Feature Article

Chitosan-based biomaterials for tissue engineering

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ABSTRACT

Derived from chitin, chitosan is a unique biopolymer that exhibits outstanding properties, beside biocompatibility and biodegradability. Most of these peculiar properties arise from the presence of primary amines along the chitosan backbone. As a consequence, this polysaccharide is a relevant candidate in the field of biomaterials, especially for tissue engineering. The current article highlights the preparation and properties of innovative chitosan-based biomaterials, with respect to their future applications. The use of chitosan in 3D-scaffolds – as gels and sponges – and in 2D-scaffolds – as films and fibers – is discussed, with a special focus on wound healing application.

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1. Introduction

Over the last years, many attempts have been made to replace petrochemical products by renewable, biosourced components. Abundant naturally occurring polymers – as starch, collagen, gelatin, alginate, cellulose and chitin [1] – represent attractive candidates as they could reduce the actual dependence on fossil fuels, and consequently have a positive environmental impact. The most challenging part of this approach is to obtain bio-based materials with properties equivalent to those of fully synthetic products, from a functional point of view. In this respect, chitosan is a quite unique bio-based polymer: its intrinsic properties are so singular and valuable that chitosan possesses no actual petrochemical equivalent. Consequently, the inherent characteristics of chitosan make it exploitable directly for itself.

2. Chitosan: structure and extraction

Chitosan is a linear, semi-crystalline polysaccharide composed of (1 → 4)-2-acetamido-2-deoxy-β-D-glucan (N-acetyl D-glucosamine) and (1 → 4)-2-amino-2-deoxy-β-D-glucan (D-glucosamine) units [2]. The structure of this polymer is depicted in Fig. 1. As such, chitosan is not extensively present in the environment – however, it can be easily derived from the partial deacetylation of a natural polymer: the chitin (Fig. 2). The deacetylation degree (DD) of chitosan, giving indication of the number of amino groups along the chains, is calculated as the ratio of D-glucosamine to the sum of D-glucosamine and N-acetyl D-glucosamine. To be named “chitosan”, the deacetylated chitin should contain at least 60% of D-glucosamine residues [3,4] (which corresponds to a deacetylation degree of 60). The deacetylation of chitin is conducted by chemical hydrolysis under severe alkaline conditions or by enzymatic hydrolysis in the presence of particular enzymes, among of chitin deacetylase [5,6].

After cellulose, chitin is the second most abundant biopolymer [2] and is commonly found in invertebrates – as crustacean shells or insect cuticles – but also in some mushrooms envelopes, green algae cell walls, and yeasts [7–9]. At industrial scale, the two main sources of chitosan are crustaceans and fungal mycelia; the animal source shows however some drawbacks as seasonal, of limited supplies and with product variability which can lead to inconsistent physicochemical characteristics [10]. The mushroom source offers the advantage of a controlled production environment all year round that insures a better reproducibility of the resulting chitosan [11], the physical properties of extracted chitosan being notably related to the growth substrate composition. Moreover, the vegetable source is generally preferred to the crustacean one from an allergenic point of view – the produced chitosan being safer for biomedical and healthcare applications [12]. The mushroom-extracted chitosan typically presents a narrower molecular mass distribution than the chitosan produced from seafood [12], and may also differ in terms of molecular mass, DD and distribution of deacetylated groups [11,13]. Chitosan DD greatly varies between 60 and 100% while its molecular weight typically ranges from 300 to 1000 kDa [1], depending on the source and preparation. Chitosan oligomers can be prepared by degradation of chitosan using specific enzyme [15] or reagent as hydrogen peroxide [16].

At the time of its production, it is difficult to predict chitosan characteristics. Therefore, the current approach consists in analyzing the resulting product composition and properties rather than in targeting predefined characteristics by controlling the process of production. The characterization of chitosan is thus quite of importance but it is not easygoing nor straightforward. Being a natural-based compound, chitosan may be contaminated by organic and inorganic impurities, and presents broad polydispersity, this is why producers mainly refer to samples viscosity rather than molecular mass. Chitosan is also poorly soluble, except in acidic medium (as it will be discussed in the following paragraph) which makes analyzes difficult to perform. Different methods including pH-potentiometric titration, IR-spectroscopy, 1H NMR spectroscopy, but also UV-spectroscopy, colloidal titration and enzymatic degradation are reported in the literature for the determination of chitosan deacetylation degree [17], while its molecular mass is typically deduced from viscosimetry or determined by size exclusion chromatography [2].

