Chemically modified chitin and chitosan as biomaterials

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Abstract

Recent studies of the chemical modification of chitin and chitosan are discussed from the viewpoint of biomedical applications. Special emphasis is placed on the role of individual functional groups in applications of modified chitosan. The modifications discussed here include chitosan attached to sugars, dendrimers, cyclodextrins, crown ethers, and glass beads. Among these derivatives, sugar-modified chitosans are excellent candidates for drug delivery systems or cell culture owing to their specificity. Chitosan–dendrimer hybrids are interesting multifunctional macromolecules. Chitosan and its derivatives are useful as carriers in drug delivery systems, as antibacterial agents, and in other medical applications.

Keywords: Chitosan; Sugar; Dendrimer; Cyclodextrin; Crown ether; Grafting; Medical applications

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1. Introduction

Studies on chitin and chitosan have been intensified since 1990 because these polysaccharides show

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excellent biological properties such as biodegradation in the human body [1,2], and immunological [3,4], antibacterial [5,6], and wound-healing activity [7–9]. In recent studies, especially, chitosan has been found to be a good candidate as a support material for gene delivery [10], cell culture [11], and tissue engineering [12,13]. Therefore, chitin and chitosan are receiving greater attention as novel functional materials. Despite their interesting biological properties, utilization has been scarcely developed.

In the meantime, commercial or practical use of chitin and chitosan (including monomer and oligomers) has been confined to the unmodified forms. For a breakthrough in utilization, chemical modification to introduce a variety of functional groups will be a key point. For this purpose, more fundamental studies on chemical modification will be required. By comparison, the chemical modification of cellulose is well studied and is still an active field. Until now, much work has been reported on the chemical modification of chitin and chitosan. Most studies have been published in reviews and books [14–16]. In this review, we describe the recent studies on biomedical aspects of chitin and chitosan.

2. Sugar-modified chitosan

The first report on the modification of chitosan with sugars was by Hall and Yalpani (Scheme 1) in 1980 [17,18]. They synthesized sugar-bound chitosan by reductive \( N \)-alkylation using \( \text{NaCNBH}_3 \) and unmodified sugar (1: method A) or a sugar-aldehyde derivative (2: method B). At that time, the sugar-bound chitosans had been investigated mainly in rheological studies; but since the specific recognition of cells, viruses, and bacteria by sugars was discovered, this type of modification has generally been used to introduce cell-specific sugars into chitosan. Morimoto reported the synthesis of sugar-bound chitosans, such as those with D- and L-fucose, and their specific interactions with lectin and cells [19–22]. Kato also prepared lactosaminated \( N \)-succinyl-chitosan (3: Scheme 2) and its fluorescein thiocarbanyl derivative as a liver-specific drug carrier in mice through a sialoglycoprotein receptor [23]. Moreover, derivative 3 was found to be a good drug carrier for mitomycin C in treatment of liver metastasis [24]. Galactosylated chitosan (4: Scheme 3) prepared from lactobionic acid and chitosan.

![Image of Scheme 1](image-url)
with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxysuccinimide (NHS) showed promise as a synthetic extracellular matrix for hepatocyte attachment [25]. A sponge type complex of cationic 4 and anionic alginate also showed spheroid formation and viability of hepatocytes [26]. Furthermore, graft copolymers of 4 with poly(ethylene glycol) or poly(vinyl pyrrolidone) were useful as hepatocyte-targeting DNA carriers [27,28].

Sialic acid is the most prevalent sugar of the glycolipids and glycoproteins on the mammalian cell surface and is the key epitope recognized as essential
for a number of pathogenic infections. Moreover, sialic acid-containing polymers have been shown to be potent inhibitors of hemagglutination of human erythrocytes by influenza viruses [29–33]. We prepared sialic acid bound chitosan (6: Scheme 4) as a new family of sialic acid containing polymers using p-formylphenyl-α-sialoside (5) [34] by reductive N-alkylation [35]. Since derivative 6 was insoluble in water, successive N-succinylations were carried out to obtain the water-soluble derivative (7). Specific binding of wheat germ agglutinin with lectin was shown in the presence of derivative 7.

