Part One
General Aspects of Chitosan

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Chemical and Technological Advances in Chitins and Chitosans Useful for the Formulation of Biopharmaceuticals

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1.1 Introduction

Chitin is the first polysaccharide discovered (1811): its bicentennial has been celebrated in a review article by Muzzarelli et al. [1] that traces the origin of the modern carbohydrate polymers science. In a more recent time, chitosans and their derivatives have been studied for formulations that enhance the absorption of macromolecular biotherapeutics (peptides, protein therapeutics and antigens, as well as plasmid DNA) and for the preparation of particulate drug-targeting systems. The number of yearly published papers dealing with this topic during the period 2000–2009 has been growing at the following impressive rate: 90, 110, 120, 150, 245, 320, 420, 470, 670, and 705. Some review articles are cited here for readers seeking complementary information. Kean and Thanou [2] published an overview about the biodegradation, biodistribution, and toxicity of chitosan-based delivery systems as well as the current status of chitosan drug formulations and underlined that, despite the high number of published studies, chitosan is not approved by the US Food and Drug Administration for any product in drug delivery. Nevertheless, chitosan is used as a generally regarded as safe (GRAS) material. It was explained that when a hydrophobic moiety is conjugated to a chitosan unit, the resulting amphiphile forms self-assembled as nanoparticles that encapsulate a quantity of drugs and deliver them to specific sites. Chemical attachment of drugs to chitosan throughout a functional linker may also produce useful prodrugs, exhibiting the appropriate biological activity at the target site.

The advanced development of chitosan hydrogels has led to new drug delivery systems that release drugs under varying environmental stimuli. The development of intelligent drug delivery devices requires a foundation in the chemical and physical characteristics of chitosan-based hydrogels, as well as the therapeutics

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to be delivered. In their review article, Bhattarai *et al.* [3] reported on the developments in chitosan hydrogel preparation and defined the design parameters in the development of physically and chemically cross-linked hydrogels. Carreira *et al.* [4] addressed smart polymers derived from chitosan, including particulate carrier systems, hydrogels, and film-based materials that are responsive to stimuli such as temperature and pH, and summarized recent developments in graft modification of chitosan by living radical polymerization.

Recent developments of chitosan nanosystems for delivery of hydrophilic and lipophilic drugs and polynucleotides into the eye surface were reviewed by de la Fuente *et al.* [5], who took into consideration that nanoscience and nanotechnology promoted important breakthroughs. In particular, the application of nanotechnology in ophthalmology has led to the development of novel strategies for the treatment of ocular disorders. Indeed, the association of an active compound to a nanocarrier allows the compound to intimately interact with specific ocular structures, to overcome ocular barriers, and to prolong its residence in the target tissue. Chitosan nanosystems have been specifically adapted for the delivery of hydrophilic and lipophilic drugs and also polynucleotides onto the eye surface, but these still demand a full preclinical evaluation. Oyarzun-Ampuero *et al.* [6] reviewed chitosan structures as transmucosal delivery vehicles for complex macromolecules, and as carriers of anticancer drugs. In the core-coating nanostructures, the core consists of a lipid (Miglyol 812 liquid; or tripalmitin, solid) surrounded by a chitosan layer. These nanostructures display outstanding properties in relation to the favored transport of peptides and vaccines across the nasal and intestinal barriers, as well as the targeted intracellular delivery of anticancer drugs into tumor cells. Reviews on oral delivery topics have also been published by Bowman and Leong [7], Kim *et al.* [8], Chopra *et al.* [9], Werle *et al.* [10], and Muzzarelli and Muzzarelli [11]. Reviews on the repair of wounded human and animal tissues, on the chemistry of chitosan hydrogels, and on the cross-linking with a safe plant compound are those by Muzzarelli [12–14], who also published reviews on the regeneration of bone [15,16] and on the enhanced biochemical efficacy of oligomeric and partially depolymerized chitosans [17]. Information of more general interest about the presence of chitin in the earliest forms of life can be found in reference [18].

Among additional review articles on chitins and chitosans, those most directly involving chemical approaches were coauthored by Jayakumar *et al.* [19], who focused on the preparation and applications of carboxymethyl and succinyl derivatives of chitin and chitosan with particular attention to their biomedical applications; by Liu *et al.* [20], who centered on the hydrophobic modifications of chitosans mainly for gene delivery in comparison with polyethyleneimine and polylysine; by Kristiansen *et al.* [21], who pointed out selected examples of periodate oxidation of alginates, chitosans, hyaluronan, scleroglucan and schizophyllan, and cellulose; and by Hamman [22], who focused on periodate oxidation of polysaccharides for modification of chemical and physical properties. More general treatments can be found in review articles by Kumari *et al.* [23], Jayakumar *et al.* [24], and Zhang *et al.* [25].

The scope of the present chapter is therefore to bring to the reader's attention certain chemical approaches that are being developed today, and that seem to be prone to offer further chances to chitins and chitosans to act as protagonists in the scenario of drug and gene delivery, as well as in related fields. More detailed knowledge about the chemical aspects of the upgrading, processing, and exploitation technologies will help researchers to understand the interdisciplinary aspects of the ongoing applied research.

1.2 Safety of Chitins and Chitosans

A large body of knowledge exists today on the use of chitosans as safe biomaterials, and more specifically as drug carriers for a variety of applications. Lack of interdisciplinarity and limited perception of the importance of the chemical and biochemical characteristics of the isolated chitins or chitosans for the replication of experiments and optimization of results, however, are recurring faults in recent literature dealing with the biomedical applications of chitosan.

Articles that assessed the safety and biocompatibility aspects never reported intolerance or allergy in individuals and animals (with very few exceptions; see below), even when the quantities of chitosan used in single experiments were quite large. Therefore, crab, shrimp, prawn, and lobster chitins, as well as chitosans of all grades, once purified, should not be considered as "crustacean derivatives," because the isolation procedures have removed proteins, fats, and other contaminants to such an extent as to allow them to be classified as chemicals regardless of their origin.

Isolated purified chitins and chitosans are linear polysaccharides that, at the nano level, have highly associated structures, recently refined in terms of regularity, nature of bonds, degree of crystallinity, and unusual colloidal behavior [26,27]. Chitins and modified chitins exert a number of beneficial actions, namely: (1) they stimulate macrophages by interacting with receptors on the macrophage surface that mediate the internalization of chitin particles to be degraded by lysozyme and *N*-acetyl-β-glucosaminidase (such as Nod-like, Toll-like, lectin, Dectin-1, leukotriene, and mannose receptors); (2) they stimulate macrophages to produce cytokines and other compounds that confer nonspecific host resistance against bacterial and viral infections, and antitumor activity; (3) chitin is a strong Th1 adjuvant that up-regulates Th1 immunity induced by heat-killed *Mycobacterium bovis*, while down-regulating Th2 immunity induced by mycobacterial protein; (4) direct intranasal administration of chitin microparticles into the lung was also able to significantly down-regulate allergic response to *Dermatophagoids pteronyssinus* and *Aspergillus fumigatus* in a murine model of allergy; (5) chitin microparticles had a beneficial effect in preventing and treating histopathologic changes in the airways of asthmatic mice; and (6) authors support the fact that chitin depresses the development of adaptive type 2 allergic responses [28]. Moreover, Da Silva *et al.* [29] explicitly recognized that chitin particles are multifaceted immune adjuvants.

