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Chitosan based-nanoparticles and nanocapsules: Overview, physicochemical features, applications of a nanofibrous scaffold, and bioprinting

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Abstract

Recent advancements in the synthesis, properties, and applications of chitosan as the second after cellulose available biopolymer in nature were discussed in this review. A general overview of processing and production procedures from A to Z was highlighted. Chitosan exists in three polymorphic forms which differ in degree of crystallinity (α , β , and γ). Thus, the degree of deacetylation, crystallinity, surface area, and molecular mass significantly affect most applications. Otherwise, the synthesis of chitosan nanofibers is suffering from many drawbacks that were recently treated by co-electrospun with other polymers such as polyvinyl alcohol (PVA), polyethylene oxide (PEO), and polycaprolactone (PCL). Ultimately, this review focuses on the area of new trend utilization of chitosan nanoparticles as nanospheres and nanocapsules, in cartilage and bone regenerative medicine. Owing to its biocompatibility, bioavailability, biodegradability, and costless synthesis, chitosan is a promising biopolymeric structure for water remediation, drug delivery, antimicrobials, and tissue engineering.

Keywords: Chitosan; chitosan production; the degree of deacetylation; chitosan physicochemical properties; chitosan nanofibers; bioprinting.

1. Chitosan source

Chitosan consists of residues of 2-acetamido-2-deoxy- β -D-glucopyranose and 2-amino-2-deoxy- β -D-glucopyranose (Fig. 1) and it is the second prevalent biopolymer in nature after cellulose. The -NH₂ and -OH in the chitosan structure are the main functional groups responsible for chitosan properties and its unlimited application. In 1859, Rouget discovered chitosan when he studied the deacetylated forms of chitin from crustacean marine [1]. Since the first international conference of chitin and chitosan (Boston, USA, 1977), scientific and industrial communities have shown exponential interest in chitin and chitosan as a costless biopolymeric structural form [2].

Crustaceans, insects, mollusks, and fungi are the main sources of chitosan. In industry, chitosan is made from the external chitinous exoskeleton of marine crustaceans such as crabs, shrimps, lobsters, crayfish, krill, and woodlice which are waste products that originated from the fishing industry [3]. First, the crude material is subjected to demineralization, deproteinization, deodorization, and drying to extract chitin. Then, the chitin is being deacetylated, cleaned, and left to dry to gather chitosan. This method is economically efficient since raw materials are not associated with any costs and could include the extraction of Astaxanthin. Currently, chitosan is commercially produced in the entire world and its price in powder form depends on the application, properties, and purity. The main characteristics that ascertain the biopolymer of chitosan are its molecular weight and the degree of deacetylation. Other properties are important, such as crystallinity, surface area, particle size, air humidity, and ash content [4, 5]. These characteristics are important in controlling the properties of chitosan, i.e. solubility, polycation nature, antimicrobial activity, biocompatibility, and bioadhesion. Consequently, different shapes and physical forms of chitosan such as films, nanofibers, nanoparticles, nanocapsules, microparticles, membranes, sponges, scaffolds, and hydrogels determine their possible utilization in water treatment, food processing, cosmetics, agriculture, and biomedicine [6-8].

1.1. Processing and production of chitosan

In general, two main processes are used for chitosan production: the ordinary process and the fermentation process [9]. In the ordinary method, the sources are crustaceans, insects, or mollusks. First, chitin is isolated from the raw materials using various chemical treatments that remove ash, proteins, color, and flavor. Chitin is converted to chitosan by alkaline deacetylation. Several phases are performed to purify chitosan such as dissolution, precipitation, centrifugation, and finally, the drying phase. In the fermentation process, chitosan is obtained from fungi, i.e. fungal biomass containing glucan complexes with chitin or chitosan is obtained by fermentation, [10]. Then the biomass is separated from the fermentation medium by filtration and drying and the next successive steps are performed to extract the chitosan [11]. Fig. 2 shows the main steps of the ordinary method to produce chitosan. This process usually begins with the demineralization phase. The main goal of this step is to reduce the ash and mineral content in the crustacean waste. Ash mainly consists of inorganic traces such as carbonates, phosphates, and other mineral salts [12]. Demineralization occurs mainly in dilute HCl solutions (1-10%) assisted by stirring at room temperature for short periods of nearly 1-3 hours [13]. This treatment guarantees complete removal of the mineral content without destroying the polymer chains of the chitin. Other acids like nitric acid, sulfuric acid, formic, acetic, and EDTA can be also used [14]. Unfortunately, these nominated acids are effective, but they can damage the structure of chitin in the depolymerization process. The second step is deproteinization that includes the removal of proteins residues adhered to the demineralized powder. It proceeds with alkalis including sodium hydroxide, sodium bicarbonate, sodium carbonate, potassium carbonate, potassium hydroxide, or calcium hydroxide. The most common option is the use of sodium hydroxide (1-10%) with a gentle stirring at a temperature that reaches 100 °C and the period between 2 and 72 hours. A deproteinization requires effective control of time and temperature, as high temperatures and longer periods can lead to depolymerization and deacetylation. On the other hand, enzymatic treatment with proteases enzymes such as pepsin and trypsin is an alternative form of deproteinization but less efficient than alkaline treatment [15].

The third step in the isolation of chitin is deodorization/depigmentation responsible for removing pigments and odors. For this purpose, ethanol, acetone, potassium permanganate, sodium hypochlorite, or hydrogen peroxide are generally used [16]. It is important to notify that the above-mentioned phases are followed by several successive washing processes up to obtaining a controlled neutral pH value. The final product of demineralization, deproteinization,

and deodorization/depigmentation is chitin. It can then be dried for later use or immediately deacetylated.

The deacetylation reaction is the most important step in this processing, at this stage, chitin turns into chitosan. During the deacetylation of the chitin C-2 position, the acetamide group is converted into amino groups ($-NH_2$) [17]. When the percentage of amino groups attains around 50% or more, the polymer becomes soluble in aqueous acidic media, and in this case, chitosan can be obtained [18]. Alkaline hydrolysis with sodium hydroxide (40-50%) at high temperatures (80-120 °C) is the most common method for extracting chitosan from chitin [19]. The main factors influencing the deacetylation reaction are the alkali concentration, reaction time, solid/liquid ratio, temperature, and the size of the chitin particles. Several alternative methods have been studied to improve or replace conventional alkaline hydrolysis, including enzymatic agents, vapor explosion, microwave deacetylation, and ultrasonic deacetylation [20].

The purification step can be performed to produce high purity chitosan. In this case, the deacetylation product is dissolved in an acidic medium, obtaining a viscous solution with an insoluble fraction consisting of impurities, mainly ash, which has not been completely removed in the demineralization step. This viscous solution is centrifuged or filtered, and the insoluble fraction is discarded. The alkali is then added to the viscous solution, and consequently, a suspension with precipitated chitosan is formed. The suspension is centrifuged again to obtain pure chitosan (90-95%) in the form of a paste. The previous steps (dissolution / centrifugation / precipitation / centrifugation) can be repeated several times to obtain chitosan with high purity (purity greater than 99.9%) [21].

Drying is the last step and is necessary to obtain pure chitosan. This process is important to produce chitosan and to provide the moisture content which is required to conserve the product without changing the material. Typically, after drying, the desired product should have a moisture content of less than 10% (in the wet state) to provide good physicochemical and microbiological aspects after long-term storage. Polymerization reactions and Maillard reactions are the most important changes that should be avoided during the drying operation. Some methods, such as convection, floor drying, drying in the nozzle layer, spray drying, sun drying, oven drying, infrared drying, freeze-drying, and low-pressure overheating have been used to dry chitosan. All

these methods have their advantages and disadvantages, while air, temperature, and residence time are the key factors that must be controlled to obtain a good quality of chitosan product [22].

1.2. Chitosan characteristics

The main characteristics that determine the quality and flow rate of chitosan are the molecular weight, degree of deacetylation, crystallinity, surface area, and size of the particles as mentioned before [23]. Herein, the molecular mass of chitosan is a characteristic associated with the number of monomer units of a biopolymer. The evaluation and modification of this property are very important since molecular weight influences the properties like viscosity and solubility [24]. In general, the chitosan molecular weight ranges from 20 to 1200 kDa. Based on the molecular weight, chitosan can be classified as low, medium, and high molecular weight. Its molecular weight can be determined by HPLC and light diffusion but the viscometry method is the most common and simplest one [25]. In general, the molecular weight can be modified starting from a high molecular weight. Therefore, depolymerization methods are used to obtain low molecular weight edible chitosan films which more effective than high molecular weight in preserving papaya fruit and fish during their storage. The effect of the medium molecular weight of the polymer on the chitosan protein interaction was studied [26]. This study stated that increasing chitosan molecular weight from 15 to 200 kDa increases the binding constant between bovine serum albumin and chitosan in complexes.

