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MINI-REVIEW

Chitosan and its derivatives: synthesis, biotechnological applications, and future challenges

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Abstract

Chitosan is a naturally occurring biodegradable as well as a non-toxic polymer generated from chitin through alkaline deacetylation reaction, and it is insoluble in organic/inorganic solvents and water. Furthermore, chitosan is one of the most plentiful cationic polymers in natural surroundings. Due to its non-toxicity and biocompatibility, chitosan is extensively employed in industrial, biomedical, food, pharmaceutical, environmental, and agricultural industry. Chitosan-based biomaterials exhibit great potential in various biotechnological applications, such as anti-hypertensive therapy, anti-oxidant, anti-microbial, anti-allergic, immunostimulant, cancer therapy, delivery of genetic materials, delivery of bone morphogenetic type-2, wound healing, treatment of wastewater, hypocholesterolemic, and bio-imaging. Therefore, this review mainly focuses on the biotechnological potential of chitosan and its derivatives as well as presents the potential of chitosan-based biomaterial/pharmaceutical for the prevention of various life-threating chronic disorders.

Keywords Chitosan and its derivative · Biotechnological potentials · Anti-cancerous · Anti-oxidant · Bio-imaging

Introduction

Chitosan is one of the major naturally occurring cationic polymers (Domard and Cartier 1992). Chitosan is the most abundant component of the shell of crawfish, shrimp, and crab. It is a non-toxic naturally occurring biopolymer, which is manufactured by deacetylation of chitin. It is water-insoluble, which is a most important limiting factor for its utilization in biological systems. A number of chitosan derivatives are prepared by chemical or enzymatic hydrolysis of chitin or chitosan (Fig. 1) (Qin et al. 2014). Due to its non-toxic, non-allergic, and biocompatible nature, chitosan and its derivatives are used extensively in water treatment, waste treatment, agriculture, food, medicine, and cosmetics field (Muzzarelli 2009). As a result of a unique biological effect, the chitosan has attained considerable attention as a biomedical material and it can be used as an anti-obesity (Pan et al. 2018), anti-hypertensive (Auwal et al. 2017), anti-bacterial (Shahzad et al. 2015), anti-tumor (Ravi et al. 2018), anti-inflammatory (Paramasivan et al. 2014), and anti-oxidant agent (Guo et al. 2015). Chitosan possesses amino/actamido groups along with both primary and secondary hydroxyl functional reactive groups at C-2, C-3, and C-6 positions, respectively. The arrangement and distribution of these functional groups are the main factors contributing to the chitosan structural, physical, and chemical properties (Younes and Rinaudo 2015).

Chitosan can be used as a genetic material carrier during gene therapy due to their biocompatibility, biodegradability,

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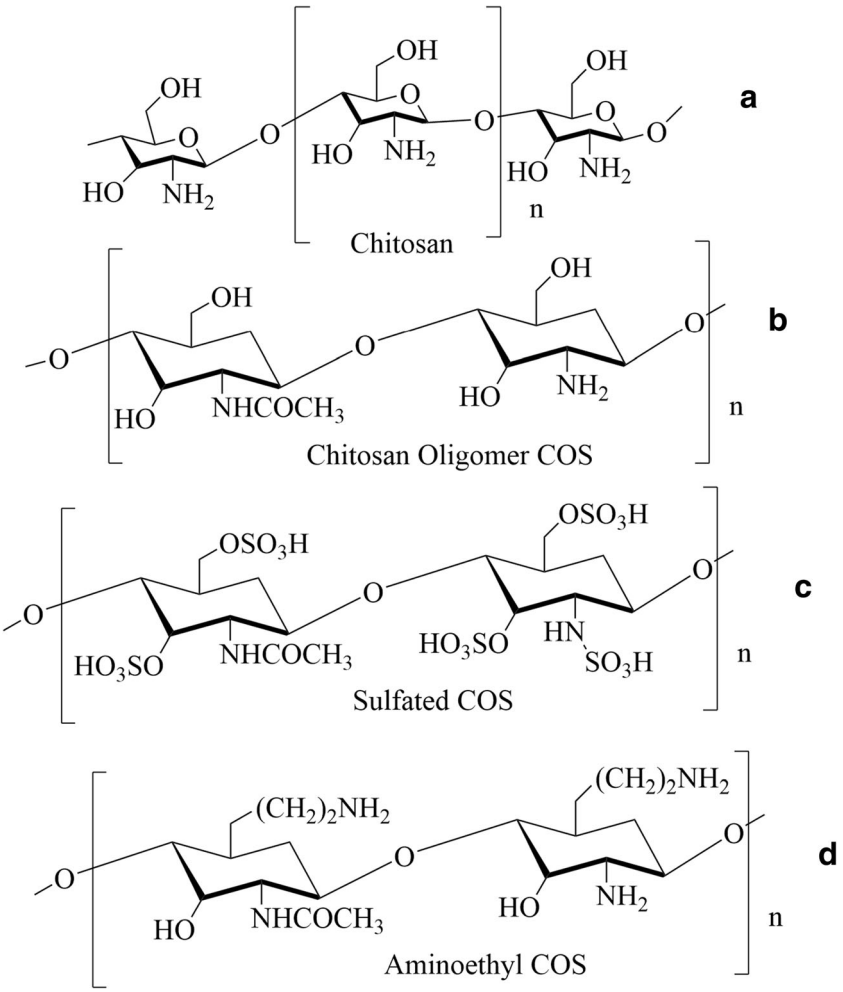

Fig. 1 Structure of chitosan (**a**), chitosan oligomer (COS) (**b**), sulfated COS (**c**), and aminoethyl COS (**d**)

and non-toxicity nature. Furthermore, the various non-viral cationic natural polymers can be derived from chitosan, which could be used for a number of biomedical, functional food, and pharmaceutical application in various industries (Chandy and Sharma 1990; Cui and Mumper 2001; Zhao et al. 2006). Chitosan is intensely compressed with negatively charged genetic materials, which protect genetic materials against nuclease degradation due to positively charged chitosan (Cui and Mumper 2001). DNA and chitosan complexes have been reported to transfect into various cells types, such as fibroblast cells (Niu et al. 2008), cervical cancer cells (Kiang et al. 2004), and human embryonic kidney cells (Kiang et al. 2004).

Chitosan and its derivatives are envisaged as a unique therapeutic strategy to prevent various chronic disorders, though improvement of the physiological function is still necessary. Therefore, this review article focuses on the synthesis of chitosan and its derivatives and their biological effects, as well as their application as an ingredient of pharmaceuticals and functional foods to treat various chronic disorders. Moreover, this review discusses novel strategies to utilize chitosan and its derivatives as carriers of genetic material in gene therapy.