3. Structure–properties relationship

The presence of amino groups in the chitosan structure (Fig. 1) differentiates chitosan from chitin, and gives to this polymer many peculiar properties. Indeed, the amino groups of the D-glucosamine residues might be protonated providing solubility in diluted acidic aqueous solutions (pH < 6) [14]. In contrast, practical applications of chitin are extremely limited due to its poor solubility, if any...
Interestingly, the aqueous solubility of chitosan is pH-dependent allowing processability under mild conditions [4], which opens prospects to a wide range of applications, particularly in the field of cosmetics [2].

Due to these amino groups, chitosan also efficiently complexes various species, such as metal ions, and therefore is often used for the treatment of waste waters, purifying them by recovering heavy metals [2]. The complexing ability of chitosan is further exploited for beverages (wine, juices...) clarification [19].

Chitosan with protonated amino groups becomes a polycation that can subsequently form ionic complexes with a wide variety of natural or synthetic anionic species [4], such as lipids, proteins, DNA and some negatively charged synthetic polymers as poly(acrylic acid) [4,18,20,21]. As a matter of fact, chitosan is the only positively charged, naturally occurring polysaccharide [18]. As a polyelectrolyte, chitosan can notably be employed for the preparation of multilayered films, using layer-by-layer deposition technique [22]. This particular case will be detailed later on.

The amino and alcohol functions along chitosan chains enable this polysaccharide to form stable covalent bonds with other species. Non-specific reactions can be performed on the alcohol groups, as etherification and esterification reactions [2] but remarkably, the amino group of D-glucosamine residues may be specifically quaternized or reacted with aldehyde functions under mild conditions through reductive amination [2]. Various functionalizations can be introduced along chitosan backbone by this technique to further extend chitosan field of applications.

Besides, chitosan exhibits other remarkable intrinsic properties: this polysaccharide exhibits antibacterial activity [23,24], along with antifungal [9], mucoadhesive [25], analgesic [9] and haemostatic properties [26]. It can be bio-degraded into non-toxic residues [27,28] – the rate of its degradation being highly related to the molecular mass of the polymer and its deacetylation degree – and has proved to some extent biocompatibility with physiological medium [29,30]. All these singular features make chitosan an outstanding candidate for biomedical applications.

4. Remarkable properties of chitosan as biomaterial

As already mentioned, several remarkable properties of chitosan offered unique opportunities to the development of biomedical applications. The elucidation of their mechanism will lead to a better understanding of chitosan medical and pharmaceutical interest.

The presence of the protonable amino group along D-glucosamine residues allows to elucidate most of chitosan properties. Indeed, the mucoadhesion of chitosan for example, can be explained by the presence of negatively charged residues (sialic acid) in the mucin – the glycoprotein that composes the mucus. In acidic medium, chitosan amino groups are positively charged and can thus interact with the mucin. This mucoadhesion is directly related to the DD of chitosan: actually, if chitosan DD increases, the number of positive charges also increases, which leads to improved mucoadhesive properties [31].

The haemostatic activity of chitosan can also be related to the presence of positive charges on chitosan backbone. Indeed, red blood cells membranes are negatively charged, and can thus interact with the positively charged chitosan. Besides, chitin shows less effective haemostatic activity than chitosan, which tends to confirm this explanation [32–34].

Due to its positive charges, chitosan can also interact with the negative part of cells membrane, which can lead to reorganization and an opening of the tight junction proteins, explaining the permeation enhancing property of this polysaccharide. As for mucoadhesion, if chitosan DD increases, the permeation ability also increases [35].

The case of chitosan antimicrobial activity is slightly more complex; two main mechanisms have been reported in the literature to explain chitosan antibacterial and antifungal activities. In the first proposed mechanism, positively charged chitosan can interact with negatively charged groups at the surface of cells, and as a consequence, alter its permeability. This would prevent essential materials to enter the cells or/and lead to the leaking of fundamental solutes out of the cell. The second mechanism involves the binding of chitosan with the cell DNA (still via protonated amino groups), which would lead to the inhibition of the microbial RNA synthesis. Chitosan antimicrobial property might in fact result from a combination of both mechanisms [23,36].

The polycationic nature of chitosan also allows explaining chitosan analgesic effects. Indeed, the amino groups of the D-glucosamine residues can protonate in the presence of proton ions that are released in the inflammatory area, resulting in an analgesic effect [37].