1.2.1. Degree of deacetylation

The degree of deacetylation (DD) is the ratio between 2-acetamido-2-deoxy- β -D-glucopyranose and 2-amino-2-deoxy- β -D-glucopyranose. When all the monomer units are 2-amino-2-deoxy- β -D-glucopyranose, the biopolymer is completely deacetylated and DD is 100%. When the percentage of 2-amino-2-deoxy- β -D-glucopyranose units reaches 50% or more, the polymer being normally soluble in aqueous acid media is called chitosan [27]. In the same way, like MW, DD influences the properties and applications of chitosan. For example, an increase in DD contributes to an increase in free amino groups in the chitosan chain. Therefore, the solubility and the polycation quality of chitosan also increase. The DD can be tested using spectroscopic methods such as Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and nuclear magnetic resonance (NMR) [28]. Chitosan with different DD can be obtained by varying

the deacetylation reaction conditions. The adsorption of anionic methyl orange dye by using chitosan with different degrees of deacetylation was reported. The results showed an increase in DD to 84% directly proportional to the adsorption capacity from 266 to 373 mg / g [29]. Similar findings were also obtained elsewhere for the adsorption of food dyes by chitosan in the binary system [30]. The DD has been shown in the literature to influence the mechanical properties (elongation and tensile strength) of chitosan films [31].

1.2.2. Crystal structure

The crystallinity of chitosan is the ratio between the crystalline and amorphous fractions of a biopolymer. This property is quantified as a crystallinity index (CI) which is determined by the relation between the characteristic peaks of X-ray diffraction (XRD). In the solid-state, chitosan is a semi-crystalline and polymorphic biopolymer, its unit cell is orthorhombic and contains two antiparallel chains free from water molecules [32]. Additionally, chitosan is found in three polymorphic phases that differ in the degree of crystallinity (α , β , and γ), depending on the source and arrangement of the chains. The commercial chitosan is a semi-crystalline polymer and CI is a function of DD. Also, the quantitative assessment of CI is important because this property affects the swelling, porosity, hydration, and absorption properties. Thakhiew's et al. [33] works showed that different methods of drying affect the physical and mechanical properties of chitosan films. They used hot air drying (HD) and low-pressure steam drying (LPSSD). They found that LPSSD provides films with a lower CI value (3-5%) than films made from HD (10-16%). As a result, the swelling degree was extended to 48-100% (LSSPD) and 75-110% (HD) [26, 34].

1.2.3. Surface area and particle size features

The surface area and size of the particles depend on the source and the extraction process associated with the porosity, pore-volume, and pore size distribution of the chitosan particles. Chitosan powder or flakes are known to have a reduced surface area (less than 10 m²/g) being non-porous materials. Particles smaller than 1 mm is commonly used in most applications. The surface area and particle size are crucial for applications such as adsorption and immobilization of enzymes since many accessible sites and porous structures are required in these vital fields [35, 36]. Some modifications have been made on chitosan to increase surface area because naturally, it is a non-porous material. The surface area is usually determined by N₂ adsorption-

desorption isotherms by the Brunauer-Emmett-Teller (BET) method. Particle size can be obtained using scanning electron microscopy (SEM), sieving test, or using a particle size analyzer. The effect of the particle size, surface area, and pore volume of chitosan on the adsorption of some food azo dyes FD&C red 2 and FD&C yellow 5 was studied before [37]. The particle sizes used were 0.10, 0.18, and 0.26 mm with surfaces of 4.2, 3.4, or 1.6 m²/g. Accordingly, the results indicated that the adsorption potential is increased by an increase in surface area and a decrease in particle size.

1.3. Properties of chitosan

The properties of chitosan depend on its characteristics which are therefore responsible for the quality and extent of use of chitosan. Among the most important properties of chitosan are solubility, polycationic nature, film formation, antimicrobial activity, and biocompatibility [38, 39].

1.3.1. Solubility and polycationic nature

The solubility and polycationic nature of chitosan are fundamental properties to produce more derivatives and it is usually a strong base with primary amino groups that have a pKa of 6.3. Therefore, this biopolymer is soluble in acid solutions diluted to pH 6.0. At low pH values, the H⁺ ions in solution protonate the -NH₂ groups, which leads to the formation of (NH₃)⁺. Therefore, chitosan is converted into a polycationic biopolymer structure. On the other hand, when the pH is above 6.0, deprotonation occurs and chitosan becomes insoluble [40]. An insoluble-soluble transition occurs when pKa with a pH is between 6.0 and 6.5. Formic acid is the most suitable solvent, while acetic acid is used more often. Other alternatives are hydrochloric acid and nitric acid. Chitosan is insoluble in sulfuric and phosphoric acids as well as concentrated acids are not indicated since they can lead to depolymerization and deformation of the polymeric chains of chitosan [41]. The solubility of chitosan and chitosan-based materials depends on several important factors, including time, temperature, DD, MW, particle size, type, the concentration of acid, and reduction methods.

1.3.2. Antimicrobial properties

Chitosan provides antimicrobial activity observed in fungi, viruses, and bacteria (Gram-negative and gram-positive). This is a key property that allows it to be used in areas such as food storage, pharmacy, and biomedicine more than other materials [42-45]. Previous studies showed that the antibacterial property of chitosan is due to its polycationic nature. The polycationic chitosan molecule strongly interacts with the surface of the microbial cells, leading to something like a progressive shrinkage in the cell membrane and eventually to cell death. At the same concern, chitosan bactericidal activity is attributed to the electrostatic interaction between the $(\text{NH}_3)^+$ chitosan groups and phosphoryl groups of the cell membrane component, phospholipid [46]. In general, the polycationic nature of the chitosan molecule communicates with the components of the microorganism's anionic surface antigens (lipopolysaccharides and proteins), which contributes to the release of intracellular response: to fluctuations in the permeability barrier. Also, it prevents bacterial growth by stopping the entry of nutrients into the cell; once in the cell, binds to DNA and thus inhibits the synthesis of proteins and RNA [44, 47, 48].

In some cases, a modification of chitosan is beneficial for developing a nanoantibiotic system able to antagonize both opportunistic and obligatory pathogenic bacteria (Fig. 3). In this study, the authors developed functionalized chitosan with 3,5-dinitro salicylic acid and hooked in antibiotic linezolid. This combination is tough on Methicillin-Resistant *Staphylococcus Aureus* (MRSA) and other microbes [49].

1.3.3. Biocompatibility

Biocompatibility can even be categorized as "compatibility with living tissues or a living system because it is non-toxic, physiologically reactive and does not cause immune rejection". One of the principal characteristics of chitosan is its biocompatibility which allows for the use of chitosan in tissue engineering, wound dressing, bone regeneration, and other related areas [50-53]. *In vitro* and *in vivo* assays have been used to evaluate the cytotoxicity, safety, and biocompatibility of chitosan inside the body [54].

1.4. Chitosan hydrogel

Hydrogels can be represented as a 3-dimensional polymeric matrix composed of natural or synthetic materials that would have a high degree of flexibility depending on their high water content [55, 56]. They can hold significant amounts of water and are distinguished by a

consistency such as soft rubber-like living tissue, making them a suitable material for different utilization. For different applications, chitosan can be used as a hydrogel but the compressive strength, in this case, is fragile. Therefore, the combination of chitosan with other materials is usually used to produce hydrogels. Some methods of producing such hydrogel include radiation crosslinking technology, freeze-thawing, dissolution/lyophilization, and others [57]. The quality of the hydrogels is checked using SEM, FTIR, XRD, DSC / TGA in the same way as other chitosan-based composites. Also, depending on the application, specific features like swelling, elastic modulus, gel resistance, mechanical properties were previously studied. Cheng et al. [58], developed the heat-sensitive chitosan-based hydrogel as a local eye drug delivery system of latanoprost for the treatment of glaucoma. Hydrogel has been characterized based on its rheological properties, the release of drugs *in vitro*, its biocompatibility *in vivo*, and *in vitro* study. The release of latanoprost from the developed hydrogel was achieved and showed a prolonged release profile. In the case of the rabbit glaucoma model, the Triamcinolone-Induced Intraocular Pressure Elevation was significantly reduced over one week. In addition, a weekly topical dose of 250 µg / ml containing latanoprost hydrogel remained within normal limits until the end of the study period. The hydrogel has desirable characteristics which correlate to its delayed latanoprost release. This new composition of chitosan in the future could be a non-invasive alternative to traditional eye drops for long-term intraocular pressure control. Fan et al. [59] produced a chitosan /gelatin/ polyvinyl alcohol (PVA) hydrogel for wound dressings utility. The gelatin was blended in a combination of PVA and chitosan using radical initiator γ -radiation. Various experimental conditions were modified, and hydrogels were widely characterized. The Maximum tensile force was 2.2 MPa. All the hydrogels had a strong clotting effect which further indicated that the hydrogels' hemostatic effect was optimal. Hydrogels have demonstrated strong resilience to water pH, swelling and evaporation rates.

2. Chitosan nanoparticles and nanocapsules form

Chitosan and its derivatives are widely used to develop nanoparticles that increase the bioavailability of the drug when taken orally. They have a good mucoadhesive capacity, enhanced bioavailability, and dissolution rate of hydrophobic drugs. Besides, chitosan improves the stability of labile drugs, the bioavailability, and the controlled release of the drug, as the particles are beyond the nanoscale range to effectively overcome barriers and enhance

permeability. However, this polysaccharide as a drug release system has some disadvantages. Its low solubility at physiological pH affects the intake and causes a pre-systemic metabolism with oral administration of drugs. The ability of chitosan alone to encapsulate water-insoluble drugs is also harmful because the loading efficiency is generally quite low. Various chitosan derivatives have been created and have been proven to be effective, especially when they are in nanoparticle form to remedy these deficiencies [60].

