is obtained by hydrolytic deacetylation. Both polysaccharides are renewable resources, simply and cost-effectively extracted from waste material of fish industry, mainly crab and shrimp shells. Research over the past five decades has revealed that chitosan, in particular, possesses unique and useful characteristics such as chemical versatility, polyelectrolyte properties, gel- and film-forming ability, high adsorption capacity, antimicrobial and antioxidative properties, low toxicity, and biocompatibility and biodegradability features. A plethora of chemical chitosan derivatives have been synthesized yielding improved materials with suggested or effective applications in water treatment, biosensor engineering, agriculture, food processing and storage, textile additives, cosmetics fabrication, and in veterinary and human medicine. The number of studies in this research field has exploded particularly during the last two decades. Here, we review recent advances in utilizing chitosan and chitosan derivatives in different technical, agricultural, and biomedical fields.

### 14.1 Introduction

Chitosan, a polymer of  $\beta(1-4)$ -linked glucosamine (2-amino-2-deoxy-O-glucose) units, is a biopolymer with unique characteristics due to the presence of free amino groups on its backbone. It is obtained by partial deacetylation of chitin, which is found in the cell walls of unicellular and filamentous fungi and in

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541

extracellular matrices and skeletal deposits of many protozoan and metazoan organisms including algae, choanoflagellates, sponges, corals, cephalopods, and arthropods. Commercially, chitin is extracted from the waste shells of marine crustaceans such as shrimp and crab. A significant proportion is used to produce chitosan, which, in contrast to chitin, is soluble in water at a slightly acidic pH and is easy to modify chemically to increase solubility at neutral pH and to add new functionalities. Chitosan and its derivatives have many desirable properties such as antioxidative and antimicrobial effects, mucoadhesiveness, biodegradability, and biocompatibility and can be manufactured in various formulations including hydrogels, films, membranes, porous sponges, nanoparticles, and nanofibers. Moreover, chitosan is considered a harmless compound, as it has received the generally recognized as safe (GRAS) status by the US Food and Drug Administration (FDA), and it has been approved as a food additive in several Asian countries (No et al. 2007). In the European Union, chitosan is registered as a basic substance, and the use of chitosan hydrochloride is considered by the European Food Safety Authority (EFSA) as having neither harmful effects on human or animal health nor any negative effects on the environment (European Commission 2014). Therefore, chitosan-based materials have been adopted worldwide in numerous applications in water treatment; food, cosmetic, and textile industry; biosensor engineering; plant protection; pharmaceutical industry; and regenerative medicine. They are used as flocculants, ion exchangers, chelating agents, coating materials, drug carriers, and scaffolds for tissue engineering. During the past years, many companies have started to develop chitosan-based products, and some have already successfully launched them for commercial purposes. This review is intended to summarize recent developments in the use of chitosan-based materials for potential and effective applications in different technical, environmental, agricultural, and biomedical fields.

## 14.2 Chitosan-Based Flocculants and Hydrogels Used in Water Treatment

Pollutants in water, industrial wastewater, and reclaimed wastewater for crop irrigation have presented severe environmental and medical problems all over the world. Such contaminants include various heavy metal ions (copper, cobalt, manganese, chromium, mercury, lead, arsenic, cadmium, and nickel), dyes (mainly azo dyes like malachite green, methyl violet, or methylene blue), oil spills, and a variety of pharmaceuticals and endocrine-disrupting compounds. Among the various methods used as remedial measures to treat polluted water and wastewater, the potential of chitosan-based composites as efficient adsorbent, flocculating and chelating agents has been widely investigated.

The presence of free hydroxyls and amino groups in many structural forms of chitosan-derived composites facilitates adsorption of pollutants such as dyes, metals, and organic compounds. Chitosan derivatives like carboxymethyl chitosan and graft

polymerization are a prevalent strategy to add a variety of functional groups to the composite. Magnetic particles are embedded usually as nanoparticles in the complex core to facilitate regeneration and reuse of adsorbent composites by applying external magnetic field.

### 14.2.1 Removal of Heavy Metal Ions

A large number of chitosan-based composites were investigated for removal of metal ions from aqueous solutions. They include chitosan-polymer macromolecular complexes (as cellulose, cellulosic matrix like cotton fibers, alginate, polyvinyl alcohol, polyvinyl chloride), chitosan ceramics, as well as clay and silicate composites (bentonite, montmorillonite, perlite, and zeolite) (Wan Ngah et al. 2011). Due to the vast number of scientific publications on chitosan-based adsorption that have been published, only a representative sample is depicted for Cr(VI) and Cu(II). Cognate composites were devised as adsorbents of other metal ions (Cd, As, Fe, Pb, Co, Pb, Hg, Ni, Zn, U) that can be found in the detailed reviews of Reddy and Lee (2013), Liu and Bai (2014), Wang and Chen (2014), Kyzas and Bikiaris (2015), Salehi et al. (2016), and Wang and Wang (2016).

**Chromium (VI)** The mutagenic and carcinogenic Cr(VI) is considered as a dangerous pollutant for humans and marine ecosystems. Composites of chitin and chitosan nano-hydroxyapatite hybrids removed Cr(VI) from aqueous solution by electrostatic interactions and reduction to Cr(III) via electron-donating groups present in the scaffold (Kousalya et al. 2010). A nanocomposite cross-linked hybrid of chitosan-alginate was able to remove Cr(VI) from water waste (Gokila et al. 2017). A more complex scaffold resin, where chitosan was mixed with magnetic particles ( $\text{Fe}_3\text{O}_4$ ), modified by ethylenediamine and stabilized by glutaraldehyde as cross-linker, was established as an effective adsorbent of Cr(VI) (Hu et al. 2011). Reducing toxic Cr(VI) to nontoxic Cr(III) was accomplished by zero-valent iron [Fe(0)] embedded in chitosan beads (Geng et al. 2009). The oxidized iron Fe(III) formed a precipitately complex with Cr(III), thus enabling the regeneration of the adsorbing complex. Another method used ceramic aluminum coated with chitosan to remove Cr(VI) by electrostatic attraction of the hydrogen chromate ions to the positively charged amino groups of chitosan (Boddu et al. 2003).

**Copper(II)** Like chromium,  $\text{Cu}^{2+}$  ions found particularly in industrial wastewater are hazardous to human health and the ecosystems. Ingenious absorbance methods using a variety of organic and inorganic compounds have been devised to adsorb and remove the toxic ions. Among them are promising measures based on chitosan composite supra-macromolecular structures. Chitosan-based composites with various organic and inorganic compounds were examined as Cu(II) adsorbents. A recyclable complex composed of L-arginine-chitosan- $\text{Fe}_3\text{O}_4$  for removal of Cu (II) ions (Wu et al. 2016) and magnetic cellulose-chitosan composite microspheres

was capable to adsorb heavy metals like Cu(II) but also Cd(II) and Pb(II) from aqueous solutions (Peng et al. 2014). Chitosan-algal biomass composite microbeads (Sargin et al. 2016b), a binary chitosan/silk fibroin composite (Ramya and Sudha 2013), and cotton fibers functionalized by triethylenetetramine (TETA) and carboxymethyl chitosan form composites and hybrids for adsorption of Cu (II) from water (Niu et al. 2017). Microcapsules composed of phytopathogenic (*Ustilago* sp.) fungal spores immobilized in cross-linked chitosan matrix (Sargin et al. 2016a) and a binary complex of chitosan and emu egg shells (Anantha and Kota 2016) were shown to remove copper ions from aqueous solutions.

Chitosan complexed with clays, ceramic minerals, and carbon-based materials was used to enhance absorbance of heavy metals from aqueous solutions. A nanocomposite that consisted of chitosan-montmorillonite (Pereira et al. 2013) and silica gel/chitin and chitosan with nano-hydroxyapatite was used as adsorbents for Cu(II) (Rajiv Gandhi et al. 2011). Nanocomposites containing chitosan-poly (vinyl alcohol)-attapulgitite were also used for removal of Cu(II) from aqueous solutions (Wang and Wang 2016). Furthermore, a recyclable magnetic microsphere composed of cross-linked chitosan-rectorite (a clay mineral) and  $\text{Fe}_3\text{O}_4$  was studied for adsorption of Cu(II) and Cd(II) (Xie et al. 2015), and chitosan-zeolite composite hydrogel beads were examined for Cu(II) sorption (Djelad et al. 2016).

A particular interesting recyclable composite with chelating capacity consists of core magnetic ( $\text{Fe}_3\text{O}_4$ )-silica particles combined with cross-linked chitosan. Its porous and highly specific surface area contributed by activated carbon carrier showed an excellent adsorption capability for  $\text{Cu}^{2+}$  ions (Li et al. 2017). A recyclable nanocomposite with a core xanthated  $\text{Fe}_3\text{O}_4$  chitosan grafted on graphene oxide introduced sulfur groups to the composite using carbon disulfide (Liu et al. 2016a).

Other sorbent composites that were prepared and studied are a recyclable composites containing chitosan grafted on a core of  $\text{Fe}_3\text{O}_4$ -hexadecyl trimethoxysilane (Liu et al. 2016b), a flocculant composed of poly(acrylic acid) grafted on chitosan (Saleh et al. 2017) or beads containing chitosan-poly(vinyl alcohol) and ZnO (Xu et al. 2017a). A sophisticated composite was prepared by using magnetic nanoparticles on the surface of polystyrene as core, coated with chitosan cross-linked by glutaraldehyde followed by grafting polyethylenimine on the complex surface (Xiao et al. 2017). This submicron composite is recyclable and exhibits good adsorption capacity for Cu(II) ions.

Highly selective adsorption of copper ions from aqueous solutions was achieved by the ion-imprinting polymer method (Kong et al. 2017). Microspheres of magnetic cores of  $\text{Fe}_3\text{O}_4$  with a shell of cross-linked chitosan and graphene oxide were used to imprint  $\text{Cu}^{2+}$  ions. Zarghami et al. (2014) prepared Cu(II) ion-imprinted membranes composed of cross-linked chitosan/poly(vinyl alcohol) for adsorption of the metal from aqueous solutions. A similar ion-imprinted technique was reported for selective adsorption of Pb(II) from a recycling wastewater unit (Hande et al. 2016).

## 14.2.2 Removal of Man-Made Environmental Pollutants

### 14.2.2.1 Industrial Dyes

Textile, leather, paper, and food industries discharge a plethora of environmental pollutants such as synthetic dyes. A variety of chitosan-based composites was examined as promising adsorbents of hard to remove industrial dyes. Chitosan per se contains functional groups for interaction with pollutants including dyes. Adding more functional groups by modifying chitosan (cross-linking of chitosan layers, direct chemical modification, or graft polymerization – see Chapter 3) improves adsorption capability. Molecular imprinting technique was devised as selective adsorbent of pollutants. Composites' core of iron oxide magnetic nanoparticles like maghemite ( $\gamma\text{-Fe}_2\text{O}_3$ ) and magnetite ( $\text{Fe}_3\text{O}_4$ ) offers a way to recover the adsorbent scaffolds for reuse. Again, since the published articles are enormous in number, only essential parameters and basic blocks of adsorbing chitosan-based composites are included.

Methyl orange as a model anionic azo dye was adsorbed by films of cross-linked chitosan/nanonized maghemite from aqueous solution (Jiang et al. 2012). Improved adsorption of the same anionic dye was achieved by preparing a magnetic chitosan grafted with multi-walled carbon nanotubes (Zhu et al. 2010), and magnetic chitosan grafted with graphite oxide nanocomposite was able to adsorb the toxic azo dye, Reactive Black 5 (Travlou et al. 2013). Chitosan modified by ethylenediamine (Zhou et al. 2011) or polyaniline (Abbasian et al. 2017) grafting was able to adsorb other anionic azo dyes like Orange 7, Acid Orange 10 acid and red 4 and direct red 23, respectively. A magnetic complex of chitosan and zirconium oxide was a potent adsorbent of food anionic azo dyes like amaranth and tetrazine (Jiang et al. 2013a). Moreover, a complex composite adsorbent was prepared by grafting chitosan with poly[poly(ethylene glycol) methyl ether methacrylate] (Tsai et al. 2017). The functionalized groups added to chitosan contributed to improved removal of the azo dye Reactive Orange 16 from water.

Recyclable composite microspheres composed of cross-linked chitosan grafted with glutamic acid and having a core of  $\text{Fe}_3\text{O}_4$  nanoparticles coated with silica adsorb cationic dyes like methylene blue, crystal violet, and light yellow 7GL (Yan et al. 2013). Similarly, an amphiphilic *N*-benzyl-*O*-carboxymethyl chitosan composite with a core of iron oxide nanoparticles was prepared for adsorption of methylene blue, crystal violet, and malachite green (Debrassi et al. 2012). The cyclic oligosaccharide  $\beta$ -cyclodextrin ( $\beta$ -CD) was added to chitosan-based composites as it provides a hydrophobic inner cavity and a hydrophilic exterior. Magnetic chitosan- $\beta$ -CD with grafted graphene oxide to enlarge surface area exhibited an improved adsorption of methylene blue as a model dye from water (Fan et al. 2013). Molecular imprinting technique is of interest to selectively remove dyes from aqueous solutions. The molecule or ion used as templates will be subsequently removed, and a recognition site is generated. Alizarin red served as template molecule, and imprinted magnetic chitosan nanoparticles showed improved adsorption of the dye (Fan et al. 2012).

#### 14.2.2.2 Removal of Micropollutants (Pharmaceuticals, Endocrine Disruptors)

Pharmaceutical, endocrine-disrupting compounds and personal care products have become a new class of hazardous environmental pollutants (Grassi et al. 2013) and have emerged as an extensive global concern. They are discharged as municipal and hospital effluents, from manufacturing industries, and found in water, reclaimed wastewater, and even in crops irrigated by reclaimed water (Paltiel et al. 2016). Pharmaceuticals, endocrine disruptors, and personal care products and their chemical transformation derivatives are characterized as stable, persistent compounds that are biologically active at very low concentrations.

The challenging goal has been to completely remove the above micropollutants from wastewater following conventional cleaning methods. Laboratory research including adsorption by chitosan-based composites has been high on the agenda (Amouzgar and Salamatinia 2015). Zhang et al. (2014) used a rather simple cross-linked magnetic chitosan-Fe<sub>3</sub>O<sub>4</sub> composite to examine the sorption of three pharmaceutical compounds from contaminated water. The absorbance analysis showed effective sorption of diclofenac (a nonsteroidal anti-inflammatory drug) and clofibrac acid (an antilipemic agent) but not of carbamazepine (an antiepileptic medication). Pharmaceuticals in water can be present as cationic, anionic, and neutral forms at different pH values. Thus, Zhang et al. (2016) in a more recent study devised an innovative, more complex three-dimensional chitosan-based scaffold. A magnetic core of chitosan-Fe<sub>3</sub>O<sub>4</sub> was grafted with polymeric arms of either the polycation [poly(2-methyl acryoxyethyl trimethyl ammonium chloride)], the polyanion poly(acrylic acid), or the neutral polymer poly(methylmethacrylate). The polycationic extension was cost-effective in removal of diclofenac from water due to charge attraction (Zhang et al. 2016). Further, magnetic composite pellets with grafted clay (bentonite) and activated carbon were prepared to examine possible cost-effective removal of cationic and anionic pharmaceuticals (Arya and Philip 2016). The composite was effective as a sorbent for the beta-blocker (atenolol), the antibiotic (ciprofloxacin), and the lipid regulator (gemfibrozil).

A variety of chitosan composites have been tested for the removal of other drugs. Cross-linked chitosan grafted with sulfonate or *N*-(2-carboxymethyl) groups was used as a sorbent to remove the dopamine agonist pramipexole dihydrochloride from polluted water (Kyzas et al. 2013). Chitosan-poly(acrylic acid)-graphite oxide nanocomposite showed adsorption of dorsolamide, a carbonic anhydrase inhibitor for eye treatment (Kyzas et al. 2014). Adsorption of nonsteroidal anti-inflammatory drugs ibuprofen and ketoprofen was studied using porous composite beads prepared of Chitosan-MIL 101 (Cr) (Zhuo et al. 2017). Using the antiepileptic carbamazepine as template, the magnetic molecular imprinted technique, based on chitosan-Fe<sub>3</sub>O<sub>4</sub> nanoparticles, was applied for selective sorption of the drug (Zhang et al. 2013c).

Chlorophenols are endocrine-disrupting chemicals, used inter alia in manufacturing pharmaceuticals that are found in wastewaters (Sin et al. 2012). Excellent adsorbing capability was demonstrated using a cross-linked chitosan-salicylic

acid- $\beta$ -CD composite. Composites of chitosan- $\gamma$ -CD were capable of adsorbing the endocrine disruptors, polychlorophenols, and bisphenol A (Duri and Tran 2013). Composite films prepared by blending microporous carbon fibers with cross-linked chitosan/polyvinyl alcohol were examined as sorbents of bisphenol A from water (Bilgin Simsek et al. 2017).

Finally, Soares et al. (2017b) proposed an interesting and unusual concept of using low-cost magnetic chitosan-based scaffold for absorbing and removing oil spills following initial skimming from water. In addition, the composite, which had a core of magnetic nanoparticles with a shell of chitosan-silica hybrid, effectively adsorbs nonpolar organic solvents.

### 14.3 Biosensors

Biosensors are essentially analytical devices that convert biological reactions or interactions into measurable signals. Basically, the biosensors' constructs consist of a biological sensing element associated and intimately interfaced with a transducer that converts a signal in one form of energy to a signal of another form. Such signals should be proportional to the amount of analyte within a certain concentration range. Electrochemical biosensor devices, for example, possess advantages as being simple and relatively cheap while offering rapid detection and high sensitivity and further being amenable to miniaturization. Biosensors have been developed not only as analytical tools for medical purposes of clinical detection but also for applications in food industry and environmental monitoring.

Chitosan, and to a much lesser extent chitin, has several advantageous qualities in the design of biosensors. The polysaccharides are biocompatible, have functional groups pliable to chemical modification, and can be easily deposited on the surface of the transducer as adhesive thin films for the immobilization of recognition elements (enzymes, antibodies, DNA, whole cells, and cell organelles). Addition of carbon tubes, graphite, and graphene oxide to the composite increases electron transfer to the transducer and enhanced mechanical strength as well as water permeability and retention. Since there is a vast array of biosensors based on chitosan in their constructs, the following provide only representative devices.

Glucose detection and monitoring is of paramount importance in the medical field. A variety of biosensors, constructed with chitosan and using immobilized glucose oxidase for the detection of glucose levels, were reported. A glucose electrochemical sensor was prepared with glucose oxidase immobilized on the composite of chitosan-carbon nanotubes (Liu et al. 2005). An amperometric glucose biosensor composed of multilayered chitosan biofilms-gold nanoparticles-glucose oxidase on platinum (Pt) electrode was devised (Wu et al. 2007). The biocompatible gold nanoparticles helped in directing the transfer of electrons to the transducer. Yang et al. (2009) devised a different glucose biosensor composed of Pt electrode-glucose oxidase- $\text{Fe}_3\text{O}_4$ -chitosan-nafion. Zhang et al. (2015c) prepared an electrochemical biosensor for glucose with chitosan-graphite composite and the



addition of magnetic  $\text{Fe}_3\text{O}_4$  nanoparticles on Pt-coated indium tin oxide (ITO) glass electrode. Shrestha et al. (2016) devised a glucose biosensor with a glassy carbon electrode on which a nanocomposite film of glucose oxidase immobilized on chitosan and on which a graft of polypyrrole-nafion and multi-walled carbon nanotubes was deposited.