Biotechnological applications of chitosan and its derivatives

The anti-hypertensive potential of chitosan

The main risk factor in the advance of cardiovascular disease is hypertension, which in turn threatens the human health (Mohamed 2014). In animal blood, there is an angiotensin-1 converting enzyme (ACE), which controls blood pressure by converting inactive angiotensin-1 into its active form angiotensin-11. This angiotensin-11 in turn plays an important role in narrowing the blood vessels and results in an increase in blood pressure. Therefore, an inhibition of ACE activity is a vital objective on the path of curing of hypertension (Rai et al. 2017). Chitosan oligomers (COS) have various functional groups at C-2, C-3, and C-6 positions (Fig. 1). Based on these functional groups, chitosan has been applied in copious biological actions, such as ACE inhibition (Qin et al. 2014). A recent study shows that chitosan oligomers act as ACE inhibitors by binding to chloride ions, which helps to activate the ACE, and thus, is very helpful to reduce the severity of

cardiovascular diseases. In this regard, Auwal and Zarei (2018) and Nguyen et al. (2013) showed that the molecular weight and degree of deacetylation (DDA) are important factors for the inhibition of ACE activity. For example, chitosan derivatives with ACE inhibitor activity were synthesized by carboxylation with $–COCH_2CH_2COO–$ and subsequent grafting with 2-chloroethylamino hydrochloride (Huang et al. 2005; Ngo et al. 2008a, b).

Sulfated-chitosan oligomers (S-COS) were prepared by grafting chitosan oligomers with sulfate to enhance its ACE inhibitor activity (Park et al. 2003). It is well known that the hydroxyl group of pyranose moieties has different chemical activities at various positions, even with the same reagents. For the synthesis of S-COS, it is possible to effectively substitute the hydroxyl group of COS at the C-6 position selectively by sulfate group while the structure of COS is conserved, because the C-6 hydroxyl groups have the highest reactivity for sulfates. For example, Qian et al. (2010) prepared three types of S-COS (3–1, 5–3, and 10–5 kDa) with great potential as ACE inhibitor. All three S-COS showed no cytotoxicity effect on human lung fibroblast cells (MRC-5) (Qian et al. 2010), while the best ACE inhibition performance was found for the 5–3 kDa S-COS. The results of a recent investigation illustrate that the substitution of a hydrogen atom at the C-6 position by sulfate group promotes the ACE inhibitor activity of COS and S-COS. Hence, chitosan and its derivatives are potent candidates for additives for pharmaceuticals and nutraceuticals, engineered to treat hypertension and related disorders.

Anti-oxidant potential of chitosan

It was confirmed that the oxidative damage by different stimuli is the main cause of cardiovascular disease (Xu and Huang 2007). At any time, the human body is affected by various free radicals, such as reactive oxygen species (ROS), which are generated inside the body as well as in the surrounding environment, during the metabolism. The oxidative stress may accidentally activate an enzyme, which in turn damages the cellular macromolecules and leads to various life-threating disorders, such as cancer, neurodegenerative diseases, aging, and cardiovascular diseases (Liochev 2013; Riaz Rajoka et al. 2018). Anti-oxidant agents, such as chitosan and its derivatives, are applied to scavenge free radicals or to inhibit the oxidative destruction caused by free radicals. Therefore, these agents have the potential to protect the human body from the deterioration induced by various free radicals (Fig. 2) (Valko et al. 2007; Zhao et al. 2015b). For example, chitosan was found to possess an anti-oxidant activity in rapeseed oil at a concentration of 0.02% (*w*/*v*), yet the anti-oxidant activity was lower than ascorbic acid (Anraku et al. 2014), which is a standard anti-oxidant agent (Ngo and Kim 2014). The addition of chitosan considerably improves the activity of anti-oxidant enzymes (catalase, glutathione peroxidase, and superoxide dismutase) and reduces the malondialdehyde as well as serum-free fatty acid. Therefore, lipid peroxidation can be reduced while anti-oxidant enzyme activities can be controlled by addition of chitosan. Furthermore, in the food industry, chitosan and its derivatives have been used as a food preservative and functional factor for drug carriers, immune-stimulating effects, as well as anti-microbials (Xia et al. 2011).

Chitin oligomers are an enzymatic, chemical, physical hydrolysis product of chitin and are water-soluble. Therefore, these oligomers can be easily used both in in vivo and in vitro condition. The presence of chitin oligomers (229.21 to 593.12 Da) influenced DNA damage and protein oxidation in mice macrophages RAW 264.7 cells. Similarly, chitin oligomers affected myeloperoxidase activity in myeloid HL60 cells (Ngo et al. 2008a; Oh et al. 2016). A recent study shows that two kinds of chitin oligomers, having different molecular weight, exhibited an inhibitory effect toward protein oxidation and DNA (Ngo et al. 2009). Furthermore, the high molecular weight chitosan (100 kDa and 99% DD) reduced uremic toxin and lipid hydroperoxides in the gastrointestinal tract of a human. Therefore, the high molecular weight chitosan is able to prevent the advance of oxidative stress in systematic circulation of the human body. Moreover, the anti-oxidant effect of high molecular weight chitosan differs from the anti-oxidant effect of conventional anti-oxidant agents such as vitamins and thus is unique (Anraku et al. 2011). Therefore, the results of this study suggested that chitosan can be employed as a new approach for anti-oxidative treatment in case of various disorders. To date, the exact mechanism of free radical scavenging by chitosan is not clear. However, it was suggested that the unsteady free radicals react with the hydroxyl group as well as the amino group at C-2, C-3, and C-6 position of the pyranose ring to produce a steady macromolecule. Furthermore, the anti-oxidant potential of chitosan can be used to treat various oxidative disorders.

