Chapter 9

Overview of Electrospinned Chitosan Nanofiber Composites for Wound Dressings

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Additional information is available at the end of the chapter

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Abstract

Chitosan has a medical application because of its natural origin and properties of biodegradability, biocompatibility, nontoxicity, and antimicrobial capacity. Electrospinning produces non-woven nanofibers to wound dressing with high specific surface area and small pores. These properties are favorable for absorption of exudates and prevent the penetration of bacteria, thus promoting wound healing. For this reason, chitosan blends are used to produce nanofiber dressings, and the characterization of the structural, mechanical, and biological properties is very promising for further studies. Nowadays, the researchers are seeking for biomaterials that provide modern dressings with many qualities, which are designed to promote wound healing. In this chapter, the electrospinning parameters that affect the nanofiber properties based on chitosan to prepare wound dressings are highlighted.

Keywords: electrospinning, chitosan, dressings, nanofibers, biomaterials

1. Introduction

Chitin is a polymer composed of *N*-acetylglucosamine that has been recognized as one of the most abundant natural polysaccharides and has been found in crustaceans, fungi, and insects [1]. To improve its solubility in water, it is partially deacetylated and converted into chitosan, which is a linear polymer of glucosamine and *N*-acetylglucosamine [2]. Due to its properties, chitosan has many applications in food preservation, medicine, biotechnology, agriculture, and water treatment. Among the properties of interest in biomedicine are its biodegradability, biocompatibility, nontoxicity, and high antimicrobial activity [3]. Thus, chitosan preparations have

been used as gels, microparticles, films, and coating agents. Medical products based on chitosan have been studied as dietary supplements, wound healing agents, hemostatic devices, and drug delivery [4].

The appropriate selection of dressings depends on the characteristics of the wound and its mode of action. The purposes of the dressings are to facilitate the healing process, control symptoms, achieve an esthetic healing, keep the wound moist, absorb exudate without leakage, prevent infection, avoid trauma when dressing needs to be changed, not present toxic skin irritation, and maintain gaseous exchange [5]. Currently, it is necessary to find these conditions in a single product, with chitosan and other biomaterials being the most studied [6].

The electrospinning process is a technique that emerged in the 1970s with the purpose of producing fibers with a size smaller than 100 nm called nanofibers [7, 8]. Specifically, the electrospinning system is recognized for its ability to produce nanofibers from a wide variety of polymers of natural or synthetic origin and natural proteins [9].

The electrospinning system faces several problems during the elaboration of nanofibers for biomedical applications, particularly, the optimization of the process conditions for each polymer [10]. This chapter presents a detailed review of the influence of electrospinning parameters on the properties of the biocomposites of chitosan in mixture with other polymers to produce dressings, as well as some in vitro and in vivo assays of their application as wound dressings.

2. Electrospinning technique for production of nanofibers

The electrospinning process is a recognized system for obtaining polymer fibers by creating an electric field. To do this, a jet of the polymer is injected through a charged needle, and the solvent where the polymer is dissolved evaporates, thus allowing the deposition of solid polymer fibers on the collector. As a result, the electrospinning system equipment consists of a high voltage source, syringe pump, a syringe loaded with the polymer solution, and a collector (**Figure 1**).

During the process, high-voltage loads are applied to the polymer solution that is injected. The increase in the electric field formed causes the repulsion interactions between the same charges and attraction between the oppositely charges in the liquid. Whereas the collector that is grounded exerts forces of attraction on the injected drop, once the electric field increases, it will reach a point where the electrostatic forces reach the equilibrium with the surface tension, deforming the cone to a drop, which it is called Taylor cone [11].

Many polymers and operating parameters have been reported for the electrospinning process. The electrospinning system is affected by polymer characteristics such as concentration, viscosity, molecular weight, and surface tension. Other parameters that influence are the components and processing parameters of the system, specifically the applied voltage, injection flow, distance between the needle and the tip, and the type of collector used [12], presented in **Table 1**.