Polymeric nanoparticles are commonly defined as solid colloidal particles of between 10 nm and 1 μ m in size [61]. Nanoparticles are used as a method for the integration and storage of active substances in different fields and consist of macromolecular and/or molecular structures in which active substances such as drugs are dissolved, stored, encapsulated, or even adsorbed, or maintained on the external interface. Nanoparticles can be classified into two major groups based on their morphology: nanospheres, as a rule, are solid structures with a homogeneous matrix structure in which resources are distributed uniformly; and nanocapsules with a traditional hollow shell structure consisting of a polymer membrane and an internal core of the included drug [62].

Among the various types of innovations in nanotechnology that can be found in the nanoparticles and nanocapsules field, it is important to emphasize that both materials are the main target of the inclusion of active substances, have a high carrying capacity, and provide controlled release. The fundamental significance in the use of nanoparticles/nanocapsules in biotechnological applications instead of microparticle systems lies in their ability to increase the absorption rate, boost bioavailability and ensure delivery of targeted drugs for cancer treatment and IV distribution systems, and so on [63]. These systems, as a means of drug release, ensure the targeted release of drugs to cells instead of nonspecific diffusion and improve the solubilization and release of poorly water-soluble drugs, ensure the delivery of two or more drugs together and protect the drugs from undesirable degradations [64].

2.1. Chitosan nanoparticles and nanocapsules preparation methods

A production method in which the chitosan particle size was reduced to a nanometer size was first described in 1994 by Ohya et al [65]. The processes used to produce nanoparticles can be divided into different categories: (i) physicochemical processes in which the nanoparticles are precipitated by using preformed polymers, with yield induced by emulsification-solvent

evaporation, diffusion, or reverses salting-out, [25]; (ii) by in-situ chemical synthesis of macromolecules which lead to polymerizations or interfacial polycondensation reactions; and (iii) mechanical processes using high energy devices such as high-pressure homogenizers, ultrasonic devices, or wet high energy milling. The main methods for producing chitosan nanoparticles and nanocapsules are ionic gelation, emulsification and crosslinking, complexation with polyelectrolytes, self-assembly and drying processes. Some features of each method are listed below with emphasis on the main aspects of the processes.

2.1.1. Ionic gelation

Ionic gelatin is probably the most common method of producing chitosan-based nanoparticles. Chitosan is a cationic polysaccharide able to gel on contact with polyvalent polyanions as tripolyphosphate (TPP). This interaction led to the formation of electrostatic interactions between opposite charges of the components [63, 66]. At acidic pH, it can spontaneously transform into particles in the submicron size. This process seemed to be very interesting because reproducible nanoparticles can be obtained in a soft medium (aqueous medium) and avoid the use of organic solvents and high temperatures. Using chitosan TPP the nanoparticles were prepared by dissolving it in a diluted solution of glacial $\text{-CH}_3\text{COOH}$. The resulting solution was magnetically stirred at room temperature until completely dissolved. Therefore, the pH increased to the expected value by adding NaOH. A TPP solution can be prepared in various concentrations by dissolving the TPP in high purity water. Both solutions are filtered through a small pore filter. Then, the TPP solution is added dropwise to the chitosan solution with constant stirring until it forms an opaque suspension, which indicates the formation of particles with average diameter controlled by the molar ratio of chitosan/TPP [67]. The mechanism of chitosan nanoparticles formation by ionic gelation using TPP as well as particle internal structure and surface topology are determined by the following parameters the degree of acetylation and MW of chitosan, intrinsic viscosity, concentration, and the molar ratio of -NH_3^+ /TPP [68]. Other factors may also influence the properties of the nanoparticles, such as operating temperature, stirring speed, and flow rate of TPP addition. This can affect particle size and polydispersity, as they appear to significantly reduce the amount of nanoparticle aggregation. Smaller particles can be isolated from larger particles by filtration and ultracentrifugation [69].

This method effectively captures small hydrophobic and hydrophilic molecules, as well as peptides, proteins, and genes. The formation of electrostatic interactions within the components of the matrix is considered to be the main retention mechanism of these biologically active compounds. In addition to TPP, other polyanions can be used for crosslinking, for example, the active molecule adenosine triphosphate (ATP), fucoidan, and sodium metabisulphite ($\text{Na}_2\text{S}_2\text{O}_5$) [70]. Catechol-containing molecules such as hydrocaffeic acid have demonstrated the ability to increase the mucoadhesion of various polymers, including chitosan. Chitosan/TPP hydrocaffeic conjugated nanoparticles obtained by ionic gelation showed 6 times greater mucoadhesion in the rabbit's small intestine than the unmodified nanoparticles [71]. Besides, by the same technique, chitosan/calcium pyrophosphate hybrid microflowers shape was prepared and efficiently applied in dye removal and enzymatic catalysis (Fig. 4) [72].

2.1.2. Polyelectrolyte complex (PEC)

PEC's forming of chitosan nanoparticles is based on the ionic interaction of chitosan with negatively charged polymers. The process occurs when polymers are mixed with opposite charges and lead to self-assembly or the formation of strong reversible electrostatic bonds in self-assembly systems. These direct interactions between the polymer chains result in the formation of complex polyelectrolyte networks with proportional structures, thus avoiding the use of covalent crosslinking agents. In particular, these polymer network systems are commonly used as biocompatible hydrogels and are sensitive to changes in surrounded environmental conditions [73]. The emergence and stability of the PEC are based on several factors including the degree of ionization within each polyelectrolyte with an opposite charge, density of the charges present in the structure of each polyelectrolyte, concentration, and proportion of polyelectrolytes in the mixture, a sequence of the mixture and the type and location of ionic groups. In polymer chains, the stability is also correlated with the MW of the polyelectrolytes, the flexibility of the polymer chain as well as the temperature, ionic resistance, and pH of the reaction medium [74]. In the presence of a common solvent, the excess polyelectrolytes charges (positive or negative) are consolidated, and a non-stoichiometric complex is formed. In contrast, the stoichiometric polyelectrolytic complex contains equal quantities of each opposite charge, and the total charge in the resulting complex is zero. In the case of chitosan, various polyelectrolyte complexes can

form when the positively charged surface of chitosan interacts with anionic biopolymers such as sodium alginate, pectin, carboxymethylcellulose, chondroitin sulfate, dextran sulfate [75], etc.

2.1.3. Drying processes

This process is frequently used in drugs, chemicals, cosmetics, materials, and food science technology. The first patent for this technology originated in the early 1870s. Even during the drying process, the water/solvents are separated through evaporation from liquids, solids, or semi-solids and the resulting vapor is collected by vacuum. Hot air, a microwave oven, spray drying, freezing, supercritical drying, and natural drying with air are common methods for drying. The techniques of spray drying and supercritical drying are often used in the production of chitosan particle systems because they are fast, light, continuous, reproducible, and adapt well to the active material. They are scaled-down in one phase without modification. These techniques are important and can achieve particles of various sizes and high stability [76]. Spray drying has been reported to be used to produce dry granules and powders from mixtures of drugs and excipients in the form of a solution or suspension. The versatility of this approach has been developed to include the synthesis of microparticles from various protein-filled polymeric materials, vaccine antigens, and therapies. Spray drying provides a comprehensive, convenient, one-step, and protein-friendly method for protein-loaded chitosan micro/nanoparticles [77]. Besides, it is widely used in compounds including heat-sensitive substances such as extracts, pigments, drugs, aromatic oils, and flavors in various types of conveyors, which lead to the formation of polymeric nanoparticles or microparticles as well as nanocomposites [78]. The fundamental theory of spray drying is dependent on the use of a flux of hot air to dry spray drops. This method requires the preparation of an aqueous chitosan solution that is sprayed into a drying chamber through a nozzle to achieve the desired particles. The resulting particles have a smooth surface, a spherical shape, and a reduced size distribution, and they also exhibit good drug stability with high efficiency when the drug is turned on [79]. Other benefits of this process include the ability to attach the surface and cover the balls in the stages of development which can increase stabilization and control the release of the drug. The size of the particles produced is affected by the velocity of the sprayed flow, the degree of crosslinking, and the flow rate of the airflow [80].

2.1.4. Emulsion system (diffusion process of oil/water emulsion)

Nanoparticles can be obtained using nanoemulsion in various ways. Emulsification processes usually require high mechanical energy to obtain small droplet sizes. The low power method should be considered according to the physical behavior of the system based on the mixing of oil and water in the presence of surfactants [81]. Firstly, a dispersion of chitosan in a glacial acetic acid solution is prepared as an aqueous component. Then, a drop of a hydrophobic solvent, such as cyclohexane, cyclohexanol, soybean oil, or another solvent with similar properties is added to the chitosan solution, all mixing continuously. The resulting mixture is treated with a surfactant, for example, Triton X-100 or Tween with constant stirring until the mixture becomes translucent, which indicates the formation of emulsion containing nanoparticles [82]. Another option is to pre-mix the surfactant in one of the solutions, saving a step in the process. In general, the particles formed in the emulsion system have nanocapsules structures, high drug loading efficiency, and better bioavailability [83].