Electrochemical biosensors using other oxidases and various constructs were fabricated to monitor food and medically important compounds. For instance, a lactate biosensor was generated using lactate oxidase and a nanocomposite structure of chitosan-polyvinylimidazole-Os-carbon nanotubes (Cui et al. 2007). Glutamate and xanthine oxidases as recognition elements immobilized on chitosan/graphene oxide-polymerized riboflavin were constructed as glutamate and hypoxanthine biosensors (Celiesiute et al. 2017). In addition, a xanthine biosensor based on immobilization of xanthine oxidase on chitosan-polypyrrole-gold nanoparticles was fabricated by Dervisevic et al. (2017). Tkac et al. (2007) developed a selective galactose biosensor with a rather simple configuration of chitosan-single-walled carbon nanotubes and immobilized galactose oxidase. A sensitive amperometric nanocomposite biosensor for cholesterol detection was constructed using a matrix of Pt nanoparticles deposited on multi-walled chitosan-carbon nanotubes complexes with immobilized cholesterol oxidase (Tsai et al. 2008). A similar construct was proposed by Medyantseva et al. (2014) for the detection of antidepressant monoamine drugs using immobilized monoamine oxidase. Dai et al. (2010) developed an electro-chemiluminescent biosensor to detect choline by immobilizing choline oxidase on a chitosan/titanate nanotubes composite film. Finally, a biosensor for measuring ethanol was prepared using alcohol oxidase immobilized on chitosan-eggshell film (Wen et al. 2007). The biosensor monitored the decrease in oxygen level vs ethanol concentration.

A number of electrochemical biosensors were similarly constructed to immobilize various dehydrogenase enzymes (Zhang et al. 2004). The nanocomposite scaffold film, attached predominantly to glassy carbon electrodes, consists of chitosan, multi-walled carbon nanotubes, and  $\text{NAD}^+$  as cofactor. The signal current is based essentially on electrooxidation of the formed NADH. Among the large list of enzymes suffice it to mention NAD-dependent alcohol (Lee and Tsai 2009; Zhang and Gorski 2011), lactate (Tsai et al. 2007) and glutamate (Hughes et al. 2015) dehydrogenases, and FAD-dependent glucose dehydrogenase (Monosik et al. 2012).

In contrast to the above enzyme-based biosensors, a nonenzymatic electrochemical device for monitoring glucose was formulated (Al-Mokaram et al. 2017). The construct, which was based on a nanocomposite film composed of polypyrrole-chitosan-titanium dioxide nanoparticles on ITO glass electrodes, involved redox reactions and exhibited improved glucose oxidation and high electron transfer kinetics.

Other biosensors detecting and measuring diverse compounds were formulated, for example, nitrite biosensor based on Cu-containing nitrite reductase immobilized on viologen-chitosan that catalyzes the reduction of nitrite (Quan and Shin 2010). Horseradish peroxidase immobilized on alumina nanoparticles-chitosan composite was devised to detect phenolic compounds (Liu et al. 2011). Wang et al. (2003) developed a biosensor to detect and measure glucose, galactose, and glutamate in



human blood by using their corresponding oxidases immobilized on chitosan-Prussian blue composite film. The biosensor used Prussian blue as a good catalyst to form hydrogen peroxide by electroreduction. Biosensors to detect catechol as well as other phenolic compounds were based on immobilized tyrosinase on a film of chitosan-nickel nanoparticles (Yang et al. 2012a). A biosensor for detection of chlorophenol that includes immobilized laccase on ZnO-chitosan nanocomposite was prepared by Mendes et al. (2017). Nanocomposite of functionalized graphene oxide (enriched with carboxylic moieties)-polypyrrole-chitosan film was constructed to detect hydrogen peroxide using screen-printed carbon electrodes (Akhtar et al. 2017). Such a device was able to electro-catalyze the reduction of hydrogen peroxide. Teepoo et al. (2017) constructed an electrochemical biosensor to detect and monitor hydrogen peroxide by using horseradish peroxidase immobilized on a chitin-gelatin nanofiber composite. Another biosensor for hydrogen peroxide that used immobilized catalase on chitosan- $\beta$ -cyclodextrin (with ferrocene in its cavity) was fabricated by Dong et al. (2017). It was based on chitosan-functionalized graphene oxide (enriched with carboxylic moieties)-polypyrrole nanocomposite able to electrocatalytically reduce hydrogen peroxide.

Detection and quantification of trace amounts of carcinogenic and toxic metallic ions are of great challenge and importance. A cross-linked chitosan-carbon nanotube sensor was developed for the determination of Cd(II) and Hg(II) (Janegitz et al. 2011). Sugunan et al. (2005) prepared a biosensor made of chitosan-gold nanoparticles to detect Cu(II) and Zn(II), and Ahmed and Fekry (2013) used a construct of chitosan- $\alpha$ -Fe<sub>3</sub>O<sub>4</sub> nanoparticles sensor to detect Ni(II), As(II), and Pb (II). Biosensors were developed to detect and determine organophosphorus (OP) pesticides as well. For instance, Stoytcheva et al. (2018) prepared a device based on OP hydrolase immobilization on a chitosan-carbon-nanoparticles-hydroxyapatite nanocomposite. A nanocomposite immunosensor to monitor the OP compound, chlorpyrifos, is based on immobilized anti-chlorpyrifos monoclonal antibody on multi-walled carbon nanotubes-chitosan-thionine (as electronic mediator) (Sun et al. 2012b). An intricate electrochemical immunosensor for the detection and monitoring of the fungal hepatocarcinogen, aflatoxin B1, as model antigen was developed by Masoomi et al. (2013). The construct scaffold involved chitosan-gold nanoparticles, immobilized polyclonal anti-aflatoxin B1, and a magnetite core that can enable regeneration of the immunosensor.

Biosensors based on chitosan/multi-walled carbon nanotubes hybrid films were developed largely by Babaei and colleagues to determine and quantitate drugs and neurotransmitters: acetaminophen and mefenamic acid (Babaei et al. 2010), dopamine and morphine (Babaei et al. 2011a), paracetamol (Babaei et al. 2011b), L-DOPA (Babaei and Babazadeh 2011), and 5-hydroxytryptamine and dopamine (Xu et al. 2015).

The polycationic nature of chitosan films in immunobiosensors is also exploited to immobilize polyanionic polymers such as nucleic acid sequences and proteins. Singh et al. (2013) devised an electrochemical DNA biosensor to detect typhoid which was constructed by surface immobilizing *Salmonella typhi* single-stranded (ss) DNA on graphene oxide/chitosan/ITO nanocomposite as a bioelectrode. The biosensor was

capable of distinguishing between complementary, noncomplementary, and one base mismatch sequences. A similar electrochemical DNA biosensor was developed for the detection of *Escherichia coli* 0157:H7 (Xu et al. 2017b). It was prepared with immobilized *E. coli* ss-DNA using a graphene oxide/chitosan hybrid nanocomposite. An electrochemical immunobiosensor to detect botulism neurotoxin A was reported by Afkhami et al. (2017). The sensor consisted of a gold nanoparticles/chitosan/graphene nanocomposite with immobilized antibodies to quantify the bound neurotoxin. To detect  $\alpha$ -fetoprotein in human serum, an immunosensor was fabricated in which the  $\alpha$ -fetoprotein antigen was immobilized on a film of a gold nanoparticles/carbon nanotubes/chitosan nanocomplex to quantify protein levels using a competitive immunoassay format (Lin et al. 2009). Giannetto et al. (2017) fabricated a competitive electrochemical immunosensor to detect HIV1-related capsid protein p24 in human serum. The p24 antigen was immobilized on gold-free single-walled carbon nanotube-chitosan complex for the interaction with a mouse monoclonal anti-p24, which was used for competitive immunodetection. Liu et al. (2009) developed an immunosensor to detect carcinoembryonic antigen, which is based on corresponding antibodies immobilized on chitosan-gold nanoparticles. Finally, Qiu et al. (2009) reported an immunosensor to detect hepatitis B surface antigen, which was constructed on the basis of a gold nanoparticles/chitosan/ferrocene biofilm with immobilized hepatitis B antibodies.

## 14.4 Beneficial Properties of Chitosan for Possible Use in Agriculture, Food, and Textile Industry

The wide-ranging antimicrobial, antiviral, and antioxidant activities, induction of defense systems in plants, and stimulation of plant growth by chitosan, chitosan oligomers, chemically modified chitosan and their composites have indicated their potential use in agricultural practices (El Hadrami et al. 2010; Malerba and Cerana 2016). Pre- and postharvest treatment of coating seeds, fruits, and vegetables by edible chitosan-based films effectively improve germination and plant vigor and prolonged shelf life and storage quality of food products (No et al. 2007). Preservation by chitosan-based coating also expanded to include meat, eggs, dairy products, and seafood (Friedman and Juneja 2010). Other promising practices such as delivery and slow and sustained release of chitosan-based encapsulated agrochemicals (fertilizers, micronutrients, pest control agents, and genetic materials) have been widely investigated (Malerba and Cerana 2016).

### 14.4.1 Antimicrobial and Antioxidant Activities

There are several comprehensive reviews that summarize the potential use of chitosan, its derivatives, and chitooligosaccharides in agriculture as related to their

broad-spectrum antimicrobial and antioxidant activities (Aider 2010; Cota-Arriola et al. 2013; Li et al. 2013a; Xing et al. 2015; Liaqat and Eltem 2018). Such beneficial activities were demonstrated in a variety of agricultural products like preservation of vegetables, fruits, cereals, dairy products, eggs, meat, and seafood (No et al. 2007; Friedman and Juneja 2010). Chitosan per se has antimicrobial activity that depends on higher degree of deacetylation, low molecular weight (its oligosaccharides), increased protonation at low pH, and the type of microorganisms (Katiyar et al. 2014). The antimicrobial efficiency is enhanced by adding essential oils (extracted from lemon, lemon grass, cinnamon, or rosemary) (Duan and Zhang 2013; Xing et al. 2016; Yuan et al. 2016) or by adding metal ions like silver or copper (An et al. 2011; Brunel et al. 2013; Kumar-Krishnan et al. 2015; Choudhary et al. 2017a; Sharma 2017) particularly to chitosan-based nanoparticles (Friedman and Juneja 2010; Cota-Arriola et al. 2013). The mode of action is mainly attributed to electrochemical interactions between the positively charged chitosan and the negative surface charge of bacterial cells leading to membranes disruption (Xing et al. 2015). In addition, penetration and binding of nanochitosan with microbial DNA that impact mRNA and protein synthesis were proposed (Rabea et al. 2003, Malerba and Cerana 2016).

Scavenging of free radical and reactive oxygen species by chitosan and its derivatives is responsible for its antioxidative effects (Guo et al. 2005; Ngo and Kim 2014). Scavenging of superoxide and hydroxyl radicals by chitosan and its derivatives was demonstrated by several studies (Xie et al. 2001; Guo et al. 2005; Yen et al. 2008; Wan et al. 2013). Furthermore, chitosan acts as a biogenic elicitor of various enzymes that detoxify reactive oxygen species (Malerba and Cerana 2016) and induces the formation of antioxidant and fungicidal phytoalexins (Yamada et al. 1993; Hadwiger 2013; Xing et al. 2015).

#### ***14.4.2 Eliciting Defense Responses in Plants***

Chitosan and its derivatives were shown to activate plant immunity enzymes (catalase, peroxidase, superoxide dismutase, phenyl oxidase, phenylalanine ammonia lyase) that are capable of detoxifying reactive oxygen species (Hadwiger 2013; Xing et al. 2015; Malerba and Cerana 2016). Such activation engages different signal transduction pathways that involve a variety of second messengers. Other defense responses include pathogenesis-related proteins, phytoalexins, proteinase inhibitors, lignin synthesis, or callose formation (El Hadrami et al. 2010; Hadwiger 2013). Induction of programmed cell death and hypersensitivity-associated responses by chitosan and chito-oligosaccharides was documented (Zuppini et al. 2004; Vasilév et al. 2009; Zhang et al. 2012), as well as activation of plant defense genes via the octadecanoid pathway leading to jasmonate synthesis (Doares et al. 1995; Rakwal et al. 2002). Chitosan induces hydrolase enzymes such as chitinase and  $\beta$ -1,3 glucanase able to destroy chitin/glucan-containing fungal cell walls (Ma et al. 2013b; Xing et al. 2015).

### 14.4.3 Plant Protection and Food Preservation

Controlled and sustained release of chitosan-encapsulated agrochemical such as fertilizers, micronutrient, pesticides, and genetic materials was demonstrated by a plethora of investigations (Kashyap et al. 2015). Food products coating by films of edible chitosan derivatives (plus a variety of additives) prolong their shelf life with concomitant improvements in storage quality (Xing et al. 2016; Yuan et al. 2016).

#### 14.4.3.1 Pesticides

A number of examples linked to chitosan-coated pesticides given below indicate the potential of the eco-friendly techniques in plant protection against phytopathogens, insects, and weeds: controlled release of insecticides like the botanicals azadirachtin being encapsulated in the complex carboxymethyl chitosan-ricinoleic acid (Feng and Peng 2012) and rotenone wrapped in oleoyl carboxymethyl chitosan (Kamari and Aljafree 2017); nanoparticulate chitosan- $\beta$ -cyclodextrin, which encapsulated carvacrol and exhibited high acaricidal and repellency activities (Campos et al. 2018); and controlled release of avermectin conjugated to *N,O*-carboxymethyl chitosan (Li et al. 2016) or avermectin coated by silica cross-linked chitosan composite (He et al. 2013). Encapsulation of the neonicotinoids imidacloprid (Li et al. 2012a; Lim and Ahmad 2017) and acetamiprid (Yan et al. 2014), malathion, and spinosad (El Badawy et al. 2016) by chitosan-alginate capsules exhibited prolonged release of the insecticides. Slow release of the fungicide carbendazim against the phytopathogens *Sclerotinia sclerotiorum* using chitosan/ $\beta$ -CD-epichlorohydrin (Wang et al. 2017a) and hexaconazole encapsulated by chitosan nanoparticles against *Rhizoctonia solani* (Chauhan et al. 2017) was demonstrated. Ilk et al. (2017) reported the antifungal and antioxidant activities of kaempferol encapsulated in lecithin-chitosan nanoparticles against *Fusarium oxysporum*.

In addition to their slow release property, chitosan composites also protect pesticides from photodegradation. Nanoparticles of chitosan-beeswax protected deltamethrin from photodegradation (Nguyen et al. 2012), and a similar protective effect of avermectin was demonstrated for a silica/chitosan copolymer (He et al. 2013). Likewise, composites of chitosan with a variety of clays (montmorillonite, attapulgite, bentonite, and kaolinite), safe anionic dyes (Fast Green and Naphthol Yellow S), and photo-stabilized fungal conidia of the insect biocontrol agent *Aschersonia* spp. were reported (Cohen et al. 2003). Chitosan composites were found to be useful carriers of herbicides facilitating soil sorption as in the case of paraquat associated with chitosan-alginate nanoparticles (Silva Mdos et al. 2011) or slow release of paraquat encapsulated in tripolyphosphate-generated chitosan nanoparticles (Grillo et al. 2014). Moreover, encapsulation of metolachlor in blended gel beads of cross-linked carboxymethyl cellulose and carboxymethyl chitosan was effective in slow release of the herbicide as a model compound (Dong et al. 2012). Finally, slow release of atrazine encapsulated in carboxymethyl chitosan/bentonite gel was demonstrated (Li et al. 2012a).

### 14.4.3.2 Fertilizers

The modulated release of encapsulated fertilizers is important for enhanced growth of plants while reducing environmental problems of their excessive use. Experiments were accompanied by swelling rates of composites, fertilizer loads, and kinetics of release. Examples are chitosan-xanthan tablets (Melaj and Daraio 2013) or chitosan-starch beads (Perez and Francois 2016) as carriers of potassium nitrate that serve as model fertilizer; slow release of NPK fertilizers aggregated on chitosan nanoparticles (Corradini et al. 2010) and application on leaf surfaces enables translocation via stomata into the phloem (Abdel-Aziz et al. 2016); efficient controlled slow release of water soluble NPK fertilizers coated by chitosan with an additional outer coating by poly (acrylic acid-co-acrylamide) (Wu and Liu 2008). This composite also exhibited improved water absorption and retention. Noppakundilokrat et al. (2015) examined the controlled release of NPK fertilizer granules embedded in a hydrogel composed of poly(vinyl alcohol) and then chitosan and a third layer of acrylamide and acrylic acid following cross-linking of chitosan by glutaraldehyde. Controlled release of urea by a variety of chitosan-based composites was established. Urea dispersed with humic substances in chitosan (Araújo et al. 2017), urea encapsulated in chitosan-acryamide (Siafu 2017), urea release from adduct of silk fibroin-gelatin-chitosan hydrogels (Rattanamanee et al. 2015), urea smectite clay chitosan composite (Puspita et al. 2017), and urea-kaolinite mixed with chitosan (Roshanravan et al. 2015) were tested for controlled release of the fertilizer.

### 14.4.3.3 Chitosan-Coated Plant Materials

#### 14.4.3.3.1 Preharvest

Beneficial effects of preharvest chitosan-based seed coating and foliar treatment were reported by El Hadrami et al. (2010). Chitosan-coated artichoke seeds, for example, induced better germination, stimulated root system growth, and were effective against a number of pathogenic fungi (Ziani et al. 2010). Bhaskara Reddy et al. (1999) demonstrated induced resistance to seed-borne *Fusarium graminearum* followed by improved germination and vigor in wheat seeds coated with chitosan. Soybean seeds coated by chitosan had anti-feeding effects and protected against several insect pests (Zeng et al. 2012), and coating rice seeds increased antifungal effect, stimulated seeding growth, improved root system, and increased crop yield (Zeng and Shi 2009). Tomato seeds coated with chitosan resulted in resistance to infection by inducing plant defense mechanisms (Benhamou et al. 1994). Chickpea seeds treated with chitosan-silver nanoparticles promoted germination and increased biomass, chlorophyll, carotenoids, and protein contents as well as amylase activity and defense enzyme activities (Anusuya and Banu 2016). Similar effects were demonstrated in maize seeds coated with Cu/chitosan nanoparticles (Saharan et al. 2016; Choudhary et al. 2017b).