Anti-microbial potential of chitosan

The anti-microbial activity of chitosan has been scrutinized towards a wide variety of microbes (Li et al. 2019a, b). The anti-microbial activity of chitosan and its derivatives is influenced by various factors, including the degree of polymerization, molecular weight, and the degree of acetylation (DA) (Hassan et al. 2018). Sabaa et al. (2018) showed that the influence of Mw on the anti-microbial activity is greater than the effect of DA. Moreover, the anti-microbial activity of chitosan is influenced by pH, as chitosan is only soluble under an acidic condition having pH lower than 7 (Jeon et al. 2014; Raafat et al. 2008). The modification of the 3D structure of chitosan under acidic pH conditions permits the compounds solubilization and helps to maintain its anti-microbial activity (Chavez de Paz et al. 2011; Kong et al. 2008). In addition,

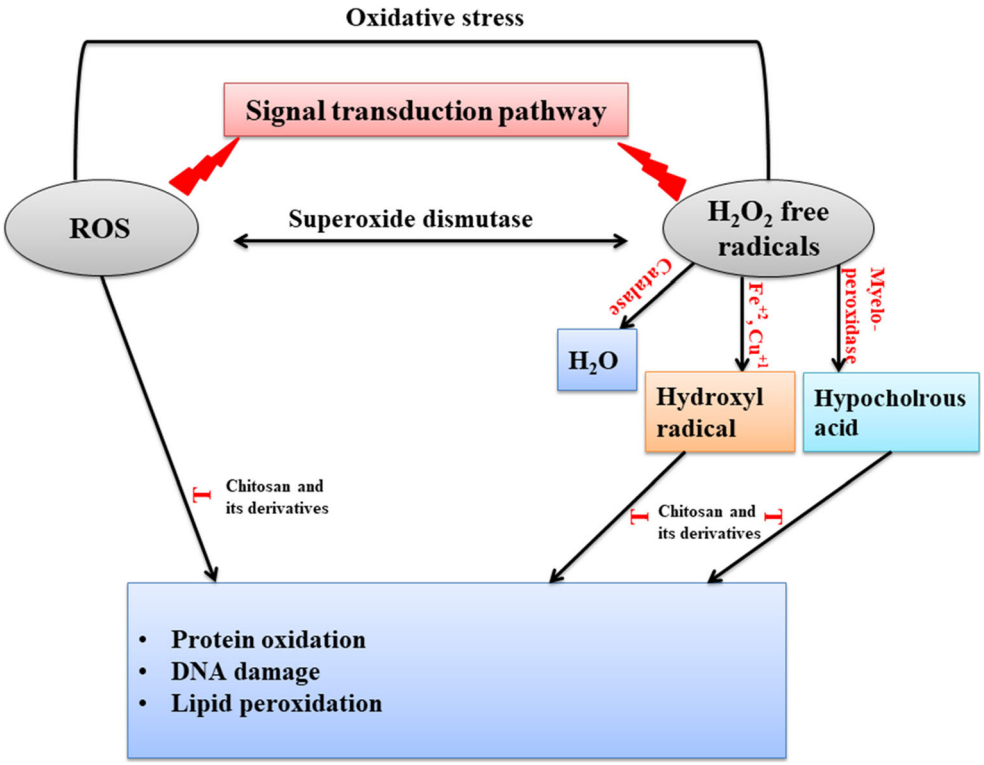

Fig. 2 Antioxidant mechanism of chitosan and its derivative. The host is affected by various oxidative stress which in turn invaderents the enzyme activation. As a result, this oxidative stress can cause the damage of macromolecules. The antioxidant agents such as chitosan and its derivative have the ability to protect the host against the oxidative stress damage by interrupting the oxidation chain reaction

the anti-microbial activity of chitosan can be enhanced by the structural modification of various functional groups at different position (Jeon et al. 2016; Li et al. 2016).

Various mechanisms of the anti-microbial mode of action of chitosan have been described in literature. For example, Perinelli et al. (2018) proposed that binding of chitosan to bacterial DNA leads to inhibition of mRNA as well as the interaction with the bacteria cell surface results in the anti-microbial activity. The potential of chitosan to bind with DNA was commonly useful/investigated for the gene delivery. On the other hand, the influence of such capability in anti-microbial action is still uncertain, since chitosan would not stretch to a target in the cytoplasm (Bowman and Leong 2006). Je and Kim (2006) proposed in another possible mechanism that chitosan binds to bacterial membrane and agitate it. The size of hydrated chitosan is, however, too big to cross the cell membrane. Thus, Raafat et al. (2008) suggested that it can interact directly with the cell membrane.

The most known supposition for anti-microbial activity of chitosan is the polycationic nature due to the presence of amine groups of glucosamine, which might be a crucial feature enabling its capability to bind to negatively charged surface constituents of numerous microbes. This binding may trigger broad modifications to the cell surface, which leads to trickle of intracellular constituents that consequences in cell death (Ganan et al. 2009). In this way, the teichoic acid in Gram-positive bacteria and lipopolysaccharide in Gram-negative bacteria play a vital role in binding of chitosan, and the modification of cell membrane function is a result of disturbance of cell wall dynamics, as shown in Fig. 3 (Raafat et al. 2008).

It is suggested that chitosan binds to Gram-positive bacteria diversely. A study inspected the effect of medium molecular weight chitosan (90 to 300 kDa) towards Gram-negative pathogens of warm water fish. The results suggested that at a concentration of 0.8% (*w*/*v*) of chitosan is needed to inhibit the growth of *Aeromonas hydrophila*; hence, a concentration of 0.4% chitosan is needed to inhibit the growth of *Flavobacterium columnare* and *Edwardsiella ictaluri*. It was suggested that the different negative charge distribution and hydrophobicity of the surface of bacteria are the main causes for the different inhibition responses of chitosan (Yildirim-Aksoy and Beck 2017). A study by Chung et al. (2004) showed that a bacteria exhibiting a stronger negatively charged surface had more contact with the polycationic configuration of chitosan. However, another study did not witness the greater liability against Gram-negative bacteria at various degrees of deacetylation (Venkatrajah et al. 2013). In contrast to the study by Chung et al., a study conducted by No et al. (2002) observed that the Gram-positive bacteria were less resistant to Gram-negative bacteria.

The anti-allergy potential of chitosan

All over the world, inflammatory and allergic diseases are widespread diseases (Kotlyar et al. 2014). The most common