Changes in the methodology can contribute to the formation of nanocapsules, the use of ultrasound and an increase in the reaction temperature can collaborate with the process. There are various modifications in the preparation of an emulsion using water/ oil or oil in the water system, for example, the addition of gelling agents such as chlorides, TPP, or polycations is performed [84]. Although the emulsification method facilitates particle size control, strong crosslinking agents are used in this process and complete removal of unreacted crosslinking agents can be an issue [85].

2.1.5. Self-organized nanoparticles

Self-assembled nanoparticles can be formed in aqueous media of amphiphilic polymers. Hydrophobic interactions between amphiphilic polymer components tend to minimize interfacial energy and allow nanoparticle formation. Although chitosan is not an amphiphilic biopolymer, the self-organizing nanoparticles can be obtained by structural modification of chitosan to bind a compound such as a palmitoyl, which causes self-aggregation of chitosan molecules [86]. The hydrophobicity of chitosan is increased with the introduction of phthaloyl, polyester, fatty acids, cholesterol [87].

Structural changes in chitosan derivatives can make it amphiphilic, resulting in nanoparticles self-assembling and be used as drug delivery vehicles. Besides, the quaternary chitosan

derivatives were synthesized using (5-pentyl) trimethylammonium and dodecyl aldehyde. Auto-aggregates formed in an aqueous solution have a dimensional distribution of 250 to 500 nm and have a hydrophobic core, which can be used as a carrier for hydrophobic substances [88].

2.2. Chitosan nanoparticles and nanocapsules characterization techniques

Dimensions and size distribution are important tools because of their ability to recognize the physicochemical and pharmaceutical behavior of the compounds present in the systems. Depending on the form of preparation, laser diffraction and a coulter counter can be used to determine particle size. However, the most common method is dynamic light scattering (DLS), which is used for measuring particle size distribution. The uniformity of the particle size is obtained by using polydispersity (PDI), with PDI values between 0.10 and 0.25 indicating a reduced dimensional distribution and higher values indicating a wide dimensional distribution [89]. Particles containing chitosan or other charged molecules show a zeta potential which corresponds to the electrical potential represented by the surface of the material in the solvent and is highly dependent on the particle's composition. The zeta potential uses the electrophoresis principle that reflects the electrical charges on the surface of the particles. Electrical fields are induced from scattered particles, whereby the particles move to the oppositely - charged electrode at a rate comparable to the zeta potential value. For a dispersion system, a high level of negative or positive zeta potential indicates the stability of the particles towards aggregation, which means a stable system. This parameter can be associated with particle stability and the zeta potential values less than ± 30 mV contribute to low stability leading to a possible aggregation of the particles [90]. Various microscopy methods can be used to confirm particle size, to observe and characterize the morphology and structure of these nanoparticles. Atomic force microscopy (AFM) and SEM are some examples of devices used for this purpose, although transmission electron microscopy (TEM) is the most used method since provides information on the structure, shape, and actual particle size as well as an estimation of the wall thickness in capsules [91]. The structural modifications of chitosan were mainly characterized by FTIR, ^1H -NMR, and ^{13}C -NMR. These modifications are generally used to illustrate the physicochemical properties of the resulting nanoparticles, to detect interaction with active substances, and study how chitosan binds with specific cellular receptors [91].

The specific surface characteristics of the resulting particle system can be integrated by X-ray photoelectron spectroscopy (XPS). This technique provides details on the elements through which particles, chemical bonds, and extensive high sensitivity molecular information derive. Besides, XPS extends a detailed composition of the surface and the proximal surface chemistry analysis of the particles [92].

2.3. Applications of chitosan nanoparticles and nanocapsules

Nanosystems based on chitosan can be used in various fields of applications, depending on the system's preferred structure and chemical aspects of biopolymers, such as pKa, degree of deacetylation. Chitosan has two types of hydroxyl groups in its structure: the primary hydroxyl group in the Carbon-6 position and the secondary hydroxyl group in the Carbon-3 position in the glucosamine unit. Additionally, the Carbon-2 position works for a group $-NH_2$. Therefore, the structure of chitosan can be manipulated and modified by inserting new functional groups or through cross-linking reactions, whereby the obtained derivatives are appropriated for the desired application. Chitosan nanoparticles modified with acetophenone by Schiff-based condensation were fabricated by ionic gelation in the presence of TPP as a gelling agent. The excellent adsorption capacity was observed towards various divalent metals: Cu^{2+} , Cd^{2+} , Hg^{2+} , and Pb^{2+} at pH 7.0. It was being used to eliminate these metal ions contaminating tap water, seawater, and industrial wastewater, as a result of which the adsorption balance is established within 30 minutes after the contact time between the absorbent and metal ions [93]. Also, the 100 nm chitosan nanoparticles can remove the hexavalent chromium (Cr^{6+}) from water, which requires the presence of TPP for the efficient adsorption of the metals. Cr^{6+} is adsorbed on the surface of chitosan/ TPP/nanoparticles and reduces Cr^{6+} to Cr^{3+} , the reduced form of Cr^{3+} is less or non-toxic. Consequently, this system can be effectively used for detoxification and purification of water from Cr^{6+} [94]. Co^{2+} , Ni^{2+} , and Cu^{2+} can also be removed from water using modified chitosan nanoparticles by preparing chitosan -poly(methacrylate) using PEC technique with particle size from 100 to 120 nm [95]. Recently, modified chitosan nanoparticles were used to eliminate Cr^{6+} and/or Rhodamine B (RHB) dye from a complex water system. About 98.4% of Cr^{6+} and 96.2% of RHB was adsorbed on the surface of modified chitosan nanoparticles. The mechanism was disused based on hydrogen bonding interaction, electrostatic attraction, and π - π stacking force (Fig. 5). Accordingly, this nanoadsorbent system is preferential adsorbent

compared to others reported materials dealing with Cr^{6+} and RHB pollutants owing to the functionality of the unsubstituted amino group in the chitosan structure. Besides, green microwave method was adopted in this paper which save cost, time, and required low energy for complete the functionality process [96].

In another study, chitosan or starch were added to poly (vinyl alcohol) NPs (PVA_n) as nano adsorbent for oil spills from wastewater [61]. The optimal adsorption amount ($q_{\text{g/g}}$) of PVAn blended chitosan was 33.6, 73.96, 93.1 g/g for hydraulic oil, kerosene, and toluene oils, respectively. This study reveals the correlation between different types of oil and their adsorption efficiency, and the mechanism was illustrated based on the diffusion mechanism. The incorporation of chitosan enhanced the reusability performance of the binary blend even after six cycles. This property produces adequate recoveries between 71.8 to 96.3% correlating to the high mechanical strength of the nanoblends which can be used efficiently in the practical application of oil spill recovery (Fig. 6).

In agriculture, chitosan nanoparticles have been used as herbicidal and pesticidal formulations of such compounds as paraquat, clomazone, and acetamiprid. Paraquat is a non-selective herbicide that is often used to control weeds. However, its solubility in water and soil absorption can cause toxicity in humans. This herbicide has been encapsulated in chitosan /alginate and chitosan/TPP nanoparticles produced by Ca(II) complexation [97] and by the ionic gelation technique [98], respectively, with an obtained diameter of about 635 and 300 nm. These systems have shown less toxicity than a pure compound, which can reduce adverse effects on human health and the surrounded environment. Clomazone is a main component in rice fields and has been encapsulated in chitosan/alginate nanoparticles with a particle size of about 200 nm [99].

Polyphenols (PPH) are common in vegetables, fruit, tea, and coffee. PPH is of great importance for human nutrition because of their nutritional value and their impact on the prevention of chronic diseases. Furthermore, they have been examined for their antiallergic, antibacterial, anti-inflammatory, antitumor, antiviral action, and above all, for their strong antioxidant activity [100]. Nanoparticles based on chitosan have been used to encapsulate polyphenols extracted from various sources. The polyphenols in tea extract have been successfully encapsulated with chitosan nanoparticles with an average size of 150-350 nm [101].

Chitosan is used in the nutrition and food industry to protect food or certain nutrients for human consumption via packaging and control of foodborne pathogens. The nisin-containing chitosan -carrageenan nanocapsules were obtained using the PEC method with the smallest particle size of approximately 500 nm. This method was tested *in vitro* to assess the antimicrobial effects against *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Enterobacter aerogenes*, followed by an antibacterial activity test in tomato juice [102].