#### 14.4.3.3.2 Postharvest

The antimicrobial activity of chitosan was targeted for use to improve preservation of a large variety of vegetable and fruit crops as well as of eggs, meat, and dairy products (Devlieghere et al. 2004; Friedman and Juneja 2010; Yuan et al. 2016). Chitosan with added compounds such as plant materials and animal proteins (formulations of chitosan with additions of tapioca starch, hydroxypropyl cellulose, pectin, and fish gelatin) was used to develop edible films. Such films in addition to their antimicrobial and antioxidant activities also keep food products from loss of moisture and oxygen penetration (Aider 2010; Duan and Zhang 2013). Postharvest coating of vegetables and fruits with chitosan and additional essential oils (extracts from lemon, rosemary, lemon grass, bergamont, cinnamon, oregano, and thymine), which by themselves exert antimicrobial and antioxidant activities, improved storage quality and prolonged the shelf life of products (Xing et al. 2016). Controlling postharvest decay during storage was reported also for additives such as olive oil, glacial acetic acid, green tea extract, and lactic acid (Xing et al. 2016; Yuan et al. 2016).

#### 14.4.3.4 Technical Applications in Food Packaging

Microbial contaminations are a serious problem in food industry, because food-borne bacteria and fungi are associated with food spoilage and food poisoning leading to economic losses and human health risks. Using appropriate food packaging materials with antimicrobial properties may prevent or at least slow down bacterial and fungal growth. For this reason, a variety of biopolymers has been tested to identify alternative materials to the classical nondegradable plastic packaging materials, which have caused serious environmental issues due to their inappropriate disposal. Optimal alternative materials should be environmentally safe due to biodegradability and biocompatibility. As chitosan-based material combine antimicrobial properties with biodegradability and biocompatibility, they are the focus of research in food packaging. Moreover, chitosan-based materials have food-preserving antioxidant activity and film-forming ability, which allows the production of transparent foils and bags. Different methods have been established during the past decades to fabricate chitosan films including casting, coating, extrusion, and layer-by-layer synthesis, and the resulting materials have been evaluated for their antimicrobial and antioxidant activity and for their optical, mechanical, barrier, and thermal characteristics. Chitosan has also been combined with other functional materials resulting in composite films with tremendous preservative properties that can be utilized for the packaging of different foods such as vegetables, fruit, and meat. For a comprehensive overview on this topic, the reader is referred to an excellent review article published recently by Wang et al. (2018).

Pure chitosan films are frequently based on dispersions of chitosan nanoparticles (Ali et al. 2014), to which plasticizers, such as glycol (Leceta et al. 2013), and/or surfactants, such as Tween 80 (Martins et al. 2012), are added to modify the mechanical properties and to emulsify auxiliary compounds. In addition, chitosan nanofibers have been fabricated as a packaging material and tested for their antimicrobial activity. For instance, Arkoun et al. (2017) examined the antimicrobial activity of chitosan/polyethylene oxide nanofibers produced by an electrospinning process. They showed that the chitosan nanofibers were efficient in inhibiting growth of *E. coli*, *Staphylococcus aureus*, *Listeria innocua*, and *S. typhimurium*, however at pH 5.8, which was below the pKa of chitosan, limiting the applicability to slightly acidic food. Importantly, the authors demonstrated that the antibacterial effects were irreversible, suggesting a bactericidal rather than bacteriostatic mechanism.

Combinations of chitosan and other natural polysaccharides have been frequently used to fabricate functional films with applications in food packaging. These biopolymers comprise of cellulose and various cellulose derivatives, alginate, cyclodextrin, glucan, mannan, pectin, starch, and xylan. Chitosan/cellulose films revealed improved mechanical properties while maintaining excellent antimicrobial properties (Xiao et al. 2013). Also chitosan/hydroxypropyl methylcellulose (HPMC) films exhibit significant antimicrobial activity. For instance, Möller et al. (2004) examined the antimicrobial effects of chitosan/HPMC films against *Listeria monocytogenes* and found that bacterial growth was completely inhibited on the film. Similarly, chitosan/carboxymethyl cellulose films showed superb food preservation properties when tested on packaged cheese (Youssef et al. 2016). Antimicrobial chitosan-alginate films have a great potential for food packaging as well, particularly because they show improved gas exchange and water vapor permeability properties when prepared by a layer-by-layer electrostatic deposition approach (Poverenov et al. 2014a). Martiñon et al. (2014) studied the effectiveness of antimicrobial multilayered coatings consisting of chitosan, pectin, and *trans*-cinnamaldehyde at different concentrations to extend the shelf life of fresh-cut cantaloupe and found that certain compositions were effective in preventing bacterial growth and spoilage. Lorevice et al. (2016) produced chitosan nanoparticles and combined them with different methyl pectin matrices to generate nanocomposite films and tested the mechanical, thermal, and barrier properties. The results showed that the nanocomposite film improved mechanical characteristics when compared with conventionally produced pectin films, making these novel materials promising for food packaging production. Similarly, chitosan/cyclodextrin films with inclusions of essential oil have been reported to possess desirable mechanical properties for food packaging (Sun et al. 2014). Moreover, this material showed significant antimicrobial activities against a variety of pathogenic bacteria.

Chitosan films have been also combined with a variety of proteins including casein (Khwaldia et al. 2014), gelatin (Poverenov et al. 2014b; Noorbakhsh-Soltani et al. 2018), collagen (Ahmad et al. 2016), kidney bean protein (Ma et al. 2013a), lactoferrin (Brown et al. 2008), and lysozyme (Yuceer and Caner 2014), as well as with antibacterial peptides such as nisin (Wang et al. 2015). In addition, chitosan was



blended with antimicrobial and antioxidant extracts from bee wax (Velickova et al. 2013) and plants, such as citrus (Iturriaga et al. 2014), thyme (Talon et al. 2017), and maqui berry (Genskowsky et al. 2015), as well as with essential oils including clove bud oil, cinnamon oil, and star anise oil (Wang et al. 2011).

Other approaches in fabricating chitosan-based films employed grafts, blends, or casts using synthetic polymers such as poly(vinyl alcohol) (Wang et al. 2015), poly(lactic acid) (Pal and Katiyar 2016), poly(ethylene) (Reesha et al. 2015), poly(ethylene oxide) (Kohsari et al. 2016), poly(styrene) (Lopez-Carballo et al. 2013), poly(propylene) (Cavallo et al. 2014), poly(caprolactone) (Alix et al. 2013), and poly(acrylonitrile-co-acrylamide) (Kumar et al. 2018) that led to improved mechanical and thermal properties. However, these synthetic polymers are not readily degraded in nature; hence concerns regarding the environmental safety have been raised. Guo et al. (2015) developed new edible antimicrobial films using microemulsions in combination with high-pressure homogenization processing. The films were made of chitosan, allyl isothiocyanide, and barley straw arabinoxylan, which were used as film-forming, antimicrobial, and emulsifying agents, respectively. The material was tested to be efficient in preventing growth of *L. innocua*.

To improve antibacterial activity, chitosan-based films were synthesized as composites with metals, minerals, and other inorganic compounds. Youssef et al. (2014) produced chitosan-silver and chitosan-gold (CS-Au) nanocomposites films, which showed enhanced antimicrobial activity against Gram-positive (*S. aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa*), fungi (*Aspergillus niger*), and yeast (*Candida albicans*). In another study published by Al-Naamani et al. (2016), poly(ethylene) films were coated with zinc oxide/chitosan nanocomposite, which completely inactivated and prevented the growth of food pathogens. In an approach based on a solution cast method, Sanuja et al. (2015) fabricated a chitosan-based nanocomposite film using nano zinc oxide and neem essential oil, which improved mechanical, physical, barrier, and optical properties. Moreover, Zhang et al. (2017) prepared chitosan/titanium dioxide composite films, which were found to possess significant antimicrobial activity against *E. coli*, *S. aureus*, *C. albicans*, and *A. niger*. Xu et al. (2017c) employed a different strategy by synthesizing chitosan/graphene oxide nanocomposites with titanium dioxide and analyzed their antimicrobial and food-preserving efficacies. They showed that the material effectively prevented *Bacillus subtilis* and *A. niger* biofilm formation presumably by disrupting cellular membranes. In addition, they demonstrated that the nano-coating could be applied as a cling film, which delays loss of moisture in fruits and vegetables and inhibits polyphenol oxidase activity and thus enzymatic browning but increases superoxide dismutase activity, which protects against reactive oxygen species. Next to these materials, chitosan-montmorillonite composites, chitosan/nanosilica films, and manifold combinations of chitin, metals, and minerals have been tested. In addition, numerous chemical chitosan derivatives have been explored for their properties to screen for new films suitable in food packaging. These derivatives include carboxymethyl chitosan and quaternized chitosan such as (2-*N*-Hydroxypropyl-3-trimethylammonium chloride) chitosan (Hu et al. 2016).

#### 14.4.4 *The Use of Chitosan in the Textile Sector*

Due to their versatile and unique physicochemical and biological properties, chitosan, its multiple derivatives, and their adjunct complexes (addition of functional groups) have attracted considerable attention for possible use of eco-friendly materials in the textile industry. They are relatively inexpensive, biocompatible, biodegradable, and nontoxic and readily adhere to textile fabrics and usually demonstrate antibacterial activity. Certain formulations retain moisture as well as impart thermal stability and UV protection.

Chitosan per se or blends of chitosan-based composites deposited onto textiles fabrics were mostly tested for durable antibacterial activity (nearly all antibacterial studies include *E. coli* and *S. aureus* that represent correspondingly Gram-negative and Gram-positive bacteria). Coated Thai silk fabric with chitosan using radio frequencies plasma treatment exhibited antibacterial effects (Wongsawaeng et al. 2017), and polyester/cotton fabric treated with chitosan can be used as an alternative to the antibacterial triclosan (Ranganath and Sarkar 2014). Chitosan grafted on cotton (Ferrero et al. 2015) or on wool (Periolatto and Ferrero 2013) fabrics using UV irradiation bestowed antibacterial activity after many washing cycles. Chitosan reduced to nanoparticles and applied onto wool fabric imparted durable antibacterial and bestowed shrink proofing (Yang et al. 2010). Nanonized chitosan applied onto cotton exhibited, in addition to antibacterial activity, also thermal stability, UV protection, as well as improved dye-binding ability (Hebeish et al. 2013). Periolatto et al. (2012) demonstrated antibacterial effects and laundry durability of cotton and silk fabrics by UV curing with 2-hydroxy-2-methylpropylpropane-1-one as photoinitiator of the photochemical reaction.

Chitosan possesses abundant potential, in particular, for use in medical textiles and sportswear. For example, a blend of chitosan (short fibers) with cotton (long fibers) yarn by spinning technology is desirable for medical applications (Lam et al. 2017). Gauze bandages for wound dressing were prepared by electrospinning of chitosan nanofibers and cotton fabric (Nawalakhe et al. 2015). Plasma treatment was applied to improve adhesion by increased cross-linking between the two fiber systems imparting subsequent durability (Nawalakhe et al. 2015). Pure chitosan microfibers produced by wet spinning process was aimed for possible stable 3-D scaffold woven or nonwoven textile fabrics to be used in regenerative medicine such as bone and cartilage engineering (Toskas et al. 2013). Lam et al. (2018) examined a blend of chitin fibrils with cotton jersey fabric and showed reduced rigidity that may provide comfort to patients with epidermolysis bullosa skin disease. Likewise, chitosan-coated textile fabrics improved atopic dermatitis disease by restraining skin microbiome (Lopes et al. 2015). Sonochemical deposition was used by Petkova et al. (2014) to coat cotton fabrics with a hybrid of chitosan and ZnO nanoparticles. This complex showed improved antibacterial activity, slow release of the metal and washing stability, and postulated as effective treatment for hospital textiles to prevent transfer of pathogens. Similarly, the hybrid of chitosan and silver nanoparticles deposited onto cotton fabric

demonstrated antibacterial effects and laundry durability befitting their possible use for medical textiles and sportswear (Xu et al. 2016). Ali et al. (2011) proposed to use chitosan nanoparticles that are able to pick and retain silver ions in medical textile applications. A polyester fabric coated with this complex hybrid imparted enhanced antibacterial activity. Nanonized chitosan applied onto cotton exhibited in addition to antibacterial activity, also thermal stability, UV protection as well as improved dye-ability (Hebeish et al. 2013).

A large number of publications signified and reported beneficial properties of chitosan and chitosan-based formulations with possibly great potential to treat textile fabrics. Such valuable features include protecting a variety of fabrics with emphasis on medical textiles, production of aromatic and flame-retarding fabrics, as well as dye removal and treatment of textile wastewater. Table 14.1 summarizes inter alia nanochitosan, chitosan nanometal complexes, or chitosan derivative composites with metals and other substances, which were treated onto textile fabrics (notably cotton), and depicts their conceivable potential for the textile industry.

## 14.5 Utilization of Chitosan in Cosmetics

Chitin and, in particular, chitosan and its derivatives provide advantageous properties in the cosmetic area. They are biocompatible and adhere to surface components of the skin and hair, forming elastic films with moisturizing and water retention capabilities. They can serve as vehicles for encapsulated cosmetic ingredients and their controlled delivery and release and formation of gels in mixtures with water and alcohol and have some antimicrobial, antioxidant, and anti-inflammatory activities, with the additional important benefit of low cytotoxicity (Lee et al. 2013; Jimtaisong and Saewan 2014; Aranaz et al. 2018).

Chitosan and its derivatives are included in cosmetic formulations and products for mainly care and protection of the skin and hair but inter alia in tooth enamel and tooth lacquer, nail lacquer, lipsticks, cleansing and bath materials, toothpaste, mouthwash, chewing gum, deodorants, and breath refreshers (Dutta et al. 2004). Aging of the skin, viewed as wrinkling, dryness, loss of elasticity, dehydration, and hyperpigmentation, is the result of long-term exposure to sunlight UV, which mainly forms reactive oxygen species. Protection from photoaging is a major drive in the cosmetic industry. For example, chitoooligosaccharides per se were able to protect UV-irradiated hairless mouse skin from photoaging damage (Kong et al. 2018). Gel formulation of chitosan microparticles served as a delivery system for the sustained release of the hydrophilic sunscreen, phenylbenzimidazole sulphonic acid (Gomaa et al. 2010). The cosmetic gel formulation of blended chitosan, collagen, and *Aloe vera*, with antibacterial and antioxidant effects, proved useful in the regeneration and rejuvenation of the skin using cultured mouse fibroblast (Rajashree and Rose 2017). Microspheres composed of carboxymethyl chitosan/collagen peptides-calcium chloride protected mice skin and thymus lymphocytes from UV-B radiation damage

**Table 14.1** Possible applications of chitosan and chitosan-based composites in the textile industry

<i>Textile fabric</i>	Chitosan-based composite	Property	Reference
<b>Chitosan derivatives</b>			
<i>Cotton, silk</i>	CS-2-hydroxy-2-methylpropylpropane-1-one <sup>a</sup>	AB, laundry durability	Periolatto et al. (2012)
<i>Cotton</i>	CS nanoparticles-copper	AB, thermal stability, UV protection	Hebeish et al. (2013)
<i>Polyester</i>	CS nanoparticles-silver	AB, sustained release of silver	Ali et al. (2011)
<i>Cotton</i>	CS-silver nanoparticles	AB, laundry durability	Xu et al. (2016)
<i>Cotton</i>	CS-ZnO	AB, slow release of metal, washing stability	Petkova et al. (2014)
<i>Cotton</i>	CS-ZnO nanoparticles	AB, UV blocking	Raza et al. (2016)
<i>Cotton</i>	CS-CuO nanoparticles	AB	Dhineshbabu and Rajendran (2016)
<i>Cotton/polyester</i>	CS-ZnO, $\text{RiO}_2$ , $\text{SiO}_2$ (nanoparticles)	AB, UV protection, self-cleaning, washing durability	Ibrahim et al. (2017b)
<i>Cotton</i>	CS-silver nanoparticles, montmorillonite	AB, thermal stability, flame-retarding activity, UV protection, water retention	Rehan et al. (2018)
<i>Cotton</i>	CS-poly (N-isopropylacrylamide) –silver nanoparticles	AB, controlled release of silver	Štular et al. (2017)
<i>Cotton</i>	CS-silver-zeolite film	Antimicrobial	Scacchetti et al. (2017)
<i>Cotton</i>	LBL CS and graphene oxide	UV protection, laundering durability	Tian et al. (2016)
<i>Cotton</i>	CS-poly(2-acrylamide-2-methylpropane sulfonic acid salt). LBL film	AB	Cheng et al. (2016)
<i>Cotton</i>	CS-(N,N,N-three methyloxirane methylammonium chloride)	Antimicrobial wound dressing, moisture retention	Yin et al. (2018)
<i>Cotton</i>	CS-poly (N-isopropylacrylamide)	AB, thermosensitivity	Wang et al. (2016a)
<i>Cotton</i>	CS-N-benzyl-N,N diethyl quaternary ammonium salt	AB	Feng et al. (2016)
<i>Wool</i>	CS-poly(propylene) imine	AB, durable washings	Sadeghi-Kiakhani et al. (2013)
<i>Silk (Antheraea pernyi)</i>	CS-(N-[(2-hydroxy-3-trimethylammonium)propyl] chloride nanoparticles)	AB, durable wrinkle and shrinkage resistant, laundry durability	Lu et al. (2014)
<i>Polyester (polylactic acid)</i>	CS-poly(vinyl alcohol)	Thermally stable blend	Grande et al. (2018)

(continued)

**Table 14.1** (continued)

<i>Textile fabric</i>	Chitosan-based composite	Property	Reference
<i>Cotton</i>	CS-coating pyrazole compounds	AB	Nada et al. (2018)
<i>Wool</i>	CS-cyanuric acid	AB, improved dyeing performance	Zargarkazemi et al. (2015)
<i>Polyester</i>	CS covered by nanonized polyaniline	Electrical conductivity, water repellency, stable laundry	Tang et al. (2014)
<b><i>Plant extracts and aromatic textiles</i></b>			
<i>Cotton</i>	CS-neem seed extract	AB, antiviral	Revathi and Thambidurai (2017)
<i>Cotton</i>	CS-beeswax are impregnated with essential oils ( <i>Eucalyptus</i> , tea tree, sage)	AB, slow release of fragrant	Cerempei et al. (2015)
<i>Cotton</i>	CS microcapsules containing essential oils ( <i>Eucalyptus</i> , sandal wood)	AB	Javid et al. (2014)
<i>Cotton</i>	CS- $\beta$ -CD, inclusion of cinnamon oil	AB, slow release of fragrant	Bashari et al. (2017)
<i>Cotton</i>	CS $\beta$ -CD, inclusion of lavender oil	AB, slow release of fragrant	Singh et al. (2017)
<i>Cotton</i>	CS-vanillin microcapsules	AB, slow release and retained fragrant after wash cycles	Yang et al. (2014)
<b><i>Flame retardation</i></b>			
<i>Cotton</i>	CS-diammonium hydrogen phosphate	Durable flame retardation	El-Tahlawy (2008)
<i>Cotton</i>	LBL CS and ammonium polyphosphate	Itumescent flame effect	Fang et al. (2015)
<i>Cotton</i>	CS phosphate-TiO <sub>2</sub> nanoparticles-1,2,3,4-butane tetracarboxylic acid, hypophosphite	AB, flame retardation	El-Shafei et al. (2015)
<i>Cotton/polyester</i>	LBL CS and melamine polyphosphate	Flame retardation	Leistner et al. (2015)
<i>Cotton</i>	LBL CS and ammonium polyphosphate	Flame retardation	Jimenez et al. (2016)
<i>Polyamide 66 fabric</i>	CS-phytic acid, oxidized sodium alginate	Flame retardation	Kundu et al. (2017)
<i>Acrylic fabric</i>	LBL CS and montmorillonite	Flame retardant	Carosio and Alongi (2018)
<b><i>Textile wastewater and dye removal</i></b>			
<i>Cotton</i>	UV-grafted CS	Absorbance and removal of excess dyes	Periolatto and Ferrero (2013)
<i>Textile fabrics</i>	CS beads impregnated with ZnO	Photodecolorization of Rhodamine B & Methylene Blue dyes	Farzana and Meenakshi (2015)

(continued)

**Table 14.1** (continued)

<i>Textile fabric</i>	Chitosan-based composite	Property	Reference
<i>Textile fabrics</i>	Laccase immobilized on CS-cerium (VI) dioxide microspheres	Decolorization of methyl red and orange II reactive dyes	Lin et al. (2015)
<i>Textile fabrics</i>	CS plus ferrous sulfate	Decolorization	Kos (2016)
<i>Textile fabrics</i>	CS-coating ZnO nanoparticles-Fe <sub>3</sub> O <sub>4</sub> nanoparticles	Removal of azo dye (reactive blue 198), recyclable composite	Nguyen et al. (2015)
<i>Textile fabrics</i>	CS-coating Fe <sub>3</sub> O <sub>4</sub> nanoparticles	Removal of azo dye (Acid Red 2)	Kadam and Lee (2015)
<i>Textile fabrics</i>	Acrylic acid grafted on Jute fibers followed by immobilization of CS	Desorption of anthraquinone dye	Hassan (2015)
<i>Textile fabrics</i>	Manganese peroxidase immobilized on CS beads	Degradation and detoxification of dyes	Bilal et al. (2016)
<i>Textile fabrics</i>	Manganese doped in CS-ZnO	Photocatalytic degradation of azo dye	Nguyen et al. (2016)
<i>Textile fabrics</i>	CS-poly(methacrylic acid)-TiO <sub>2</sub> microparticles	Removal and degradation of anionic azo dyes	Škorić et al. (2016)

*AB* antibacterial effects, *CS* chitosan, *LBL* layer by layer deposition,  $\beta$ -*CD*  $\beta$ -cyclodextrin

<sup>a</sup>Photochemical reaction by UV generating cross-linked polymers

(Liu et al. 2015b). A cosmetic cream formulation composed of quaternized carboxymethyl chitosan-montmorillonite nanocomposite bestowed good UV protection and additional moisturizing and water retention effects (Chen et al. 2017). There is a possible cosmetic use of neutralized chitosan in citrate buffer film for skin exfoliation (Libio et al. 2016).