Human antibodies against the α-galactosyl epitope are responsible for acute rejection of xenotransplanted organs from lower animals. Artificial glycopolymers having an α-galactosyl epitope are of interest from the viewpoint of medical transplantation of pig liver since they can block immune rejection. This interesting epitope also contains as a family of bioactive sugar bound chitosans. Water-soluble α-galactosyl chitosan (8: Scheme 5) prepared by the same strategy as sialic acid showed specific binding against α-galactosyl specific lectin (Griffonia simplicifolia) [36]. The different type of spacer has been prepared on sialic acid or α-galactosyl epitope bound chitosans [37]. These epitope bound chitosans may be useful as potent inhibitors of influenza viruses or blocking agents for acute rejection.
3. Chitosan–dendrimer hybrid

Dendrimers are attractive molecules owing to their multifunctional properties [38–40] and have useful applications as viral and pathogenic cell adhesion inhibitors [41,42]. Increasing scientific efforts have gone into the design and synthesis of dendrimers [43–45]. Dendronized polymers, on the other hand, are also attractive because of their rodlike conformation and nanostructure [46–49]. Although several investigations have been published toward the synthesis of dendronized polymers [50,51], there are no reports on dendronized polysaccharide specifically related to chitin and chitosan backbones. We established the synthesis of a variety of chitosan–dendrimer hybrids mainly by two procedures (Scheme 6) [52–55].

In method A, corresponding dendrimers bearing aldehyde and a spacer are synthesized, and then these are reacted with chitosan by reductive N-alkylation. This procedure has the advantage of no crosslinking during the reaction. However, the generation of reactive dendrimer is limited owing to steric hindrance. On the other hand, method B, with binding of

![Scheme 5. Structure of water-soluble α-galactosyl chitosan. From Sashiwa, Thompson, Das, Shigemasa, Tripathy and Roy [36]; by permission of American Chemical Society, USA.](image1)

![Scheme 6. Synthetic strategy on chitosan–dendrimer hybrid. From Sashiwa, Shigemasa and Roy [54]; by permission of American Chemical Society, USA.](image2)
chitosan to the dendrimer surface, allows use of commercially available amino-dendrimers such as poly(amidoamine) (PAMAM) or poly(ethylene imine) dendrimers; and binding is possible even for high generations. One weak point in method B is that it has two or more binding points that may sometimes cause crosslinking. A typical example of a hybrid obtained by method A is shown in Scheme 7 [52,55]. We visualize this hybrid as a 'tree type molecule': chitosan is the trunk, the spacer is a main-branch, the dendrimer is a sub-branch, and the functional sugar represents the flower (or leaf). In this case, tetraethylene glycol was modified in 5–7 steps, to synthesize the scaffold of the dendrimer. PAMAM dendrimers of generations (G) from 1 to 3 bearing tetraethylene glycol spacers. These were attached to sialic acid by reductive N-alkylation, and finally attached to chitosan. The DS of dendrimer per sugar unit decreased with increasing generation [0.08 (G = 1), 0.04 (G = 2) and 0.02 (G = 3)] owing to steric hindrance of the dendrimers. Scheme 8 shows a different type of chitosan–dendrimer hybrid [53]. A sialic acid dendron bearing a focal aldehyde end-group was synthesized by a reiterative amide bond strategy. Trivalent (G = 1) and nanovalent (G = 2) dendrons having gallic acid as the branching unit and triethylene glycol as the spacer arm were prepared and initially attached to a sialic acid p-phenylisothiocyanate derivative. The focal aldehyde sialodendrons were then convergently attached to chitosan. The DS values of sialodendrimer were 0.13 (G = 1) and 0.06 (G = 2). Biological evaluation of these promising hybrids as inhibitors of viral pathogens, including the flu virus, is underway.