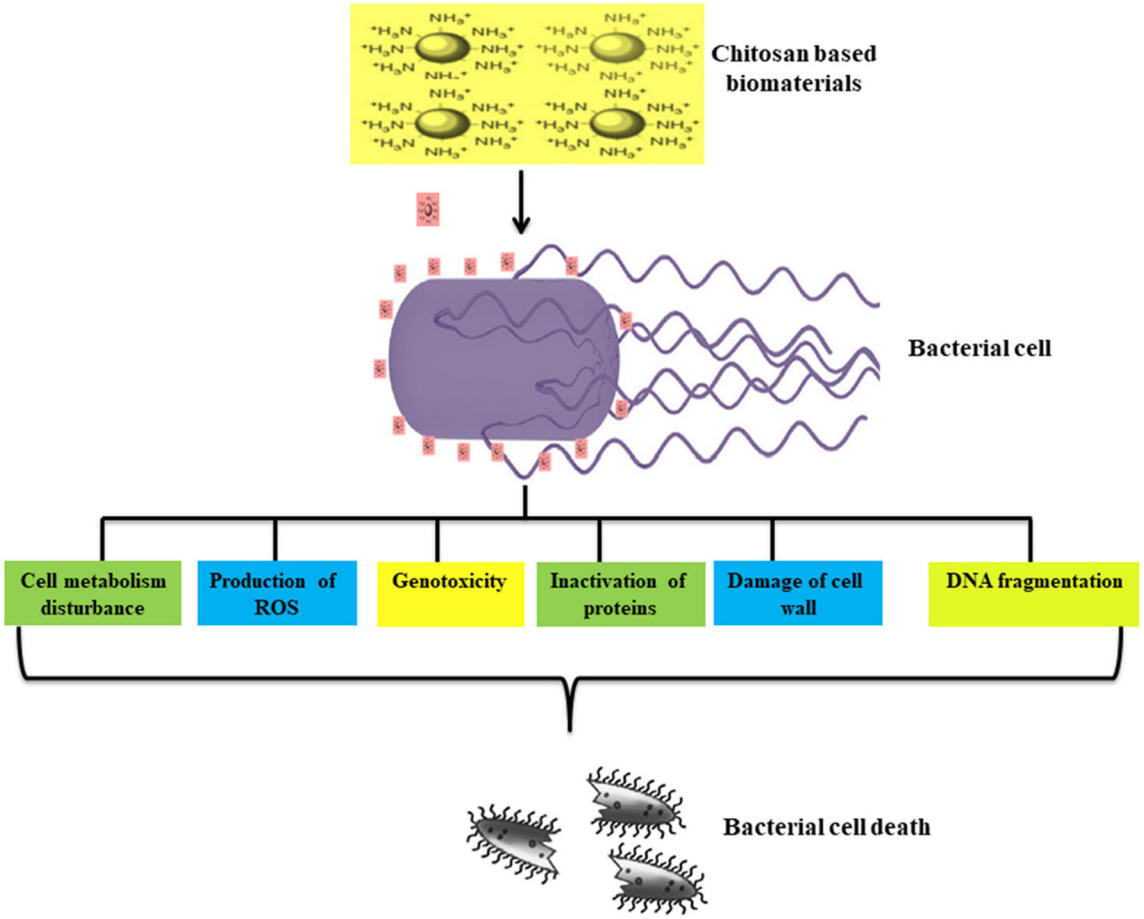

Fig. 3 Anti-microbial action of chitosan-based biomaterials

response of a host against various stimuli (immune reaction, irradiation, microbial invasion, physical damage, and ultraviolet) is inflammation. Persistent inflammation is harmful to the host, causative to the pathogenesis of numerous disorders, such as cancer, inflammatory bowel syndrome, chronic asthma, rheumatoid arthritis, and multiple sclerosis (Klimek 2008). Allergic disorder is an immune system disease, caused by the exaggerated reaction of the immune system to harmless environmental substances, including pollens, animal dander, foods, and house dust mites (Vo et al. 2012a). The mechanisms of anti-allergic disorder are shown in Fig. 4.

In allergic responses, the central event is the activation of the mast cell by immunoglobulin E–mediated stimuli. In this way, the COS with a Mw in the range of 1–3, 3–5, and 5–10 kDa were scrutinized for their ability to contradict allergic reactions in rat basophilic leukemia RBL 2H3 cells (Vo et al. 2011). A study investigated the role of COS (1–3 and 3–5 kDa) in antagonizing the activation of mast RBL-2H3 cells, which were stimulated by dinitrophenyl (DNP) bovine serum albumin antigens and DNP-specific immunoglobulin E antibody. The result of this investigation suggests that the COS significantly inhibited the degranulation of mast RBL-2H3 cells by reducing the release of β-hexosaminidase and histamine. Furthermore, the inhibition potential of COS can be evaluated by the decline in intracellular Ca^{2+} elevation. In addition, COS treatment downregulates the expression of immunoglobulin fc epsilon receptor in mast RBL-2H3 cells. However, the downregulation is dose-dependent (Vo et al. 2012a). The inhibition potential of COS on the mast RBL-2H3 cell activation suggests that the COS may find application as a novel inhibitor against allergic reactions.

Moreover, gallate oligomer chitosan (G-COS) has potential against allergic reaction in the mast RBL-2H3 cells, which were irritated by addition of dinitrophenyl (DNP) bovine serum albumin antigens and DNP-specific immunoglobulin E antibody. G-COS at a concentration of 200 μg/ml significantly inhibited the release of intracellular Ca^{2+} elevation as well as the histamine. Furthermore, G-COS suppressed the production and expression of TNF-α and IL-4. Additionally, the G-COS treatment significantly hinders the translocation of NF-kB and degradation of IkB-α, as well as the phosphorylation of MAPKs (Vo et al. 2012b). Therefore, the G-COS might be used as an inhibitor against various allergic inflammatory responses. The oral administration of COS significantly reduced the gene and protein level of TNF-α, IL-13, IL-4, and IL-3 in bronchoalveolar lavage fluid and lung tissue. Compared with