Now, to explain chitosan biodegradability, it is important to remember that chitosan is not only a polymer bearing amino groups, but also a polysaccharide, which consequently contains breakable glycosidic bonds. Chitosan is actually degraded in vivo by several proteases, and mainly lysozyme [9,38,39]. Till now, eight human chitinases have been identified, three of them possessing enzymatic activity on chitosan [40]. The biodegradation of chitosan leads to the formation of non-toxic oligosaccharides of variable length. These oligosaccharides can be incorporated in metabolic pathways or be further excreted [41]. The degradation rate of chitosan is mainly related to its degree of deacetylation, but also to the distribution of N-acetyl D-glucosamine residues and the molecular mass of chitosan [42–44].

To explain the relationship between chitosan biodegradation and DD, it is important to remember that chitosan is a semi-crystalline polymer; crystallinity is indeed maximum for a DD equal to 0 or 100% (chitin or fully deacetylated chitosan, respectively), and decreases for intermediate DD. Yet, as polymer crystallinity is inversely related to the biodegradation kinetic, when chitosan DD decreases (close to 60%), its crystallinity also decreases, which results in an increase of the biodegradation rate. Besides, the distribution of acetyl residues along chitosan will also affect its crystallinity, and consequently the biodegradation rate. Finally, it can be reasonably assumed that smaller chitosan chains will be more rapidly degraded into oligosaccharides than chitosan with higher molecular mass.
Considering all the aforementioned properties, it is not surprising that chitosan was, is and will be tested in many biomedical and pharmaceutical applications, notably for sutures, dental and bone implants and as artificial skin [2]. Within the framework of these tests, chitosan has shown biocompatibility [9, 45] and was notably approved by the Food and Drug Administration (FDA) for use in wound dressings [46].

However, the compatibility of chitosan with physiological medium depends on the preparation method (residual proteins could indeed cause allergic reactions) and on the DD – biocompatibility increases with DD increase. Chitosan actually proved to be more cytocompatible in vitro than chitin. Indeed, while the number of positive charges increases, the interaction between cells and chitosan increases as well, which tends to improve biocompatibility [47].

Besides, some chemical modifications of chitosan structure could induce toxicity [38].

After describing chitosan main properties in the previous paragraphs, the following will focus on the potential applications of chitosan as a biomaterial, and more precisely on its processing methods for uses as biomedical 3D-scaffolds for tissue engineering, and 2D-scaffolds especially for wound healing purposes.

5. Chitosan 3D-scaffolds for tissue engineering

During the fabrication of implantable scaffolds particular attention should be paid to body compatibility, mechanical properties, scaffold morphology and porosity, as well as healing and tissue replacement capacity [48]. According to literature, the main requirements for the elaboration of tissue engineering scaffolds also include that the scaffold should not induce acute or chronic response, should be biodegradable so that the cured tissue will be able to replace the biomaterial, possess surface properties that will promote cell attachment, differentiation and proliferation, have suitable mechanical properties for handling and to mimic the damaged tissue, and finally, be manufactured into variety of shapes [49, 50].

Due to its aforementioned remarkable properties, chitosan appears thus as a relevant candidate for the preparation of such biomaterials, which could substitute for missing or damaged tissue and organ [48], and allow cell attachment and proliferation [51] provided that 3D-scaffolds might be produced. Such development relies thus on robust processing methods for chitosan making able to adjust the scaffold properties at best to the diseased tissue requirements. Therefore, methodologies have been developed to finely shape chitosan hydrogels and foams (or sponges) as pertinent 3D-scaffolds applicable for tissue engineering. These will be reviewed in the following sections.

5.1. Chitosan hydrogels

Gels are composed of a solid phase, typically representing less than 10% of the total volume of the gel, and a liquid phase. In hydrogels, the liquid phase is water (and sometimes adjuvants). The solid phase ensures the consistency of the gel, making it able to soak up/absorb large quantities of water while remaining insoluble in the liquid phase [12]. Hydrogels are interesting biomaterials as their high water content makes them compatible with a majority of living tissues. Moreover, they are soft and bendable, which minimizes the damage to the surrounding tissue during and after implantation in the patient [38]. The mechanical properties of hydrogels tend to mimic those of the soft body-tissues, which allows the gels to insure both functional and morphological characteristics of the tissue to be repaired [38]. This is why hydrogels are often used as biomedical scaffolds for tissue replacements, but also for other biomedical applications such as drug and growth factor delivery [52–54].