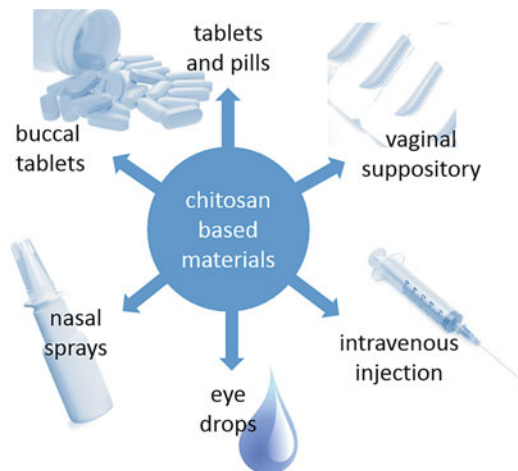
Chitosan and various chitosan derivatives, as active ingredients, were examined in cosmetic hair care products like shampoos, permanent wave agents, hair conditioner, styling lotions, rinses, hair colorant, hair sprays, and hair tonics (Dutta et al. 2004; Aranaz et al. 2018). They can adhere to the negatively charged hair keratin forming a transparent elastic film that covers hair fibers endowing smoothness, softness, and also mechanical strength (Dutta et al. 2004). A blend of chitosan and two other biopolymers like collagen and hyaluronic acid that forms a thin film over hair surface provides enhanced mechanical strength and improved conditioning of the treated hair (Sionkowska et al. 2017). Chitosan as a targeting vehicle to hair follicles in the skin was demonstrated with entrapped minoxidil, a medication to treat hair loss (Gelfuso et al. 2011; Matos et al. 2015). Microparticles and nanoparticles of chitosan-encapsulating minoxidil enabled its controlled release.

A large number of possible applications of cosmetic formulations containing chitosan and its derivatives have been patented. It is noteworthy that formulations containing chitosan are already in the busy cosmetic market.

## 14.6 Biomedical Applications of Chitosan Derivatives

Because chitosan and many of its derivatives are nontoxic, biocompatible, biodegradable, and highly versatile polymers, a large assortment of possible biomedical applications have been explored, of which some were implemented into therapeutic strategies by the pharmaceutical industry. To obtain optimal materials for the delivery of drugs, several factors have to be considered including the stability of the bioactive agents, absorption properties and mucoadhesiveness, gelling properties, particle sizes, permeation and transfection-enhancing properties, efflux pump inhibition, tissue targeting, residual toxicity of the final products, as well as release kinetic profiles. Chitosan derivatives have been developed into different kinds of pharmaceutical excipients used for the production of tablets and capsules (Illum 1998; Werle and Bernkop-Schnurch 2008), suppositories (Caramella et al. 2015), sprays (Osman et al. 2013), ointments (Kang et al. 2016), eye drops (Basaran and Yazan 2012), and wound dressings (Bano et al. 2017). The drugs are usually encapsulated by ionotropic gelation, spray drying, emulsion solvent evaporation, and coacervation (Panos et al. 2008). Chitosan-based excipients have been found useful in tablet disintegration and drug dissolution (Illum 1998) and in enhancing penetration and absorption properties (Thanou et al. 2001; van der Merwe et al. 2004; Sahni et al. 2008). Most importantly, certain dosage forms allow the controlled release of drugs (Jennings et al. 2015; Fonseca-Santos and Chorilli 2017). These include chitosan-based hydrogels (Knapczyk 1993; Kristl et al. 1993; Berger et al. 2004; Ishihara et al. 2006; Elviri et al. 2017) and micro-/nanoparticles for drug delivery (Hamman 2010). Here, we will focus on the applications of chitosan-based matrices in drug delivery for cancer, immune, and gene therapy, and we will summarize some recent advances in tissue engineering (Fig. 14.1).

**Fig. 14.1** Overview on biomedical applications of chitosan-based materials in cancer therapy and tissue engineering





### 14.6.1 Chitosan-Based Drug Carrier Systems

Chitosan-based materials can be used in various forms as drug delivery system (Fig. 14.2). Tablets are probably the most favorable and accurate dosage form, which are moreover easy to fabricate and handle. A simple method for their production is homogenization of the drug and chitosan and compressing the resulting mixture to tablets. However, it has to be considered that due to the alkaline conditions in the distal intestine, drug absorption is restricted to the more proximal regions of the gastrointestinal tract when pure chitosan is used which precipitates at an alkaline pH (Sakkinen et al. 2004; Dhaliwal et al. 2008). Therefore, more pH-insensitive formulations using higher-charged chitosan derivatives such as trimethylated chitosans or thiolated chitosan conjugates have improved absorption properties along the gastrointestinal tract. Although there is still a lack of robust data in human volunteers, some studies indicate that tablet formulations using higher-charged chitosan derivatives increase bioavailability due to improved mucoadhesiveness and better protection of the drug from degrading enzymes (van der Merwe et al. 2004). Chitosan-based tablets have been also examined for their use in vaginal drug delivery, mainly as carriers for antiviral and antifungal therapeutics (El-Kamel et al. 2002; Senyigit et al. 2014; Frank et al. 2017). However, the antimicrobial properties of chitosan may negatively affect the vaginal microflora, and hence long-term treatment should be critically evaluated (Raafat and Sahl 2009).

As chitosan-based hydrogels facilitate equal distribution and increase mucoadhesiveness, permeation, and bioavailability, they are effective formulations for eye drops to administer therapeutic drugs in ophthalmology (Krishnaswami et al. 2018). Chitosan-based formulations used in eye care include hydrogels, nanoparticles, and liposomal and colloidal systems (De Campos et al. 2001; De

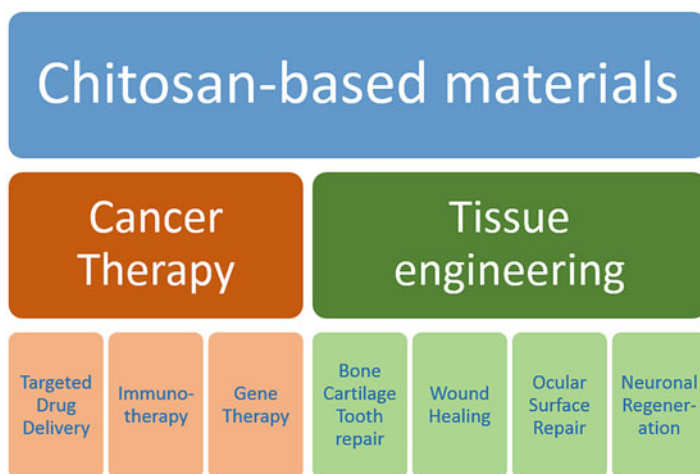


Fig. 14.2 Chitosan-based drug delivery systems

Campos et al. 2003; Diebold et al. 2007; Gupta et al. 2010). For similar reasons, they are also in use for nasal drug delivery, which is impaired by high turnover and secretion rates (Illum 2003). Notably, chitosan-coated lipid micro- and nanoparticles have been developed for nose-to-brain delivery of a variety of therapeutic drugs (Casettari and Illum 2014; Sarvaiya and Agrawal 2015). Chitosan-based nanoparticles are also a promising carrier for buccal drug delivery, which has the advantage of avoiding the hepatic first-pass metabolism and degradation in the gastrointestinal system (Sandri et al. 2005). Polymeric carriers generally have the potential advantage of prolonged release times of low-molecular-weight drugs. Because chitosan is additionally susceptible to hydrolysis by lysozyme in the blood serum, which facilitates drug release, and exhibits no toxic or hemolytic effects when applied parenteral (Nordtveit et al. 1994; Richardson et al. 1999), chitosan-based formulations are also suitable carriers for controlled drug release when administered by intravenous injection (Thanoo et al. 1992).

#### ***14.6.2 Chitosan-Based Drug Delivery Systems in Chemotherapy***

Conventional chemotherapeutics are frequently not very effective in reaching the tumor cells, as solid tumors are not well supplied with blood, and lack lymphatic vessels, which results in a decreased convective flow in the interstitial fluid. To overcome these problems, novel drug delivery systems have been designed. These carriers are capable of encapsulating high concentrations of the cytotoxic compound within a macromolecular matrix that specifically targets the cargo to the tumor cells where the drugs are finally released in a controlled manner. This concept profits from the EPR (enhanced permeability and retention) effect, the phenomenon that macromolecules preferentially accumulate in solid tumors, probably because they have a defective vasculature and lack effective lymphatic drainage (Matsumura and Maeda 1986). Chitosan-based nanoparticles have many properties that make them suitable carriers for anticancer drugs. Next to their great chemical flexibility, allowing the design of selective carriers, chitosan-based materials evidently exhibit also the EPR effect depending on the tumor microenvironment (Yhee et al. 2017). Moreover, they are degraded inside the body into fragments which can be cleared by the kidney (Kean and Thanou 2010), and several studies suggested that chitosan itself has antitumor effects (Qi and Xu 2006; Yao et al. 2013a), making this polymer a highly suitable supplementary antitumor drug and drug carrier. Indeed, chitosan-based nanocomposites can be used to deliver hydrophilic and hydrophobic drugs such as doxorubicin hydrochloride and paclitaxel, respectively (Kim et al. 2006; Yousefpour et al. 2011). Studies analyzing chitosan-based drug delivery systems for cancer treatment are summarized in Table 14.2.

To target tumor cells by the EPR effect passively, Mitra et al. (2001) fabricated chitosan-based nanoparticles of about 100 nm carrying a dextran-doxorubicin

**Table 14.2** In vitro and in vivo studies using chitosan-based nanoparticles in various cancer treatments

<i>Drug/ targeting</i>	Chitosan-based composite	Experimental system, effects	Reference
<b><i>Chemotherapeutic drug delivery</i></b>			
<i>Doxorubicin</i>	CS-dextrane conjugate	Mice, AT, prolonged circulation	Mitra et al. (2001)
<i>Doxorubicin/ trastuzumab</i>	CS cross-linked by succinic anhydride, Lys thiolation	SKOV-3 cells, AT, targets HER2+ receptors, enhanced uptake	Hebeish et al. (2013)
<i>Doxorubicin</i>	CS-pluronic F127 micelles	MCF7 cells, AT, high drug loading capacity	Naruphontjirakul and Viravaidya-Pasuwat (2011)
<i>Doxorubicin/ luteinizing hormone RH</i>	CS-/poly(methyl vinyl ether maleic acid, magnetic nanoparticles	MCF7 cells, AT, increased cytotoxicity, targeting LHRH receptors	Varshosaz et al. (2016)
<i>Doxorubicin/ folate</i>	CS-coated magnetic nanoparticles	U87 cells in athymic mice, AT, guide by magnetic field, decreased tumor growth	Yang et al. (2017)
<i>Doxorubicin</i>	Aluminosilicate zeolite (ZSM-5) CS core-shell nanodisks	Mice, AT, pH-dependent drug release, reduced Tu growth and increased apoptosis	Yang et al. (2018)
<i>Doxorubicin</i>	CS-cobalt-ferrite-titanium oxide nanofibers	B16F10 cells, AT, fast drug release at low pH and alternating magnetic field	Radmansouri et al. (2018)
<i>Doxorubicin, verapamil/ cRGD</i>	Magnetic CS-poly(lactic acid-co-glycolic acid) nanoparticles	HepG2 and S-180 cells, Tu-bearing mice, AT, accumulation in tumor tissue	Shen et al. (2013a)
<i>Paclitaxel</i>	Glycol-CS- $\beta$ -cholanolic acid nanoparticles	Tu-bearing mice, AT, impaired tumor growth after injection	Kim et al. (2006)
<i>Paclitaxel</i>	CS-glyceryl monooleate core-shell nanopoparticles	MDA-MB-231cells, AT, 1000-fold reduction in IC50	Trickler et al. (2008)
<i>Cisplatin</i>	Glycol-CS- $\beta$ -cholanolic acid nanoparticles	Tu-bearing mice, AT, impaired tumor growth after injection, EPR	Kim et al. (2008)
<i>5-Fluorouracil</i>	CS-polyaspartic acid sodium salt	Mice, sustained drug release in vitro and in vivo	Zheng et al. (2007)
<i>5-Fluorouracil/ hyaluronidase</i>	CS-polyethyleneglycol-gelatin copolymer	COLO-205 and HT-29 cells, AT, increased cytotoxicity by uptake and controlled drug release	Rajan et al. (2013)
<i>5-Fluorouracil</i>	N-succinyl-CS-g-poly (acrylamide-co-acrylic acid)	Simulated gastric and intestinal fluids, efficient drug loading pH-dependent drug release	Bashir et al. (2017)
<i>5-Fluorouracil/folic acid</i>	cystamine conjugated CS-methoxy poly(ethylene glycol)	MCF7 cells, AT, improved hemocompatibility, high cytotoxicity to cancer cells	Antoniraj et al. (2018)

(continued)

**Table 14.2** (continued)

<i>Drug/targeting</i>	Chitosan-based composite	Experimental system, effects	Reference
<i>TNF-<math>\alpha</math>/anti-EGFR-2</i>	CS-silica hollow nanospheres	MCF-7 cells, AT, pH-dependent TNF- $\alpha$ release inside tumor	Deng et al. (2011b)
<i>Oxaliplatin/hyaluronic acid</i>	CS nanoparticles encapsulated in Eudragit S100 coated pellets	Mice, HT-29 cells, AT, specific drug delivery in the colon	Jain et al. (2010)
<i>Trans-resveratrol/Biotin, avidin</i>	CS nanoparticles	HepG2 cells, cytotoxicity highest when both, avidin and biotin, were coupled	Bu et al. (2013)
<i>Gemcitabine/anti-EGFR, anti-chitosan</i>	Glycol-CS nanobioconjugate	SW1990 cells, effective inhibition of cell proliferation, colony formation, migration, and invasion	Xiao and Yu (2017)
<b>Cancer gene therapy</b>			
<i>Survivin-siRNA/baclofen</i>	N-trimethyl CS-TPP developed for pulmonary delivery	A549 cells, bronchoalveolar lavage fluid, effective gene silencing of the survivin gene resulting in apoptosis	Ni et al. (2018)
<i>Midkine-siRNA</i>	CS combined with 2-chloroethylamine and N,N-dimethyl-2-chloroethylamine hydrochloride	HepG2 cells, efficient transfection, significant decrease of cell proliferation	Zhong et al. (2015)
<i>psiRNA-hBCL2/dendrimeric RGD</i>	Polyethyleneimine-g-CS	Tu-bearing mice, AT, efficient and specific transfection of tumor cells and silencing of anti-apoptotic hBcl2	Kim et al. (2017)
<b>Cancer immunotherapy</b>			
<i>Ovalbumin</i>	CS nanoparticles	Mice, AT, increased cytokine levels and stimulation of natural killer cells, decreased tumor growth, detection of ovalalbumin specific cytotoxic T cells	Wen et al. (2011), Highton et al. (2016)
<i>IL-12</i>	CS nanoparticles	Mice, AT, activation of cytotoxic T cells and natural killer cells, tumor regression, nor recurrence	Zaharoff et al. (2009)
<i>GRP</i>	Mannosylated CS nanoparticles	Mice, intranasal application, AT, enhanced tumor regression paralleled by anti-GRP antibody production	Yao et al. (2013b)
<i>IP-10 plasmid/fofate</i>	CS nanoparticles	Mice, AT, inhibition of cell proliferation, induction of apoptosis, suppression of angiogenesis, and inactivation of regulatory T cells	Lai et al. (2014)