2.1. Characteristics of chitosan polymer for electrospinning nanofiber process

According to Kai et al. [12], electrospinning requires a very concentrated polymer solution. The low concentrated solutions cause the formation of weak chains, as well as insufficient electrostatic

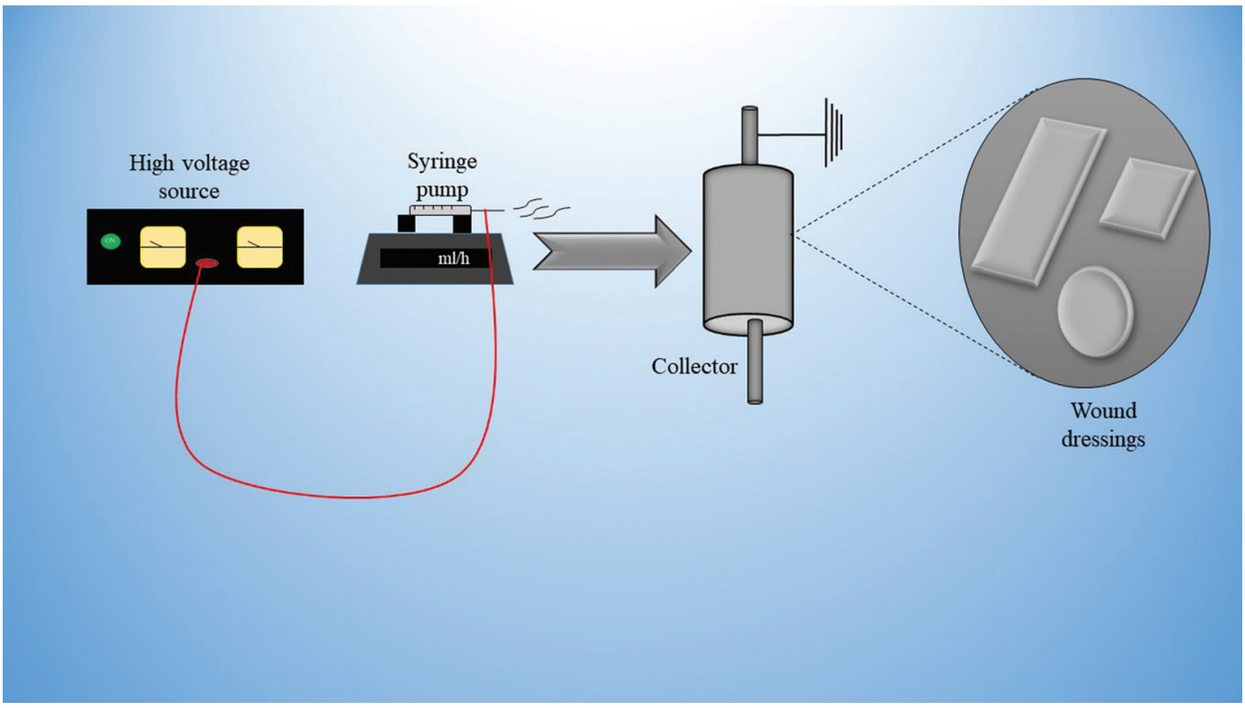

Figure 1. Components of the electrospinning system.

repulsive forces, giving rise to the appearance of droplets in the collector and the incomplete formation of the fibers.

Surface tension and viscosity are parameters of the polymer solution that mainly affect the diameter of the fiber [10]. These authors agree with Sill and von Recum [11], who mentioned that if the solution is very diluted the polymeric fiber will break into droplets before reaching the collector due to surface tension. When the solution is very concentrated does not complete the fiber formation is not obtained due to the high viscosity, which makes difficult the flow during injection.

The molecular weight of the polymer is another parameter that affects electrospinning. Low molecular mass polymers lead to bead formation, while high molecular weight polymers produce fibers with larger diameter [12]. Likewise, Cui et al. [24] found that to produce pectin nanofibers by electrospinning the molecular weight of the polymer should be in the range of 200–950 kDa.

2.2. Components and processing parameters of the electrospinning technique

Haider et al. [25] reported that the critical amount of applied voltage varies according to the polymer. Also, the formation of nanofibers with small diameters is due to the increase in the applied voltage, attributed to the stretching of the Taylor cone, and the formation of the jet (**Figure 2**). However, the increase in the critical voltage value forms beads in the nanofibers, which is caused by the decrease in the size of the Taylor cone and an increase in the flow velocity.