Small interfering RNA (siRNA) may be an effective candidate for disrupting genes in undesirable eukaryotic cells that are associated with diseases such as cancer, viral infections, and other genetic conditions. The siRNA consists of 21–23 base pairs that can induce a reduction in target genes [103]. Due to its positive natural properties, chitosan-based nanoparticles provide electrostatic interaction with negatively charged siRNA and thus improve their bioavailability. Therefore, siRNA is destructive for DNA and achieving a high level of safe gene silencing [104]. Studies conducted on a mouse model of ovarian cancer have led to significant transfection and *in vivo* accumulation of cells, leading to effective inhibition of uncontrolled proliferation *in vivo*. In this study, the nanoparticles-lactate of polyethylene glycol / folic acid chitosan oligosaccharide were obtained by ion gelation and showed an average size of about 200 nm. Various other studies have shown that chitosan-based nanoparticles are useful for the encapsulation and release of siRNA [105]. The small interfering RNA was successfully incorporated by ionic gelation into the chitosan TPP with a particle size of 78-200 nm [106]. These systems are *in vitro* or *in vivo* tested against melanoma B16 cells [107]; Lung cancer [105]; Cardiomyoblast cells [108]; breast cancer [109]. According to the drug delivery line established by chitosan and modified chitosan nanoparticles, the treatment of brain diseases (tumor diseases, neurodegenerative, cerebrovascular) in which drugs must penetrate the brain has been improved for these systems. Indeed most of the compounds do not reach the cerebral blood flow barrier due to the protection of the brain guaranteed by the regulation of the blood-brain barrier (BBB) [110]. The drugs were administered via the nasal passage to overcome the limitation imposed by the BBB. They settled in the respiratory epithelium, entered the system circulation and therefore the BBB could be crossed [111].

Chitosan exhibits mucoadhesive properties, which have been carefully studied and improved by modifying the structure of chitosan. Various systems have been used to incorporate many

drugs, such as a chitosan-coated lipid matrix containing lipophilic dye Nile Red and near-infrared dye Dir [112]; Buspirone hydrochloride encapsulated in chitosan nanoparticles thiolate obtained by ionic gelation (200-600 nm) [113]. The intranasal pathways using chitosan nanoparticles have shown effective biocompatibility and biodistribution through the human bronchial epithelium (16HBE14p) [114]. Also, chitosan nanoparticles have been used in various diseases, for example, anxiety disorders [115]; and Neuroprotection in Alzheimer's disease [116]. Further, the mucoadhesive property of chitosan may also be useful for the administration of drugs for controlled oral, nasal, ocular [84], vaginal [117] local, and transdermal administration [81].

The mechanism responsible for the penetration effect is based on its positive charges. However, a more pronounced cationic property was obtained by trimethylation of the primary amino group without improved permeability properties indicating a more complex mechanism than expected [118]. High molecular weight and a high degree of deacetylation improve epithelial permeability. PEGlation, N-succinylation, and thiolation can also significantly improve their mucoadhesive properties. Thiolated chitosan forms disulfide bonds with glycoproteins in the mucosa and the effect can be increased using mercaptonicotinamide [119].

3. Chitosan nanofibers

Nanofibrous scaffolds have attracted an intensive interest in the last years due to their feasibility to be used for numerous applications including water filtration, wound healing, dressing, and medical clothing [120]. It might be noticed that the obtained accompanied properties of produced nanofibers are susceptible to the preparation conditions. These conditions include the type of polymer, viscosity, injection rate, voltage, orientation, and even the humidity [121]. The obtained properties might be changed upon variation of the former conditions involving fibers' diameters, porosity content, and surface roughness. Therefore, the selection of the polymeric phase with adequate conditions could lead to a formation of nanofibrous scaffolds with appropriate behaviors. For instance, both fibers' diameters and content of porosity are the main controlling factors of mechanical properties. However, using these scaffolds for tissue engineering applications is preferred to be conducted with a highly porous surface. This is because high interconnected porosity could introduce a simple way for vascularization besides nutrients and oxygen transformation. Furthermore, keeping the moisture of injured skin could

encourage fast healing; therefore, tailoring the properties of the fabricated electrospun nanofibrous scaffolds should meet the requirement of the suggested application.

It can be mentioned that chitosan has been used intensively for heavy metals removal from aqueous solutions and preferred rather than different nanocomposites in the removal efficiency [122-126]. The presence of amino and hydroxyl anions in chitosan structure might induce electrostatic forces towards the targeted ions, other physical interactions help for adsorption of ions through a porous surface. Otherwise, besides nanofibers, chitosan is produced in numerous shapes for various utilizations including balls, micro/nano-particles, or sponges[127].

3.1. Production of chitosan nanofibers

There are several methods for producing chitosan nanofibers, such as electrospinning forcing, blowing a solution, coaxial electrospinning, centrifugal electrospinning [128]. Herein, we introduce a comprehensive description of techniques used for the preparation of chitosan nanofibers and their applications in different fields of technology.

3.1.1. Electrospinning

Nanofibers might be produced via different techniques, however, one of the most facile and low costed ones is the electrospinning technique [129, 130]. The technique is based on applying a high voltage on a polymeric solution to be elongated and sputtered on a moving/fixed substrate to produce nanofibers. The optimum behavior of electrospun nanofibers could be assigned to the high surface to mass ratio ($\sim 40\text{-}100\text{ m}^2/\text{g}$), which can be controlled considerably [131].

The electrospun nanofibers configuration is established on a uniaxial tension for a viscoelastic gel. A typical electrical torsion formation contains three main components: a capillary tube gathered to a needle, a high voltage source, and a substrate for fibers' collector, which is illustrated in Fig. 7 [132].

It is obvious that in the electrospinning technique, a high electric field is required and applied over the distance between the syringe tip and the collection plate. The polymeric viscous gel is placed in a syringe and injected into a pump, which creates a steady flow. As soon as the electric field reaches a certain critical value that the repulsive electrostatic forces exceed the surface tension, the flow of charged fluid is expelled from the conical protrusion, which is known as the

Taylor cone. Finally, the jet lengthens and breaks constantly, as a result of the nanofibrous scaffolds are deposited on the collector with instantly fast solvent evaporation [133].

The important parameters in the electrospinning process are solution parameters and process parameters. The parameters of the solution are determined dependent on the characteristics of the solvent and the polymer, including type and concentration of the polymer, molecular weight, viscosity, surface tension, dielectric constant, and conductivity. The process parameters include the voltage, distance between the tip of the nozzle and collector, flow rate of the polymer solution, design and position of the nozzle, composition of the collector, geometry, and speed of rotation. Environmental parameters include temperature, humidity, and air velocity [134, 135].

Concerning solution parameters, the increase in polymer viscosity or concentration contributes to increased fiber diameter. However, if the viscosity of the polymer solution is too low, the ejected jet can decompose into droplets and lead to electrospray [136]. As a rule, low surface tension values lead to the formation of pearl less fibers, consequently, low stresses can be applied during electrospinning. Also, low conductivity polymer solutions cannot be welded, as there is no surface charge on a drop of liquid needed to form a Taylor cone. On the other hand, high electrical conductivity causes a decrease in the tangential electric field across the surface of a drop of liquid thus inhibits Taylor's cones from forming. Electrospinning is a successful machine for generating controlled electric voltage on a polymer solution dissolved in a solvent with high dielectricity constants that led to the formation of thinner nanofibers [137]. Applying high voltage on the charged solution boosts the electrostatic force, which increases the jet's elongation, resulting in a thinner diameter of the fiber. Voltages between 5 and 40 kV are generally applied during electrospinning according to most of the automatically established machines. A larger distance between the row and the collector can increase the elongation time due to the obtained thinner fiber length. Some high volatility solvents, high temperatures, and low humidity will support solvent evaporation and causing porous fiber surface [138]. However, in some cases some of non-polymer molecules can be electrospun without the use of polymer as a carrier [139].

Chitosan has proven to be a problem for the electrospun. Unfortunately, the polycationic nature of chitosan in acidic aqueous solution with a high number of amino groups in its backbone prevents chitosan to be electrospun. To date, nanofiber structures have been successfully obtained

by electroforming chitosan solutions in 90 wt. % aqueous acetic acid or by using a certain solvent such as trifluoroacetic acid (TFA), dichloromethane (DCM), and formic acid. However, traces of solvent or toxic organic acid in the products are harmful, especially if applied to human skin or injured tissues [140].

Recently, the nanofiber mats have been made of electrospun chitosan with biodegradable synthetic polymers such as PVA, polyethylene oxide (PEO), poly (vinylpyrrolidone) (PVP), poly (lactic acid) (PLA). Polycaprolactone (PCL) and polyethylene terephthalate (PET). The biocompatible, mechanical, antimicrobial activity, and other properties of chitosan nanofibers have been significantly improved with the addition of PVA, PLA, PCL, PEO, PET, and PVP [141]. To improve the biological efficiency of chitosan-derived nanofibers, researchers sought to squeeze chitosan using natural polymers such as collagen, gelatin, and elastin [142].

3.1.2. Coaxial electrospinning

Coaxial electrospinning is a modern and economical approach for producing coaxially structured nanofibers. The typical coaxial configuration consists of two concentric capillaries (two different solutions), the solutions of the core and the shell of which they come out and join at the end, forming a core-shell nanofiber (Fig. 8). The key benefit of this approach is to strengthen material that is less rotating using a highly rotating material [143].

As a result, various materials including inorganic and organic compounds have been successfully combined as functional nanocomposites for high added value. The technique has been widely used to control secondary fibrous structures, to encapsulate drugs or biological agents in fibers, to produce nanofibers from non-fibrous materials, and to incorporate functional fluids into the fiber matrix. Various biologically active drugs and substances, such as DNA, proteins, antibiotics, can be entrapped directly into the nucleus that is protected by the formation of the upper layer [144].