Notwithstanding these amply accepted points, a small number of articles raised concern about the presumed allergenicity of chitosan, but a close scrutiny of those few papers dealing with chitin and allergy revealed that conceptual and methodological errors are unfortunately present, such as omitted consideration of mammalian chitinase and/or chitotriosidase secretion (accompanied by inactive chitinase-like proteins) as an ancestral defensive means against invasion, capable of preventing the insurgence of allergy; omitted consideration of the fact that the mammalian organisms recognize more promptly the secreted water-soluble chitinase produced by a pathogen, rather than the insoluble and well-protected chitin within the pathogen itself; and omitted mention of the potent allergen from crustacean flesh, tropomyosine, in superficial and incomplete reports on chitin as an allergen. On the other hand, the wide majority of the investigators have recognized that chitosan-based delivery systems have immunoadjuvant properties and therefore are useful to enhance the absorption and/or cellular uptake of peptides and proteins across mucosal sites. Therapeutic peptides and proteins and protein-based antigens are chemically and structurally labile compounds, which are almost exclusively administered by parenteral injections. Non-invasive mucosal routes have attracted interest for administration of these biotherapeutics. Chitosan is a mucoadhesive polysaccharide capable of opening the tight junctions between epithelial cells and has functional groups for chemical modification, which has resulted in a large variety of chitosan derivatives with tunable properties for the nasal and pulmonary administration of protein therapeutics and antigens [30].

The general safety of chitosan as a pharmaceutical excipient has been recently found quite satisfactory in a review article by Baldrick [31]. Toxicological issues of chitosan and derivatives are further discussed in Chapter 23.

1.3 Ionic Liquids: New Solvents and Reaction Media

The true solvents reported to dissolve chitin include solvent systems of *N,N*-dimethylacetamide with 5% lithium chloride, alkali and ice mixtures (sodium hydroxide either alone or together with urea or thiourea), some strong acids such as methanesulfonic acid, and some fluorinated solvents such as hexafluoro-2-propanol. These solvents, which are generally volatile and/or corrosive, pose certain health hazards, while the resulting

chitin solutions are unstable due to hydrolysis under strong acid or basic conditions. Ionic liquids capable of dissolving biopolymers were regarded as green solvents to replace the volatile organic compounds (VOCs) commonly used in various chemical industries. The first, pioneering work was reported by Reichert *et al.* [32,33] and by others of the same team [34]. Typical ionic liquids in this context are 1-allyl-3-methylimidazolium chloride (AmiCl), 1-butyl-3-methylimidazolium chloride (BmiCl), and 1-butyl-3-methylimidazolium acetate (BmiAc).

Because attention was immediately captured by cellulose, which is similarly dissolved by ionic liquids [35], not so much information on the dissolution of chitin in ionic liquids is present in the literature yet. Xie *et al.* [36] used BmiCl and declared that up to 10% (*w/w*) of chitin could be dissolved within 5 h at 110 °C; however, this finding was questioned because of diverging results presumably due to the diversity of chitin in terms of polymorphic form, different origin, molecular weight (MW), and degree of acetylation (DA), thus much more work is obviously needed in order to optimize ionic liquids for different chitins.

Cellulose and chitin solutions are reciprocally compatible upon mixing; for example, an acidic cellulose + chitin gel electrolyte made of cellulose, chitin, 1-butyl-3-methylimidazolium, 1-allyl-3-methylimidazolium bromide (AmiBr), and an aqueous H_2SO_4 solution was investigated for electric double-layer capacitors with activated carbon fiber cloth electrodes [37,38]. The gel electrolyte showed high ionic conductivity comparable to that of an aqueous 2 M H_2SO_4 solution at 0–80 °C (57.8 S/m at 25 °C, which is also comparable to 61.9 S/m of 2 M H_2SO_4). These results indicate that the acidic cellulose–chitin hybrid gel electrolyte has practical applicability to an advanced electric double-layer capacitor with excellent stability and working performance.

An even more significant example was provided by Takegawa *et al.* [39], who prepared chitin and cellulose composite gels and films using the two ionic liquids, AmiBr and BmiCl. The two polysaccharides were dissolved separately, and then the two liquids were mixed in various ratios at 100 °C to give homogeneous mixtures. The gels were obtained after 4 days. On the other hand, the films were obtained by casting the mixtures on glass plates, followed by soaking in water and drying. Rheological evaluations on the clear solution of 5% (*w/w*) chitin in AmiBr (obtained at 100 °C for 48 h) showed that it behaved like weak gels [40].

Wu *et al.* [41] proposed BmiAc as a good solvent for isolated chitins with different origins and MW. BmiAc could dissolve α- and β-chitins of various MW to desirable concentrations at relatively low temperatures. Cooling the chitin in BmiAc solutions to ambient temperature resulted in chitin gels, from which a chitin sponge or film were regenerated using water or methanol. Weakened hydrogen bonds were reported in the regenerated α-chitins from BmiAc as compared with the parent α-chitin. The regenerated α-chitins from the BmiAc solvent suffered a remarkable decrease in the crystallinity degree as compared to the parent α-chitin. The regenerated β-chitin from BmiAc had a crystal structure close to that of the regenerated α-chitins, implying that a transition from β-chitin (a metastable crystalline form) to α-chitin (a stable crystalline form) may take place during the dissolution and the recovering processes. The regenerated chitins are thermally more stable than the parent ones. On the basis of these results, it was possible to establish a new biomass + ionic liquid platform for new processing and homogeneous chemical modifications of native chitin. Such an approach could also be used as a pretreatment technique for many heterogeneous chemical reactions and enzyme degradations in chitin-based polysaccharides. To make a practical example, a known weight of chitin was dispersed into 4.0 g of BmiAc in a flask, and the mixture was heated at 100 °C and stirred until completely transparent. Then, it was cast onto a mold and allowed to cool; the gel formed in the ionic liquid was then coagulated in a methanol bath, and rinsed with portions of fresh solvent to eliminate the ionic liquid. The obtained semitransparent regenerated gel was finally oven-dried at 60 °C to obtain a pure chitin film. As an alternative, different coagulants (water or methanol) and drying methods (oven-drying or freeze-drying) can be adopted. The ionic liquid in the coagulants was recovered by evaporating water or methanol and dried in a vacuum oven, and the purity was confirmed by ^{1}H nuclear magnetic resonance (NMR) before recycling. The efficacy of BmiAc is apparent if the data in Table 1.1 are compared [41].