Three main types of chitosan hydrogels have been developed, presenting either reversible or irreversible gelation. Chitosan can indeed be either physically associated, coordinated with metal ions or irreversibly/chemically cross-linked into hydrogels [38].

5.1.1. Physically associated chitosan hydrogels

The formation of “physical” hydrogels is based on the reversible interactions that can occur between polymer chains. Those interactions have a non-covalent nature, such as electrostatic interactions, hydrophobic interactions or hydrogen bondings [55, 56]. Those interactions can be dependent of various parameters as pH, concentration, temperature…., which make them not very stable, exhibiting reversible gelation. The swelling of those hydrogels can be tuned by adjusting the nature and the quantities of each component, in order to increase or decrease the number of interactions. As a rule, fewer interactions will lead to a softer gel while a higher number of interactions will give a tighter and stiffer gel.

It is remarkable to observe that chitosan is able to form a gel, all by itself, without the need of any additive. The process is based on the neutralization of chitosan amino groups and thus the inhibition of the repulsion between chitosan chains. The formation of the hydrogel then occurs via hydrogen bonds, hydrophobic interactions and chitosan crystallites [38, 70], as illustrated in Fig. 3.

Further on, chitosan hydrogels can be formed by blending chitosan with other water-soluble non-ionic polymers, as poly(vinyl alcohol) (PVA) [55, 71]. Thermo-sensitive chitosan hydrogels can be obtained by blending chitosan with polyol salts, as glycero phosphate disodium salt [72]. Chitosan structure can also be modified to form physical hydrogels: chitosan-g-poly(ethylene glycol) (PEG) graft copolymer is able of self-organization depending on the temperature to form stable hydrogels [73].

Thanks to the polycationic nature of chitosan in acidic conditions, formation of chitosan hydrogels through electrostatic interactions involving small-size polyanions but also polyelectrolytes appears straightforward (Fig. 4a).

Positively charged chitosan will interact with negatively charged molecules, such as phosphates, sulfates and citrates ions [57, 58], to form hydrogels. Varying concentration and size of the anionic species as well as the numbers of D-glucosamine units vs. N-acetyl-D-glucosamine units allows fine tuning of the swelling of the obtained hydrogel.
Larger negatively charged molecules, natural and synthetic polyanions, can also give gels with chitosan. In the natural polyanion category, proteins (as gelatin, collagen, keratin, albumin and fibroin) [59–61], anionic polysaccharides (as hyaluronic acid, alginate, pectin, heparin, xanthan, dextran sulfate, chondroitin sulfate, fucoidan) [62–65], carboxymethyl cellulose and glycosaminoglycans [62,66] have been reported for such purpose. Synthetic polyanions – as poly(acrylic acid) (PAA) – were also used to form hydrogels [67]. The charge density of chitosan and of the polyanion will play a major role in the swelling and stability of the formed hydrogels. Proper adjustment of the pH of the medium is thus important.

It should be noted that electrostatic interactions can occur with other secondary interactions, as for example hydrogen bonding [68,69]. However, the interactions between charged chitosan and anions or polyanions are stronger than these secondary bonding. The preparation of ionic chitosan hydrogels avoids the use of catalyst or toxic reacting agent, which is a prerequisite for biomedical applications.

The easiness of gelation without the need of toxic additives, in conditions compatible with the human body led to the development of injectable solutions that exhibit a sol/gel transition upon injection in the body, and offer the unique opportunity to shape the gel within the diseased...
tissue used as mold or template, perfectly adjusting the scaffold to the defect. However, the mechanical resistance/strength of the aforementioned hydrogels is quite limited and uncontrolled dissolution of the gels can occur [74]. Besides, it is difficult to accurately control the size of the hydrogel pores. Therefore, irreversible chemical cross-linking of the hydrogels have been investigated.

5.1.2. Chitosan cross-linked by coordination complex

Coordinate-covalent bonds can also occur with chitosan via metal ions as Pt (II), Pd (II), and Mo (VI) [68,69] leading to the formation of another type of hydrogels, but less suited for biomedical use. This gelation is depicted in Fig. 4b.

5.1.3. Chemically cross-linked chitosan hydrogels

The formation of those hydrogels occurs via covalent bonding between polymer chains (Fig. 4c). The obtained hydrogels are much more stable than the previous physically associated hydrogels since the gelation is irreversible. However, this approach requires the chemical modification of the primary structure of chitosan, which could alter its initial properties, particularly if amino groups are involved in the reaction. In addition, the involved reaction might be a source of contamination by toxic residual reactants or catalyst traces.