AT anti-tumor effects, CS chitosan, Tu tumor

conjugate and examined the antitumor effects *in vivo* in macrophage tumor cells implanted into BALB/c mice. The authors observed an improved therapeutic efficacy of dextran-doxorubicin loaded chitosan nanoparticles, which is probably due to the prolonged circulation time and/or drug accumulation at the tumor sites. In another study published by Yousefpour et al. (2011), doxorubicin was conjugated to chitosan using succinic anhydride as a cross-linker. In a second step, the resulting self-assembled chitosan-doxorubicin conjugate nanoparticles were conjugated with trastuzumab, a monoclonal antibody to the human epidermal growth factor receptor 2+ (Her2+), via lysine thiolation and subsequent linking of the derived thiols to chitosan. The Trastuzumab conjugated chitosan-doxorubicin nanoparticles selectively targeted Her2+ cancer cells resulting in enhanced uptake when compared to chitosan-doxorubicin particles and the free drugs. In another study, pluronic F127, a block copolymer of hydrophobic polyoxypropylene flanked by two chains of hydrophilic polyoxyethylene, was grafted onto chitosan to generate a copolymer micelle that can encapsulate doxorubicin (Naruphontjirakul and Viravaidya-Pasuwat 2011). The resulting chitosan-pluronic micelles carrying doxorubicin showed a high drug loading capacity and revealed a higher cytotoxic activity to MCF7 breast cancer cell lines *in vitro* than the free drug. Another approach to deliver doxorubicin specifically to cancer cells was reported by Varshosaz et al. (2016). The research team fabricated dual targeted nanoparticles loaded with doxorubicin and magnetic nanoparticles to treat breast cancer. For this purpose, the nanoparticles were produced via a layer-by-layer technique and functionalized with a bioconjugate of chitosan/poly(methyl vinyl ether maleic acid) and luteinizing hormone-releasing hormone (LHRH) to target corresponding receptors on the surface of MCF7 breast cancer cells, which presumably take up the particles by endocytosis. The targeted nanoparticles increased the cytotoxicity of doxorubicin about twofold in LHRH-positive cancer cells. In a similar approach, folate-grafted chitosan-coated magnetic nanoparticles were loaded with doxorubicin to target human glioblastoma U87 cells in athymic BALB/c nude mice in a subcutaneous tumor model system (Yang et al. 2017). Guiding the injected nanoparticles to the tumor by a magnetic field significantly decreased tumor growth by controlled delivery of doxorubicin to the cancer cells and demonstrated the feasibility of magnetic nanoparticles to direct the localization of drug release. Mesoporous aluminosilicate zeolite (ZSM-5) chitosan core-shell nanodisks loaded with doxorubicin were used as pH-responsive drug delivery systems against osteosarcoma that release the drug after upon endosomal acidification (Yang et al. 2018). Recently, Radmansouri et al. (2018) showed that doxorubicin-loaded electrospun chitosan/cobalt ferrite/titanium oxide nanofibers could be used for localized melanoma cancer therapy. The fastest release of doxorubicin from prepared magnetic nanofibers was observed at acidic pH when an alternating magnetic field was applied. As mentioned above, chitosan-based nanoparticles can also be modified to carry hydrophobic drugs such as paclitaxel. For this purpose, hydrophobic side chains are grafted onto chitosan. For instance, Kim et al. (2006) used glycol chitosan nanoparticles that were hydrophobically modified with  $\beta$ -cholanolic acid and incorporated paclitaxel. The resulting nanoparticles showed sustained drug release, and following injection into the tail

vein of tumor-bearing mice, tumor growth was impaired. In a subsequent study, the same research team used this system as carrier for cisplatin, which is also poorly soluble in water. The hydrophobically modified glycol chitosan nanoparticles loaded with cisplatin exhibited the EPR effect, as they accumulated in solid tumors, and was proven to have a high antitumor efficacy in a tumor-bearing mice model (Kim et al. 2008). Paclitaxel was also encapsulated in chitosan-containing glyceryl monooleate core-shell nanoparticles, which were generated by the emulsification/evaporation technique (Trickler et al. 2008). Using this drug delivery system, the authors observed a 1000-fold increase in cytotoxicity, when determining the  $IC_{50}$  values in a human breast cancer cell line. Another common hydrophobic anticancer drug is 5-fluorouracil, which has been widely used to treat different kinds of solid tumors. In a study by Zheng et al. (2007), polyelectrolyte nanoparticles based on chitosan and polyaspartic acid sodium salt were used to encapsulate 5-fluorouracil testing various conditions for nanoparticle preparation such as temperature, ionic strength, pH and cross-linker concentration, and different loading methods. The optimized nanoparticles showed sustained drug release in vitro and in vivo. Rajan et al. (2013) prepared hyaluronidase-5-fluorouracil-loaded chitosan-polyethyleneglycol-gelatin copolymers as a targeted drug delivery system and examined particle size, distribution, morphology, and drug loading capacity. The nanoparticles showed less cytotoxicity than free 5-fluorouracil when applied to colon cancer cells for a few hours. Another approach for controlled drug delivery used molecular surface imprinted graft copolymer of chitosan with methyl methacrylate, which was prepared by free-radical polymerization with 5-fluorouracil as template molecule (Zheng et al. 2016). The pH dependency and the kinetics of drug release suggested that this chitosan-based carrier is optimal for orally applied colon-specific drug delivery. A similar strategy to achieve colon specificity was used recently by Bashir et al. (2017). They synthesized pH-responsive semi-interpenetrating network hydrogels of *N*-succinyl-chitosan via Schiff base mechanism using glutaraldehyde as a cross-linking agent and embedded poly(acrylamide-co-acrylic acid). The hydrogel exhibited a porous structure and pH-dependent swelling properties. The hydrogel was effectively loaded with 5-fluorouracil, and the determined drug release was pH-dependent as well, with high release rates at pH 7.4 and low rates at pH 1.2.

In many cases, chitosan nanoparticles have been conjugated with tumor-specific ligands to mediate active targeting of cancer cells, which is expected to increase therapeutic efficacy, accelerate drug release to selected sites, prevent unwanted drug release before arrival at the target sites, and diminish adverse side effects of chemotherapeutic drugs. Active targeting can be accomplished by functionalizing chitosan-based nanoparticles and hydrogels using tumor-targeting ligands, which bind to specific receptors that are specifically present on the surface of cancer cells. Proper ligands of such kind include cytokines, peptides, folic acid, hyaluronic acid, biotin or avidin, and antibodies (Prabaharan 2015). Here, we will discuss only a one example for each of these ligands to illustrate active targeting.

Deng et al. (2011b) synthesized monodispersed and pH-sensitive chitosan-silica hollow nanospheres, loaded them with antitumorigenic tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), and conjugated them with an antibody to epidermal growth factor receptor

2, which is overexpressed in about 20% of all women suffering from breast cancer (Owens et al. 2004). Subsequent drug release studies demonstrated that the nanospheres delivered cytotoxic TNF- $\alpha$  to MCF-7 breast cancer cells and suppressed tumor with high therapeutic efficacy. Due to the acidic microenvironment inside solid tumors, TNF- $\alpha$  is gradually released from the nanospheres, binds to the TNF- $\alpha$  receptor, and activates a signaling cascade which induces programmed cell death.

In a study published by Shen et al. (2013a), doxorubicin and verapamil were combined in chitosan nanoparticles to achieve an integrated treatment for cancer and doxorubicin-induced cardiomyopathy in the process of cancer therapy. For this purpose, chitosan shells coated on magnetic nanoparticles were loaded with both drugs and entrapped into poly(lactic acid-co-glycolic acid) nanoparticles conjugated with a cyclo(Arg-Gly-Asp-D-Phe-Lys) (cRGD) peptide targeting  $\alpha_v\beta_3$  integrin, which is highly expressed on activated endothelial cells of newborn vessels during tumor angiogenesis as well as in some tumor cells (Liu et al. 2008). Near-infrared laser irradiation was sufficient to trigger drug release within an acidic microenvironment. Cytotoxicity assays performed *in vitro* suggested that cRGD-conjugated nanoparticles exhibited a greater growth inhibitory potential in cancer cell lines than the free drug or control nanoparticles likely due to cRGD-mediated targeting of tumor cells. *In vivo* imaging and biodistribution studies further showed that the nanoparticles preferentially accumulated in the tumor tissue under magnetic guidance. Finally, *in vivo* data for tumor regression along with electrocardiogram recordings and histopathology observations indicated that the cRGD-conjugated polymer-coated magnetic nanoparticles could have a high therapeutic potential as a dual-drug delivery system for the treatment of both cancer and doxorubicin-mediated cardiotoxicity.

Recently, a novel disulfide-linked chitosan-g-methoxy poly(ethylene glycol) copolymer was successfully synthesized, which was suggested to have excellent properties for redox-responsive drug delivery (Antoniraj et al. 2018). Redox-responsive 5-fluorouracil-loaded nanoparticles were synthesized by ionic gelation method, and folic acid was used to functionalize the nanoparticles for receptor-targeted drug delivery, as cancer cells commonly express high-affinity folate receptors on their surface. The 5-fluorouracil-free nanoparticles showed improved hemocompatibility, and the 5-fluorouracil-loaded nanoparticles conjugated with folic acid had a high cytotoxicity to MCF7 breast cancer cells, presumably due to intracellular internalization because of folic acid conjugation, which is expected to enhance the cellular uptake of the nanoparticles.

Many types of cancer cells overexpress different isoforms of hyaluronic acid receptors, which leads to enhanced binding and internalization of hyaluronic acid, as reported for instance in breast tumor cells (Bourguignon et al. 2000). To exploit this fact for targeting tumor cells, Jain et al. (2010) prepared hyaluronic acid-conjugated chitosan nanoparticles loaded with oxaliplatin and encapsulated in Eudragit S100-coated pellets for effective delivery to colorectal tumors. In immunodeficient C57BL mice model with HT-29 cancer cells injected into the ascending colon, relatively high local drug concentrations were found in the colon tumors after oral administration of the oxaliplatin nanoparticles, and the concentrations increased with



prolonged exposure time. Coupling of hyaluronic acid onto the surface of chitosan nanoparticles was found to make them more specific for delivery of the anticancer drug to the tumor of the colon.

Several studies revealed that also biotin and avidin possess tumor-targeting properties. Biotin receptors are overexpressed in many tumor types characterized by rapid division rates and aggressive growth (Russell-Jones et al. 2004), and avidin (a highly glycosylated protein) is recognized by lectins expressed on the surface of tumor cells (Yao et al. 1998). For this reason, Bu et al. (2013) prepared chitosan nanoparticles conjugated with either biotin or both biotin and avidin as tumor-targeted carrier system for the delivery of *trans*-resveratrol. Pharmacokinetic experiments revealed that avidin-biotin-loaded nanoparticles rapidly accumulated in the liver after injection, while the delivery nanoparticles conjugated only with biotin was attenuated. Cytotoxicity assays using HepG2 cells further uncovered that compared to *trans*-resveratrol solution and unconjugated chitosan nanoparticles, both biotin and avidin-biotin loaded nanoparticles significantly improved anticancer activity, but the latter combination exhibited a higher cytotoxicity. Thus, it was proposed that the synthesized nanoparticles conjugated with avidin and biotin may be a potent drug delivery system particularly to targeting hepatic carcinoma.

Finally, Xiao and Yu (2017) developed a glycol/chitosan nanobioconjugate loaded with gemcitabine and conjugated with anti-EGFR and anti-chitosan antibodies to target pancreatic cancer cells and cause aggregation. Administration of the chitosan conjugates efficiently blocked tumor growth and metastatic spread in human pancreatic cancer cells.

### 14.6.3 Chitosan-Based Vectors for Gene Therapy

Gene therapy requires the transmission of nucleic acids (DNA or RNA) into the target cell to mediate expression of therapeutic genes or to silence gene expression by RNA interference. However, negatively charged phosphates of nucleic acids impair permeation through the plasma membrane, which is negatively charged as well. In addition, unprotected nucleic acids are highly susceptible to degradation by nucleases. Hence, delivery of nucleic acids into cells relies on non-viral or viral vectors, which drastically improves transfection and protects from enzymatic degradation (Wivel and Wilson 1998). As viral vectors have the risk of causing adverse side effects such as immune reactions and malignant transformation, many efforts have been made to develop non-viral vectors for gene delivery, among them are ample examples of different chitosan-based nanoparticles.

Actually, unmodified chitosan is not an effective carrier for the transfer of nucleic acids due to its low solubility in water and instability of DNA/RNA chitosan complexes at physiological pH. Thus, chitosan requires chemical modification or grafting to convey appropriate physicochemical properties to the resulting complex. Chitosan modifications that have been used to design chitosan-based carriers for gene or siRNA delivery include quaternization by alkylation of tertiary amines,

reaction with 2-chloroethylamine hydrochloride and *N,N*-dimethyl-2-chloroethylamine hydrochloride, conjugation with polyethylene glycol, poly(amidoamine) or RGD dendrimer grafting, modification with phosphatidylcholine, or combinations of these modifications.

Among the quaternized chitosan derivatives, *N*-trimethyl chitosan and its derivatives have been extensively studied for their suitability in gene delivery, because they are reasonably soluble in water, have comparably little tendency to form aggregates, and exhibit a high loading capacity for nucleic acid under physiological conditions. In a systematic study published by Germershaus et al. (2008), the physicochemical properties of chitosan, *N*-trimethyl chitosan, and poly(ethylenglycol)-*N*-trimethyl chitosan were analyzed and compared. Using cell lines derived from mouse embryonic fibroblasts as a transfection system for plasmid DNA, the authors observed a significant increase in transfection efficiency when *N*-trimethyl chitosan nanoparticles were used to deliver plasmid DNA instead of chitosan nanoparticles. In addition, grafting poly(ethylenglycol) onto *N*-trimethyl chitosan further improved transfection efficiency, stabilized the particles, decreased particle size, and reduced cytotoxicity when compared to unmodified *N*-trimethyl chitosan nanoparticles. Zheng et al. (2009) prepared folate-conjugated *N*-trimethyl chitosan nanoparticles and compared cellular uptake and transfection of plasmid DNA (pDNA) in vitro with non-conjugated *N*-trimethyl chitosan nanoparticles using folate overexpressing KB and SKOV3 cells and folate receptor-deficient A549 and NIH/3T3 cells. The folate-*N*-trimethyl chitosan/pDNA complex showed a decrease in cytotoxicity in comparison to pDNA complexes made of polyethylenimine. Moreover, folate conjugation increased transfection efficiency and folate receptor-mediated endocytosis by KB cells and SKOV3 cells when compared to non-conjugated *N*-trimethyl chitosan nanoparticles.

Exploring further possible improvements of *N*-trimethyl chitosan-based gene delivery systems, Zheng et al. (2015) synthesized arginine, cysteine, and histidine-modified trimethyl chitosan nanoparticles to form complexes with pDNA. Using HEK 239 cells, they evaluated stability, cellular uptake, endosomal escape, release behavior, nuclear localization, and in vitro and in vivo transfection efficiencies. The cysteine-modified *N*-trimethyl chitosan nanoparticles turned out to be the most promising candidates for gene delivery due to sufficient stability, high cellular uptake, and glutathione-responsive release-favoring mechanism in combination with preferable nuclear distribution. Addition of sodium tripolyphosphate to the cysteine-modified nanoparticles was further effective to compromise certain disadvantageous attributes for pDNA delivery. *N*-trimethyl chitosan nanoparticles were also employed in drug-siRNA co-delivery using a metastatic breast cancer cell line (Eivazy et al. 2017). In this study, the authors tested simultaneous delivery of siRNA to silence the gene encoding the high mobility antigen (HMGA-2) and the anticancer drug doxorubicin to boost therapeutic anticancer effects. They found that dual delivery of HMGA-2 siRNA and doxorubicin by trimethyl chitosan nanoparticles significantly inhibited breast cancer cells growth.

A very recent study developed novel strategies in fighting lung cancer by RNA interference mediated gene silencing of the gene encoding the anti-apoptotic protein,

Survivin. For this purpose, Ni et al. (2018) designed nanoparticles consisting of baclofen functionalized *N*-trimethyl chitosan as polymeric carriers, TPP as ionic cross-linker, and siRNA to Survivin. Baclofen was used to target the nanoparticles to non-small lung cancer cells that overexpress the GABA<sub>B</sub> receptor, which specifically binds baclofen (Zhang et al. 2013b). The siRNA-loaded nanoparticles increased the uptake of Survivin-siRNA through the interaction with GABA<sub>B</sub> receptor and efficiently induced apoptosis and gene silencing. The authors further encapsulated the siRNA-loaded nanoparticles into mannitol microparticles for dispersion in the HFA-134a aerosol to allow administration by pressurized metered-dose inhalers. Pulmonary delivery of siRNA is expected to avoid serum-induced degradation, reduce systemic side effects, and improve therapeutic efficacy.

Zhong et al. (2015) hypothesized that the addition of amino residues to chitosan could improve stable complex formation with negatively charged siRNA enhancing transfection and gene silencing efficiency. For this purpose, they prepared a novel chitosan derivative (MixNCH) combining 2-chloroethylamine hydrochloride and *N,N*-dimethyl-2-chloroethylamine hydrochloride with chitosan and examined the physicochemical properties of the resulting nanoparticles. Using a hepatocellular carcinoma cell line (HepG2), gene transfection efficiency of MixNCH/midkine-siRNA nanoparticles and inhibition of HepG2 cell proliferation were analyzed. They found that midkine-siRNA delivered by MixNCH nanoparticles was able to significantly reduce both mRNA and protein levels of the midkine growth factor, resulting in a significant decrease of cell proliferation in HepG2 cells.

Guzman-Villanueva et al. (2014) evaluated the capability of different-sized chitosan derivative-based polyplexes to carry, internalize, and release siRNA in human adenocarcinomic epithelial cells. For this purpose, they first prepared *N*-phthaloyl-chitosan or *N*-phthaloyl-oligochitosan, reacted them with polyethylene glycol and hydroxybenzotriazole in DMF, and then cross-linked the polymers using ECD. Finally, the *N*-phthalimido groups were removed by the reaction with hydrazine monohydrate, and the products were purified by dialysis against water and ethanol. Both the chitosan- and oligochitosan-based polyplexes exhibited biodegradability, low cytotoxicity, and resistance to enzymatic degradation up to 24 h. When loaded with siRNA, the oligochitosan-based polyplexes drastically increased cellular internalization of the siRNA and gene silencing compared to naked siRNA. To improve the transfection efficiency of chitosan-based gene delivery systems, Deng et al. (2011a) fabricated a dendronized chitosan derivative using a copper-catalyzed azide alkyne cyclization reaction of propargyl focal point poly(amidoamine) dendron with 6-azido-6-deoxy-chitosan. The resulting dendronized chitosan nanoparticles exhibited higher water solubility and buffering capacity than native chitosan and showed lower cytotoxicity and enhanced transfection efficiency in transformed human embryonic kidney and nasopharyngeal carcinoma cell lines than commonly used polyethylenimine.

As already mentioned above, the RGD motif can be used for targeting chitosan-based nanoparticles to tumor sites via the interaction with integrin  $\alpha_v\beta_3$ . Utilizing this fact, Kim et al. (2017) produced a dendrimeric RGD peptide/polyethylenimine grafted chitosan copolymer, which was soluble in water. The copolymer was

nontoxic to mammalian cells and erythrocytes in the absence and presence of plasmid DNA. Moreover, it was found to transfect cells involving microtubule-dependent macropinocytosis and clathrin-mediated endocytosis. Finally, injecting copolymers complexed with psiRNA-hBCL2 to silence the gene for the human anti-apoptotic Bcl2 protein into BALB/c-nu mice carrying a PC3 prostate tumor xenografts, markedly inhibited tumor growth. Thus, the copolymer was suggested to be a good candidate to develop a specific targeted gene delivery system.