A Chitosan–dendrimer hybrid prepared by method B is shown in Scheme 9 [54]. As the construction of the hybrid was difficult from the original chitosan, a derivative, N-methoxycarbonylchitosan (21), was used as the chitosan backbone. PAMAM dendrimers (G = 1–5) having a 1,4-diaminobutane core were
attached to 21 by amidation under conditions that prevented crosslinking. The hybrids 22 could be prepared even at high generations (G = 4 or 5), although the DS of dendrimer was decreased with increasing generation of dendrimer from 0.53 (G = 1) to 0.17 (G = 4) or 0.11 (G = 5). Since this hybrid was soluble in acidic solutions, undesired crosslinking did not occur. However, two or more intermolecular binding points were observed. Finally, sialic acid was successfully attached to the primary amine of the dendrimer with DS ranging from 0.7 to 1.4 per glucosamine unit, which indicates a highly convergent synthesis of sialic acid in the chitosan backbone. Given the fact that flu virus hemagglutinins exist as clusters of trimers (200–300/virions), it is likely that the novel dendronized chitosan–sialic acid hybrids prepared by method B will present added beneficial architectures not present in previously reported sialodendrimers [56–58]. Preliminary biological evaluation of analogous hyperbranched sialodendrimers has already shown increased inhibitory properties [41].

4. Cyclodextrin-linked chitosan

Cyclodextrin (CD) has gained prominence in recent years because its hydrophobic cavity is capable of binding aromatic and other small organic molecules, and therefore provides ideal binding sites. CD-linked chitosan is interesting for the viewpoint of pharmaceutics, including drug delivery, cosmetics, and analytical chemistry. Although functionalization at the 6-position of OH in CD is relatively easy, the secondary 2 or 3 position is shown to be the more important site of cyclodextrin in binding studies. Sakairi prepared α-CD linked chitosan (Scheme 10, 37) using 2-O-formylmethyl-α-CD by reductive N-alkylation and confirmed the host-guest complex 37 with p-nitrophenol [59]. Tosylated β-CD is also
useful to link chitosan at the 2-position of CD (38), as reported by Chen et al. [60]. They applied 38 for slow release of radioactive iodine \( ^{131}\text{I}_2 \) in rats. The amount of \(^{131}\text{I}_2 \) in the blood of rats was still approximately half the maximum after 70 days, and the organs of rats retained much higher radioactivity than with the inclusion complex of \( \beta \)-CD with \(^{131}\text{I}_2 \). CD linked chitosan could also be prepared via the intermediate of its monochlorotriazinyl derivative (39) [61]. This compound was used for decontamination of waters containing textile dyes. An insoluble crosslinked chitosan bearing \( \beta \)-CD was prepared using \( N \)-succinyl chitosan and aminated-\( \beta \)-CD via amide bond formation [62]. The \( \beta \)-CD linked chitosan using 1,6-hexamethylene diisocyanate as a spacer was also prepared by Sreenivasan [63]. This material showed interaction with cholesterol and was useful as an adsorbent matrix.

5. Crown ether bound chitosan

Crown ethers have particular molecular structures that cause them to exhibit complexing selectivity for metal ions. Novel polymers containing double structures and properties of both chitosan and crown ethers form stronger complexes with metal salts and show better selectivity for metal ions because of the synergistic effect of high molecular weight. Tang et al. prepared a crown ether bound chitosan of \( N \)-Schiff base-type (40) and its reduced analog (41) in Scheme 11 [64]. Their chemical
structures were characterized by elemental analysis, together with IR, X-ray, and solid-state $^{13}$C NMR analyses. Crown ether bound chitosans not only had good adsorption capacities for noble metal ions $\text{Pd}^{2+}$, $\text{Au}^{3+}$, and $\text{Ag}^{+}$, but also high selectivity for adsorption of $\text{Pd}^{2+}$ in the presence of $\text{Cu}^{2+}$ and $\text{Hg}^{2+}$. Crosslinked crown ether bound chitosans were also reported (Scheme 12) [65]. These crosslinked derivatives have space net structures with embedded crown ethers, and each mesh has a certain space volume. When the original chitosan was reacted with 4,4'- dibromobenzo-18-crown-6-crown ether, the crosslinked product between 6-OH and NH$_2$ was obtained (42). However, this product included heterogeneous crosslinked structures between 6-OH and 6-OH, or NH$_2$ and NH$_2$. While, benzylidene-protected chitosan (CTB) produced homogeneous crosslinked structures between 6-OH and 6-OH (43).
These crown ether bound chitosans may be useful for the separation and preconcentration of heavy or precious metal ions in aqueous environments.