Both the amino and the hydroxyl groups of chitosan can form a variety of linkages, as amide and ester bonding, but also Schiff base formation [55,75], which can lead to chitosan hydrogels formation. Small multifunctional molecules or polymeric cross-linker can both be used to react with chitosan and induce cross-linking. Chitosan can also be firstly modified by activated functional groups before inducing cross-linking by photo-induced reaction or enzymatic catalyzed reactions [38].

A straightforward way to obtain reversible chitosan hydrogels is the use of dialdehyde cross-linkers, such as glyoxal and glutaraldehyde, which fastly react with the amino groups of the chitosan D-glucosamine units and, to a lesser extent, with the hydroxyl groups of chitosan [62]. Triplyphosphate, ethylene glycol, diglycidyl ether and diisocyanate can also play the role of cross-linker to obtain chitosan hydrogels. However, all the aforementioned cross-linkers may impart toxicity to the formed hydrogels, which is a major concern given the future application of the hydrogels as biomaterials [76].

Lately, a natural derived cross-linker, the genipin (C11H14O5, isolated from Genipa Americana in 1960 and then from Gardenia jasminoides Ellis) has been used as an alternative cross-linking agent [62]. This cross-linker degrades more slowly than other cross-linkers (such as glutaraldehyde) and its toxicity has not yet been established [62,77]. Genipin can react with primary amine and thus cross-link polymers containing such amino groups [77,78], like proteins and chitosan.

The properties of the resulting hydrogels will rely on the cross-linking density and the ratio of cross-linker molecules to the moles of polymer repeating units [79].

It is important to note that chemical cross-linking of chitosan that involves chemical modifications of chitosan primary amine might induce modifications of chitosan properties. However, these groups being more reactive than the hydroxyl groups, they are mostly used for this purpose since the cross-linking degree might consume only few of the amino groups initially present on chitosan. Chitosan with high DD is then of interest as starting material.

5.2. Chitosan sponges

Sponges are nothing else than foams with an open porosity. These solid structures are able to absorb high amount of fluids (more than 20 times their dry weight), due to their micro-porosity. They typically offer good cell interaction, still being soft and flexible [80].

Chitosan sponges are mainly used as wound healing materials, as they can soak up the wound exudates, while helping the tissue regeneration. Chitosan sponges also find application in bone tissue engineering, as a filling material [81].

These sponges are mainly obtained by freeze drying (lyophilization), a simple efficient process consisting in freezing a solution of chitosan followed by sublimation of the solvent under reduced pressure (a SEM image of a chitosan sponge prepared by this technique is presented in Fig. 5). For example, chitosan [54], chitosan/tricalcium phosphate (TCP) [81,82], and chitosan/collagen sponges [83] were prepared by this technique and used as scaffolds for bone regeneration. Chitosan-ZnO composite sponges also prepared by freeze-drying [80] showed good swelling, antibacterial and haemostatic activities, confirming their potential healing in wound dressing application.

Cross-linked chitosan sponges loaded with a model of antibiotic drug – the norfloxacin – were prepared by solvent evaporation technique [84]. Fibrillar structure was obtained and those sponges showed promising use for wound dressing application.

Recently, efforts were dedicated to the use of supercritical carbon dioxide (scCO2) as a green medium to induce porosity to chitosan scaffolds [85]. The scCO2 method allows the preparation of porous chitosan scaffolds, suitable for cell cultures directly upon depressurization.

6. Chitosan 2D-scaffolds for wound dressing

In the particular case of wound dressing, specific requirements have to be met to encounter efficiency. The
wound dressing should be non-toxic and non-allergenic, allow gas exchanges, keep moist environment, protect the wound against microbial organisms and absorb wound exudates [80]. As mentioned above, chitosan sponges have been found to fit quite well these requirements. Nevertheless, as far as skin tissues are concerned, more 2D-shaped scaffolds, such as films and porous membranes, are supplementary candidates, spreading the panel of structures available to tackle the challenges addressed in skin repair.

6.1. Chitosan films

Chitosan films can be easily prepared by wet casting from chitosan salt solutions, followed by drying (typically using oven or infrared (IR) drying [86]). For example, Hem-Con® bandage is an engineered chitosan acetate preparation designed as a haemostatic dressing [80].