To confer membrane-like properties to chitosan-based gene delivery systems, Li et al. (2015) grafted phosphorylcholine and macrocyclic polyamine onto chitosan to obtain water-soluble nanoparticles. Chitosan grafted with both compounds were more efficient in binding and protecting plasmid DNA than chitosan grafted only with phosphorylcholine or macrocyclic polyamine. The authors also demonstrated that phosphorylcholine and macrocyclic, polyamine-grafted chitosan had a positive net charge and can, therefore, wrap DNA to yield nanoparticles of about 100 nm in diameter. Finally, the DNA-loaded nanoparticles significantly increased cellular uptake and transfection rates in transformed human embryonic kidney cells when compared to chitosan/DNA complexes. A similar transfection strategy was published by Picola et al. (2016), who inserted phosphorylcholine and increasing numbers of diethylaminoethyl (DEAE) groups into the polymer. The resulting chitosan nanoparticles were water soluble at physiological pH and less cytotoxic than lipofectamine, a commonly used transfection reagent. They further could form complexes with plasmid DNA, and the transfection efficiencies of the nanoparticles with high DEAE substitution rates tested in HeLa cells were in the same range as determined for lipofectamine. When the nanoparticles were loaded with siRNA, they were able to induce gene silencing, with efficiencies highly dependent on the N/P ratio.

#### ***14.6.4 Chitosan-Based Adjuvants for Vaccine in Immunotherapy***

Chitosan-based materials have been recognized to be potent adjuvants for immunotherapy, because they non-specifically stimulate immune responses in the host organism and therefore have antiviral, antimicrobial, and antitumor properties (Li et al. 2013b). The adjuvant potency of chitosan is comparable to incomplete Freund's adjuvant, and it has stronger immune-stimulatory effect than aluminum hydroxide, which is frequently used in vaccines though it shows adverse side effects such as neurotoxicity (Zaharoff et al. 2007). The mechanism of how chitosan triggers immune responses involves phagocytosis-dependent activation of the NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome, which finally induces a robust interleukin-1 $\beta$  response (Bueter et al. 2011, 2014). Carroll et al. (2016) described another mechanism by which chitosan stimulates the activation of dendritic cells inducing cellular immunity. They found that chitosan

promotes the intracellular release of DNA, which involves the cGAS-STING pathway. As a result, type I interferon is secreted activating the expression of interferon-controlled genes. Due to the release of the cytokines that stimulate dendritic cells, the cellular immune system is elicited.

Due to its mucoadhesiveness, chitosan and its derivatives are considered effective for mucosal administration, which includes oral, nasal, as well as ocular antigen-delivery routes. However, other routes are also anticipated to be effective in provoking immune response including subcutaneous (Borges et al. 2008; Scherliess et al. 2013), intraperitoneal (Chang et al. 2010), intravenous (Shi et al. 2011), and intratumoral injections (Zaharoff et al. 2010). Evidently, innate immune responses are stimulated by chitin, chitosan, or derivatives (Peluso et al. 1994; Tokura et al. 1999; Lee et al. 2008; Lee 2009). This includes the activation of alveolar macrophages with the release of cytokines such as interleukin (IL)-12, tumor necrosis factor- $\alpha$ , or IL-18, leading to INF- $\gamma$  production predominantly released by natural killer cells (Shibata et al. 1997a, b). However, also humoral and cellular adaptive immune responses are triggered by some antigens when co-administered or encapsulated in chitosan-containing micro- and nanoparticles (Tokura et al. 1999; van der Lubben et al. 2001; Arca et al. 2009; Mori et al. 2012). In particular, Wen et al. (2011) found that the stimulatory effect on the humoral and cellular immune system by chitosan results in a balanced Th1/Th2 response. However, care has to be taken in assessing the properties of chitosan-based adjuvants, as many studies do not provide sufficient data on the chemical and physical characteristics, preparation and formulation procedures, as well as potential impurities (Vasiliev 2015). This is particularly critical, as immune responses appear to depend on these parameters as uncovered by Scherliess et al. (2013), who reported that the degree of immune response varied when chitosans of different qualities were used. The immune-stimulatory effect of chitosan is affected by the combination of molecular weight, solubility, particle size, and viscosity as well as deacetylation degree.

Preclinical studies performed predominantly in mice models suggested that chitosan-containing antigen-delivery systems are promising adjuvant platforms for mucosal vaccination against human pathogenic viruses such as influenza (Read et al. 2005; Svindland et al. 2012; Sawaengsak et al. 2014; Liu et al. 2015a); hepatitis A, B, and E (Jiang et al. 2007; Tao et al. 2017a; Tao et al. 2017b; Soares et al. 2018); human papilloma virus (Ma et al. 2015); and poliovirus (Ghendon et al. 2011). Combinations of chitosan and heat-inactivated human herpes viruses (HSV) were further tested as an immunomodulating adjuvant in T cells and antigen-presenting cells in HSV-infected mice (Choi et al. 2016). Using chitosan nanoparticles targeted to dendritic cells via antibodies to the DEC-205 surface receptor, Raghuwanshi et al. (2012) successfully delivered plasmid DNA carrying the cDNA for the N protein to trigger immunization against the severe acute respiratory syndrome coronavirus (SARS-CoV). Simultaneous comparison of targeted formulations using intramuscular and intranasal routes revealed that intramuscular administration induced a more potent systemic IgG response compared to intranasal administration. Solid evidence substantiating the advantages of chitosan as an efficient adjuvant for nasal vaccination originates from clinical examinations on a norovirus vaccine, which

demonstrated the ability of a chitosan/monophosphoryl lipid-based antigen delivery system (ChiSys®) to induce immunity against the gastroenteric norovirus infections after immunization (Smith et al. 2014).

Chitosan-derived adjuvants were also used in combination with antigens derived from bacterial toxins, such as diphtheria toxoids (McNeela et al. 2000; Schipper et al. 2017), tetanus toxoids (Ahire et al. 2007; Pirouzmand et al. 2017), and dermonecrotxin (Jiang et al. 2004). In addition, the potential of various vaccine formulations against anthrax were evaluated in female BALB/c mice (Malik et al. 2018). Encapsulating protective antigens (PA) in trimethyl-chitosan nanoparticles and administering them by subcutaneous, intramuscular, and intraperitoneal injections resulted in a strong IgG antibody response (Th1-biased) when combined with immune-stimulatory CpG oligodeoxynucleotides or polyinosinic-polycytidylic acids. Interestingly, without the immune-stimulatory nucleic acids, the PA-loaded trimethyl-chitosan nanoparticles led to a Th2-biased immune response.

Many studies have explored the adjuvant properties of chitosan in vaccines against cancer. In a study published by Wen et al. (2011), the effects of chitosan nanoparticles on the immune response triggered by an ovalbumin antigen in mice were analyzed. As the administration of the chitosan nanoparticles did not only increase cytokine levels of Th1 (IL-2 and IFN- $\gamma$ ) and Th2 (IL-10) cells, but also stimulated natural killer cells, the authors suggested that chitosan is a promising adjuvant for cancer immunotherapy by promoting both humoral and cellular immune responses. This hypothesis was confirmed by Highton et al. (2016), who demonstrated that immunization with an ovalbumin/chitosan hydrogel had antitumor effects in an intracaecal mice cancer model. After subcutaneous injection of the ovalbumin/chitosan vaccine, the authors detected CD8<sup>+</sup> T memory cells specific for ovalbumin and observed decreased tumor growth in contrast to unvaccinated control mice or mice that were vaccinated with dendritic cells and ovalbumin.

Zaharoff et al. (2009) analyzed antitumor effects in mice using a bioluminescent orthotopic bladder cancer model, after repeatedly administering chitosan/IL-12 into the bladder and comparing the antitumor efficacy of this treatment with that of an established adjuvant therapy applied to treat bladder cancer based on attenuated mycobacteria (bacillus Calmette-Guerin therapy). Determination of the urinary cytokine spectrum and immunohistochemical analysis resulted in the identification of cytotoxic T cells and natural killer cells as effector cells responsible for tumor regression. In contrast to the *Bacillus* Calmette-Guerin therapy, chitosan/IL-12 treatment utterly prevented recurrence of the disease.

More recently, Yao et al. (2013b) prepared mannosylated chitosan nanoparticles and loaded it with a plasmid to produce a vaccine against gastrin-releasing peptide (GRP), whose receptor is overexpressed in various cancer cells. The nanoparticles were intranasally administered in a subcutaneous mice prostate carcinoma model to evaluate the efficacy on inhibition of the growth of tumor cells. Cell binding and cellular uptake assays revealed that the mannosylated chitosan nanoparticles facilitate targeting to antigen-presenting cells, promoting receptor-mediated endocytosis

via the mannose receptor. Due to antigen representation, enhanced tumor regression was observed as a result of the production of high titers of anti-GRP antibodies. A similar strategy was finally used by Lai et al. (2014) to test an immune therapy against hepatocellular carcinoma in a H22 tumor-bearing mice model. They synthesized folate-conjugated chitosan nanoparticles and loaded them with a plasmid-encoding mouse interferon- $\gamma$ -inducible protein-10 (IP-10). They found that IP-10 plasmid exhibited efficient antitumor activity, prolonging the survival time in H22 tumor-bearing mice. The antitumor effects were likely due to different effects. Next to the secretion of IFN- $\gamma$  and IP-10, inhibition of regulatory T cells, suppression of angiogenesis, inhibition of cancer cell proliferation, and activation of apoptosis contributed to tumor growth inhibition.

### ***14.6.5 Tissue Engineering***

Tissue engineering is an increasingly important interdisciplinary field in regenerative medicine, which aims to create replacements for damaged tissues based on the combined knowledge provided by physicians, biologists, and engineers. Most approaches employ scaffolds made from biocompatible polymers, which are colonized by cells of the respective tissue. Ideally, the scaffold increases adherence, proliferation, and differentiation of colonizing cells. Chitosan and its derivatives offer ample benefits to generate cell and tissue supporting matrices, which include chemical versatility, antimicrobial activity, biocompatibility, biodegradability, and negligible toxicity (Ahsan et al. 2018). Chitosan can be produced to form sponge-like scaffolds using rather simple phase separation techniques including freeze-drying (Aranaz et al. 2014), gas foaming (Kaynak Bayrak et al. 2017), and electrospinning procedures (Qasim et al. 2018). The presence of a system of interconnected pores with appropriate diameters facilitates vascularization and tissue integration. Moreover, chitosan-based scaffolds can be synthesized in combinations with ample natural and synthetic polymers resulting in matrices exhibiting special characteristics. Due to their positive surface charges they open the possibility to fabricate polyelectrolyte complexes with anionic polymers such as glutamic acid (Fang et al. 2014), hyaluronic acid (Lalevee et al. 2016), dextrane sulfate (Kulkarni et al. 2016), heparin (Almodovar and Kipper 2011), dermatan sulfate (Rasente et al. 2016), and chondroitin sulfate (Tsai et al. 2011). Particularly the presence of glycosaminoglycans, which are naturally found in extracellular matrices, is known to modulate the activity of cytokines and growth factors by binding to the polymers (Zaman et al. 2016). Otherwise, chitosan-based scaffolds can be loaded with cytokines and growth factors to attract cells and stimulate tissue regeneration (Sun et al. 2012a; Bader et al. 2015; Choi et al. 2015). Finally, they further open the possibility of controlled degradation and resorption in physiological environments, designing mechanical properties that match the conditions found in the respective tissue, and determination of desired sizes and shape by easy fabrication procedures. Therefore,



chitosan-based scaffolds have numerous applications in tissue engineering, and we will review recent progress in using these materials for tissue regeneration and wound healing.

#### 14.6.5.1 Bone, Cartilage, and Tooth Repair

Bone defects can either be congenital or result from trauma, infection, cancer, or failed orthopedic surgical procedures (Venkatesan and Kim 2010). The grafts used to bridge bone defects can be autografts (bone material from other body regions of the same patient) or allografts (bone material from decedents). However, both materials have disadvantages: autografts require bone harvesting from healthy tissues and may cause complications of wound healing and pain, and allografts may result in immunogenic rejection and have the risk of transmitting viral diseases from the donor to the recipient. Due to these concerns, scientists around the world are searching for alternative materials as bone graft substitutes. As described before, chitosan-based materials have valuable properties for orthopedic applications. Chitosan itself has the capacity to increase bone regeneration rates (Muzzarelli et al. 1993b); however, it cannot fully substitute natural bone material. Therefore, different composite scaffolds have been developed to assure porosity for vascularization and nutrition, facilitate the formation of new bone material (osteoconductivity), guarantee structural integrity during ingrowth at the site of implantation, and orchestrate biodegradation with bone regeneration (Venkatesan and Kim 2010). In addition, chitosan-based composites can be loaded with cells and growth factors that promote osteoconductivity and hence facilitate bone regeneration. One of the most important chitosan grafting that has been used in bone tissue engineering is hydroxyapatite, which by itself stimulates bone regeneration, provided that the scaffold has a microporous structure (Woodard et al. 2007). Hydroxyapatite grafting of chitosan can be easily achieved by coprecipitation from homogeneous mixtures of precursor (Deepthi et al. 2016, and references therein). One of the first researchers who tested combinations of chitosan and hydroxyapatite was Michio Ito, who examined the use of chitosan-bonded hydroxyapatite paste for treatment of periodontal defects (Ito 1991). In 2004, Ge et al. (2004) published a remarkable study, in which they tested different combinations of air- and freeze-dried chitosan/hydroxyapatite materials that were colonized by osteoblasts and implanted into rats. The material was found to be nontoxic and biodegradable and to stimulate mineralization. The explanted material that was colonized by osteoblasts before implantation showed newly formed bone material containing proliferating osteoblasts that recruited surrounding tissue to grow in. In another study published by Oliveira et al. (2006), three-dimensional macroporous hydroxyapatite/chitosan bilayered scaffolds of inorganic and organic deposits were produced in a stepwise procedure and examined with regard to their mechanical properties and cytotoxicity to mouse fibroblast-like cells. Moreover, *in vitro* cell culture studies using goat marrow stromal cells revealed that the macroporous hydroxyapatite/chitosan

composite is a suitable material that promotes attachment, proliferation, and differentiation into osteoblasts and chondrocytes. Additionally, three-layered porous materials of collagen, hydroxyapatite, and chitosan were produced and characterized as an artificial bone matrix (Wang et al. 2008b). When testing murine pre-osteoblast cell line (MC3T3-E1) grown on this matrix, the cells proliferated significantly more rapidly than cells grown on a pure chitosan matrix (Teng et al. 2008). In addition, higher levels of alkaline phosphatase (secreted by osteoblasts) were determined, which is indicative for bone regeneration. Similar results were obtained when osteoblasts were cultured on hydroxyapatite/chitosan nanocomposites and osteocalcin as a marker for late osteoblastic differentiation, and mineralized bone matrix formation was determined (Chesnutt et al. 2009). Further studies characterized hydroxyapatite/chitosan hybrids with additional blend materials such as montmorillonite (Katti et al. 2008), polylactic acid (Cai et al. 2009), cellulose and carboxymethyl cellulose (Liuyun et al. 2009; Jiang et al. 2013b), gelatin (Sellgren and Ma 2012; Maji et al. 2015; Lee et al. 2017), nylon 66 (Huang et al. 2011), polygalacturonic acid (Khanna et al. 2011), marine sponge collagen (Pallela et al. 2012), collagen (Wang et al. 2009), alginate (Jin et al. 2012; Kim et al. 2015; Liao et al. 2018), chondroitin sulfate (Venkatesan et al. 2012a; Hu et al. 2017), hyaluronic acid (Hu et al. 2017), fibroin (Lima et al. 2013; Ran et al. 2016; Ye et al. 2017), poly-3-hydroxybutyrate-co-3-hydroxyvalerate (Zhang et al. 2015b), fucoidan (Lowe et al. 2016),  $\beta$ -tricalcium phosphate (Shavandi et al. 2015; Oryan et al. 2017), graphene oxide (Yu et al. 2017),  $\beta$ -cyclodextrin (Shakir et al. 2016),  $\beta$ -1,3-glucan (Przekora and Ginalska 2017; Przekora et al. 2017), whitlockite (Zhou et al. 2017a), zoledronic acid (Lu et al. 2018), and zirconium dioxide (Balagangadharan et al. 2018). Also, three-dimensional hydroxyapatite/chitosan-carbon nanotube scaffolds were shown to be promising materials for bone regeneration (Im et al. 2012). Naturally, many of these combinations have been tested also in the absence of hydroxyapatite (Park et al. 2000a; Li et al. 2005; Jiang et al. 2006; Arpornmaeklong et al. 2008; Venkatesan et al. 2012b; Deng et al. 2013; Azevedo et al. 2014; Dinescu et al. 2014; Listoni et al. 2015; Georgopoulou et al. 2018; Koç Demir et al. 2018).

Several studies showed that various growth factors such as transforming growth factors (TGFs), vascular endothelial growth factors (VEGFs), bone morphogenic proteins (BMPs), insulin-like growth factors (IGFs), and platelet-derived growth factors (PDGFs) have major impacts on vascularization and osteoblast activities and thus have been employed to stimulate bone regeneration (Yun et al. 2012). However, there are limitations in maintaining therapeutic concentrations due to the short half-life of the growth factors in vivo. This can be effectively prevented by the controlled release of the growth factors from porous chitosan composite matrices, which have been demonstrated to stimulate bone formation. For instance, PDGF-BB is an important osteogenic growth factor in the process of bone regeneration, as it stimulates mesenchymal cell proliferation and differentiation and mediates chemotaxis of osteoblast. Park et al. (2000a, b) produced a porous chitosan or chondroitin-4-sulfate/chitosan sponges releasing PDGF-BB to stimulate bone regeneration. The release rate of PDGF-BB increased proportionally with increasing concentrations loaded onto the sponge, and PDGF-BB retained its chemotactic activity regardless of

being loaded onto the sponge or added freely to test solution (Park et al. 2000a). Finally, osteoblast proliferation was found to be stimulated in PDGF-BB-loaded chondroitin-4-sulfate/chitosan sponge compared with that of the unloaded sponge. PGFs have also been used in combination with other growth factors. As VEGF is known to prolong cell survival, osteoblast proliferation, differentiation, and migration next to its effects on angiogenesis, it has been suggested that the combined action of VEGF and PDGF can accelerate the bone healing process even more efficiently. De la Riva et al. (2010) established a system based on brushite-chitosan capable of controlling release kinetics for these two growth factors. After implanting the chitosan scaffolds loaded with the growth factors into rabbits with femur defects, release kinetics and tissue distribution of radiolabeled VEGF and PDGF were determined. Analyzing bone repair histologically revealed that the combined use of VEGF and PDGF promoted bone regeneration most effectively.

Also, the controlled release of IGF-1 and BMP-2 by the enzymatic degradation of the porous chitosan scaffold stimulates bone healing and regeneration in rabbits considerably (Nandi et al. 2013). When the chitosan particles were loaded only with one of the two growth factors, the effect was found to be more pronounced for IGF-1 than for BMP-2 infiltrated matrices. In a very recent study, chitosan/biphasic calcium phosphate scaffolds functionalized with BMP-2-encapsulated nanoparticles and the RGD tripeptide were produced using a desolvation technique (Gan et al. 2018). In vitro cell culture and in vivo implantation tests demonstrated that RGD and BMP-2 synergistically increased cell adhesion and spreading via integrin binding triggering differentiation of osteoblasts. Increased bone healing was also observed, when porous chitosan scaffolds were loaded with resolvin D1, a potent lipid immune modulator derived from both eicosapentaenoic acid, and implanted into rats with a femur defect (Vasconcelos et al. 2018). Obviously, resolving D1 administration in the acute phase of the innate immune response to the bio-implant had beneficial effects during bone tissue repair.