Table 1.1 *Solubility of isolated chitins and chitosan in three ionic liquids (Elaborated from [41]).*

Polymer	Origin and viscosity	Solubility % (*w/w*) at 110 °C		
		AmiCl	BmiCl	BmiAc
α-Chitin	Crab, 35 cP	Insoluble	Partly soluble	Soluble, 6
β-Chitin	Squid pen, 15 cP	Insoluble	Partly soluble	Soluble, 7
β-Chitin	Squid pen, 278 cP	Insoluble	Insoluble	Soluble, 3
Chitosan	Crab, MW 97 kDa	Soluble, 8	Soluble, 10	Soluble, 12

AmiCl: 1-allyl-3-methylimidazolium chloride; BmiCl: 1-butyl-3-methylimidazolium chloride; and BmiAc: 1-butyl-3-methylimidazolium acetate.

Likewise, the dissolution behavior of chitin in a series of ionic liquids containing alkylimidazolium chloride, alkylimidazolium dimethyl phosphate, and 1-allyl-3-methylimidazolium acetate has been studied by Wang *et al.* [42]. The dissolution behavior of chitin in ionic liquids was affected by the DA, the degree of crystallinity, and the MW of chitin, as well as by the nature of the anion of the ionic liquid. Moreover, 1-ethyl-3-methylimidazolium acetate can dissolve raw crustacean shells completely, leading to the recovery of chitin of high purity and high MW, amenable to powder, films, and fibers directly spinnable from the extract solution [43].

Several chemical and enzymatic reactions can be carried out on chitins dissolved in ionic liquids. Zhang *et al.* [44] reported that hydrolysis of chitosan in imidazolium-based ionic liquids with good total reducing sugars yields over 60% in the presence of 6.0% HCl at 100 °C, within 7 h. Acetylation of α-chitin, using acetic anhydride in AmiBr, was performed by Mine *et al.* [45]. The 2% mixture of chitin and AmiBr was heated at 100 °C for 24 h for dissolution; then, acetic anhydride (5–20 Eq) was added, the mixture was heated with stirring for 24 h, and the product was insolubilized by pouring the reaction mixture into methanol. The product with the highest degree of substitution (1.86) was obtained by using 20 Eq of acetic anhydride at 100 °C, being soluble in dimethyl sulfoxide (DMSO). Its structure was further confirmed by ^{1}H NMR spectroscopy in DMSO and by infrared spectrometry.

N-methylimidazole, a component of 1-alkyl-3-methylimidazolium ionic liquids, accelerated remarkably the trans-esterification reaction catalyzed by lipase from *Candida antarctica* [46]. An ionic liquid composite material based on chitosan and 1-butyl-3-methylimidazolium tetrafluoroborate ($BmiBF_4$) was readily used as an immobilization matrix to entrap proteins and enzymes. A pair of well-defined, quasi-reversible redox peaks of hemoglobin (Hb) was recorded at the glassy carbon electrode modified with chitosan–$BmiBF_4$–Hb film, by direct electron transfer between the protein and the electrode. Dramatic enhancement of the biocatalytic activity was evidenced at the chitosan–$BmiBF_4$–Hb electrode by the reduction of oxygen and trichloroacetic acid. The chitosan–$BmiBF_4$–Hb film was also characterized by UV-visible spectra, indicating excellent stability in solution and good biocompatibility for protein. The unique composite material based on polymer and ionic liquid might find applications in electrochemistry, biosensors, and biocatalysis [47]. In fact, the lipase-catalyzed enantioselective acylation of allylic alcohols in an ionic liquid was demonstrated by Itoh *et al.* [48]. The reaction was significantly dependent on the counter anion of the imidazolium salt, and good results were obtained with 1-butyl-3-methylimidazolium hexafluorophosphate ($BmiPF_6$) or $BmiBF_4$. The lipase-catalyzed trans-esterification was then investigated using methyl esters as acyl donors, especially under reduced pressure in $BmiPF_6$. The trans-esterification of 5-phenyl-1-penten-3-ol took place smoothly at 20 Torr and 40 °C when methyl phenylthioacetate was used as the acyl donor in $BmiPF_6$. The acylated compound was obtained in optically pure form, thus making possible the repeated use of the lipase, because there was no drop in the reaction rate after various repetitions of the process.

The usual syntheses of 6-*O*-L-ascorbyl fatty acid esters via esterification catalyzed by *Candida antarctica* lipase have lows levels of efficiency due to either the scarce solubility of L-ascorbic acid in nonpolar organic solvents, or inadequate enzymatic activity in polar organic solvents. Park *et al.* [49] reported that replacing

organic solvents with ionic liquids such as 1-alkyl-3-methylimidazolium tetrafluoroborates makes this synthesis more efficient and greener for three reasons. First, like polar organic solvents, ionic liquids dissolve polar substrates such as ascorbic acid (around 130 g/l in $BmiBF_4$ at 60 °C) but, unlike polar organic solvents, ionic liquids do not inactivate lipase. For this reason, an ionic liquid gives faster reaction and higher yield. Second, it eliminates toxic organic solvents that easily evaporate. Third, since ionic liquids are not volatile, a vacuum could be used to drive the equilibrium toward product formation, thus eliminating the need to use an excess of acyl donor or an activated acyl donor. The lipase-catalyzed direct esterification of stoichiometric amounts of ascorbic acid and oleic acid gave a high conversion (83%). The product 6-*O*-L-ascorbyl oleate was isolated using only water and ethanol or methanol in 61% yield, and was found useful to protect fats and oils from oxidation [49].

1.4 Chitin and Chitosan Nanofibrils

In the course of 2008–2010, certain scientific and technological advances were made in the area of the isolation of nanofibrils (otherwise called chitin nanocrystals or whiskers). In particular, articles were published dealing with the following: (1) nanochitin isolated mechanically in the presence of minor amounts of acetic acid, (2) nanochitin isolated after hydrolysis with hydrochloric acid, and (3) nanochitosan obtained either from partially deacetylated chitin or from partially deacetylated chitosan. Said advances are of such importance as to put in the shadow the technology developed during previous years, for reasons that will be apparent below [11].