On the other hand, calixarenes have demonstrated outstanding complexation ability towards ions, organic molecules, etc. and are considered the third best host molecules, after cyclodextrins and crown ethers. Li reported the first synthesis of calixarene-modified chitosan (Scheme 13) [66]. The adsorption properties of calixarene-modified chitosan (I and II) varied greatly as compared with the original chitosan, especially the adsorption capacity toward $\text{Ag}^{+}$.
and Hg\(^{2+}\), because of the presence of the calixarene moiety. These derivatives did not dissolve in common organic solvents and could easily be powdered, thus making them easier to use as adsorbents than unmodified chitosan.

6. Chemical grafting of chitosan

Graft copolymerization is important for the functionalization of chitin and chitosan and development of practically useful derivatives. Many routes for grafting have been investigated, such as ceric ion, Fenton’s reagent, gamma-irradiation, various radicals, and ring-opening [67]. An interesting feature of polyoxazoline chains is the fact that they are regarded as pseudopeptides having considerable chain flexibility [68]. It has also been disclosed that oxazoline grafted chitosan (Scheme 14) has the capability of incorporating lipase P and catalase and shows increased hydrolytic activity compared with free enzymes [69,70]. The molecular shape of watersoluble grafted chitosan 45 was evaluated by atomic force microscopy (AFM), cryo-transmission electron microscopy (cryo-TEM), and small-angle neutron scattering (SANS) analyses. Grafted chitosan bearing short graft chains formed a unimolecular ring structure 40–60 nm in diameter, but medium length graft chains led to monodisperse spherical structures. With still longer graft chains intermolecular aggregation occurred to provide larger particles. These studies should be useful in devising strategies to regulate molecular design and guest-binding properties of water-soluble grafted chitosan.

Homopolymers and copolymers based on lactic acid have been widely used in sutures and drug-release systems owing to their biodegradability. Furthermore, pH-sensitive polymer gels have potential use in the delivery of drugs to specific regions of the gastrointestinal tract. Novel pH-sensitive, physically crosslinked hydrogels were synthesized without a catalyst by grafting D,L-lactic acid onto amino groups in chitosan (Scheme 15: 46) [71]. The pH-sensitivity was due to the aggregation of the hydrophobic side chains. The specific solution content of hydrogels decreased when the pH and ionic strength were increased. Although grafting on chitin and chitosan has been performed by high-energy irradiation or the addition of initiators such as cerium (IV) and a redox system, these methods cause degradation of the polysaccharide backbone, thus giving rise to grafted products with complicated and uncertain structures. Kurita synthesized graft copolymers on chitin by using the mercapto group

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Scheme 14. Oxazoline grafted chitosan (DDA = 50%). From Aoi, Takasu, Okada and Imae [70]; by permission of Wiley, USA.

Scheme 15. D,L-lactic acid grafted chitosan. From Qu, Wirsen and Albertsson [71]; by permission of Wiley, USA.
Methyl methacrylate (MMA) was efficiently grafted on mercaptochitin in DMSO, the grafting percentage reaching 1300%. Although the side-chain ester groups were resistant to aq. NaOH alone, hydrolysis of ester could be achieved with a mixture of aqueous NaOH and DMSO. The moisture absorption and lysozyme susceptibility was much greater for the graft product (47) than for chitin.