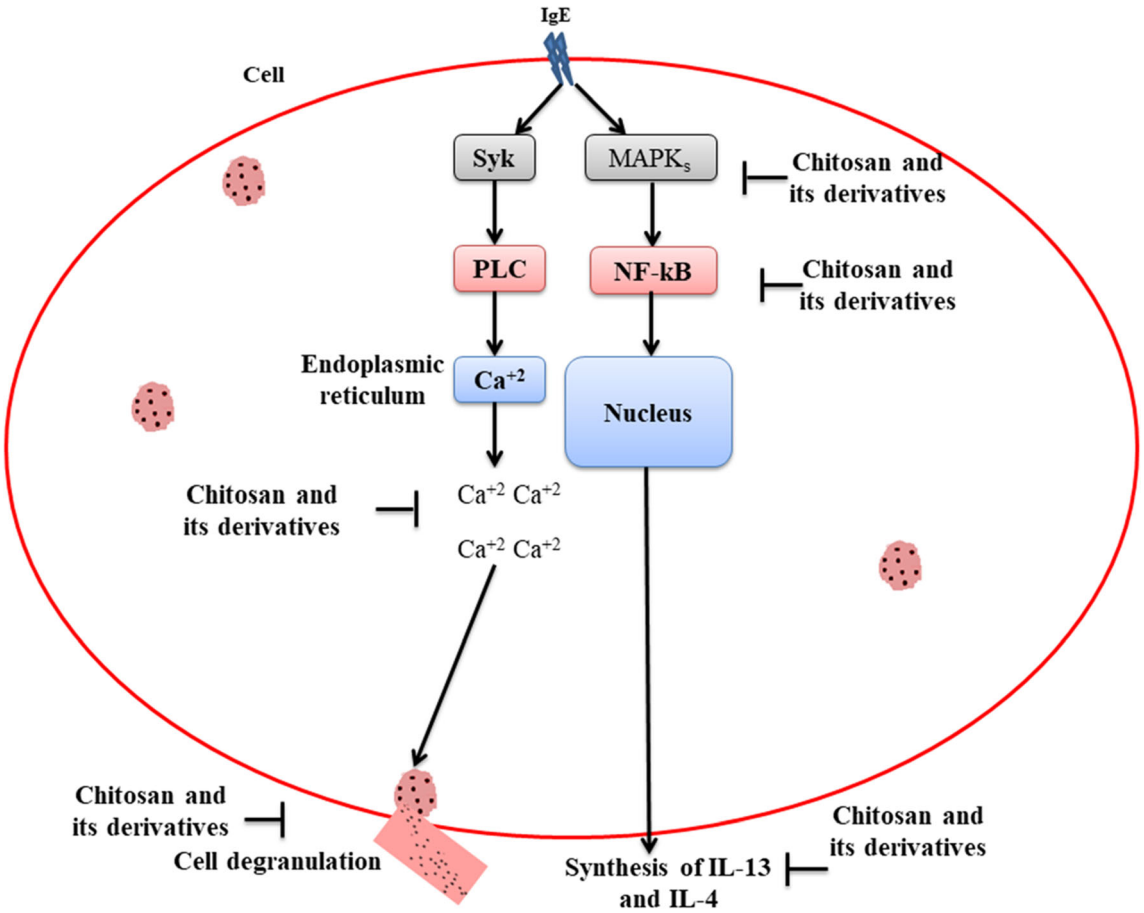

Fig. 4 Anti-allergic mechanisms of chitosan and its derivatives

the control group, the protein level of TNF-α, IL-13, and IL-4 in bronchoalveolar lavage fluid was reduced by a factor of 9.9, 3.0, and 5.8, respectively. These results suggest that the COS can be used for the development of therapeutic agents antagonizing the mast cell–mediated allergic inflammatory response (Chung et al. 2012).

The immunological potential of chitosan

The immunological potential of chitosan derivatives and their consequence as an adjuvant has been evaluated in three main eras previously (Li et al. 2013). A study conducted by Kawada et al. (2007) reported the effect of chitosan and their derivatives on Th1 adjuvant to induce viral-specific immunity. Chitosan and its derivatives have the potential to initiate the adaptive Th1, Th2, and Th17 immune response. Both in vivo and in vitro models were reported by Da Silva et al. (2010); 5 different mice together with IL17A-, TLR2-, MyD88-, and TLR4-deficient mice and C57BL/6 wild-type mice as a control were employed in this study. Each mouse in a group received two intraperitoneal doses along with alum plus or ovalbumin (OVA) to compare the adjuvant potential. The mixture of chitin and alum was together correspondingly initiated to be adjuvant for OVA-induced BAL and tissue inflammation, eosinophilic infiltration, and OVA-specific IgE initiation. Furthermore, IL-17 and MyD88 play prominent roles in chitosan-mediated response. On the other hand, adjuvant effects of chitosan and their derivatives on the proliferation of T cell were mediated with the help of TLR2. The production of IL-13, IL-5, and IL-4 was detected in both OVA-alum primed and OVA-chitin primed T cells (Da Silva et al. 2010; Elieh Ali Komi et al. 2018).

Furthermore, chitosan and its derivatives have been widely used as an adjuvant in DNA/protein-based vaccines (Levitz et al. 2015). In these vaccines, chitosan and its derivative can benefit the host through various mechanisms, including depot formation and antigen protection, as well as the enhancement in antigen presentation and uptake. Moreover, chitosan has the ability to directly restrain the immune response for its adjuvant capability when used as mucosal and injectable vaccine (Carroll et al. 2016).

The anti-cancerous potential of chitosan

The most terrible human disease is cancer, which is an increasing threat nowadays due to changing nutrition, global

warming, and lifestyle (Jayde and Boughton 2016). The natural anti-cancer drugs, such as chemopreventive compounds and free radicals, have gained abundant courtesy in the cure of various cancer species (Gurib-Fakim 2006). A study conducted by (Karagozlu et al. 2010) evaluated the ability of amino ethylated COS (800–3000 Da) to inhibit the proliferation of human gastric adenocarcinoma (AGS) cells. All amino derivative COS induced significant cell death in human gastric adenocarcinoma cells, which was observed by distinguishing the cell viability, change in cell morphology, and the change in nuclear morphology. Moreover, amino derivative COS inhibit the proliferation of human gastric adenocarcinoma cells via regulating the expression level of an important gene involved in apoptosis, including p21, bcl-2, p53, and bax genes.

The amino ethylated COS (AE-COS) inhibited the proliferation of AGS cells in a dose-dependent manner. For example, at a concentration of 50 to 500 μg/ml, the AE-COS inhibited the proliferation of AGS cells by 22 to 85%, respectively. Therefore, it is concluded that the amino derivative COS possess a positive potential as an anti-cancer drug and can be used as cancer preventive chemotherapy (Karagozlu et al. 2012). Moreover, the low Mw chitosan and COS can be used as anti-tumor drugs, as these compounds induce the apoptosis in Ehrlich ascites tumor cells via the DNA fragmentation (Harish Prashanth and Tharanathan 2005).

Highly charged COS reduced the cell viability of three different cancerous cell lines, including cervix cancer (HeLa), liver cancer (Hep3B), and colon cancer (SW480) cell lines. By DNA fragmentation as well as by the fluorescence microscopy, it was shown that the anti-cancerous effect of highly charged COS is due to their ability to induce necrosis (Huang et al. 2006).