One of the electrospinning parameters with greater influence on the mechanical properties is the type of collector. During the electrospinning process, the fibers are deposited in a lower potential electrode known as a rotating or static collector. Thus, the fibers can be guided through the electric field formed between the tip of the needle loaded with the polymer solution and the high voltage source [26].

Polymer	Electrospinning parameters	Collector	References
PVA	AV (14.5–17.5 kV) TCD (125 mm) FR (6 μL/min)	Stainless steel collector	[13]
CS/PVA	AV (18 kV) TDC (15 cm) FR (0.35 ml/h)		[14]
CS/HA	AV (15 kV) TCD (15 cm) FR (1.2 ml/h)	Rectangular 6 × 2 cm aluminum collecting plate	[15]
PVA/SA	AV (15 kV) TCD (15 cm)		[16]
CS/PVA	AV (15 kV) TCD (15 cm)	Copper plate wrapped with aluminum foil	[17]
SA/PEO	AV (20 kV) TCD (20 cm)		[18]
CS/PEO	AV (15–35 kV) TCD (15 cm) FR (0.1–2 mL/h)	Aluminum foil attached to a drum collector	[19]
SA/PVA	AV (17 kV) TCD (5 cm) FR (0.2 ml/h)	Aluminum target	[20]
SA/PVA	AV (17 kV) TCD (10 cm) FR (2 μl/min)	Rectangular nickel collector with a glass microscope slide taped to its surface	[21]
Ge	AV (12 kV) TCD (10 cm) FR (0.003 ml/min)	Stainless steel mandrel (30 cm length and 5 cm in diameter) and rotational speeds (1700, 2400, 3100, 3800, and 4500 rpm)	[22]
PL/CS	AV (5 kV) TCD (12.5 cm) FR (0.6 ml h^{-1})		[23]

PVA, Poly(vinyl alcohol); CS, chitosan; HA, hydroxyapatite; PEO, poly(ethylene oxide); SA, sodium alginate; PL, polycaprolactone; Ge, gelatin; AV, applied voltage; TCD, tip-to-collector distance; FR, flow rate

Table 1. Polymers and parameters used in electrospinning.

The collectors of the first electrospinning equipment lacked movement, but over time they have been modified in order to improve the alignment of the fibers and modify their surface. The use of rotating cylindrical collectors has helped in the alignment of the fibers. At the same

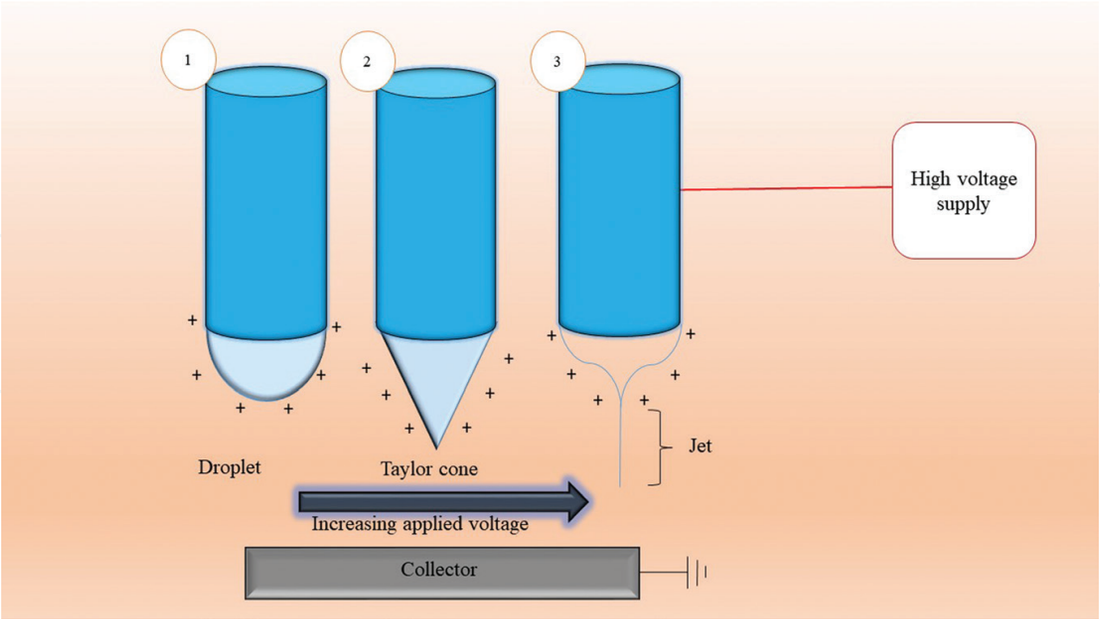

Figure 2. Taylor cone formation stages during voltage application.