7. Enzymatic modification of chitosan

The enzymatic approach to the modification of chitin and chitosan is interesting owing to its specificity and environmental advantages compared with chemical modification. With respect to health and safety, enzymes offer the potential of eliminating the hazards associated with reactive reagents. Payne et al. reported enzymatic grafting of phenolic compounds onto chitosan to confer water solubility under basic conditions (Scheme 17) [73]. Tyrosinase converts a wide range of phenolic substrates into electrophilic \( \text{o} \)-quinones. In slightly acidic media (pH 6), chitosan could be modified under homogeneous conditions with the natural product chlorogenic acid. The modified chitosan was soluble under both acid and basic conditions, even when the degree of modification was low. The chemistry of quinones, however, remains poorly characterized because of its complexity. Quinones can undergo two different reactions to yield either Schiff bases (48) or Michael type adducts (49). Since it is possible for quinones to undergo either or both type of reactions with amines, as well as oligomer-forming reactions with other quinones, it is common for reactions between quinones and amines to yield complex mixtures of products. Chen et al. grafted hexyloxyphenol to chitosan by tyrosinase [74]. On the basis of contact angle measurements, the heterogeneous modification of chitosan film was found to produce a hydrophobic surface due to the substituent. While, homogeneously modified chitosan exhibited rheological properties characteristic of associating water-soluble polymers.
From the biochemically relevant quinones studied so far, it would seem possible to prepare materials of medical interest. For instance, menadione, a synthetic naphthoquinone derivative having the physiological properties of vitamin K is particularly prone to rapid reaction with chitosans, greatly modifying its spectral characteristics and increasing the surface hydrophobicity of treated chitosan films [75]. Research under way will provide information on the biological properties of these enzymatically modified chitosans.

8. Medical applications of chitosan derivatives

8.1. Drug delivery system

Colloidal systems have found numerous applications as promising delivery vehicles for drugs, proteins, antigens, and genes due to their low toxic side effects and enhanced therapeutic effects. Polymeric self-assembly systems, (SA’s) are one type of colloidal system that has been widely investigated in terms of micellar behavior in the areas of biotechnology and pharmaceutics. Precise control of size and structure is a critical design parameter of micellar system for drug delivery applications. To control the size of an SA, chitosan was depolymerized with sodium nitrite, and hydrophobically modified with deoxycholic acid to form the SA in aqueous media (Scheme 18) [76]. The size of the SA could be varied from 130 to 300 nm in diameter. Because of the chain rigidity of chitosan, the SA was suggested to have a cylindrical bamboo-like structure, which could form only a very poor spherical form in a bird’s nest-like structure. In a test of the potential application of the SA as a gene delivery carrier, a significant enhancement of transfection efficiency by the SA was observed against COS-1 cells (up to a factor of 10). This approach to control the size and structure of the chitosan-derived SA may find a wide range of applications in gene delivery as well as in general drug delivery applications. Lee et al. reported the delivery of adriamycin (ADR) using the SA of the deoxycholic acid-modified chitosan (50) [77]. Deoxycholic acid was covalently conjugated to chitosan via an EDC-mediated reaction to generate SA nanoparticles. ADR was physically trapped inside the SA and slow release of ADR was thereby achieved.

The formation of hydrogels from polymers using noncovalent crosslinking is a useful method of preparing hydrogels for drug delivery. These gels are likely to be biocompatible as gel formation does not require the use of organic solvents or chemical reactions, which may be potentially deleterious to the drug load. Such physically crosslinked chitosan based gels are formed by exploiting either hydrogen bonding or hydrophobic attractions. Martin et al. have focused on the use of pendant hydrophobic groups to achieve noncovalent crosslinking [78]. Palmitoyl glycol chitosan (GCP, Scheme 19) hydrogel has been evaluated as an erodible controlled release system for the delivery of hydrophilic macromolecules. Fluorescein isothiocyanate (FITC)-dextran, and/or amphiphilic derivatives Gelucire 50/13 and vitamin E [d-α-tocopherol poly(ethylene glycol) succinate]

Scheme 18. Deoxycholic acid modified chitosan. From Kim, Gihm, and Park [76]; by permission of American Chemical Society, USA.
were used as model macromolecules. Hydration and erosion were governed by the hydrophobicity of the gel and the presence of the amphiphilic additives. The controlled release of FITC-dextran was governed by the hydrophobicity of the gel. In a subsequent study, GCP hydrogel was evaluated for buccal delivery of the hydrophobic drug denbufylline [79]. The buccal route has been advocated as a possible means for administration of drugs which undergo extensive hepatic first-pass metabolism or which are susceptible to degradation in the gastrointestinal tract.