For cancer patients, the generation of metastases is the primary cause of death all around the world. The materialization of fresh capillary vessels from the pre-exiting vessels is called angiogenesis, and it is mandatory for tumor and normal vasculature. Throughout the later life, the angiogenesis happens only due to normal physiology reasons, such as wound healing and ovary cycling. However, angiogenesis plays a very vital role in the pathology of some life-threating disease including inflammatory disease, rheumatoid arthritis, and tumor growth. Specifically, in cancer, the metastasis and growth of cancerous tissue depends upon the degree of angiogenesis (Ramjiawan et al. 2017; Wang et al. 2015). At a concentration of 100 μg/ml, COS induced the fabrication of H_2O_2 and superoxide by the neutrophils. In the meantime, the COS treatment prompts the apoptosis of peritoneal neutrophils. On the other hand, the MPO production was increased after treatment with COS (Dou et al. 2009). The COS have the potential to inhibit the angiogenesis process. Therefore, COS can be used in the treatment of cancer. Furthermore, COS inhibit the chicken chorioallantoic membrane (CAM) angiogenesis, migration, and tubed formation of induced HUVECs cells. However, COS have no effect on normal HUVECs. Thus, it is concluded that the COS have anti-angiogenic potential as well as it can antagonize the effect of stimulation of hepatoma carcinoma cell culture fluid on endothelial cell at a certain level (Wu et al. 2008).

COS extracted from fungi have been investigated for its potency as anti-metastasis and anti-tumor drug as well as the related pathways. The results show that COS treatment decreased the rate of DNA synthesis and the percentage of S phage as well as inhibited the proliferation of human hepatocellular carcinoma (HepG2) cells. Furthermore, COS treatment upregulated (p21) and downregulated (cyclin A, cdk-2, and PCNA) the expression of cell cycle–related genes (Shen et al. 2009). All these results suggest that chitosan and its derivatives can be helpful to suppress the cancer cell proliferation and metastasis generation and might act as a potential candidate for cancer treatment drugs (Shen et al. 2009).

The genetic material delivery potential of chitosan

In an acidic environment, amino groups of chitosan are positively charged. This charge is responsible for a strong electrostatic interaction with negatively charged macromolecules, including DNA or RNA (Hejazi and Amiji 2003). Therefore, during the gene therapy procedure, chitosan might be used as a genetic material delivery agent in order to shelter genetic material in contradiction of nuclease and to enrich the transfection efficacy, although, the most important factors under consideration when using chitosan as a genetic material delivery agent are its Mw and degree of deacetylation (DA). In recent years, various studies were conducted to unravel the effect of Mw and DA chitosan on the meritorious transfer of genetic material into cells. Both of these properties (Mw and DDA) of chitosan have a great impact on its physical, chemical, and biological properties (Aiba 1992; Lavertu et al. 2006; Thevarajah et al. 2017). The increase of Mw and DDA is also accompanied by an enhancement in transfection efficacy and binding ability to DNA (Maurstad et al. 2013). Therefore, the high Mw (approximately 100 kDa) chitosan has many applications in delivery processes of genetic material, including delaying the release of genetic material, forming stable physical shapes of polyplexes, and forming stable polyplexes with genetic materials (Köping-Höggård et al. 2004).

On the other hand, this high Mw chitosan has some pharmaceutical disadvantage, including high viscosity when used as in vivo delivery, slow dissociation of genetic material, and low solubility at physiological pH (Hejazi and Amiji 2003; Köping-Höggård et al. 2004). To improve the latter, low Mw and water-soluble chitosan (LMWSC) has been prepared by salt removal methods. The resulting LMWSC was successfully used in food and pharmaceutical industry (Jang et al. 2002). Furthermore, the formation of steady polyplexes without clump generation, effortlessly dissociated polyplexes, reduced

viscosity and solubility at neutral pH of low Mw chitosan, was investigated for gene therapy (Strand et al. 2010). For in vitro transfection of an adenocarcinoma (MAT-LyLu) cell line, a pDNA-loaded chitosan microsphere was prepared using precipitation techniques. The amounts of the plasmid, as well as the Mw of chitosan, affect the transfection efficacy in cells. The low Mw pDNA-loaded chitosan is endowed with a slightly higher transfection efficacy than the high Mw chitosan (Akbuğa et al. 2004). Furthermore, 3–4 kDa chitosan forms a steady complex and affords a higher level of gene expression than high Mw chitosan (Köping-Höggård et al. 2003).

The transfection efficacy and stability of chitosan polyplexes is not only depended on the Mw and DDA of chitosan but also on the pH of transfection medium (Lavertu et al. 2006; Nimesh et al. 2010), the concentration of genetic material (Strand et al. 2010), and the concentration of serum (Yuan et al. 2009). Therefore, for an effective gene delivery system based on chitosan, the optimal parameters have to be determined, including serum stability, DDA, MW, medium pH, and concentration of genetic material.

Bone morphogenetic protein-2 delivery potential of chitosan

The amine groups of chitosan can be additionally chemically modified to expand the application of chitosan in biological and biomedical fields as well as to enhance its solubility. Furthermore, chitosan can be modified and converted into various physical forms including films, powders, fibers, and even pastes (Balagangadharan et al. 2017). Chitosan scaffolds can be prepared by following various methods including phase separation, gas foaming, solvent casting, and the freeze-drying (Qi et al. 2014). Among them, the freeze-drying method is the most suitable method for the preparation of chitosan-based scaffolds for bone tissue engineering with chitosan (Nandi et al. 2013). In recent years, various studies have been conducted, indicating the role of chitosan and its derivatives in drug delivery systems. A very recent study shows that water-soluble chitosan in rabbit models has the ability to promote osteoblast differentiation along with the expression of bone morphogenetic protein-2 (BMP-2). Moreover, it can incline the calcium concentration in serum as well as the alkaline phosphatase activity (Ruijin et al. 2016).

In recent years, chitosan has attained great attention as a BMP-2 delivery agent. In the development of approaches for bone tissue regeneration, chitosan gels were used as a transporter medium for delivery of rhBMP-2. In a study, the quantity and quality of bone formation in a group of 36 rats with life-threatening bone weakness were scrutinized (Issa et al. 2008). In Sprague Dawley rats, chitosan nanoparticles containing collagen material were used to deliver the rhBMP-2. The results show that the group of rats treated with rhBMP-2 along with chitosan nanoparticles and collagen exhibited greater bone formation rate than the group treated without rhBMP-2. The effect of accelerated bone formation was augmented with the increased treatment and analysis time (Lai et al. 2013).

Nowadays, porous chitosan scaffolds (70 to 900 μm; pore size) supplemented with insulin like growth factor-1 and BMP-2 can be used for bone healing. These porous chitosan scaffolds have approximately 87% BMP-2 absorption efficiency. In contrast to solely chitosan, vascularization and cell proliferation were far greater when chitosan scaffolds were supplemented with BMP-2 (Nandi et al. 2013). At the presence of chitosan, cellular proliferation and growth were observed even as a BMP-2 film was absent. However, on the other hand, the combination of chitosan with BMP-2 has the capability to differentiate myoblastic mouse cell lines on to the osteoblastic types (López-Lacomba et al. 2006). A nonfibrous chitosan BMP-2 membrane was utilized for bone tissue regeneration, and it assisted enhancement of calcium deposition, alkaline phosphatase activity, and cell proliferation as well as the osteoblast cell attachment (Park et al. 2006).