Impairment of the articular cartilage is frequently due to sport-related injury, disease, trauma, and tumor. If not treated successfully, it may result in osteoarthritis, which increasingly affects also younger individuals (Muzzarelli et al. 2012). In contrast to bone regeneration, cartilage healing is limited by the lack of vascularization and poor proliferation rates of chondrocytes. Injection of hyaluronan into the joints of arthritic patients is known to improve their function, as it restores viscoelasticity and flow of the synovial fluid, helps to normalize hyaluronan production, and finally reduces pain and inflammation. Chitosan easily forms polyelectrolyte complexes with hyaluronan and chondroitin sulfate, which are important building blocks particularly of the hyaline cartilage found on the surface of joints. Therefore, combinations of chitosan and hyaluronan and/or chondroitin sulfate may be useful in cartilage healing. In a first attempt to realize this idea, Kuo et al. (2015) synthesized a highly elastic, macroporous, and chitosan-containing gelatin/chondroitin-6-sulfate/hyaluronan (GCH) cryogel scaffold, which mimics the extracellular matrix composition of the cartilage. Furthermore, in vitro cell culture studies suggest that chondrocytes proliferate and redifferentiate within the porous matrix of the cryogels. Although chitosan reduces cell proliferation, it stimulates the secretion of sulfated

glycosaminoglycans and type II collagen. In addition, they performed *in vivo* studies culturing chondrocytes on the GCH chitosan cryogel and implanting the material into rabbits with an articular cartilage defect (Kuo et al. 2015). After 3 months, the defect in the chondrocytes/cryogel group was completely covered with semitransparent tissue, which had similar characteristics as the native cartilage. A large variety of other chitosan-based materials lacking glycosaminoglycans have been suggested as suitable scaffolds for cartilage tissue engineering and some of them have been tested as mesenchymal stem cell carriers. Such materials include scaffolds containing *N,N*-dicarboxymethyl chitosan (Mattioli-Belmonte et al. 1999), chitosan/gelatin and/or alginate complex (Xia et al. 2004; Li and Zhang 2005; Bhat et al. 2011), poly(L-lactide)/chitosan microspheres (Lao et al. 2008; Haaparanta et al. 2014), polyethylene oxide/chitin/chitosan scaffolds (Kuo and Ku 2008), genipin-cross-linked chitosan/silk fibroin sponges (Silva et al. 2008; Vishwanath et al. 2016), chitosan/polyester-based scaffolds (Alves da Silva et al. 2010), chitosan/collagen type I scaffolds (Gong et al. 2010), chitosan/poly(epsilon-caprolactone) blend scaffolds (Neves et al. 2011; Filova et al. 2016), chitosan/poly(l-glutamic acid) scaffolds (Zhang et al. 2013a, 2015a), polyvinyl alcohol/chitosan composite hydrogels (Dashtdar et al. 2015), poly(*N*-isopropylacrylamide)/chitosan hydrogels (Mellati et al. 2016), viscoelastic silk/chitosan microcomposite scaffolds (Chameettachal et al. 2017), and chitosan/graphene oxide polymer nanofibers (Cao et al. 2017).

As in the case of bone repair, strategies using various chitosan-based scaffolds loaded with growth factors such as TGFs (Kim et al. 2003; Choi et al. 2015), IGFs (Zhao et al. 2010), BMPs (Mattioli-Belmonte et al. 1999), and basic fibroblast growth factor (bFGF) (Tan et al. 2007) have been tested for cartilage repair. In an interesting pilot study, Qi et al. (2013) produced an injectable chitosan/polyvinyl alcohol gel and examined its structure and physicochemical properties. The resulting material exhibited low cytotoxicity and good biocompatibility. Next, the gel was mixed with rabbit bone marrow stromal cells (BMSCs) that were transfected with an adenovirus to produce TGF- $\beta$ 1, and rabbits with cartilage defects were injected with this mixture. After 16 weeks, the defects appeared to be fully repaired. The regenerated tissue was almost indistinguishable from the native cartilage. In another study, a demineralized bone matrix was conjugated with mesenchymal stem cell (MSC) E7 affinity peptide (EPLQLKM) and combined with a chitosan hydrogel for cartilage engineering. Cell culture and implantation experiments demonstrated that the developed material has a high chondrogenic capacity facilitating tissue repair of cartilage defects (Meng et al. 2015). In a more recently published study, the proliferation and differentiation of multipotent dental pulp stem cells into chondrocytes were investigated to generate cartilage-like material. In this case, a porous chitosan-xanthan gum matrix was employed as a scaffold and loaded with kartogenin to promote chondrogenic differentiation (Westin et al. 2017). The manufactured scaffold exhibited favorable characteristics for cartilage tissue engineering, such as high porosity, low cytotoxicity, and mechanical properties compatible with those characteristic of cartilage.

Very recently, Agrawal et al. (2018) reported the *in vitro* generation of cartilage-like material by seeding human mesenchymal stem cells on freeze-dried porous silk fibroin/chitosan scaffolds and culturing them in a spinner flask bioreactor under dynamic conditions. The team was successful in preparing a cartilage construct of 5 mm thickness, which roughly corresponds to the thickness of a native articular cartilage.

Due to its unique properties, chitosan has also emerged as a scaffold for potential applications in dental medicine. Chitosan-based hydrogels and nanocomposites have been used as anti-erosive and enamel-repairing additives in dentifrices and chewing gums (Shibasaki et al. 1994; Arnaud et al. 2010; Ganss et al. 2011; Ruan et al. 2014), for reduction of dental bacterial biofilm formation (Jahanizadeh et al. 2017), for guided tissue regeneration to treat periodontal diseases such as periodontitis (Ma et al. 2014; Lotfi et al. 2016), as dentin-bonding agent (Fawzy et al. 2013), as modification of dental restorative materials and implants (Petri et al. 2007; Ali et al. 2017; Ibrahim et al. 2017a), and as scaffold for stem cell-based tissue regeneration (Yang et al. 2012b; Asghari Sana et al. 2017; Soares et al. 2017a). Periodontitis is a chronic inflammation of the gum, which ultimately may lead to the loss of periodontal tissues and teeth (Pihlstrom et al. 2005). Current therapeutic strategies mainly rely on good oral hygiene (brushing and flossing), plaque removal, and in more severe cases local application of antibiotics and surgical intervention including open flap debridement, osseous surgery, as well as guided tissue and bone regeneration. Chitosan-based materials turned out to be very useful for periodontal tissue regeneration. Such materials comprise methylpyrrolidinone chitosan (Muzzarelli et al. 1993a), chitosan scaffolds coated with a bioactive hydroxyapatite (Ang et al. 2002; Coimbra et al. 2011; Fraga et al. 2011; Miranda et al. 2016), injectable thermosensitive chitosan/ $\beta$ -glycerophosphate/hydroxyapatite hydrogels (Chen et al. 2016), chitosan-based risedronate/zinc-hydroxyapatite intrapocket dental films (Khajuria et al. 2018), asymmetric chitosan/tripolyphosphate cross-linked membranes (Ma et al. 2014), porous chitosan/collagen scaffolds (Yang et al. 2012b), mucoadhesive electrospun chitosan and thiolated chitosan nanofibers (Samprasit et al. 2015), chitosan-coated titanium surfaces (Campos et al. 2015), chitosan modified glass ionomer restoratives (Petri et al. 2007), chitosan-intercalated montmorillonite/poly(vinyl alcohol) nanofibers (Ghasemi Hamidabadi et al. 2017), and polyhydroxybutyrate/chitosan/nano-bioglass nanofiber scaffolds (Hashemi-Beni et al. 2018).

In one of the first studies that used growth factors to promote dental pulp stem cell differentiation, porous chitosan/collagen scaffolds prepared by freeze-drying were used and loaded with a plasmid vector encoding the human BMP-7 gene (Yang et al. 2012b). The stem cells grown in this scaffold were successfully transfected by the plasmid vector, which led to the formation of BMP-7 triggering odontoblastic differentiation as indicated by the activation of specific marker genes encoding osteocalcin, bone sialoprotein, dentin sialophosphoprotein, and dentin matrix protein 1. The chitosan/collagen scaffolds with stem cells were subcutaneously implanted into the back of BALB/c mice. After 4 weeks, the material was explanted and evaluated by immunohistochemistry. In the gene-activated scaffold group, there were still transfected cells detectable showing the upregulated gene expression when compared to pure scaffold groups.

#### 14.6.5.2 Cutaneous Wound Healing

Cutaneous wound healing is a complex process in which the skin repairs itself. The process is divided into different stages, which include blood clotting (hemostasis), inflammation, cell migration and proliferation, and tissue remodeling (maturation). The wound healing process may be delayed or completely fail leading to non-healing chronic wounds, which are frequently found in patients with diabetes, venous or arterial diseases, infections, and age-related metabolic deficiencies. Bedsore and burns behave differently, as the healing process is complicated by coagulation, necrosis, and infections. Small, non-severe wounds may be treated with chitosan-containing ointments (Kweon et al. 2003), topical gels (Alsarra 2009), and/or wound dressings (Jayakumar et al. 2011). For instance, Kang et al. (2016) reported the synthesis of silver chloride nanoparticles stabilized with chitosan oligomer for an ointment that was tested on burn wound healing in a rat model. Burn wound healing of rats treated with this ointment was superior to rats treated with pure Vaseline or chitosan ointments. More severe wounds may require removal of necrotic tissue and surgical wound closure using suturing techniques. If the defects are too large to be covered in this way, autologous avascular mesh grafts, microvascular flap grafts, mikroskin grafts, and/or cultured epithelial grafts are transplanted to the wound site (Chua et al. 2016). However, surgical intervention is only possible up to a critical size. To cover large-sized skin defects, artificial grafts produced by tissue engineering techniques are required. This type of wound dressings must protect from infections, absorb excess exudates, and facilitate oxygen and nutrient exchange. In addition, the material must be nontoxic, non-allergenic, non-adherent, and biocompatible.

Chitosan-based materials are a good choice for wound dressings, as they fulfill most of the criteria mentioned above, and they are known to promote wound healing by activating platelets when getting into contact with blood (Periyah et al. 2013). Moreover, chitosan-based scaffolds can be loaded with growth factors to facilitate skin repair by promoting cell adhesion and proliferation (Lu et al. 2016). Several *in vitro*, preclinical and clinical studies actually demonstrated that chitosan-based hydrogels films, powders, and dressings, as well as artificial skins, accelerate wound healing and reepithelialization (Patrulea et al. 2015). However, the precise mechanism of action in promoting wound healing is still under debate. Next to chitosan-mediated immunomodulation, the type of functionalization contributes to wound healing.

Chitosan-based scaffolds used to promote wound healing comprise hydrogels, films, micro- and nanoparticles, nanocomposite materials, and micelles, and many biocompatible chitosan derivatives have been tested including N-carboxybutyl chitosan (Dias et al. 2010), hydroxybutyl chitosan (Hu et al. 2018), fluorinated methacrylamide chitosan (Wijekoon et al. 2013; Patil et al. 2016; Akula et al. 2017), and chitosan/polyvinyl alcohol materials (Charernsriwilaiwat et al. 2014; Wang et al. 2016b).

In addition, numerous chitosan hybrid materials have been synthesized for wound healing, which comprise chitosan or carboxymethyl chitosan/gelatin hydrogels (Huang et al. 2013; Patel et al. 2018), chitosan/heparin/poly( $\gamma$ -glutamic acid) composite hydrogels (Zhang et al. 2018), chitosan-hyaluronan composite sponge scaffolds (Sanad and Abdel-Bar 2017; Tamer et al. 2018), heparin-chitosan complexes (Kratz et al. 1997; Kweon et al. 2003), chitosan-fibrin nanocomposites (Vedakumari et al. 2015), polyvinyl alcohol/chitosan/fibroin-blended sponges (Yeo et al. 2000), polyvinyl alcohol/starch/chitosan hydrogels with nano zinc oxide (Baghaie et al. 2017), poly(caprolactone)/chitosan/poly(vinyl alcohol) nanofibrous sponges (Gholipour-Kanani et al. 2014), chitosan/poly(ethylene glycol)-tyramine hydrogels (Lih et al. 2012), chitosan-alginate polyelectrolyte complexes (Wang et al. 2002; Hong et al. 2008; Caetano et al. 2015; Kong et al. 2016), porous keratin/chitosan scaffolds without and with zinc oxide (Tan et al. 2015; Zhai et al. 2018), nano-titanium oxide/chitosan complexes (Peng et al. 2008), chitosan/collagen hydrogels and sponges (Wang et al. 2008a; Cui et al. 2011; Ti et al. 2015), chitosan green tea polyphenol complexes (Qin et al. 2010, 2013), dextran hydrogels loaded with chitosan microparticles (Ribeiro et al. 2013), chitosan/polycaprolactone scaffolds (Bai et al. 2014; Zhou et al. 2017b), chitosan oleate ionic micelles (Dellera et al. 2014), castor oil polymeric films reinforced with chitosan/zinc oxide nanoparticles (Diez-Pascual and Diez-Vicente 2015), sponge-like nano-silver/zinc oxide-loaded chitosan composites (Lu et al. 2017), gellan gum-chitosan hydrogels (Shukla et al. 2016), chitosan-silica hybrid dressing materials (Park et al. 2017), chitosan/bentonite or tourmaline nanocomposites (Devi and Dutta 2017; Zou et al. 2017), chitosan/gelatin/chondroitin-4-sulfate films with and without zinc oxide (Cahu et al. 2017), chitosan/polyvinylpyrrolidone/cellulose nanowhiskers nanocomposites (Hasan et al. 2017),  $\alpha$ -tocopherol-loaded chitosan oleate nanoemulsions (Bonferoni et al. 2018), chitosan-based liposome formulations (Mengoni et al. 2017), and electrospun chitosan/polyethylene oxide/fibrinogen biocomposites (Yuan et al. 2018).

Topical application of anti-inflammatory and antioxidant curcumin, which is a component of many curry powders, has been shown to promote wound healing, significantly preventing oxidative damage in tissues (Gopinath et al. 2004). Chitosan-alginate sponges have been used to deliver curcumin for dermal wound healing in rat. Loading curcumin onto the chitosan sponge enhanced the therapeutic healing effect when compared to other carriers like cotton gauze. Similarly, injectable nanocomposite hydrogels composed of curcumin, N,O-carboxymethyl chitosan, and oxidized alginate possess many characteristics that promote wound healing including exudate absorption and immobilization and activation of growth factors. Nano-curcumin, which is released slowly from the hydrogel in a sustained manner, evidently stimulates fibroblast proliferation, angiogenesis, and collagen production, supporting the healing process when tested in a mice model (Li et al. 2012b). Wound dressings made of chitosan/poly- $\gamma$ -glutamic acid/pluronic/curcumin nanoparticles also promoted collagen formation and tissue regeneration (Lin et al. 2017), and collagen-alginate scaffolds impregnated with curcumin-loaded chitosan nanoparticles proved promising in the treatment of various pathological manifestations of diabetic wounds (Karri et al. 2016). Most recently, Zhao et al. (2018)



prepared a thermosensitive chitosan/ $\beta$ -glycerophosphate hydrogel loaded with  $\beta$ -cyclodextrin-curcumin and demonstrated improved healing of infected cutaneous wounds in rats, which may be due to the combination of antioxidative, antimicrobial, and anti-NF- $\kappa$ B signaling effects.

As silver has been demonstrated to have potent antimicrobial activities with no reports on bacterial resistances, several laboratories prepared chitosan wound dressings impregnated with silver to prevent wound infections and promote wound healing (Graham 2005). Indeed, a wound dressing composed of nano-silver and chitosan improved wound healing in rats better than a silver sulfadiazine dressing, which led to unwanted higher silver levels in the blood than the chitosan-silver dressing (Lu et al. 2008). In another study, Abdelgawad et al. (2014) combined silver nanoparticles that were embedded in chitosan with polyvinyl alcohol to produce antimicrobial nanofibrous material for wound dressing. The material with the highest chitosan-silver nanoparticle content was tested against *E. coli* and showed significant antibacterial activity. A combined antibacterial/tissue regeneration response triggered by functional chitosan-silver nanocomposites was also reported for thermal burns (Luna-Hernandez et al. 2017). To increase antibacterial activity wound dressings, several groups combined chitosan-silver-based materials with sulfadiazine, a sulfonamide antibiotic. Topical administration of chitosan-based hydrogels containing silver sulfadiazine improved burn and wound healing capacities in different studies (Nascimento et al. 2009; Chakavala et al. 2012; Aguzzi et al. 2014; Lee et al. 2014; El-Feky et al. 2017a). Besides inhibiting Gram-negative bacteria such as *E. coli*, chitosan-based dressings carrying silver sulfadiazine also inhibited the growth of Gram-positive bacteria as well as fungi such as *C. albicans* on an infected wound (El-Feky et al. 2017b).

Several studies reported that wound healing is accelerated when chitosan hydrogels are combined with adipose-derived or mesenchymal stem cells. Altman et al. (2009) grew human adipose-derived stem cells onto a chitosan/silk fibroin scaffolds and used it in a cutaneous wound healing model. They found that this regimen significantly enhanced wound healing, increasing micro-vascularization and differentiation into epidermal epithelial cells. In another study, an artificial dermis was fabricated by culturing human adipose-derived stem cells on a poly (L-glutamic acid)/chitosan scaffold (Shen et al. 2013b). Notably, the seeded stem cells maintained their capability to proliferate, produce extracellular matrix, and secrete cytokines including transforming growth factor  $\beta$ 1 and vascular endothelial growth factor. The artificial dermis was used to cover wounds that have been generated before in streptozotocin-induced diabetic mice. The artificial dermis significantly accelerated wound closure and healing in diabetic mice. Tong et al. (2016) used a different stem cell-based strategy to generate a skin substitute promoting wound healing. They manufactured a collagen-chitosan sponge scaffold to culture bone marrow-derived stem cells, which were pre-treated by hypoxia to induce the expression of pro-angiogenic cytokines. When the skin substitute was used to treat wounds generated in diabetic rats with hindlimb ischemia, wound healing was enhanced in comparison to scaffold-only controls or skin substitutes that were generated with normoxic stem cells.

A novel strategy for wound healing involving exosomes was reported recently. Exosomes are small secretory membrane vesicles that are involved in cell-to-cell communication. Stem cell-derived exosomes can improve wound healing and promote skin regeneration by stimulating cell proliferation and migration, angiogenesis, and reepithelization and modulating immune responses (Phinney and Pittenger 2017). Based on these observations, Shi et al. (2017b) isolated exosomes derived from gingival mesenchymal stem cells and encapsulated them in chitosan/silk hydrogel sponge. The combination of the exosomes and hydrogel was effective in promoting skin wound healing in a diabetic rat model by inducing reepithelialization, vascularization, and neuronalization paralleled by the remodeling of the extracellular matrix.