6.1.1. Improving properties of chitosan free-standing films

To improve the properties of those films, some physicochemical processes have been tested. For examples, (i) nitrogen or argon plasma [87] treatment of chitosan membranes increases the film surface roughness and improves the cell adhesion and proliferation (especially for films treated with nitrogen plasma at 20 W during 20 min), (ii) ozone or UV irradiation promotes surface modification of chitosan films leading to depolymerization of chitosan [88], (iii) by introducing silica particles or poly(ethylene glycol) [89] into the chitosan films, their porosity can be artificially modified with macro and micro-pores (pores size ranging 0.5–100 μm).

As far as mechanical properties are concerned, blending and chemical modifications of chitosan have proven efficiency. To improve the films ductility, chitosan can be blended (or copolymerized) with poly(ethylene glycol) (PEG), which results in a decrease of the modulus with an increase of the strain at break (for 50/50 chitosan/PEO blended film, the decrease of the modulus was 56%, while the increase of the strain at break was 125%, in comparison to pure chitosan film) [90]. Amidation of the chitosan films (up to 50%) can occur with thermal treatment; it leads to significant strengthening of the films and to a decrease of the film solubility in aqueous media [91]. Chitosan films prepared in presence of dialdehyde starch, that plays the role of cross-linking agent, show improved mechanical properties and better water-swelling (best values were obtained with a dialdehyde starch content of 5%, with a tensile strength of 113.1 MPa and an elongation at break of 27%) [92], via Michael addition reaction, chitosan/polyethylene glycol diacrylate (PEGDA) blended films were developed [93], showing enhanced swelling (the maximum swelling was observed at a ratio of CS/PEGDA equal to 40/60) and good mechanical properties (the maximum values of tensile strength and modulus were obtained at a ratio of CS/PEGDA equal to 70/30).

Other noteworthy developments are also the preparation of: (i) phosphorylated chitosan films (prepared from the reaction of orthophosphoric acid and urea in DMF), which present higher ionic conductivity than normal chitosan films (conductivity of pure chitosan film being equal to $8.3 \times 10^{-5}$ S cm$^{-1}$, and reaching $1.2 \times 10^{-3}$ S cm$^{-1}$ for a chitosan film containing $87.31$ mg/m$^2$ of phosphorus – NB: conductivity measurements were realized after hydratration of the films) [94], (ii) chitosan blends with minocycline hydrochloride, to prepare films that accelerate the healing of burns (reduction of the wound size) [95], (iii) chitosan/poly(vinyl alcohol)/alginate films by casting/solvent evaporation method, in order to create a moist environment within the framework of wound healing [96], and (iv) non-adherent film of β-glucan and chitosan [97,98].

Heterogeneous chemical modification of the chitosan film can tune the surface properties of the film; for instance, when a stearoyl group was attached to the chitosan films, they became more hydrophobic (contact angle of pure chitosan film being $89 \pm 6^\circ$, while $101 \pm 4^\circ$ was found for N-stearoylchitosan film) and promoted proteins adsorption. When chitosan films were reacted with succinic anhydride or phthalic anhydride, it resulted in more hydrophilic films (contact angles of $56 \pm 2^\circ$ and $51 \pm 7^\circ$ were found for N-succinylchitosan and N-phthalylchitosan films, respectively), which promoted lysozyme adsorption [99]. Interestingly, a thermo-sensitive film was prepared by combining thiolated chitosan, poly(N-isopropyl acrylamide) and ciprofloxacin, so that by decreasing the temperature, the film can easily be removed from wound [100].

Finally, incorporation of inorganic particles in these films allows synergistic effects of both materials leading to high performance healing. Ag nanoparticles possess identified antimicrobial activity, notably used in silver-based wound dressings [101]. Those Ag particles can be added to chitosan film, to further avoid microorganism proliferation [102–105]. Polyphosphate procoagulant can also be incorporated to the chitosan/Ag films [24]. Beside Ag, zinc oxide (ZnO) nanoparticles are non-toxic and present antibacterial and good photocatalytic activities [106]. Like the Ag nanoparticles, they can be incorporated in chitosan films. Complex films containing chitosan, Ag nanoparticles and ZnO particles were prepared [107]. They possess pronounced antibacterial activity (especially the film containing 0.1 wt.% Ag and 10 wt.% ZnO), thus promising application in wound dressing field.

6.1.2. Chitosan thin films

The properties of the wound dressing surface in contact to the diseased body are particularly of importance to provide efficient healing. Multilayer coatings and nanostructured thin films have thus also been investigated in order to tune the surface properties of various medical devices. Two methods to design chitosan thin films in order to modify the surface of a performed substrate will be shortly described: Langmuir–Blodgett (LB), and the layer-by-layer (LBL) deposition techniques. These two processes are of importance since both of them allow to control parameters such as film thickness, composition, morphology, and even roughness [18].