Chitosan potential in wound healing

The wound healing is a continuous process comprising five different consecutive and overlapping phases. These phases are maturation, proliferation, migration, inflammation, and hemostasis (Archana et al. 2015). Yet, this wound healing process does not apply for the junctures of burn wounds. Here, the process comprises of epidermis formation, proliferation, agglutination, necrosis, and infection periods. Mostly, severe skin wounds are treated by wound dressings (Aoyagi et al. 2007). Larger wounds need tissue regeneration techniques, including cell growth factors and scaffolds to accelerate the cell proliferation and adhesives, while small wounds can be repaired by surgical reconstruction (Oryan et al. 2014). Chitosan scaffolds possess macro- and micro-sized pore (Croisier and Jérôme 2013); macro-sized pores are vital for blood vessels as well as growth and migration of mesenchymal cells, while the micro-sized pores are important for cell-to-cell communication (Venkatesan and Kim 2010).

Critical wounds need the resection of necrotic tissue and are treated by skin implanting to replace the destroyed skin and to encourage the healing process (Adhikari et al. 2016). At present, there are three kinds of tissue, including dermal as well as epidermal component, and epidermal tantamount component, which can be used for the replacement of skin (Boucard et al. 2007). Due to some restrictions, including the application of numerous materials and deficiency of experimental analysis as well as the inconstant manufacturing expertise, the cutaneous tissue engineering is still in its juvenile stage (Meimandi-Parizi et al. 2013). Any technique used for wound dressings should have the ability to shelter the wound from external detrimental effects, should not be allergenic, should be comprising of biocompatible material, should

possess barrier properties towards microbes, allow the exchange of oxygen as well as nutrients, and absorb or drain excessive exudates (Archana et al. 2015; Jayakumar et al. 2011).

Chitosan is a substance used in primary wound dressing techniques and fulfills all the criteria listed above (Lu et al. 2016). Various chitosan scaffolds saturated with growth factors were employed for cutaneous wound healing process (Sivashankari and Prabaharan 2016). Furthermore, chitosan and its derivatives were utilized to antagonize bleeding and to shelter the wound from external environmental irritants (Wang et al. 2012). Chitosan stimulates the platelets during the inflammatory phase, and after that, it can boost the angiogenesis process by proliferation of endothelial cells (Venkatesan and Kim 2010). On the other hand, the secretion of IL-8 during the healing process was augmented by chitosan. Therefore, it can help in the migration and proliferation of endothelial and fibroblast cells (Saravanan et al. 2016).

In the field of tissue healing, there are some important biochemical events of chitosan including activation of fibroblast, synthesis of type-IV collagen, production of cytokine, activation of polymorphonuclear cell, and migration of giant cell. Well-known wound healing materials based on chitosan are Hemcon® (Hemcon), Chitoseal® (Abbot), and Clo-Sur® (Scion) (Muzzarelli et al. 2007). Chitosan in combination with alginate and collagen has been used as a scaffold in wound healing (Ti et al. 2015).

Chitosan and its derivatives play a vital role in wound healing by stimulating the fibroblast, osteoblast, neutrophils, and macrophages during the different stages of the wound healing process (Dai et al. 2011). Chitosan treatment percolates neutrophils at the initial stage of the inflammatory phase and enhances proliferation of fibroblast. Thus, chitosan treatment enhanced the production of collagen III during the maturation and proliferative phases of healing process (Lu et al. 2017). Chitosan along with the combination of other materials can be used to initiate the granulated tissue formation during the cutaneous wound healing process (Fouda et al. 2009; Mahmoud and Salama 2016). The treatment of wounds with chitosan powder reduced the number of inflammatory cells and rete ridges, while it enhanced the re-epithelialization at the wound site (Dai et al. 2011). Furthermore, chitosan treatment enhanced the oxygen permeability, fluid drainage, epithelialization, and inhibited the invasion of microorganisms (Paul and Sharma 2004). A dressing of chitosan acetate benefited the early closure of wounds and also reduction of a number of inflammatory cells in the wound (Dai et al. 2011).

Wastewater treatment potential of chitosan

Chitosan has shown plentiful potential in the field of wastewater treatment because of its copious benefits, including abundant sources, environmental friendliness, low cost, biodegradability, biocompatibility, non-toxic nature, higher content in amino terminal group, and good absorbent property for both organic as well as inorganic pollutants (Danilchenko et al. 2011). Chitosan and its hydrogels were employed to remove organic pollutants, inorganic pollutants, heavy metal ions, and odors and dyes from industrial wastewater (Kavya et al. 2013). Moreover, chitosan hydrogels have the potential of releasing dyes upon washing with ethanol and thus, these hydrogels can be recycled.

A recent study evaluated the ability of a chitosan-based cellulose eco-friendly hybrid film, which was made by a solvent casting technique, to adsorb heavy metal ions. The result shows that cellulose acts as a very good source for strengthening the chitosan film due to generation of hydrogen bonds among the two polysaccharides. Furthermore, the addition of cellulose particles (10%) increased the mechanical properties of chitosan films. The maximum Cd^{+2} adsorption capacity of the hybrid film was found at pH 6.0. Hence, chitosan and cellulose-based films might be used as adsorbent agents for heavy metal (Cd^{+2}) ion polluted water (Rahmi and Julinawati 2017).

Chitosan can be used as a solid support during metal catalyst reaction in order to control the dispersion stability, the formation of nanoparticles, and to prevent agglomeration of metal (nano)particles. The addition of silver nanoparticles to chitosan composite materials improved the removal of copper ions from aqueous solution. A beneficial pH of 7.0 was selected as the most suitable pH of the aqueous solution for the adsorption experiment. Thus, it can be concluded that these composite material can be considered suitable material for the removal of copper ions from aqueous solution (Azzam et al. 2016). Hence, from the above results, it is concluded that chitosan-based composite materials might be suitable for removal of metal ions and various other pollutants from aqueous solution.