Quaternized chitosan has potential as an absorption enhancer across the intestinal epithelium due to its mucoadhesive and permeability enhancing properties. Xu et al. synthesized the water-soluble derivative of chitosan, \( N \)-(2-hydroxypropyl)-3-trimethyl ammonium chitosan chloride (HTCC) [80]. HTCC nanoparticles were formed by the ionic gelation process of HTCC with sodium tripolyphosphate (TPP). Bovine serum albumin, considered as a model protein drug, was incorporated into HTCC nanoparticles with 90% encapsulation. In vitro release studies showed a burst effect followed by a slow release. Thus HTCC nanoparticles are a potential vehicle for the administration of proteins.

Porous drug-delivery devices have received much attention for use with such drugs as anticancer and peptide-based therapeutic agents. The wet phase-inversion method (phase inversion induced by immersion precipitation) is a suitable technique for preparing macroporous gels with desired morphology and pore size. Mi et al. prepared macroporous beads of chitosan and its derivatives by this method [81]. With aqueous TPP solution as a casting medium, both liquid-liquid and solid-liquid phase separation processes were responsible for the formation of high porosity chitosan beads. Following phase-inversion, the porous chitosan beads were chemically modified by introduction of quaternary ammonium (HTCC), octanoyl, and benzoyl groups. A nonsteroidal anti-inflammatory drug, indomethacin, used for the treatment of arthritis, was immobilized on the porous chitosan beads by different types of intermolecular interactions, such as electrostatic attraction or hydrophobic interaction (Scheme 20). These chemically modified chitosans showed obvious effects on the adsorption of indomethacin, thus demonstrating the possibility of using these materials in a drug delivery system.

The application of chitosan for gene transfection is being studied. The advantage of chitosan-based vectors lies not only in avoiding cytotoxicity problems that are inherent in most synthetic polymeric vehicles but also in their unique capability for transcellular transport. However, as shown with other polycation/DNA complexes, chitosan/DNA complexes are formed by electrostatic interaction between primary amino groups and phosphate groups, which is strong enough to resist DNA unpacking within cells. Anticipating that the incorporation of hydrophobic moieties might considerably increase the transfection efficiency, Liu et al. synthesized a series of alkylated chitosan (AC; Scheme 21) derivatives using alkyl bromide and investigated the stability of AC/DNA complexes [82]. With longer alkyl side
chains, the transfection efficiency was increased and leveled off when the number of carbons in the side chain exceeded eight. The higher transfection efficiency is attributed to increased entry into cells facilitated by hydrophobic interactions and easier unpacking of DNA from AC carriers due to the weakening of electrostatic attractions between DNA and AC.

Scheme 20. Schematic chemical interaction between indomethacin and chemically modified porous chitosan beads. From Mi, Shyu, Chen and Lai [81]; by permission of Elsevier Science Ltd, Oxford, UK.

Scheme 21. Synthesis of N-alkylated chitosan. From Liu, Zhang, Sun, Sun and Yao [82]; by permission of American Chemical Society, USA.
8.2. Antibacterial activity

After the discovery of the antimicrobial activity of chitosan, many researchers have continued studies in this field. The mechanism behind this activity can be summarized as follows:

1. The cationic nature of chitosan causes it to bind with sialic acid in phospholipids, consequently restraining the movement of microbiological substances.
2. Oligomeric chitosan penetrates into the cells of microorganisms and prevents the growth of cells by preventing the transformation of DNA into RNA.

However, the water-insolubility of chitosan is disadvantageous for its wide application as an antibacterial agent. Jung et al. prepared anionic side-chain-grafted, water-soluble chitosan (WSC) derivatives having zwitterionic properties [83]. To prepare these derivatives, mono(2-methacryloyloxyethyl) acid phosphate and vinylsulfonic acid sodium salt were grafted onto chitosan (Scheme 22). Antimicrobial activity against Candida albicans (Ca), Trichophyton rubrum (Tr), and Trichophyton violaceum (Tv) depended largely on the amount and type of grafted chains as well as changes of pH. The highest activity was shown at pH 5.75 against Ca and Tv, due to the difference in affinity between cell walls of fungi and the chitosan derivatives.

Recent studies have been focused on the development of antibacterial surfaces to attain high functionality and high-value products. Poly(ethylene terephthalate) (PET) is a basic material in the textile and plastics industries. Accordingly, the improvement of the antibacterial properties of PET is important for a wide range of industrial applications. Huh et al. prepared chitosan-grafted PET (C-PET) and quaternized chitosan-grafted PET (QC-PET) [84]. Against S. aureus, C-PET and QC-PET showed high growth inhibition in the range of 75–86% and still retained 48–58% bacterial growth inhibition after laundering.