1.4.1 Mechanically Isolated Nanofibrils in the Presence of Acetic Acid

α-Nanochitin has been isolated in the presence of acetic acid (pH 3): the suspension was treated first with a domestic blender, and then the slurry of 1% purified chitin was passed through a grinder (MKGA6-3; Masuko Sangyo Co., Ltd., Japan) at 1500 rpm [50]. The grinder treatment was performed with the clearance gage of −1.5 (corresponding to a 0.15 mm shift) from the zero position, which was determined as the point of slight contact between the grinding stones. In principle, there is no direct contact between grinding stones thanks to the chitin suspension. In a further work, the same team developed the concept that it would be advantageous to enhance the cationic repulsion existing between chitin fibers with the aid of acetic acid at pH 3, in order to fibrillate the chitin [51]. Results showed that protonation degrees as small as 4% or less are sufficient to weaken the hydrogen bonds that protect chitin from fibrillation. It should be recalled here that all chitins are deacetylated to a minor extent in those positions where proteins were originally linked, and that this partial deacetylation (max. 0.10) is large enough to protonate the fibers. Under these conditions, various industrial chitins, even alpha polymorph, lend themselves to the isolation of nanofibrils with 10–20 nm cross-section, having a high degree of crystallinity.

An extension of the previous work was the treatment of industrial chitin dry powders instead of "never-dried" chitins, thus removing an important limitation that prevented in the past the large-scale production of nanochitin. By doing so, the strong acid (3 M HCl) was abandoned and thus the risks inherent to its use were avoided. This method provided a significant advantage for industrial application in terms of a stable supply, storage stability, transportation costs, and storage space, since chitin nanofibers can be prepared from low-weight, low-volume, and nonperishable dried chitin. Chitin nanofibers from commercial prepurified dry chitin are advantageous for laboratory-scale investigations because a large amount of chitin could be immediately and easily obtained by a simple fibrillation process without all of the traditional purification processes (the removal of proteins, minerals, lipids, and pigments) that generally require 5 days for "never-dried" chitin [52]. Their scanning electron microscopy (SEM) images show that, in the absence of acetic acid, bundles of fibers

are obtained; instead, with acetic acid, nanofibrils are formed with a large area-to-volume ratio that is of outstanding importance for the adsorption of a wide range of drugs and other compounds. Finally, the Ifuku method does not require ultrasonication [50,51], as a point of difference from the one by Fan *et al.* [53].

According to the latter, the key factors to preparing chitin nanofibers with a high aspect ratio (3–4 nm in cross-sectional width and at least a few microns in length) are as follows: (1) squid pen β-chitin is used as the starting material, and (2) the β-chitin undergoes ultrasonication in water at pH 3–4 and 0.1–0.3% for a few minutes. No *N*-deacetylation occurs on the chitin during the nanofiber conversion; the original crystal structure of β-chitin is maintained, although the crystallinity index decreases from 0.51 to 0.37. Cationization of the C2 amino groups present on the crystallite surfaces of the squid pen β-chitin under acidic conditions is necessary for preparing the nanofibers. The mechanical disintegration is described as follows: the wet chitins are suspended in water at 0.1–0.3%. Several drops of acetic acid, a dilute hydrochloric acid solution, or a dilute NaOH solution are added to the chitin slurries to adjust their pH values from 3 to 8. Ultrasonication is applied to the slurries (15 mL each) for 2 minutes using an ultrasonic homogenizer at 19.5 kHz and 300 W output power (7 mm probe tip diameter, US-300T, Nihonseiki, Japan). The temperature increase is below 5 °C during ultrasonication [53].

These advantages can be easily appreciated if one considers the drawbacks of the previous preparation methods, which included low yield (around one half), dangers from the use of boiling HCl, disposal of the used, black-colored HCl, disposal of enormous quantities of slightly acidic water, the presence of HCl in the final product even after one week of dialysis or ultrafiltration, optional ultrasonication, difficult adjustment of the pH value because of the strength of the HCl present, overall scaling-up difficulties, and excessive costs. Even though certain authors, including Watthanaphanit *et al.* [54], used ultracentrifuges at 10 000 rpm in performing lab-scale preparations, the industrial centrifuges do not offer such performances and are precluded to HCl solutions. Nevertheless, Tzumaki *et al.* [55] used common centrifuges (3400 rpm, 15 min) but had to ultrasonicate for a long time (45 minutes) with the risk of overheating. Chang *et al.* [56] used both ultracentrifugation and ultrasonication.

1.4.2 Nanochitosan Obtained from Partially Deacetylated Chitin or Deacetylated Nanochitin

Watthanaphanit *et al.* [57] prepared nanochitosan by deacetylation of chitin nanofibrils with 50% NaOH containing 0.5% (*w/w*) sodium borohydride; as a consequence, the MW went down to 59 kDa, much lower than the one of chitosan from chitin powder under the same conditions (420 kDa). The degree of deacetylation (DD) was 0.50, and the suspensions were colloidal at 1–13%. Phongying *et al.* [58] completely destroyed their nanochitosan during their first attempt to deacetylate nanochitin. The new methods, however, opened new routes to a nanofibrillar product endowed with a better cationic charge, that is, chitosan, a more versatile polysaccharide than chitin. At the same time, Fan *et al.* [59] deacetylated the fine chitin powder in a relatively mild way, thus producing nanochitosan that underwent homogeneous dispersion at pH 3–4, with birefringence and high viscosity, while at pH 6–7 the dispersion was not homogeneous, because of inadequate protonation.

The advantageous and characteristic features of the newly developed α-chitin nanofibrils are that (1) commercially available pure α-chitins (originating from crab and shrimp shell) can be used as starting materials; (2) nanofibrils are obtained in high yields (85–90%); (3) the rod-like morphology of the nanofibrils supports the high yields, because α-chitin nanofibrils prepared by acid hydrolysis from (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO)-mediated oxidation have a spindle-like shape with a larger distribution range of widths; (4) the α-chitin nanofibril dispersions had high UV-Vis transmittance (hence high transparency), indicating that individualization of α-chitin fibrils was achieved; and (5) the α-chitin nanofibrils have a lower hurdle in terms of safety issues, compared with chemically modified materials such as TEMPO-oxidized α-chitins, hence potential applications can be expanded to functional foods, and the life science and medical fields. The α-chitin nanofibrils had an average width and length of 6.2 ± 1.1 and 250 ± 140 nm, respectively (ratio around

40). Because conversion to nanofibrils was achieved in water at pH 3–4, protonation of the amino groups on the crystalline fibril surface is the key driving force for the individualization of α-chitin fibrils [59].