To form a Langmuir [108] monolayer at the air–water interface, a solution in an organic volatile solvent of an amphiphilic water-insoluble (macro)molecule is spread on the surface of water. When the organic solvent is evaporated, the hydrophilic part of the amphiphile is anchored...
to the aqueous subphase, while the hydrophobic part is directed toward air [18] (as depicted in Fig. 6a). The Langmuir–Blodgett (LB) process [109] consists in transferring this Langmuir monolayer onto a solid support. This can be done by immersing (or emerging) a solid support in (or from) the aqueous subphase to recover the monolayer (Fig. 6b). By repeating the process, multilayered films can be obtained.

Chitosan being poorly soluble in organic solvents, chemical modification is required for this process to be performed. Chitosan modified with long alkyl chains attached to the primary hydroxyl and amino groups, was the first example of LB chitosan films formation [18,110], this derivatization making the resulting chitosan soluble in chloroform. Derivatives of chitosan pentamers were used to produce LB films [111,112], while other amphiphilic chitosans, namely, N,N-dialkyl-chitosans, were also reported [113]. All these films find application mainly in drug release and drug delivery systems, since the amphiphilic films are able to entrap various drugs with efficacy.

To facilitate the optical characterization of the LB films, amphiphilic chitosans bearing an alkyl group combined with a cinnamoyl chromophore were synthesized [114]. Mixed films of cholesterol and chloroform-soluble O,O-dipalmitoyl chitosan are an example of two-component LB films [115].

On the other hand, the layer-by-layer (LBL) deposition technique is based on the alternated adsorption of materials bearing complementary charged or functional groups [22,112], in aqueous medium. A schematic representation of LBL process is presented in Fig. 7. A large number of molecules can be used, including synthetic and natural polyelectrolytes [111], nanotubes [116] and biomolecules [117]. Such polyelectrolyte multilayered films find many applications in the field of sensors [118], filters, but also as biomaterial for tissue engineering [119].

The polycationic nature of chitosan makes this polymer well-suited for LBL processes. As a consequence, LBL chitosan-based films are involved in many applications as sensors, drug delivery systems, haemostatic devices, bone implants and wound dressings [18].

Chitosan can be LBL co-deposited with several species on metallic devices [18]. For sensing applications, enzymes, antigens, nucleic acids, carbon nanotubes, phthalo-
cyanines, and porphyrins can be trapped by this process in a chitosan matrix that helps maintaining the biomolecule activity. For coronary stents, chitosan/heparin LBL films are particularly interesting for their anticoagulation properties [18].

To improve mechanical properties of chitosan LBL films, which might be especially important when deposited in soft substrates, chitosan can be combined in the film with clays such as Na-montmorillonite [120], with carbon nanotubes [121], or with cellulose nanowhiskers [122].

LBL films entirely made of polysaccharides, such as chitosan and hyaluronic acid [123], and of chitosan and alginate [119] offer great prospect for wound dressing purpose, as they have shown abilities to accelerate the healing.

6.2. Chitosan porous nanofiber membranes

There are several ways to produce chitosan fibers; the first example was reported as early as 1926 [124,125]. Fibers were notably produced using dry [126] and wet spinning [127] from acetic acid solution. Lithium chloride/N,N-dimethylacetamide was also used as a solvent [128]. In order to decrease production cost and to improve fiber properties, blends of chitosan with other polymers were also considered: sodium alginate [129], tropocollagen [130], cellulose, sodium hyaluronate, sodium heparin, sodium chondroitin sulfate [124], poly(acrylic acid) [131] were thereby employed.

In recent years, electrospinning (ESP) [132] has distinguished as a versatile technique for the preparation of polymer fibers from a few nanometers to microns in diameter, depending on the processing conditions. ESP uses a high voltage to create an electrically charged jet of polymer solution or melt which can lead to fibers formation. Fig. 8 shows a schematic representation of this technique. The mats of fibers produced by this method possess high porosity, high specific surface area, and are able to mimic the natural extracellular matrix, which makes these mats great candidates for biomaterial applications. Chitosan (nano)fibers find many applications for the preparation of wound dressing and for tissue engineering [133].

However, as chitosan is a polyelectrolyte in acidic medium, its electrospinning turns out to be tricky. Indeed, during ESP, repulsive forces between charged species within polymer backbone arise as a consequence of the application of an important electric field. This phenomenon restricts the formation of continuous fibers and often leads to the creation of particles [133,134].