8.3. Other medical applications

Laminin is known to be involved in metastasis of tumor cells. A peptide containing the Tyr-Ile-Gly-Ser-Arg (YIGSR) sequence, corresponding to a partial sequence of laminin, inhibited angiogenesis and thus depressed tumor growth. Nishiyama et al. prepared YIGSR-chitosan conjugate and assayed antimetastatic activity (Scheme 23) [85]. One peptide was introduced per 6.3 glucosamine residues. The conjugate proved to have higher inhibitory activity against...
experimental lung metastasis of B16BL6 melanoma cells in mice than did the parent peptide (Table 1).

Apoptosis, or programmed cell death, is an essential physiological process in the normal development and homeostasis of multicellular organisms. Derangements of apoptosis have deleterious consequences, as exemplified by various human disease states, including acquired immunodeficiency syndrome, neurodegenerative disorders, and cancer. In mammals, chitosan was found to stimulate nonspecific resistance against *E. coli* infection, to suppress the growth of Meth A tumors in syngenic Balb/c mice, and to stimulate nitric oxide production in RAW 264.7 macrophages. WSC has higher reactivity than water-insoluble chitosan. Koo et al. reported the effect of high molecular weight WSC on serum starvation-induced apoptosis in human astrocytes (CCF-STTG1 cells) [86]. WSC, with an average molecular weight of 300 kDa and degree of deacetylation (DDA) over 90% (obtained from JA KWANG Co., Ansung, Korea), can be produced using a simple multistep membrane separation process. WSC significantly protected from serum starvation-induced cellular rounding up and from serum starvation-induced cell death as tested by flow cytometry. WSC also protected from serum starvation-induced p53 activation as determined by the Western blot technique (Fig. 1). From these results, it appears that WSC may prevent serum starvation-induced apoptosis of CCF-STTG1 cells via p53 inactivation.

A chronic inflammatory response associated with β-amyloid (Aβ) and interleukin-1β (IL-1β) is responsible for the pathology of Alzheimer’s disease (AD). Astrocytes are predominant neuroglial cells of the central nervous system and are actively involved in cytokine-mediated events in AD. To investigate the biological effect of WSC (DDA > 90%), Kim et al. [87] examined cytotoxicity, production of pro-inflammatory cytokines and inducible nitric-oxide synthase (iNOS) on a human astrocytoma cell line CCF STTG1 stimulated with IL-1β and Aβ fragment 25–35 (Aβ [25–35]). WSC itself had no effect on cell viability of human astrocytoma cells. The secretion and expression of pro-inflammatory cytokines, tumor necrosis factor-α, and interleukin-6 were significantly inhibited in human astrocytoma cells by pretreatment

![Scheme 23. Preparation of peptide–chitosan conjugate. From Nishiyama, Yoshioka, Ohara, Kurita, Hojo, Kamada, Tsutsumi Mayumi and Kawasaki [85]; by permission of Royal Society of Chemistry, UK.](image)

**Table 1**

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<th>Sample</th>
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<th>Inhibition (%)</th>
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<td></td>
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From Nishiyama, Yoshioka, Ohara, Kurita, Hojo, Kamada, Tsutsumi Mayumi and Kawasaki [85]; by permission of Royal Society of Chemistry, UK.

![Fig. 1. Western blot analysis. Fifty μg of total protein were resolved by 10% SDS-PAGE, transferred to nitrocellulose membrane, and analyzed by Western blotting using an anti-p53 polyclonal antibodies. Line 1, normal medium alone; line 2, normal medium plus WSC (10 μg/ml); line 3, serum starved-medium alone and line 4, serum starved-medium plus WSC (10 μg/ml). Arrows represent appearance of p53. Datum represents one of three independent experiments. From Koo, Jeong, Hong, Choi, An and Kim [86]; by permission of Elsevier Science Ltd, Oxford, UK.](image)
with WSC (Fig. 2). Expression of iNOS was induced by IL-1β and Aβ [25–35] and was partially inhibited by treatment with WCS. Kim et al. demonstrated the regulatory effects of WSC in human astrocytes for the first time and suggested that the anti-inflammatory effects of WSC may reduce and delay pathogenic events in AD.