As an early application, Hariraksapitak and Supaphol [60] developed tissue scaffolds from a 50:50 (*w/w*) blend of hyaluronan and gelatin that contained different amounts of acid-hydrolyzed α-chitin nanofibrils by a freeze-drying method. The weight ratios of the nanofibrils to the blend were up to 30%, and the average pore size of the scaffolds ranged between 139 and 166 μm, regardless of the nanofibril content, but the incorporation of 2% nanofibrils in the scaffolds doubled their tensile strength. Although the addition of 20–30% nanofibrils improved their thermal stability and resistance to biodegradation, the scaffolds with 10% were the best for supporting the proliferation of cultured human osteosarcoma cells. Several applications were immediately developed: melatonin was adsorbed on the nanofibrils by Hafner *et al.* [61] and Yerlikaya *et al.* [62], and lipoic acid was likewise treated by Kofuji *et al.* [63]. More extended studies were made by Muzzarelli *et al.* [64], who incorporated chitin nanofibrils into wound dressings made of chitosan glycolate and dibutyryl chitin, and by other authors who prepared similar composites. Glycerol plasticized-potato starch was mixed with chitin nanofibrils to prepare fully natural nanocomposites by casting and evaporation. This led to improvements in the tensile strength, storage modulus, glass transition temperature, and water vapor barrier properties of the composite. However, above 5% (*w/w*) loading, aggregation of the nanofibrils took place with negative effects [56]. On the opposite, Azeredo *et al.* [65] evaluated the effect of different concentrations of cellulose nanofibers and plasticizer (glycerol) on the tensile properties, water vapor permeability, and glass transition temperature of chitosan edible films, and established a formulation to optimize their properties: the nanocomposite film with 15% cellulose nanofibers and 18% glycerol was comparable to some synthetic polymers in terms of strength and stiffness. The incorporation of chitosan whiskers in alginate fibers was achieved by Watthanaphanit *et al.* [57] by mixing homogenized chitosan whisker colloidal suspension with 6% (*w/v*) sodium alginate aqueous solution, followed by wet spinning. The chitosan whiskers (which had a length and width of 309 and 64 nm, respectively, and an average aspect ratio around 4.8) were prepared by deacetylation of chitin whiskers obtained by acid hydrolysis of shrimp chitin. The noticeable improvement in the tensile strength of the nanocomposite yarns took place at the expense of the elongation at break. The chitosan whiskers imparted antibacterial activity to the nanocomposite yarns against *Staphylococcus aureus* and *Escherichia coli*.

Chitin nanofibrils were acetylated to modify the fiber surface [52]. The acetylation degree could be controlled from 0.99 to 2.96 by changing the reaction time. At 1 minute acetylation, the moisture content of the nanocomposite decreased from 4.0% to 2.2%. The nanofibril shape was maintained, and the thickness of the nanofibrils increased linearly with the content of the bulky acetyl groups. Composites containing the acetylated chitin nanofibrils (25%, *w/w*) were fabricated with acrylic resin [52]. Finally, nanofibers based on poly(vinyl alcohol) (PVA) as the matrix and nanocrystals of α-chitin (around 31 nm in width and around 549 nm in length) as the nanofiller have been prepared by Junkasem *et al.* [66]. The average diameters of the electrospun fibers ranged between 175 and 218 nm. The addition and increase of the amount of whiskers caused the crystallinity of PVA within the nanocomposite materials to decrease and the glass transition temperature to increase.

1.5 Electrospun Nanofibers

Electrospinning is a recent technique useful to produce chitin and chitosan nanofibers of indefinite length and some hundred nanometers in cross-section, which might find novel applications as biomedical items thanks to the high surface area and porosity of the resulting mats. At the present time, the manufacture of nanofibers is laborious and time-consuming, and presents safety issues. While the reader is referred to the review article by Jayakumar *et al.* [67] on this matter, it should be said that, generally, the manufacture of an around 300 μm

thick, 30 × 15 cm mat from a solution of plain chitosan in methylene chloride and trifluoroacetic acid solution requires approximately 4 h, while 12 h are needed for mats of the same size and around 20 μm thick by conventional electrospinning of chitosan and poly(ethylene oxide) in dilute acetic acid solution. This technique based on trifluoroacetic acid as an electrospinning solvent for chitosan yields a relatively high throughput. It relies on empirical knowledge, because by varying the viscosity, temperature, electrical potential, geometry of the collector, and other parameters, the nanofibers may assume irregular forms (lack of uniformity, beaded fibers, and interconnectivity) that prevent their use. Several remedies such as neutralization with alkaline compounds or chemical cross-linking with glutaraldehyde have been tested in order to stabilize the chitosan nanofibers, but their neutralization in aqueous media usually results in undesirable fiber contraction, leading to partial or complete loss of their features at the nano level. It should also be kept in mind that the just-mentioned solvents are toxic, and therefore their manufacture at the present time is slow and hazardous.

A body of knowledge on the thermally driven formation of amide bonds between chitosans and the organic acids that dissolve them has existed since the late 1990s. In particular, Yao *et al.* [68] presented a protocol based on the use of lactic acid for the preparation of lactamidated chitosan in the form of films that, after purification with methanol and chloroform, were tested for biocompatibility toward fibroblasts. This matter was amply studied by Toffey *et al.* [69,70] with acetic acid and propionic acid, in the frame of projects concerning the regeneration of chitin from chitosan via amide bond formation, as well as by Qu *et al.* [71,72], who also used lactic acid. This has also been discussed in review articles such as the one by Muzzarelli and Muzzarelli [73].

Therefore, one way to stabilize the nanofibers made of chitosan lactate salt is the thermal treatment that induces amide group formation, thus imparting water insolubility to the product. In fact, chitosan lactate solutions in methylene chloride and trifluoroacetic acid solution with concentrations of 3–6% (*w/w*) yielded stable, bead-free nanofibers with mean diameters of 50–350 nm, as reported by Cooper *et al.* [74]. A 4.5% (*w/w*) chitosan lactate salt solution in methylene chloride plus trifluoroacetic acid resulted in the highest electrospinning rate. The residual solvent was removed from the as-spun nanofibers by incubation at 70 °C for 12 h. The conversion of the ionic bond between amino groups of chitosan and lactate groups to a covalent amide bond was accomplished by heating under vacuum at 100 °C for 3 h, which transformed the water-soluble chitosan lactate to water-insoluble chitosan lactamide to a certain extent. The amidation was confirmed by the absorption band at 1790 cm^{-1} in the Fourier-transformed infrared (FTIR) spectra. The substitution degree was 0.165 ± 0.065, resulting in approximately $7.9 \pm 3.1\%$ (*w/w*) lactic acid in the form of amide in the nanofibers. It is not excluded that part of this lactic acid is in the form of a polylactide but, in any case, this is an initial step toward the preservation of nanofibrous shape in physiological media [74].