A few examples of pure electrospun chitosan fibers are reported; chitosan (nano)fibers were successfully produced from trifluoroacetic acid (TFA) and dichloromethane (DCM) mixtures [135,136]. It was suggested [135] that the amino groups of the chitosan form salts with TFA [137], which destroy the interactions between chitosan molecules and facilitate the ESP process. In addition, the use of DCM allows improving the homogeneity of the produced fibers. Another group used TFA in conjugation with glutaraldehyde, to produce cross-linked chitosan fibers [138]. Finally, the use of concentrated aqueous acetic acid was considered [139,140].

To facilitate the electrospinning of chitosan, some authors report on the use of modified chitosan such as hexanoyl chitosan [141] and PEGylated chitosan [142]. Nevertheless, the easiest way to produce chitosan (nano)fibers is the electrospinning of chitosan as a blend with another polymer. Many examples of such chitosan blend fibers produced by ESP are reported in the literature.

The electrospinning of chitosan in presence of poly(ethylene oxide) (PEO) [143–147] and ultrahigh-molecular-weight poly(ethylene oxide) (UHMWPEO) [148] are commonly reported. A SEM image of chitosan/PEO nanofibers is presented in Fig. 9. Poly(vinyl alcohol) (PVA) [149–151] can also be used to form chitosan (nano)fibers. The uses of poly(ethylene terephthalate (PET) [152], poly(vinyl pyrrolidone) (PVP) [153] and poly(lactic acid) (PLA) [154] were also reported.

Chitosan/PEO nanofibers prepared by ESP exhibit cellular biocompatibility [133] [147]. The structure of the fiber mats was found to promote the attachment of human cells, while preserving their morphology and viability [147]. The addition of UHMWPEO to chitosan ESP solution allows forming fibers ranging from less than an hundred nanometers to a few tens micrometers [148], while adding PVP allows decreasing the diameter of chitosan-based fibers [153]. These blends thus offer great prospects for designing tissue engineering scaffolds [133]. The joint use of PVA and chitosan allows the preparation of fibers without beaded...
Table 1
Main advantages, disadvantages and applications of the chitosan biomaterials presented.

<table>
<thead>
<tr>
<th>Biomaterial type</th>
<th>Specifications</th>
<th>Main advantage(s)</th>
<th>Main disadvantage(s)</th>
<th>Main biomedical application(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogels (3D)</td>
<td>• Physically associated (reversible)</td>
<td>Soft flexible non-toxic</td>
<td>Not stable (uncontrolled dissolution may occur)</td>
<td>Tissue replacements/engineering drug/growth factor delivery</td>
</tr>
<tr>
<td></td>
<td>• Chemically cross-linked (irreversible)</td>
<td>Soft flexible stable controlled pore size</td>
<td>May be toxic</td>
<td></td>
</tr>
<tr>
<td>Sponges (3D)</td>
<td>• Free-standing</td>
<td>High porosity</td>
<td>May shrivel</td>
<td>Tissue engineering (filling material)</td>
</tr>
<tr>
<td></td>
<td>• Thin (LB)</td>
<td>Material coating</td>
<td>Laborious for the construction of multilayers</td>
<td>Wound dressings skin substitutes</td>
</tr>
<tr>
<td>Films (2D)</td>
<td>• Thin (LB)</td>
<td>Material coating</td>
<td>Many steps</td>
<td>Coatings for a variety of scaffolds</td>
</tr>
<tr>
<td>Porous Membranes (2D)</td>
<td>Nanofibers</td>
<td>High porosity</td>
<td>ESP of pure chitosan difficult</td>
<td>Coatings for a variety of scaffolds</td>
</tr>
</tbody>
</table>

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References


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Christine Jérôme completed her master’s degree in chemical sciences from the University of Liege in 1998 at the University of Liege, Belgium and then worked as a post-doctoral researcher on developing the electrospinning process of acrylates. In 2000, she joined the University of Ulm in Germany as a recipient of the Humboldt scholarship and studied the synthesis of functional magnetic nanohybrids. She returned to the University of Liege in 2001 as Research Associate of the National Foundation of the Scientific Research in the group of Professor R. Jérôme, where she became Professor in 2006 and director of the Center for Education and Research on Macromolecules in 2007. Today full Professor, her research interests include bioceramic polymers, functional macromolecular systems and advanced biomaterials.