The hypocholesterolemic potential of chitosan

Chitosan has the potential to prevent absorption of fat as well as to bind the dietary fats (Kanauchi et al. 1995). On the other hand, chitosan is very viscos and due to its viscosity, it is challenging to use it in physiological as well as in functional food applications. Chitosan and its oligomers put forth significantly hypocholesterolemic consequences with different physiochemical characteristics. In an in vitro investigation, eleven products were examined for their swelling behavior, fat binding capability, DD, and their ability to bind bile acids (Zhou et al. 2006). However, it was not yet proven that the swelling ability, DD, and molecular weight predict the binding capacity of bile acid.

Chitosan oligomers having low Mw were more effective in enhancing lipoprotein lipase action in mice liver and plasma, while chitosan oligomers having higher DD show a significant effect on increasing the cholesterol and fecal fats in mice

(Zhang et al. 2012). Presently, there are various proposed mechanisms for cholesterol decline induced by chitosan. First of all, the viscous polymer solution entrapment is recommended to decrease the absorption of cholesterol and fats in the food. Moreover, the positive charged amino group of chitosan determined the interaction between chitosan and negatively charged substances, such as the bile acids and fatty acids (Muzzarelli et al. 2006). Thirdly, chitosan adsorption on the surface of the emulsified lipid produced a protective coating, which might stop lipase from adsorbing to the droplet surface and gaining access to the lipids (Ogawa et al. 2003).

The bio-imaging potential of chitosan

Bio-imaging is a technique, which offers several benefits over other technique, including outstanding consecutive as well as three-dimensional resolution with a real window, non-exposure to radiation, and in vivo quick acquisition of images (Yang et al. 2017). The tissue surveillance is one of the most vital techniques today, used in clinical medication to evaluate the anatomy and function of tissue. It was advanced from simple visual examination to in vivo and in vitro microscopic as well as the spectroscopic methods (Um et al. 2018). The fluorescence imaging technique is more suitable to monitor various cellular activities than the magnetic resonance imaging (MRI) technique. The fluorescent imaging technique and chitosan-based biomaterial are mostly used in the biomedical field, including bio-sensing, drug delivery, and cell imaging (Jang et al. 2006; Li et al. 2009).

Presently, various fluorescent materials (quantum dots, fluorescent proteins, and organic dyes) can be used because they have excellent excitation spectra, good bio-imaging properties, high photostability, and strong fluorescence. On the other hand, their applications are limited as they can release harmful and toxic heavy metal ions (Li et al. 2011). This problem can be solved by employing polymers, having green amino groups including poly-urea dendrimers (Restani et al. 2014), polyethylenimine (Lin et al. 2010), poly-amido-amine (Yang et al. 2011), and ploy-amino-ester (Shen et al. 2011). Due to the presence of an amino group, chitosan is a convenient choice among naturally occurring amino polymers along with good biological, physical, and chemical characteristics (Martin et al. 2009).

Chitosan can be used as biomaterial in various biomedical fields due to its non-toxicity and biodegradability (Van De Manakker et al. 2009). It is stated that telluride quantum dots embedded in chitosan nanoparticles can be used as an attractive bio-imaging agent (Ghormade et al. 2015). Being an imaging agent, the telluride quantum dots have some disadvantage because of its cytotoxicity. However, as soon as it is embedded in chitosan, it stops the release of quantum dots for a long time. Thus, it is clear that by this means, it can reduce its long-term toxicity. Furthermore, chitosan nanoparticles exhibit several advantages. For example, chitosan nanoparticles are non-degradable by hydrolytic enzyme, including lysozyme and chitinase (Ghormade et al. 2015). Chitosan embedded gadolinium nanoparticles can be synthesized and used for bio-imaging purposes, since it can be located for an extended period of time at the cancerous location, which is beneficial for detailed imaging (Kumar et al. 2004).

Chitosan can be utilized to diminish the gold non-particles to turn as photodynamic carrier and photothermal converter; while it can also allow photodynamic remedy. Furthermore, chitosan plays a vital role in the elimination of breast cancer (MCF-7) cells as well as in bio-imaging application (Hari et al. 2013). Furthermore, the integrated upconversion of nanoparticle presents some advantages, including magnetic properties as well as these upconversion prime to the technological and scientific significance (Yong et al. 2009). In addition, various upconverted nanoparticles exhibit negligible photo destruction to biological tissue, low background auto fluorescence, low toxicity, sophisticated fluorescence efficacy, high chemical stability, and deep light infiltration (Wei et al. 2014).

Chitosan and future perspective

For the future, we anticipate that composite materials based on chitosan will gain considerable attention, for example, composites based on the addition of various inorganic/organic nanoparticles (chitosan bio-nanocomposite material). In recent decades, it has been shown that chitosan plays a vital and extraordinary role in various fields, including biomedicine, agriculture, and material science (Upadhyaya et al. 2014; Zhao et al. 2015a). In the future, chitosan-based materials can be used in the form of advanced composites or fibers, for example, to accelerate tissue repairing and wound healing processes as well as to advance the existing tissue engineering expertise. Various bio-polymers, including carrageenan (Gholipour-Kanani et al. 2012), chitosan (Nadalin et al. 2006), hyaluronic acid (Yu et al. 2016), and gelatin (Chaudhari et al. 2016) are envisaged to find application in the field of green chemistry. The synthesis of nanoparticles embedded in chitosan (known as biopolymer) is a new approach to develop bio-materials with characteristics of antimicrobial activity, bio-compatibility, and long-term stability. We anticipate that these newly synthesized biopolymers have promising potential in tissue engineering techniques and in biomedical field (Jeuken et al. 2016).

Furthermore, in the future, the used of chitosan-based biopolymers could offer a way to advance the new tissue engineering materials. Beside this, chitosan-based biopolymers have shown potential in drug delivery, and it is anticipated that novel robust and biocompatible drug delivery vectors can be obtained in the future. Chitosan-based biomaterials permit more conveyance of drugs used to prevent various disorders.

Conclusions

In the present review article, we have discovered that the vital function of chitosan-based hybrid materials is extensity used in various biotechnological applications. Recent investigations have shown that chitosan-based biomaterials can be used as safe functional ingredient and are very helpful in the treatment/prevention of a wide variety of life-threating disorders. Due to the increased biotechnological potential, for example, as anti-hypertensive, anti-oxidant, anti-microbial, anti-allergic, immunological, anti-cancerous, delivery of genetic materials, delivery of bone morphogenetic type-2, wound healing, treatment of wastewater, hypocholesterolemic, and bio-imaging, chitosan biopolymers and its derivatives have promising prospective health-promoting functional foods. In addition to this, these materials are also promising candidates for pharmaceuticals and wound dressings for biomedical industries. Finally, this article pointed out various recent challenges for the use of the newly synthesized chitosan-based materials in the field of biomedical.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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