An alternative to the experiments just described is the esterification of chitosan with the use of lactide or polylactide. For example, Skotak *et al.* [75] prepared chitosan derivatives following a "one-pot" approach by grafting L-lactide oligomers via ring-opening polymerization. For the synthesis, 600 mg of chitosan were dissolved in 10 mL of methanesulfonic acid, followed by the addition of 3.05 g of the L-lactide monomer. This reaction mixture was stirred for 4 h at 40 °C under an argon atmosphere and then transferred to 100 mL of 0.2 M KH_2PO_4, 16 mL of 10 M NaOH, and comminuted ice. The side chain length had average values between 4.6 and 14 units. On average, there are two side chains of oligo-L-lactide per glucosamine ring, and their length depends on the initial reagents ratio. If this ratio increases, additional oligo-L-lactide graft might be attached to hydroxyl groups located on carbons 1 or 4 generated *in situ* during degradation of chitosan, when water content increases as a byproduct of the main esterification reaction. Indeed, L-lactide grafted chitosan samples display cytotoxicity over a range of substitution degree values, as demonstrated with fibroblast culture tests. This is supposed to depend on the oligo-L-lactide chain length, a key parameter controlling biodegradation, as it was demonstrated for star-shaped, polylactide-grafted poly(amidoamine) dendrimers. These materials might be interesting for controlled release and drug delivery, where hydrolysis rate control is of key

importance. This synthetic route renders the esterified chitosans soluble in a broad range of organic solvents, facilitating the formation of ultrafine fibers via electrospinning [75].

Electrospinning was applied to fabricate biocompatible membranes made of poly(L-lactic acid) and chitosan [76]. Thanks to their high porosity and interconnected structure, the electrospun mats were suitable scaffolds for cell or tissue growth. Injection rate, polymer concentration, and applied voltage were varied to investigate their effects on electrospun fibers. SEM images indicated that the structures with different diameters, including bead, bead-in-string, and nanofibers, would be controlled by adjusting the above variables. The diameter of strings was about 50 nm and the diameter of beads was 0.4–2.0 μm, depending on the preparation conditions. There was a critical range in the above parameters for the formation of homogeneous nanofibers. Outside the critical range, the bead-on-a-string structures became significant. When osteoblastic cells were cultured with nanofibrous membranes, the cell density was higher and the secretion of fibril was more significant as compared with the cells cultured on dense films. The results indicated that the biocompatibility of poly(L-lactic acid) and chitosan would be improved by changing their topography from smooth surface into nanoscaled structures.

Chitosan-based, defect-free nanofibers with average diameters ranging from 62 ± 9 nm to 129 ± 16 nm were fabricated via electrospinning of blended solutions of chitosan and poly(ethylene oxide) [77]. Generally, SEM imaging demonstrated that as the total concentration of both polymers increased, the number of beads decreased, and the fiber diameter decreased as chitosan concentration increased. Chitosan-poly (ethylene oxide) solutions underwent phase separation over time; as a result, blended solutions could be electrospun with no trouble within 24 h of blending. The addition of sodium chloride stabilized these solutions for a longer time before and during electrospinning. Pure chitosan nanofibers with high DD (about 80%) could not be produced: when attempting to electrospin chitosan from aqueous acetic acid at concentrations above the entanglement concentration, the electric field was insufficient to overcome the combined effect of the surface tension and viscosity of the solution. Therefore, the DD is a very important parameter that should be taken into account.

Chitosan, sodium chondroitin sulfate, and pectin–nanofibrous mats were prepared from the respective polysaccharide–poly(ethylene oxide) blend solutions by electrospray [78]. Unblended polysaccharide solutions showed low processability, namely, they could not be electrosprayed. The addition of 500 kDa poly (ethylene oxide) to chitosan solutions enhanced the formation of a fibrous structure. Sodium chondroitin sulfate–poly(ethylene oxide) and pectin–poly(ethylene oxide) blend solutions were generally too viscous to be sprayed at 25 °C, but at 70 °C the fibrous structure was formed.

1.6 Polyelectrolyte Complexes and Mucoadhesion

Cells, scaffolds, and growth factors are three main components of a tissue-engineered construct. Collagen type I (collagen-I), a major protein of the mammalian extracellular matrix, is a most suitable scaffold material for regeneration. The following examples are elegant demonstrations of results obtained with polyelectrolyte complexes involving three biopolymers.

Lin *et al.* [79] evaluated the characteristics of scaffolds composed of different ratios of collagen-I and chitosan with added hyaluronan in order to obtain optimum conditions for the manufacture of chitosan–hyaluronan–collagen-I porous scaffolds. The scaffolds were immersed in 50 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride alcoholic solution at 4 °C twice for 48 h, to generate cross-linking amide bonds. The mean pore diameters ranged from 120 to 182 μm and decreased as the chitosan composition increased. All scaffolds showed high pore interconnectivity and adsorbed 35- to 40-fold of physiological fluid without loss of shape and stability. The presence of large ratios of chitosan in the scaffold depressed its degradation by collagenase based on measurements of 4-hydroxyproline release.

The proliferation of fibroblasts cultured in the scaffolds was high. Overall, the 9:1:1 weight ratio of collagen-I, hyaluronan, and chitosan was optimal for the objectives of cartilage tissue engineering.

Wu *et al.* [80] deposited chitosan on the surface of freshly peeled mica (without $NiCl_2$ treatment), and then the fully mixed solution of collagen-I and hyaluronan (1:1 in volume) was cross-linked on the surface of chitosan with the aid of glutaraldehyde. The growth of 3T3 fibroblasts on the surface of the chitosan–hyaluronan–collagen-I complex film visualized the morphological changes of platelets during the coagulation process. The results indicated that this class of polyelectrolyte complexes possesses coagulation properties, cell compatibility, and antibacterial activity. In another report, Chen *et al.* [81] investigated the biocompatibility of chitosan–hyaluronan–collagen-I complexes with the cornea, and their suitability as substrates for growing rabbit corneal cells. Corneas were transparent and translucent. Cells seeded on chitosan–hyaluronan–collagen-I complexes were allowed to proliferate, and they partly formed confluent monolayers after 9 days in culture. Cultured cells were well attached to complexes containing 10% chitosan, 20% collagen, and 0.5% hyaluronan. The complexes had good biocompatibility with the cornea.