It has been stated that some hard-to-process polymer solutions could be co-electrospun to create ultrafine cores in the shells of other polymer materials. Chitosan nanofibers have recently been obtained by coaxial electrospinning. Core and coating nanofibers can be made of synthetic polymers with high spinning properties. Recently, produced chitosan nanofibers by coaxial electrospinning using PEO as a coating model were obtained, optimum ones, in the condition of

merging chitosan with PEO at the ratio of 80:20 shell to the core, voltage 25kV and the flow rate 3ml/hr. [145].

3.1.3. Characteristics of chitosan nanofibers

Nanofibers have a large surface area, small pore sizes, and the ability to create three-dimensional structures. Also, its chemical, physical, and biological properties attract attention in the field of materials science, mechanics, electronics, medicine, plastics, and energy. Therefore, characterization methods are needed to effectively track the size, morphology, and dimension of these nanomaterials to evaluate and develop their production process. Also, it is necessary to know the properties of nanofibers which determines whether the resulting material is suitable for a specific application, and some of these characterizations are shown in Table (1) [146].

4. Some applications of chitosan nanofibers

Chitosan nanofibers can be used in various fields mainly due to the presence of -NH₂ and -OH groups, along with their structure. Some of the possible uses of chitosan nanofibers are in the areas of drug delivery, biosensor, food packaging, water purification, tissue engineering, and wound dressing. This biopolymeric nanofibrous offers enormous structural possibilities for chemical modifications that create new properties, particularly in the biomedical field.

4.1. Purification and treatment of contaminated water

The demand for high-quality water has increased in recent decades [96, 125, 147, 148]. Drinking contaminated water can cause millions of deaths every year, mainly due to pathogen contamination. Membrane filtration is a recognized technology for reducing the contamination of drinking water. Further, bacteria can be easily killed by the ultrafiltration membrane upon using antibacterial surfaces that were made up of silver ions and other metals [149]. Also, the viruses were removed through nanofiltration membranes according to the size exclusion principle. One of the materials used for filtration is chitosan nanofibers owing to their significant properties in wastewater treatment, agriculture, pharmaceuticals, food science, textiles, and biotechnology [150]. Recently, chitosan nanofibers have been extensively studied for water treatment. This nanofiber material is described as a natural polymer, which is suitable for the elimination of heavy metals and colored dyes from water. β -cyclodextrin/chitosan/PVA nanofiber webs were

designed by electrospinning and utilized in the dynamic adsorption of organic micropollutants and heavy metal ions from water [151].

Surgutskaia et al. manufactured aqueous PEO/chitosan nanofibers to adsorb nickel (Ni^{2+}), cadmium (Cd^{2+}), lead (Pb^{2+}), and copper (Cu^{2+}) from a solution. The authors observed that the maximum adsorption capacity of Ni^{2+} , Cu^{2+} , Cd^{2+} and Pb^{2+} ions through a PEO/chitosan nanofiber membrane follow the order: $\text{Ni}^{2+} > \text{Cu}^{2+} > \text{Cd}^{2+} > \text{Pb}^{2+}$. The measured thermodynamic parameters, ΔG° , ΔH° , and ΔS° demonstrated that the adsorption of this system was feasible, spontaneous, and endothermic in nature. After five cycles of adsorption-desorption, the reusability was calculated and the results showed that the PEO/chitosan nanofibers can be widely used in the continuous adsorption process [152].

Recently, created chitosan nanofibers have been functionalized with TiO_2 NPs to remove heavy metal ions without electrospinning [153]. The authors showed that Au@TiO_2 was successfully prepared using the photo precipitation process and then loaded into a matrix of chitosan fibers, the resulting material showed plasmonic fibers effect. Photocatalytic and photoreduction efficiency were studied for many pollutants that were exposed to visible light irradiation. Plasmon fiber has a high decomposition efficiency compared to Au@TiO_2 .

4.2. Wound dressing material

The antibacterial resistance of microorganisms, as a rule, is one of the main problems in the field of wound care and their treatment, which leads to complications such as infections and delayed healing [154]. An appropriate dressing ought to be capable of absorbing exudates from the wound area and this allows water to evaporate at a certain point, preventing microbial transport [155]. Several studies are primarily developed innovative prophylactic antibiotics resistant microorganisms contaminated wounds. Titanium fibers of chitosan have been considered dressings because of their high efficiency against different bacterial strains [156].

Antimicrobial mats from colloidal nanofibers dispersions of AgNPs based on chitosan mixed with PVA for wound dressings were constructed by electrospinning [157]. This study showed that the PVA/ chitosan blend has a well antibactericidal activity against different bacterial strains. Also, the rotating webs made from the mixtures including AgNPs were better. Thus, the

involvement of AgNPs in combination with PVA/ chitosan solutions has enhanced not only electrospinning efficiency but also the antibacterial ability of electrically shaped fibers sheet.

In a recent study, honey was added to the PVA/chitosan polymer matrix before electrospinning to evaluate the ability of these nanofibers to heal a wound. The results showed that PVA/chitosan/honey has a positive effect on the healing of burn wounds. This study reported that topical use of this bio fiber displayed significant healing activity in the burning region [158].

The capabilities of 3D printed chitosan for chronic wound healing utilization were used. The results displayed that after 24 exposure to the human fibroblasts cell line, more than 90 % of cells still were viable, which demonstrated the high biocompatibility of these scaffolds and encourage their utilization for clinical applications [159]. Furthermore, N. M. Ergul et al. examined 3D printed polymeric blend containing chitosan/PVA encapsulated with hydroxyapatite (HAP) for tissue engineering. It was shown that grains size of HAP was around 1.21 μm , while the elasticity modulus of the fabricated scaffold reached around 91.14 MPa for the ratios of chitosan/PVA/HAP (15 wt. %) based on the added contents of HAP [160]. In addition, Min Zhu et al. studied the photoactivated antibacterial behavior of chitosan films doped with Ag/MoS₂. The results exhibited that antibacterial efficiency reached around 98.66% and 99.77% against *S. aureus* and *E. coli*, respectively under visible light after 20 minutes of continuous exposure [161]. Moreover, A.A. Menazea et al. investigated the effect of AuNPs embedded through a polymeric blend of chitosan/PVA for wound healing applications. They found that photoactivated antibacterial blend illustrated great potency towards both *E. coli* and *S. aureus* with inhibition zones of 13.1 $\pm$ 1.3 mm and 14.8 $\pm$ 2.4 mm for the highest additional AuNPs [162].

4.3. Tissue engineering

Polymer nanofibers are still of significant concern in regenerative medicine as building materials for restoring, preserving, or enhancing the function of human tissues damaged due to aging, sickness, injury, or birth defects. Tissue engineering can also be segregated into several segments depending on the tissue/organ class such as bone, ligaments, cartilage, tendons, liver, nerves, and skin [163]. There are some widely accepted basic requirements for the construction of polymer forests. The structure must have high porosity with the adequate distribution of pore sizes, a large surface, biodegradability, adequate mechanical properties, and biocompatibility.

Various biocompatible polymers have been developed from natural materials such as collagen, elastin, laminin, etc. [164].

Poly(ϵ -caprolactone)/chitosan nanofibrous was fabricated using an electrospinning technique to create bone and skin tissue [165]. The presence of chitosan in the chitosan /PCL structure led to a significant improvement in hydrophilicity. Further, the cytocompatibility of the chitosan /PCL framework was studied and proved to be non-toxic. All these results showed that the chitosan /PCL nanofiber structure would be an excellent system for bones and skin tissue engineering.

Another example produced chitosan /PVA fibers with CaCO_3 incorporation. The authors observed that a tensile test of fibers reinforced with CaCO_3 at content 4% by weight gave an elastic modulus sixty times higher than the value of the fibers without CaCO_3 . The biocompatibility of the CaCO_3 -loaded fibers was also tested in a 14-day incubation with simulated biological fluid (SBF) to induce the formation of apatite minerals and in a 14-day analysis of cell proliferation with ATDC5 cells. Chitosan/PVA- CaCO_3 is perhaps the most effective medium for cell growth as compared to chitosan/PVA and chitosan/PVA-apatite. CaCO_3 -enhanced chitosan / PVA can be a viable substitute for cartilage [166].

4.4. Delivery of drugs

Owing to large specific surface area nanofibers can deliver platforms for drugs, peptides, and vaccine antigens. The release of the drug may be immediate, delayed, or modified depending on the type of interaction between the polymer with the drug [167]. Usually, an immediate release is noticed when a composition of a water-soluble substance and a water-soluble polymer is used. However, the extended-release profile can be tailored by integrating the drug into other nanocarriers, such as nanoparticles, liposomes, dendrimers, then loaded into nanofibers or use hydrophobic polymers [168].

Chitosan nanofibers have recently exhibited controlled drug release affinity [169]. This material may be useful for the delivery of proteins/peptides, anti-inflammatory drugs, antibiotics, imaging agents. Fabrication of poly (d, l-lactide-glycolide) (PLGA)/ chitosan nanofiber structure by electrospinning was also assessed [170]. The results of this study showed that the drug release rate increased with increasing chitosan content, as the addition of chitosan improved

hydrophilicity. The release rate from an aligned PLGA nanofiber skeleton was also lower than that of a randomly oriented PLGA/ chitosan nanofiber skeleton.