9. Other systems

Glass beads have received attention as a preferred support material owing to their controllable narrow size dispersion in addition to their mechanical strength. Liu et al. reported a new hybrid that adsorbs transition metal ions after surface modification of nonporous beads with chitosan (Scheme 24) [88]. Glass beads were modified in three steps to obtain pendent aldehyde groups. Finally, the glass beads were modified with chitosan by reductive N-alkylation. Metal ions such as Cu²⁺, Ag⁺, Pb²⁺, Fe³⁺, and Cd²⁺ were trapped over 90% on a column of chitosan modified glass beads.

Liu et al. also reported another type of chitosan glass beads modified through a 1,3-thiazolidine linker [89]. In this case, a terminal aldehyde group (39) produced by nitrous acid degradation of chitosan was used for coupling with an L-cysteine linker to glass beads (Scheme 25). This method could be applied to a variety of silica materials, and further biological or pharmaceutical application can be expected with covalent chitosan–inorganic silica hybrids.

Chitosan fiber is a well known biofunctional fiber, but little is known about fragrant biofibers, bioyarns, and biothreads based on chitosan. Hirano and Hayashi [90] prepared novel fragrant chitosan derivatives, as fibers, and yarns via a Shiff base with fragrant aldehydes such as cinnamaldehyde, n-decylaldehyde, citral, etc. A portion of the fragrant aldehyde was slowly released from the fibers, and yarns in the open air at room temperature, but little was released in a closed dry glass vessel.

![Scheme 24. Modification of glass bead with chitosan. From Liu, Tokura, Haruki, Nishi and Sakairi [88]; by permission of Elsevier Science Ltd, Oxford, UK.](image-url)
The dissolution of chitosan in a basic aqueous medium is important, not only for biomedical applications but also for chemical and industrial uses. Muzzarelli et al. [91] obtained a rigid, transparent chitosan hydrogel by pouring chitosan salt solution into saturated aqueous ammonium hydrogen carbonate. Incubation at 20 °C for 5 days yielded chitosan carbamate ammonium salt, Chi-NHCO₂⁻NH₄⁺, a chemical species that by hydrolysis or by thermal treatment decomposed to regenerate chitosan in the free amine form. Chitosans of different degrees of acetylation, molecular sizes, and origins (squid and crustaceans) were used in the form of hydrochloride, acetate, glycolate, citrate, and lactate salts. Their hydrogels obtained in ammonium hydrogen carbonate solutions yielded chitosan solutions at pH values as high as 9.6, from which microspheres of regenerated chitosan were obtained by spray-drying.

10. Conclusion

Despite the fact that chitin and chitosan have been called our ‘last biomass resource’ and are expected to lead to new functional polymers, their utilization has scarcely been explored. Even though a variety of interesting biological activities have been in chitosan and its derivatives, practical application of these has lagged. One of the main reasons is that these biological activities are not specific to chitosan; such activities are also found in other materials. The second reason is the cost problem, since chitosan is relatively expensive (20–30 US dollars per kg). If a specific biological activity were to be found unique to chitosan materials, development of practical utilization would be encouraged despite cost, especially for biomedical use.

Chitin and chitosan are structurally similar to heparin, chondroitin sulfate, and hyaluronic acid, which are all biologically important mucopolysaccharides in all mammals. These mucopolysaccharides are anionic polymers owing to substituent carboxyl and sulfuryl groups. On the other hand, chitosan is almost the only cationic polysaccharide in nature, and it is nontoxic and biodegradable in the human body. This special property is worthy of note in regard to biomedical applications. However, since chitosan does not dissolve in neutral and basic aqueous media, its biomedical use is limited. Chemical modification of chitosan provides derivatives that are soluble at neutral and basic pH. Moreover, chemical modification can be used to attach various functional groups and to control hydrophobic, cationic, and anionic properties. Further studies and development of
chitin, chitosan, and their derivatives for biomedical applications can be expected in the 21st century.

References


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