time, Huang et al. [27] used a thin circular collector with a sharp edge and found that with this modification electric field is concentrated, favoring the alignment and collection of the fibers.

The collectors can be adjusted to various specifications from a stationary plate to a rotating cylinder. When a static collector is used, the fibers will have a random order, while aligned fibers will be generated with the rotary collector [11].

Huang et al. [27] used collectors with movement to prepare gelatin hydrofibers. They confirmed that the use of rotating collectors of cylindrical shape with high speed orientates the nanofibers. In addition, they reported that when the collector speed is lower than the alignment speed, the hydrofibers will have a random order, while high speed causes instability in the injection jet, affecting the surface morphology of the fibers.

In addition, the position of the collector influences the morphology of the fibrous materials. Haider et al. [25] reported that the diameter increases at short distances between the needle and the collector, while the diameter decreases at long distances between the needle and the collector. Also, Sill and von Recum [11] explained that the distance between the needle and the collector affects the continuous and complete formation of the fibers. Small distances are not enough to achieve the evaporation of the solvent before being deposited in the collector, resulting in the formation of loose and weak fibers that tend to stick together and make it difficult to remove from the collector.

With the intention of providing greater guidance in continuous nanofibers, Dabirian et al. [28] incorporated two injection syringes with different charges and positioned in a triangular form with respect to the initial needle of the process. Both syringes are attracted to each other, and the nanofibers are twisted and collected continuously in the form of a yarn.

3. Characteristics parameters of chitosan biopolymer

Chitosan is a linear polysaccharide consisting of *N*-acetyl-D-glucosamine (GlcNAc) and D-glucosamine (GlcN), which is produced by alkaline deacetylation of crustacean chitin [29]. It is soluble in dilute acid solutions of acetic, lactic, malic, formic, or succinic acid. It is polycationic at pH 6 and easily interacts with negatively charged molecules such as proteins, fatty acids, anionic polysaccharides, bile acids, and phospholipids and, therefore, can be used as blended solutions [30].

This polysaccharide has antibacterial, antifungal, mucoadhesive, analgesic, hemostatic, nontoxicity, biodegradable, and biocompatible activity (**Figure 3**) [31]. These biological properties have favored its topical application and implantation. Another recent medical application is controlled drug delivery systems [32]. Baldrick [33] reported the possible oral use of chitosan nanoparticles with drug carrier, as gel beads of chitosan can be degraded between 14 and 28 days after being implanted. Mansouri et al. [34] propose to chitosan as a candidate to be used in gene therapy due to its ability to bind and form complexes with DNA by the electrostatic attraction created.

Chitosan has been shown to be a nontoxic polymer, the reason why the Food and Drug Administration (FDA) has approved its use in wound dressings [35]. Likewise, in the clinical reports with chitosan-based biomaterials, inflammation or allergic reactions have not been reported [2]. Waibel et al. [36] detailed the first study of chitosan as bandages in soldiers with shellfish allergy, where all soldiers tolerate the chitosan bandage without reaction.

A very important property is biodegradation, which can be chemical or enzymatic degradation. Chemical degradation refers to acid-catalyzed degradation, such as the gastric juices of the stomach. Likewise, chitosan can be degraded by enzymes, which are responsible for