Gold nanorods (AuNRs) containing double-fiber hybrid PVA/ chitosan nanofibers were prepared using a simple electrical conjugation process. The authors found how these nanofibers are used as carriers for the doxorubicin anticancer (DOX) drug that is inserted into the nucleus of the cells. The integrated AuNRs and DOX in fibers effectively inhibit the growth and proliferation of ovarian cancer cells and, thanks to their unique optical properties, can also be used as a cell imaging medium. The nanofiber matrix, which integrates two roles of cell imaging and the delivery of drugs may have great potential for use in biomedical fields [171].

5. 3D chitosan printable scaffold

5.1. Chitosan printed in cartilage and bone regenerative medicine

The architecture of chitosan and its stability in physiological environments are very analogous to that of glycosaminoglycan. Furthermore, chitosan degradation products are often elements of cartilage synthesis. These advantages of chitosan are widely used in the construction of bones and cartilages. Also, a coating of chitosan on the PCL scaffold contributed to better fixation and penetration of the cells. It is also recommended the chitosan to be mixed with other materials, such as biological glass and collagen, to improve the chemical, osteoconductive and mechanical properties of chitosan [172]. In a previous study, a new rapid prototyping system was developed for the delivery of robots to attain chitosan/HAP structures [173]. They added the HAP/ chitosan base to improve the osteoconductivity and mechanical strength of the scaffolds. In their analysis, they employed acetic acid to create chitosan hydrogel and HAP chitosan solution, supported by extrusion to obtain a gelatinous precipitate in a bath mixture of sodium hydroxide and ethanol. The increased use of sodium hydroxide led to the rapid deposition and poor mobility of the chitosan molecules at the edges of the contact structure. Conversely, a very low concentration of sodium hydroxide reduces the deposition rate and causes poor dimensional stability. Tests on cell culture showed acceptable results as well. Rapid prototype robotic delivery (RPBOD) may therefore be an effective method for producing chitosan-based structures.

Ascertaining printing parameters such as outlet pressure, exit speed, and initial exit height was a problem in a study by Ang et al. [173]. These parameters depend on the solution's

viscosity; therefore, they differed for each composition and concentration. The macroscopic analysis, such as visual inspection, was used to solve this problem and establish coherent and uniform filaments. It is difficult to select the right binding to 3D print. It has been documented that different acids have been studied, such as citric acid, phosphoric acid, lactic acid, and sulphuric acid [174]. It was found that a shorter curing period was obtained with 20-40 wt.% of binding citric acid, and the 30-40 wt.% lactic acid [175].

Several chitosan binders were tested for 3D printing and obtained nearly identical results for lactic acid. They were double-checked using lactic acid, citric acid, and acetic acid as the binder by printing a similar composition (chitosan/HAP). They found the 40 wt. % lactic acid boosts medium viscosity, fast solidification time, and high wettability. Therefore, lactic acid has the correct structure relative to other binders.

There was an improvement in structural stability with a ratio of 20 wt. % of chitosan and 40 wt. % of binder lactic acid and after processing 40 wt. % of lactic acid. The three-dimensional printing structures, which consist of 25 wt. % of chitosan and 75 wt. % of HAP is shown in Fig. 9. The resulting printed samples showed a compressive strength of 16.32 MPa, an elastic modulus of 4.4 GPa, and a porosity of 37.1%. The low porosity is due to the destruction of the open pores due to the high concentration of lactic acid. They thought that the chitosan chemical structure allows attractive bio-stimulation. Their findings suggested that the elevated chitosan /HAP structure is a promising alternative to the bone regeneration process.

In another study, the mechanical property and cytocompatibility of a hybrid constitute synthetic polymer (polyethylene glycol diacrylate (PEGDA)) and chitosan was evaluated [176]. This study showed that chitosan molecular weight and the feeding ratio are effective parameters in the shaping and precision of the structure. The ratio of PEGDA to nutrition with chitosan was set at 5:1 and 10:1, which was the optimal range. Low chitosan molecular weight requires an excessive concentration of sodium acetate due to its higher deacetylation to neutralize its protonated amino groups. They used Stereolithography (SLA) to create frames, while a 7.5:1 ratio showed a boost printability effect. SLA can monitor the frame composition and porous structure, depending on the anatomical requirements of the patient. They designed bezels in an ear-shaped form with a laser beam of 405 nm. The presence of strengthened chitosan in the resin increases the coefficient of swelling. Adequate mechanical properties and enormous potential for

cell attachment contributed to the growth of an innovative design for the cartilage tissue engineering field with complex geometry.

Also, chitosan /alginate was chosen as the desired combination because of its similarity to natural cartilage glycosaminoglycans (GAGs) such as hyaluronic acid, keratin sulfate, and chondroitin sulfate. The compositional form can give a desirable property such as compressive strength or swelling potential. Besides, a neutral pH skeletal preparation provides a green environment that guards the combined growth factor for a drug against denaturation. They had to use a modern manufacturing process, integrating 3D printing with directional freezing to monitor building micro- and macro-channels. The macro channels were generated using 3D printing of positive and negative shapes in the methodology used, and the microchannels were developed by spatial freezing of the solution. These scaffolds with simultaneous lamellar capillaries and uprights made the cartilage quite normal for replication and showed better absorption of inflammation. Besides, better distribution of cells together with the height and appropriate diameter of the scaffolds also made a significant contribution to the process of regeneration [177]. On the other hand, chitosan was mixed with gelatin without the need for further treatments. This solution was combined with β -glycerophosphate forming hydrogel at the physiological temperature and enhanced the cellular interaction of chitosan. This solution was supplemented with hydrochloric acid and deionized water. Cells were introduced to the solution, and a method of bioprinting was carried out. Their findings showed that solid contamination, accumulation, and gelation during the 3D bioprinting can escalate to needle clogging, which can be prevented by cooling the prepared solution; centrifugation and degassing under vacuum prevent these problems during printing. They proved that even an increase in chitosan concentration results in a greater viscosity and an intensified gelation time which reduces fiber size. Furthermore, the distance between the needle and the impressed surface is an important factor to use agarose gel in this space. The diameter of the fiber decreased with the increasing speed of advance. The new bioprinter offers a simple and inexpensive way to print bi-substrates of chitosan /gelatin and at the same time maintains high cell viability [178].

It was observed that these new low stability chitosan hydrogels are easily dissolved, as mentioned in some cases in a previous study [179]. A new gelatin/sodium alginate/carboxymethyl hydroxycarbonate- chitosan composition filled with bone marrow stem

cells for three-dimensional bioprinting was reported. The addition of chitosan improves proliferation, cell adhesion, and mechanical force owing to the ionic bond between the -NH_2 group of chitosan and the -COOH group of the alginate. To enhance water solubility, chitosan was modified into carboxymethyl chitosan by dissolving gelatin in double distilled water, then alginate and carboxymethyl chitosan subsequently added to the solution and the ions were crosslinked with 2% CaCl_2 . The stem cells of the bone marrow were loaded into a hydrogel and a three-dimensional bioprint was performed. The mechanical feed system has been set to a speed range of 1 to 10 mm and a pressure range of 20 to 60 psi. They showed that the decomposition rate of hydrogels with carboxymethyl chitosan has decreased compared with hydrogels without chitosan, which means that carboxymethyl chitosan has improved the stability of the hydrogel. Furthermore, the antibacterial activity with carboxymethyl chitosan has been improved compared to the hydrogels without chitosan.

Chitosan was modified for 3D bioprinting of artificial bone tissue in a previous study [180]. In this study, chitosan was modified to N, O-carboxymethyl chitosan to react with the polyphosphate and improve the biologically active properties of the hydrogel. The N, O-carboxymethyl chitosan was dissolved in physiological solution and added polyphosphate to the solution, followed by centrifugation to remove a gas bubble. The solution was mixed with an alginate aqueous solution and then, bioprinted using a 3D bio plotter. Next, Ca was added for networking and the hardening of bio-printing structures. The proposed composition induced SaOS-2 bone cells for biomineralization. These scaffolds for bioprinting were *in vivo* studied and demonstrated significant activity of regeneration induction in lower leg defects of rats. This composition seems a very productive material for the custom design of frames in the bones.

5.2. Printed chitosan in the formation of a vascular network structure

Nowadays, chitosan has been used in multiple applications in tissue engineering and regenerative medicine, especially in the design of vascular networks. Because of its outstanding properties, chitosan is extensively used in vascular tissue engineering by the blending of chitosan with polycaprolactone. This combination shows desirable mechanical degradation and superior biological properties [165].

871 Recently, alginate and chitosan hydrogels with different content have been used to print
872 channels with a wall thickness of less than 200 microns, in which a robotic printer directly
873 printed microfluidic channels in the shape of a vessel tube. 1.0 M sodium hydroxide was used as
874 the crosslinking agent of chitosan and acetic acid to dissolve chitosan. The high content of
875 chitosan solution (4%) required higher pressure, which led to an increase in shear stress and a
876 decrease in cell viability. On the contrary, the mechanical behavior is decreased at low chitosan
877 content (2%). Therefore, chitosan with a concentration of 2.5 to 3% was chosen as the optimal
878 concentration range. On the other hand, they have shown that the distribution of polymers or the
879 rate of crosslinking affects the diameter of the channels.