#### 14.6.5.3 Ocular Surface Reconstruction

Corneal damage can be the result of different diseases and injuries and may lead to a reduction or even loss of vision. Currently, the only therapy to cure vision loss after irreversible corneal damage is a surgical procedure where the cornea is replaced by donated corneal tissue. Frequently, the entire cornea is replaced in a surgical intervention called penetrating keratoplasty. As there is a shortage of corneal donors and there is a certain risk associated with the surgery and graft rejection, new types of corneal replacements are examined including materials containing chitosan. Actually, topical application of chitosan or chitosan/*N*-acetylcysteine to the eye is known to enhance corneal epithelial proliferation and migration during the wound healing in rabbits (Fischak et al. 2017). This process appears to involve the activation of the extracellular signal-regulated kinases (ERK) pathway (Cui et al. 2017). Another study evaluated the effects on corneal epithelium regeneration by combination of exogenous recombinant human serum-derived factor-1 $\alpha$  (rhSDF-1 $\alpha$ ) with a thermosensitive chitosan/gelatin hydrogel and analyzed the underlying mechanism (Tang et al. 2017). Conducting *in vitro* experiments, the team showed that rhSDF-1 $\alpha$  enhanced stem cell proliferation, chemotaxis, and migration, as well as the expression of related genes in limbal epithelial and mesenchymal stem cells (LESCs and MSCs). *In vivo* experiment using an alkali burn-injury rat model further revealed enhanced corneal epithelium regeneration and increased local expression of growth factors known to be essential for corneal epithelium repair. The underlying mechanism by which rhSDF-1 $\alpha$  released from the chitosan/gelatin hydrogel stimulates corneal regeneration may involve activation of C-X-C chemokine receptor type 4 (CXCR4) expressing cells (LESCs and MSCs) and chemotactic attraction of these cells to the sites of lesion via the binding of rhSDF-1 $\alpha$  to the CXCR4 receptor.

Chen et al. (2005) had considered a tissue-engineering scaffold made of collagen, chitosan, and hyaluronic acid as a potential replacement for corneal tissue. To study cytocompatibility *in vitro*, they cultured rabbit limbal corneal epithelial cells, corneal endothelial cells, and keratocytes on the polymer complexes and demonstrated that the corneal cells were able to attach, migrate, and proliferate. To evaluate

biocompatibility *in vivo*, they implanted the polymer complex into the corneal stroma of rabbit eyes and inspected ocular reactions. Overall, the polymer complexes exhibited transparency and good biocompatibility, as they were degraded and absorbed within the corneal tissue while maintaining transparency. In another study, poly(ethylene glycol)-stabilized carbodiimide-cross-linked collagen-chitosan hydrogels were tested for biocompatibility and host-graft integration. For this purpose, Rafat et al. (2008) performed *in vitro* and *in vivo* studies demonstrating excellent biocompatibility when analyzing human corneal cells, dorsal root ganglia from chick embryos, or subcutaneous implants. The hydrogel scaffold was also studied as corneal substitute by implanting it into the cornea of pig eyes and monitoring them for 12 months. The substitute was seamlessly integrated into the cornea with regeneration of host corneal epithelium, stroma, and nerves. Liang et al. (2011) prepared a blend membrane composed of hydroxyethyl-chitosan, gelatin, and chondroitin sulfate. The membrane exhibited good transparency, ion and glucose permeability, and cytocompatibility for corneal endothelial cells, which formed a monolayer on the membrane in cell culture. *In vivo* animal experiments revealed that the membranes were characterized by biodegradability and a good histocompatibility suggesting that the membranes may be employed as carriers for corneal endothelial cell transplantation. Similar results were obtained for chitosan/polycaprolactone, chitosan/poly(ethylene glycol), silk fibroin/chitosan, carboxymethyl chitosan/gelatin/hyaluronic acid, as well as hydroxyethyl-chitosan blend membranes, which were tested as potential scaffolds and carriers for bovine, ovine, and rabbit corneal endothelial cells, respectively (Wang et al. 2012b; Guan et al. 2013; Ozcelik et al. 2013; Liang et al. 2014; Xu et al. 2018).

Using an allogeneic rabbit model of stromal destruction caused by bacterial keratitis, Chou et al. (2018) tested the hypothesis that intra-stromal injection of keratocyte spheroids manufactured on chitosan coatings has higher therapeutic efficacies than eye drop instillations or isolated cell injections. The results of clinical observations and histological studies performed 2 weeks after the surgical intervention showed that, in comparison to a treatment relying only on antibiotics, intra-stromal grafting of keratocytes provides additional benefits due to improved preservation of cellular phenotypes, secretion of collagen matrix, and retention of the graft.

In a stem cell therapeutic approach published by Chien et al. (2012), human corneal fibroblasts (keratocytes) were reprogrammed into human-induced pluripotent stem cells (iPSC) using a feeder cell-free culturing system. To increase iPSC delivery and engraftment, the researchers generated an injectable thermogelling carboxymethyl-hexanoyl chitosan nanogel with seeded iPSCs and showed that viability and pluripotent properties of the reprogrammed iPSCs were maintained in the hydrogel system. They further demonstrated that the reprogrammed iPSCs grown on the hydrogel could be used to enhance corneal wound healing efficiently. This strategy opens the possibility for a personalized therapy for human corneal damage when iPSCs are reprogrammed from cells derived from corneal surgical residues.

#### 14.6.5.4 Neuronal Regeneration

The plethora of favorable characteristics of chitosan outlined in this chapter prompted many researchers around the world to employ chitosan-based materials also in the reconstruction of peripheral nerves to improve healing of nerve damage caused by accidents or diseases. Although therapeutic interventions to peripheral nerve repair have yielded some progress during the past years, a full recovery of nerve function is usually not achieved. Current therapies mostly rely on microsurgical techniques, which either try to directly establish a tension-free connection between the ends of severed nerves (epineural, fascicular, and grouped fascicular repair) or bridge larger nerve defects by autologous grafts (cable grafts, trunk grafts, and vascularized nerve grafts) (Matsuyama et al. 2000; Houschyar et al. 2016). The various neurosurgical techniques used to connect nerve ends are challenging, and the therapeutic results are frequently not satisfactory. Many studies have provided evidence that various types of conduits, such as veins, pseudo-sheaths, and bioabsorbable tubes, are helpful in bridging shorter gaps by promoting nerve regeneration. After bridging nerve gaps with hollow conduits, the lumen between the nerve ends becomes filled with fibrin, and macrophages and other cells are attracted, which create a favorable microenvironment for vascularization and neuronalization.

Chitosan-based conduits have been extensively analyzed for this purpose. An early electrophysiological and histological study on nerve regeneration using rat sciatic nerve defects demonstrated that pure chitosan/collagen conduits were superior in bridging 1 cm nerve defects to that of control groups (Wei et al. 2003). The chitosan/collagen film was found to be degraded about 3 months after the surgery. In a methodologically similar study, Wang et al. (2005) generated an artificial nerve graft composed of a chitosan conduit and tested them to bridge a 3 cm dog sciatic nerve defect. In contrast to the previous study, the conduit was filled with longitudinally arranged filaments of polyglycolic acid. The team found that the sciatic nerve trunk was successfully reconstructed in dogs treated with the chitosan/polyglycolic acid graft with reinnervation of the target skeletal muscle. In a case report on a 55-year-old man with a 3 cm median nerve defect in the distal forearm, implantation of chitosan/polyglycolic acid graft promoted nerve regeneration and functional reconstruction, so that the patient was able after 36 months to fully use the injured hand during daily activities (Gu et al. 2012). Other chitosan-based conduits have been successfully used to guide and promote nerve generation in various *in vitro* and *in vivo* models. These materials include chitosan/gelatin and chitosan/poly(L-lysine) polyelectrolyte-based scaffolds (Martin-Lopez et al. 2012), chitosan-gold nanocomposites (Lin et al. 2008), chitosan/poly(lactic acid) films (Xie et al. 2008), chitosan/poly(3-hydroxybutyrate-co-3-hydroxyvalerate) nanofibers (Biazar and Heidari Keshel 2014), porous chitosan-poly(p-dioxanone)/silk fibroin copolymers (Wu et al. 2015), poly(D,L-lactide-co-glycolide) sleeves with multifilament chitosan yarn or a microcrystalline chitosan sponge core (Wlaszczuk et al. 2016), chitosan/hyaluronic acid hybrid materials (Li et al. 2018a), porous

chitosan- $\gamma$ -glycidoxypropyltrimethoxysilane hybrid membranes (Shirosaki et al. 2014), hydroxyapatite-coated tendon chitosan tubes with adsorbed laminin peptides (Itoh et al. 2003), and hyaluronic acid doped-poly(3,4-ethylenedioxythiophene) nanoparticles in a chitosan/gelatin matrix (Wang et al. 2017b).

An evident upgrade of chitosan-based conduits is luminal loading with neurotrophic factors such as nerve growth factor (NGF), ciliary neurotrophic factor (CNF), or brain-derived neurotrophic factor (BDNF), which are all secreted by Schwann cells that support growth of neuronal cells (Houschyar et al. 2016). In addition, fibroblast growth factor (FGF), glial growth factor (GGF), and vascular endothelial growth factor (VEGF) were reported to have positive effects on nerve regeneration.

Yang et al. (2011) immobilized NGF on genipin-cross-linked chitosan and tested the material for cytotoxicity using primary cultured Schwann cells and for neuronal differentiation of PC12 cells in response to NGF release. Subsequently, Wang et al. (2012a) demonstrated that genipin-cross-linked chitosan conduits loaded with NGF can be successfully used to bridge 1-cm-long sciatic nerve defects in rats as revealed by electrophysiological assessment, behavioral analysis, and histological examination 24 weeks after the surgery. Similar results were obtained, when NGF-containing microspheres were implanted into chitosan conduits to repair a 1 cm defect of the facial nerve in rabbits (Liu et al. 2013). In another NGF-based approach, Chao et al. (2016) combined an autologous vein conduit with a chitosan- $\beta$ -glycerophosphate-NGF hydrogel. The researchers surgically reconstructed a 5-mm-long defect of a rat facial nerve with an autologous vein and then injected the chitosan- $\beta$ -glycerophosphate-NGF hydrogel into the lumen of the conduit. Facial nerve regeneration was as efficient as in control groups, which were transplanted with an autologous nerve, but significantly better than in control groups where the vein conduit was injected with NGF only.

Shen et al. (2010) used a polylactic/polyglycolic acid chitosan nerve conduit loaded with CNF to repair larger canine tibial nerve defects in crossbred dogs and evaluated nerve regeneration by general inspection, electrophysiological, immunological, and histological analyses 3 months after the surgery. Nerve regeneration was significantly improved in animals that were treated with CNF-loaded polylactic/polyglycolic acid chitosan conduits when compared to groups treated with the polylactic/polyglycolic acid chitosan conduits. The results were similar to controls groups that were treated with autologous nerve grafts, suggesting that the artificial nerve conduit is a promising alternative for bridging nerve defects.

Furthermore, Zhao et al. (2014) hypothesized that tacrolimus-loaded chitosan enhances peripheral nerve regeneration through modulation of the expression profiles of neurotrophic factors. To test this hypothesis, they loaded tacrolimus onto chitosan conduits and examined nerve regeneration of sciatic nerve injury in a rat model. They found significant regeneration of sciatic nerves with normal morphology but higher density of myelinated nerve fibers in rats treated with tacrolimus-loaded chitosan. The underlying mechanism seems to involve BDNF signaling, because nerve regeneration was paralleled by an increased expression of BDNF and its corresponding receptor (TrkB) in the motor neurons in the spinal cord.

The membrane-bound cell adhesion molecule L1 is known to promote neurite growth and prevent neuronal apoptosis, a function which can be mimicked by a recombinant chimeric version of this molecule called L1-Fc (Roonprapunt et al. 2003). Loading L1-Fc to an artificial chitosan/polyglycolic acid conduit, Xu et al. (2004) studied guided regeneration of rat optic nerves. They found that the implanted chitosan/polyglycolic acid conduit was degraded and absorbed. When L1-Fc loaded conduits were implanted to bridge a defect caused by surgical intervention, axonal regeneration and remyelination were significantly improved when compared to control groups that were treated with conduits lacking L1-Fc.

Nerve regeneration can be additionally promoted using chitosan-based scaffolds as conduits seeded with stem cells that express neurotrophic factors and can differentiate into nerve cells. Zheng and Cui (2010) tested chitosan conduits of such kind combined with rat bone marrow mesenchymal stem cells to evaluate their potential for the reconstruction of 8-mm-long rat sciatic nerve defects. They demonstrated that the combination of chitosan and mesenchymal stem cells alone was sufficient to improve nerve regeneration and functional recovery. Moreover, some of the mesenchymal stem cells were found to have differentiated into neural stem cells. Similar results were obtained when injured rat sciatic nerves were treated in this way, and the nerve repair was monitored electrophysiologically and histomorphologically (Moattari et al. 2018) or by noninvasive magnetic resonance neurographic imaging (Liao et al. 2012). In addition, chitosan-coated poly-3-hydroxybutyrate conduits combined with human bone marrow mesenchymal stem cells were recently shown to be efficient in promoting nerve regeneration in this rat model of sciatic nerve injury (Ozer et al. 2018). Improved nerve regeneration was also reported for chitosan/poly(lactic-co-glycolic acid) scaffolds seeded with autologous bone marrow mesenchymal stem cells to treat injuries of dog sciatic nerves and rhesus monkey median nerves (Xue et al. 2012; Hu et al. 2013). In another approach, Zhu et al. (2015) used chitosan conduits filled with bone marrow mesenchymal stem cells and evaluated nerve regeneration and neuronal survival when injured lumbosacral nerves were bridged with this material. They found that this treatment enhanced sacral nerve regeneration and motor function 6 and 12 weeks after the surgery. Moreover, the mesenchymal stem cells prevented cell death of motor neurons in the anterior horn of the spinal cord, thereby improving the motor function in rats treated with the mesenchymal stem cell-seeded chitosan conduit. Finally, a clinical study performed with 14 patients suggests that defects in chronic spinal cord injury can be successfully bridged with peripheral nerve grafts combined with a chitosan-laminin scaffold and co-transplanted bone marrow-derived mesenchymal stem cells, which enhanced recovery (Amr et al. 2014).

Using chitosan/silk fibroin scaffolds grafts seeded with adipose-derived stem cells, Wei et al. (2011) examined regeneration of surgically injured rat sciatic nerves. Implantation of this conduit significantly improved axonal regeneration and functional recovery in comparison to control groups. The positive effect was partially attributed to the differentiation of adipose-derived stem cells into Schwann cells, which additionally secrete neurotrophic factors and prevent apoptosis. Nie et al. (2014) investigated axonal regeneration and remyelination using a chitosan/gelatin-

based conduit combined with TGF- $\beta$ 1 and Schwann cells. For this purpose, they bridged a 10-mm defect of a rat sciatic nerve and examined nerve regeneration based on functional recovery, electrophysiological measurements, retrograde labeling, and immunohistochemical analysis. The obtained data indicate satisfactory functional recovery of the injured sciatic nerve.

Meyer et al. (2016) filled chitosan (5% degree of acetylation) conduits with a gel containing hyaluronic acid and laminin (NVR-gel) and added genetically modified neonatal rat Schwann cells as cellular delivery system for neurotrophic factors. Testing the chitosan conduits in the rat sciatic nerve model revealed that the chitosan conduit, which only is filled with the NVR-gel, was insufficient to promote nerve regeneration in contrast to autologous nerve grafts. Notably, delivery of FGF by seeded Schwann cells genetically modified to overexpress this factor improved nerve regeneration significantly. Unexpectedly, Schwann cells expressing GDNF did not show positive effects in this experimental setup. Recently, Zhu et al. (2017) used skin-derived precursor Schwann cells to seed chitosan/silk scaffolds for bridging a 10-mm-long rat sciatic nerve gap. The artificial graft exhibited significant promoting effects on peripheral nerve repair and hence constitutes an alternative to other stem cell-based approach promoting nerve regeneration.

## 14.7 Concluding Remarks

Numerous studies reported favorable effects of chitosan-based materials for a wide range of applications. Doubtless, the controlled and targeted delivery of drugs to specific tissues has a great potential in biomedicine, and first clinical trials with chitosan-based drug carrier systems revealed promising results for the therapy of a variety of diseases including diabetes and cancer and also mainly because adverse side effects are reduced. The antimicrobial activity of chitosan and its derivatives is particularly important when the polymer is used for textile fabrication, food packaging, wound dressings, and tissue engineering. However, the underlying mechanism of antimicrobial activity is not fully understood. One prominent explanation is the assumed interaction of chitosan's positively charged amine groups with the negatively charged surface of bacteria and fungi, which might impair the movement of ions across membranes and hence disrupt cellular integrity. Although the proposed mechanisms seem plausible, there is a clear lack of experimental data that would provide evidence at a molecular level reminding us to continue basic research on the mode of actions. The studies conducted so far indicate that the antimicrobial activity of pure chitosan is not sufficient to prevent microbial infections completely in vivo. However, chitosan and its derivatives can be combined with other antimicrobial compounds including essential oils (Krausz et al. 2015), polyphenols (Madureira et al. 2015), tretinoin (Ridolfi et al. 2012), metal ions (Sanpui et al. 2008; Tran et al. 2010), lysozyme (Wu et al. 2017), or antibodies (Jamil et al. 2016), to prevent bacterial or fungal infections. Thus, chitosan appears to be an ideal



adjuvant polymer for design and production of new materials exhibiting intrinsic antimicrobial properties for a large variety of potential applications in the chemical, pharmaceutical, food, and textile industry. Similarly, the observed immunostimulatory effects of chitosan need further investigation, as there may be a certain risk to develop allergic or even anaphylactic reactions after oral ingestion (Kato et al. 2005). However, it has to be noted that overall the beneficial characteristics exceed possible side effects due to some allergic potential. Finally, the antitumor activity of chitosan also needs to be analyzed in more detail. Recently, Li et al. (2018b) provided some evidence indicating that chitosan activates dendritic cells, which subsequently secrete pro-inflammatory cytokines and thereby enhance immune surveillance by natural killer cells. Accordingly, the antitumor effects of chitosan can be enhanced by specifically targeting dendritic cells by attaching mannose to the surface of chitosan nanoparticles (Shi et al. 2017a). Different pattern recognition receptors that are expressed on the surface of dendritic cells are potential receptors for chitosan. This includes Toll-like receptors, C-type lectin receptors, and other molecules, which are known to recognize specific molecular patterns, particularly those associated with pathogens. However, currently it is not known how dendritic cells recognize chitosan. In summary, it has to be noted that chitosan is a highly promising material for a variety of applications in industry and medicine. While chitosan-based materials have been commercially launched as packaging and coating material in food industry, as an ingredient in cosmetics, and as ion exchanger in water treatment and are approved for human dietary use and wound dressing, their commercial applications in medicine as drug delivery systems or scaffold for tissue engineering are pending. Nevertheless, there are clinical phase 2/3 trials, and depending on their outcome, some products may reach first approval by the health authorities in near future.

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