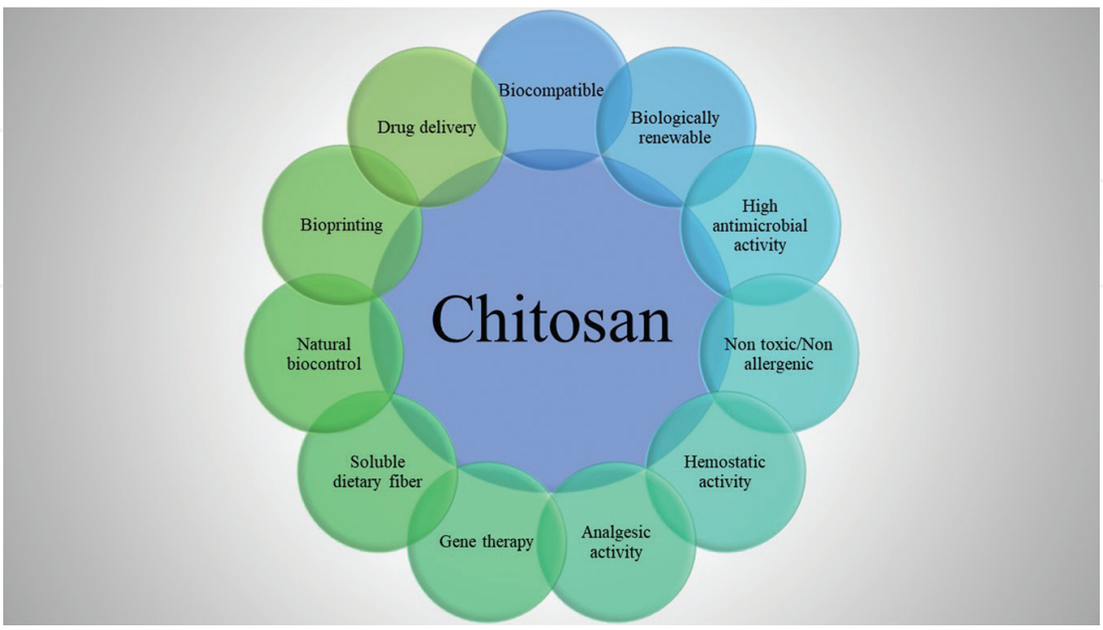

Figure 3. Promising properties of chitosan for medical use.

hydrolyzing the bonds between glucosamine-glucosamine, glucosamine-*N*-acetyl-glucosamine, and *N*-acetyl-glucosamine-*N*-acetyl-glucosamine [35]. In addition, the degradation rate of chitosan depends mainly on the molecular weight and the degree of deacetylation. However, the degradation of chitosan is not fully understood.

Ng et al. [37] reported a new emerging technology that can provide 3D nanostructures named bioprinting and highlighting for chitosan, due to its poor printability. They prepared a polyelectrolyte complex blending chitosan and gelatin. The hydrogels were customized and fabricated considering the wound size.

Due to its cationic properties, the chitin and its derivatives have demonstrated that are a potent elicitor in low concentrations. Thus can activate defense mechanisms against pathogen nematodes. Also, in plants can be used to control herbivorous insect and viral diseases [38].

Biocompatibility according to Baldrick [33] is attributed to glucosamine, which is the most abundant component in chitosan and produced in the human body from glucose. Also, it is responsible for producing glycosaminoglycan which forms a cartilage tissue in the body. Chitosan by its positive charge can bind to free fatty acids and bile salt from dietary lipid during the absorption in the gut.

The hemostatic and antimicrobial activity of chitosan is related to its polycationic nature. Therefore, it is involved in the agglutination of red cells that stimulates the formation of clots [39]. The antimicrobial activity is attributed to the electrostatic interactions between the polycationic structure of chitosan and the anionic components of the surface of microorganisms [40].

Xia et al. [41] mentioned that chitosan has free amino groups which are able to neutralize gastric juices and form a protective barrier in the stomach. On this basis, they have proposed it as a safe material with biomedical applications in the treatment of peptic ulcers, for wound healing.

In the preparation of materials for wound healing, chitosan has been used to develop nanofibers by the electrospinning system [29]. Nevertheless, according to Pakravan et al. [19], chitosan has limited electrospinnability, due to its polycationic nature in solution and rigid chemical structure. However, it is possible by blending with other polymers due to the formation of hydrogen bonds.

De Vrieze et al. [42] reported that a strong solvent such as concentrated acetic acid is necessary for chitosan electrospinning, and Geng et al. [3] demonstrated that the concentration of the solvent decreases the surface tension of the solution generating stable jet stream during electrospinning. Also, Kriegel et al. [43] reported that the increase in acetic acid concentration affects surface tension with the appearance of beads in chitosan/poly(ethylene oxide) nanofibers.