Electrostatic interactions of cationic chitosan with the negatively charged mucin have been reported as the main driving force for its strong mucosal adhesion [82]. However, Snyman *et al.* [83] examined mucoadhesive interactions of trimethyl chitosan with different degrees of quaternization and demonstrated that the presence of quaternary ammonium groups is detrimental to mucoadhesion. The authors related this adverse effect to conformational changes in chitosan. Alternatively, Sogias *et al.* [84] believe that this effect could also be due to the nature of the quaternized amino groups, which could certainly interact electrostatically with mucins but would no longer form hydrogen bonds. The charge of mucin particles as a function of pH was assessed by measuring the zeta-potential, which found it to be negative at all pH values measured (pH 1–7). At pH 7.0, the mean zeta-potential of mucin aggregates is −19.4 mV; however, acidification reduces the particles' negative charge, approaching neutrality at around pH 2.0. Addition of chitosan to mucin at pH 2.0 is accompanied by a marked increase in solution turbidity until the chitosan-to-mucin weight ratio reaches 0.05; turbidity then decreases with further polymer addition. Reducing the acetylation degree to 50% resulted in extension of the chitosan solubility to pH values higher than 7.0, but also reduced its capacity to aggregate mucin. Sogias *et al.* [84] also demonstrated that electrostatic attraction between chitosan and gastric mucin can be suppressed by 0.2 M NaCl; however, this does not prevent the aggregation of mucin particles in the presence of this biopolymer. The presence of 8 M urea or 10% (*v/v*) ethanol also affects mucin aggregation in the presence of chitosan, demonstrating the respective roles of hydrogen bonding and hydrophobic effects in mucoadhesion.

Large surface area, porous endothelial basement membrane, and high total blood flow of the nasal mucosa ensure a rapid absorption of compounds; however, a major problem of nasal drug delivery is the mucociliary clearance that rapidly removes poorly mucoadhesive formulations from the absorption site. Mucoadhesive polymers must be used to prevent rapid clearance, and, along this line, Luppi *et al.* [85] studied the preparation and characterization of mucoadhesive nasal inserts based on chitosan–hyaluronan polyelectrolyte complexes precipitated at various pH values and with different molar ratios. *In vitro* mucoadhesion studies were performed according to the method of Bertram and Bodmeier [86]: a hot agar plus mucin solution (phosphate buffer pH 7.4) was cast on a Petri dish and left at 4–8 °C for 3 h in order to form a gel. The inserts (100 mg) were placed on top of the gel, and after 10 min the plate was attached to a disintegration test apparatus, and moved up and down in a pH 7.4 buffer at 25 °C. Key findings indicated that the selection of the preparative conditions allows modulation of insert swelling and mucoadhesion. The ionization of vancomycin and insulin molecules, according to their isoelectric points, did not affect the release behavior. Therefore, chitosan–hyaluronan complexes can be used for the formulation of mucoadhesive nasal inserts for the delivery of peptide and protein drugs.

Perioli *et al.* [87] prepared hydrogels by using hydroxyethyl cellulose together with chitosan or 5-methylpyrrolidinone-chitosan (MP–chitosan: MW 17 kDa, DA 0.09, and degree of substitution of 0.20) and loaded with the antibacterial drug metronidazole (0.75%). The structure of MP–chitosan is depicted in

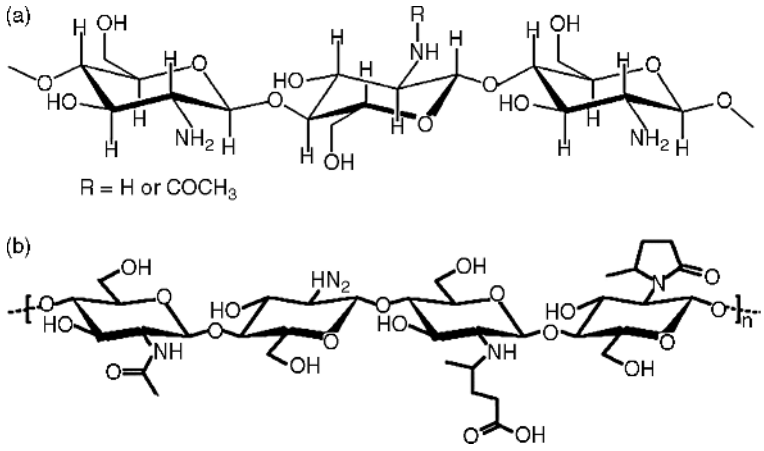

Figure 1.1 *(a) Chitosan, where the degree of deacetylation (DA) is determined by the value of R; the structure is dictated by the alternate orientation of each repeating unit, so that one couple of β(1–4) linked repeating units form one structural unit. (b) 5-methylpyrrolidinone-chitosan (MP–chitosan) where one of the units is shown in the carboxybutyl form before cyclization to the 5-methylpyrrolidinone form. Reprinted from reference [87], Copyright (2008), with permission from Elsevier.*

Figure 1.1. All formulations showed pseudoplastic flow and the viscosity increase linearly dependent on the polysaccharide content. All the preparations were able to release higher drug amounts than Zidoval®, a marketed formulation. MP–Chitosan improved the gel characteristics in terms of mucoadhesion force, rheological behavior. and drug release, pointing out that this modified chitosan is quite suitable for the preparation of more acceptable vaginal formulations [87]. Also, ampicillin-loaded M–chitosan microspheres were prepared and characterized by Giunchedi *et al.* [88]. Still, in the study by Perioli *et al.* [87], the *in vivo* mucoadhesion force was 0.106 ± 0.006 N, 123 ± 0.017 N, and 0.196 ± 0.017 N for MP–chitosan contents of 0.5, 1.0, and 2.0, respectively, with constant 4% hydroxyethyl cellulose for all; while for Zidoval®, it was 0.060 ± 0.011 N. The gels showed higher viscosity at lower temperature while getting less pasty with the increase of temperature, a desirable rheological behavior that favors good storage at room temperature and easy application *in loco*. Also, tablets formulated with plain chitosan (DA 0.03, MW 100 kDa) admixed with either polycarbophil (Noveon® AA-1, MW $>$ 1000 kDa) or polyvinylpyrrolidone (MW 1.3×10^6) adhered immediately to the mucosal surface and showed high adhesion times (21–70 h) [89]. In particular, polycarbophil offered prolonged contact between mucosa and tablets when mixed with equal amounts of chitosan.