880 Higher delivery speeds obtained by extrusion led to thicker walls and build wider channels.
881 The printed alginate microfluidic channels were continual and more homogeneous based on their
882 findings, but the printed chitosan samples were more fragile and easily breakable. Even though
883 the concentration and delivery speed of selected materials are effective factors during
884 microchannel printing. Hence, the viability of the cells is increased in the form of a single
885 channel or mass incorporated in the hydrogel and this process is considered a promising
886 production route for the development of the vascular network [181].

887 **5.3. Printed chitosan in skin regeneration**

888 Chitosan has attracted great attention in the areas of dressings and skin regeneration
889 because of its antimicrobial and hemostatic properties. It may even be used as an adjuvant for the
890 recruitment of polymorphonuclear leukocytes or neutrophils in the wound area, fibroblast
891 proliferation, hyaluronic acid synthesis, angiogenesis, and deposition of collagen. It contributes
892 to accelerating the process of wound healing and preventing scar formation. The fragile attitude
893 of chitosan in skin tissue engineering can sometimes be also rectified by mixing with collagen,
894 alginate, or other polymers [182].

895 **5.4. Cast chitosan for tissue engineering applications**

896 Hard tissues in mammals consist of two main constituents: hydroxyapatite (HAP) as a
897 mineral part and collagen fibers as the biological contributor. It might be reported that HAP
898 seems to be configured in nanorods with diameters in the range of 5-10 nm and length about 30-
899 50 nm. Furthermore, these rods are embedded vertically through the nanofibers of collagen. It

could be concluded that while HAP represents the predominant component to resist compression stresses, collagen fibrous contribute mainly to deal with tensile stresses. In other words, the great mechanical properties of hard tissues could be assigned to their hierarchical structure. Therefore, a design of bone possesses the upper hand in mechanical properties than the types of constituents. Besides, HAP is a distinct inorganic agent with a hexagonal structure. Besides its high biocompatibility, bioactivity, low cytotoxicity, and osteoconductivity, it possesses numerous crystallographic sites that are able to be substituted partially whereas crystal structure is remaining. The biological HAP is usually substituted with versatile ions with different contents such as carbonate which comes from the ambient atmosphere. Therefore, mimicking of hard tissues in mammals might be reached via designing a multi-doped HAP to be embedded through a bio-polymeric agent, which could introduce adequate mechanical behaviors and avoid annealing procedures. In this issue, Mg/Ag co-doped HAP was encapsulated into chitosan using a casted technique. The morphological features of the obtained scaffolds were shown in Fig. 10 (a-f) [183].

6. Advantages, drawbacks, and future trends of chitosan 3D printing

3D bioprinting is a modern fabrication process to place biological materials, cells, and biomolecules accurately on scaffolds. The production control facilitates the approximation of natural textiles with customized biological and mechanical properties suitable for repairing tissues [184]. Though 3D printing was a breakthrough in the development of scaffolding, it still represents a long way to organ reproduction. One of 3D printing's most significant problems is the development of a functional organ filled with vascular lumens of different sizes. All cell types need a network of vessels to gain access to nutrients and oxygen, as well as to expel waste. Therefore, the creation of a complete and complex network of vascularization in the printed structure remains a mystery [185]. However, 3D printing technology has more possibilities than traditional scaffolding methods for developing the boundaries of the vascular system by checking the parameters. Interestingly, the impression of the native organ has many problems. Biological imitation of the whole organ, considering its various types of cells and microstructures, is a rigorous procedure that requires great effort.

Up to date, there is an important issue that should be taken into consideration which is the economic factor for the preparation of 3D printed chitosan, in addition to the preparation

drawbacks such as the huge time of the crosslinking process and poor mechanical features. Such issues limit the utilization of chitosan 3D printing for most medical purposes (tissue engineering). These issues can be avoided *via* adding new substances such as gelatin compounds which can act as mediators for the creation of new functional groups [186]. Based on that, such gelatin has been added to enhance the printability of chitosan. Additionally, gelation has been used to enhance the thermal stability of chitosan decomposition occurred around 220°C. The amazing features of modified chitosan facilitate its application in many biomedical purposes due to its proper biocompatibility and adhesion with the cells. Therefore, it is necessary to explore the most parameters and materials that can be used for changing the undesired features of chitosan [187]. Furthermore, the added compounds represent an important factor that affects the shape and precision of the structure of interest. It has been reported that chitosan with low molecular weight is more favorable than the chitosan with high molecular weight. To get high properties, chitosan with low molecular weight should be then used. Moreover, chitosan with high molecular weight forms a rigid crystalline structure owing to the presence of huge inter and intramolecular hydrogen bonds along with its structure, which in turn, restricts its application in SLA bioprinting. Besides, acetic acid which is used for dissolving chitosan can be used to overcome partially the formation of free radicals. In a short, in SLA of chitosan, the photoinitiator concentration is a very important aspect. It has been reported that the utilization of photoinitiator has a threshold value. Above the threshold value, the photoinitiator can lead to poor cell adhesion and cell viability [180]. Based on the aforementioned findings, it was observed that the concentration of both solvent and materials is a very important factor affecting the mold structure in terms of mechanical features and stability. Moreover, different parameters such as physical or chemical agent impacts on the process and the crosslinking agent have been also checked, in turn, leading to an increment in the diameter of both core and channels. Thus, 3D printing produced chitosan with high viscosity and the presence of crosslinking agents with high concentration can create drawbacks such as limiting the feeding, transportation of oxygen, and cell migration. For that, viscosity and crosslinking concentration are highly appreciable for the formation of good chitosan 3D printing [188]. On contrary, it is clarified that 3D chitosan can swell after the printing process leading to decreasing the diameter and struts of the pores. Thus, for decreasing the swelling factor of 3D chitosan, a crosslinking agent should be added in a high concentration [189].

Previously, there is much-published work documented the development of a superior method (solvent-free) such as laser absorption of two photons and single-photon during shear deformation for the generating of chitosan with various molecular weights and allyl substituted derivatives scaffolds. The advantage of using such a method concerns the avoidance of organic and aqueous solvents with no problem associated with viscosities and distributors [190].

As previously mentioned, the evaluation of cytotoxicity for 3D chitosan illustrated the viability of cells and cell growth formation. However, there is a lack of studies about other factors that should be extensively examined. Some of these factors are biodegradability, absorption of water, and mechanical features of the formed 3D prints. All these categories will be discussed in detail in future studies. Moving to our discussion about the amazing properties of 3D printing structures; such morphological structure is a very important issue that should be accompanied by the prepared scaffolds that affect cellular behaviors. The 3D printing is more favorable to be prepared with a morphological structure containing huge porous structures with small sizes to be efficient during the penetration of cells. It has been documented in previous studies that 3D printed chitosan has been prepared with a pore size of less than 250 μm . Indeed, chitosan with a positive charge is more desired for cartilage tissue engineering domains [191].

The rapid gelation of a negatively charged polyanion in an aqueous medium acted as an inhibitor of the diffusion of various parts of bioprinting. As in the study which reported that chitosan has a sufficient gelling time, acceptable for 3D bioprinting. Besides, it has maximum printing precision, long life, and is the cheapest gel compared to other approved materials used before in the materials for clinical purposes. Although bioprinting skin tissues developed with chitosan and chitosan /collagen have not received FDA approval. Given the similarity of composites with other approved materials, the regulatory approval may be relatively simple in the future [192].

It could be observed that upon variation of compositional contributions, the surface morphology has been changed considerably. HAP with dimensions of 4 μm has varied to be 1.25-5 μm upon the changing of dopant concentrations. Furthermore, HAP grain was scattered through chitosan fiber which has diameters in the range of 2.5-5 μm . The compressive strength was conducted and displayed that additional Mg (II) might introduce high resistance to compressive stresses. The optimum compressive value was achieved at around 15.0 MPa [183].

Conclusion

The materials based on chitosan have several technological applications that have already been investigated and many others that are possible. For an effective investigation of chitosan-based materials, it must be considered that all areas of applications depend on the properties of the biopolymer. The properties, in turn, depend on the production process, which depends on the raw materials. Therefore, a series of clearly defined steps must be followed by the raw material to produce chitosan. Also, chitosan is easily modified in various ways to obtain special properties that are used in different trends. Traditional and advanced analytical methods should be used in all these phases to verify the main results. Besides, in this review, the chitosan nanofibers are limited owing to the cationic property and solubility of chitosan in acids and this also was discussed. To evaluate and develop the chitosan nanofibers production process, it is necessary to analyze its morphology, crystallinity, chemical composition, structural changes, and mechanical properties. Since chitosan is a natural polymer with useful biological properties, its nanofibers hold promise for a variety of applications. The presence of $-NH_2$ and $-OH$ groups in chitosan nanofibers allows their use in fields such as biotechnology, wastewater treatment, medicine, and pharmaceuticals.

Declaration of Competing Interest

All authors declare that there is no conflict of interest.

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