4. Myriad nanocomposites for wound dressings

The development of biocomposites from biodegradable polymers has attracted great interest due to their capacity to completely degrade and not produce toxic effects [44]. Also, the main challenge in science of biomaterials and tissue engineering is to create matrices either

of natural origin, synthetic, or blends that are suitable for the development of medical prototypes with improved properties.

In biomedical area, they are interested in developing polymers as biomaterials, because more complex structures need to be achieved on the requirements for their different applications [45].

According to the National Institute of Health (NIH), biomaterials are defined as "any substance (other than a drug) or combination of substances synthetic or natural in origin, which can be used for any period of time, as a whole or part of a system which treats, augments, or replaces tissue, organ, or function of the body " [46].

4.1. Natural and synthetic polymeric nanofibers

In biomedicine, the natural polymeric nanofibers are polysaccharides, collagen, keratin, silk, tubulin, actin, cellulose, chitin [47]. Likewise, there is great variety polymers such as poly(lactic acid-co-glycolic acid) (PLGA), poly(lactic acid) (PLLA), polycaprolactone (PCL), poly(ethylene oxide), (PEO), and poly(vinyl alcohol) (PVA). All these have been used in wound dressings [7, 45], as shown in **Table 1**.

4.2. Wound dressing theory

Traditional dressings used on wound treatment were made from natural or synthetic materials. In the past, the main function of the dressings was to keep the wound dry through the evaporation of the exudates and to avoid the introduction of dangerous microorganisms to the wound [48]. However, over time this idea has been modified, according to Newman et al. [49]; nowadays, an ideal wound dressing must have the objective of providing an optimum moisture, accelerate the healing process, absorb large amounts of exudates, and prevent tissue maceration around the wound, which would cause a second injury. Also, Caló and Khutoryanskiy [50] reported that the dressings are designed with the purpose of maintaining a moist environment between the wound and the dressing, favoring the healing of wounds. The healing process is a dynamic process which allows a complex sequence of events, which include homeostasis, inflammation, proliferation, and remodeling [25].

A wound is defined as a defect or a break in the skin resulting from trauma or medical/physiological conditions. Traditionally, wounds can be classified depending on the number of layers of skin and affected area. When only the epidermis is damaged, the wound is superficial. If, epidermis and deeper layers are affected, is named partial thickness. Moreover, when the subcutaneous fat and deeper tissue have been affected, the wound is named full thickness [50].

On the other hand, Zahedi et al. [51] mentioned that the dressings can be classified from other aspects. Therefore, these authors classified them into three groups called passive, interactive, and bioactive dressings. Passive dressings are ordinarily used for common wound coverage such as cotton gauze. Interactive dressings are characterized for being transparent and permeable. Bioactive dressings have the advantage that requires long periods to be removed and are made from biopolymers such as collagen, chitosan, alginate, and elastin. In the **Figure 4** are presented commercial dressing such as hydrocolloids, alginates, collagens and hydrofibers.