1.6.1 Chitosan Polyelectrolyte Complexes Soluble in Alkaline Medium

To overcome the well-known solubility limitations of chitosan, the intermediate chitosan carbamate ammonium salt was developed by Muzzarelli *et al.* [90], who originally observed the spontaneous dissolution of chitosan in ammonium hydrogen carbonate. Upon mixing the chitosan carbamate solution with a polyuronan solution, both in NH_4HCO_3, no precipitation occurred thanks to the provisional anionic nature of chitosan carbamate. The polyuronans tested were alginate, hyaluronan, polygalacturonate, xanthan, gum Arabic, carboxymethyl cellulose, carboxymethyl guar, pectin, and 6-oxychitin, besides κ-carrageenan (a sulfated polysaccharide) and the neutral polysaccharide guar. In all cases, no precipitation occurred at pH 10.4 imparted by the excess NH_4HCO_3. Each homogeneous solution containing one chitosan polyuronan complex was found suitable for spray drying because during the latter process (1) the excess NH_4HCO_3 decomposes at 70–107 °C into the volatile compounds carbon dioxide, water, and ammonia; (2) chitosan

carbamate decomposes to yield plain chitosan, carbon dioxide, and water; and (3) chitosan and polyuronans form polyelectrolyte complexes in the form of microspheres collected at 45 °C, while the warm air stream takes away the said volatile compounds that, of course, may be recovered. Acidic drugs soluble in NH_4HCO_3 may be added to the complex prior to spray drying, thus allowing for the preparation of drug-loaded microspheres, as reported by Muzzarelli *et al.* [91] and Gavini *et al.* [92]. The MP–chitosan in the form of films for protein delivery was also studied by Colonna *et al.* [93].

1.6.2 Polyelectrolyte Complexes of Regioselectively Oxidized Chitin

Among the chitin derivatives mentioned above, 6-oxychitin is peculiar insofar as it is a polysaccharide similar to hyaluronan for certain chemical aspects, such as the presence of functional groups, but with a much lower MW. The radical TEMPO permits one to regioselectively oxidize chitin at the O6 position with a very simple experimental set-up. The 6-oxychitin sodium salt is fully soluble, and its low MW makes it promptly reactive with chitosan itself, yielding a polyelectrolyte complex of two different aminated polysaccharides, both obtained from the same parent chitin. This complex has been used in bone regeneration studies [94,95].

1.6.3 Polyelectrolyte Complexes of Chitosan with Bacterial Cell Wall Components

The antibacterial activity of chitosans has been clearly associated with polyelectrolyte complex formation by cationic chitosans (or amphoteric chitosans with prevailing cationic character) with anionic cell wall components, such as teichoic acid and phospholipids. This has been unequivocally documented by microscopy; for example, flocs of *N*-carboxybutyl chitosan were seen to adhere to the cell wall while an important cellular decay is quite noticeable. The consequent damages are mainly due to (1) mechanical stress imposed by the complex, leading to leakage of proteins, increased membrane permeability, and lysis (loss of cytoplasmic β-galactosidase demonstrated and measured by biochemical methods); (2) chelation of transition metal ions and destabilization of metal–enzymes, and alteration of ionic equilibria in the ion channels; (3) enzyme inhibition; (4) DNA complexation by internalized chitosan oligomers leading to poor RNA synthesis; (5) agglutination of endocellular organelles, accompanied by abnormal expansion of the periplasmic space, also supported by microscopic evidence; and (6) nucleic acid loss by the damaged cells, as documented by spectrophotometric data at 260 nm [96–101]. Thus, bacteria are either killed or inactivated, depending on the strain, but no general rule can be established even for a single genus. Moreover, the measurement by Davydova *et al.* [102] of the zeta-potential and the particle size of bacterial lipopolysaccharides (LPSs) and their chitosan complexes has recently provided a further explanation of the antibacterial activity of chitosans, as shown in Table 1.2, where, as a consequence of complexation, the definitely negative zeta-potential values become positive. Also, in two cases out of three, the size increases impressively.

Table 1.2 *Zeta-potential and particle size for lipopolysaccharides (LPS) and their chitosan complexes (Elaborated from [102]). Results presented as mean ± standard deviation*

	Zeta-potential (mV)	Size (nm)
Proteus vulgaris LPS	-32.7 ± 2.4	350.3 ± 31.9
P. vulgaris LPS complexed with chitosan	$+27.9 \pm 2.5$	300.5 ± 37.5
Yersinia pseudotuberculosis LPS	$-31.4 \pm$ NA	32.2 ± 2.5
Y. pseudotuberculosis LPS complexed with chitosan	$+21.2 \pm 0.6$	137 ± 11.9
Escherichia coli LPS	-14.5 ± 2.0	107.8 ± 7.3
E. coli LPS complexed with chitosan	$+0.83 \pm 0.09$	887.0 ± 75.3

NA: not available.

In the initial steps of its candidacidal action, chitosan prevents the adhesion capacity of *Candida albicans* to various supports and its biofilm development. Based on observations like this one, it can be said that the cell wall is an attractive target for the development of strategies to combat biofilm-associated infections. Medical devices (e.g., suture filaments, and catheters) treated with antimicrobial agents before they are implanted in patients have been proposed. Chitosan has been demonstrated suitable for this purpose and, for example, it is effective against *Cryptococcus neoformans* responsible for life-threatening meningoencephalitis. In fact, chitosan prevents *C. neoformans* biofilm formation as shown by SEM imaging: while untreated *C. neoformans* cells are connected by copious amounts of polysaccharide, the same grown in the presence of chitosan (0.04 mg/mL) show no exo- or capsular polysaccharide [103]. Further results along the same line were presented by Liu *et al.* [104] on the inhibition of bacterial adherence on the internal surface of biliary stents modified with chitosan. In order to develop an antimicrobial suture, polyacrylate was grafted onto the surface of polypropylene suture monofilament, followed by chitosan immobilization [105]. Of course, polypropylene was activated by O_2 plasma exposure (180 s, 100 W). The chitosan immobilization conditions were as follows: carbodiimide in citrate buffer pH 4.8 (10 mg/mL) at 4 °C for 30 min, and chitosan in acetate buffer pH 4.6 (0.4%) at 4 °C for 24 h. Chitosan was covalently linked to the polyacrylate with the aid of carbodiimide, which promotes the amide bond formation between the amino group and the carboxyl group. The chitosan quantity linked to the surface was linearly proportional to the degree of polyacrylate grafting onto the underlying polypropylene suture. In this way, biofilm formation was prevented thanks to the polyelectrolyte complexation of immobilized chitosan with pathogen cells, and infections consequent to the use of said experimental sutures were curbed most significantly, this being a major achievement based on the study of chitosan polyelectrolyte complexes.

Finally, it is worth mentioning briefly that chitosan has suddenly become a protagonist in the area of DNA and genetic material delivery, because it forms polyelectrolyte complexes whose stability is tunable for loading as well as for release to the cell nucleus. Very recent reviews on this matter are available [106–108]. Also, Chapters 15 and 16 provide further details on the subject.

1.7 Conclusions and Future Perspectives

There is interest in being up to date with the most recent chemical and technological developments of chitins and chitosans necessary for biomedical applications. The trends examined in this chapter, never discussed before in the frame of a review chapter, are certainly leading to more elaborated products that will make possible new achievements in the exploitation of the inherent peculiar properties of this class of polysaccharides.

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