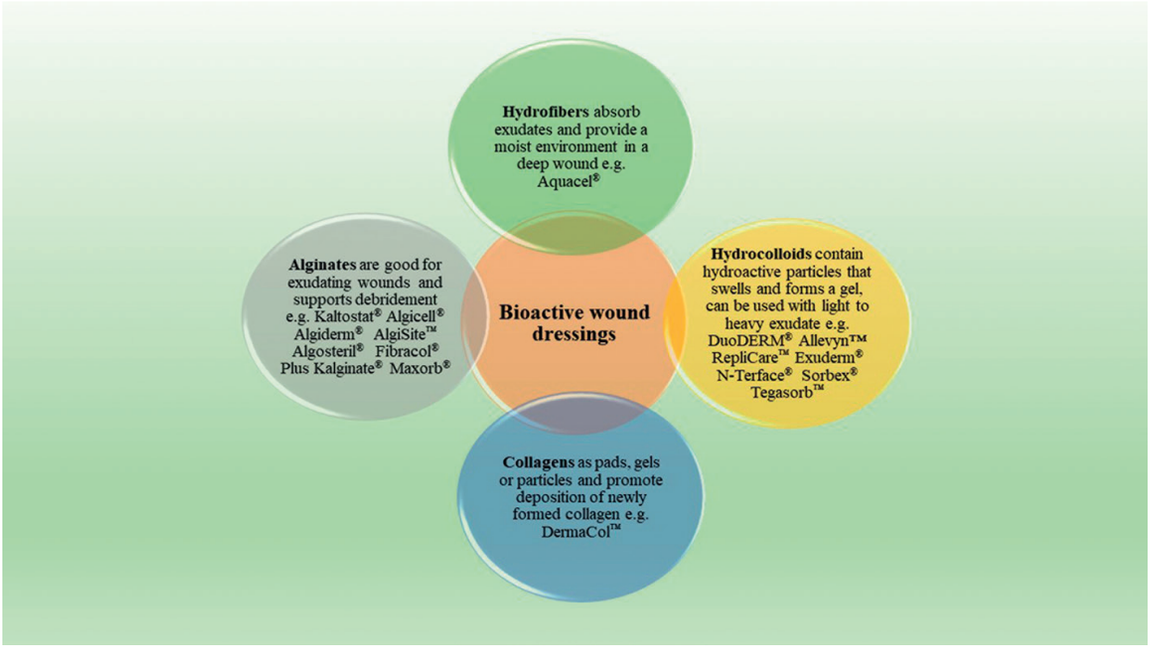

Figure 4. Commercial products of bioactive products.

According to Newsom et al. [52], hydrogels are effective for necrotic wounds because they hydrate wounds and reduce postoperative pain. However, they have disadvantages in wounds with large amounts of exudates and hemorrhages; that is why the use of alginates is recommended. Hydrocolloids promote a moist environment, autolytic tissue debridement, also as protection against microorganisms and are used in postoperative wounds. Hydrofibers are recognized for accelerating the wound healing process and improving the appearance of the final healing.

This wide variety of modern dressings has the advantage of creating an optimal environment that allows the proliferation of epithelial cells and improves the treatment of wounds [48].

5. Properties of electrospinned chitosan nanofibers/composites for wound dressings

The properties of the dressings are optimized through the modification of the electrospinning parameters. Most studies mentioned that the composition of the polymer solution such as solvent, concentration, viscosity, and surface tension affects the morphological structure and mechanical properties of nanofibers [12].

5.1. Superficial morphology

The structural morphology is visually evaluated with scanning electron microscopy (SEM), which also allows the measurement of the diameter of the nanofibers.

According to Bhardwaj and Kundu [8], the viscosity of the polymer solution determines the size and surface morphology of the nanofibers. Also, Bhattarai et al. [53] indicated that electrical conductivity is a physical property that modifies the final diameter of nanofibers.

Haider et al. [54] mentioned that the changes in the surface of nanofibers are attributed to the parameters of electrospinning and concentration of the solution and solvent (trifluoroacetic acid). Haider et al. [25] mentioned that selection of the solvent is very important for the formation of fibers with smooth and bead-free surface formation, considering the solvent capacity to dissolve the polymer and moderate boiling temperature. Also, Geng et al. [3] mentioned that a higher concentration of acetic acid decreases the surface tension and increases the charge density of the jet, improving the morphology of the nanofiber.

Pakravan et al. [19] prepared chitosan nanofibers with poly(ethylene oxide) and found that chitosan solutions, due to the polycationic nature, are more conductive compared to poly(ethylene oxide). Specifically, poly(ethylene oxide) reduces the protonation of the chitosan and avoid the formation of hydrogen bonds between amino groups of chitosan and other groups. The diameters of the chitosan and poly(polyethylene) oxide nanofibers are in the range of 60 to 120 nm.

Similarly, Bhattarai et al. [53] worked with chitosan and poly(ethylene oxide). They reported cylindrical nanofibers with a diameter of 75 nm and mentioned that viscosity is the parameter that modifies the electrospinning and the morphology of the nanofiber. They also indicated that the viscosity is attributed to the interactions between the chains of both polymers.

Lu et al. [55] prepared nanofibers of poly(ethylene oxide) with a low concentration of sodium alginate, showing smooth surfaces with a diameter of 250 nm; this was achieved by increasing the viscosity and decreasing the electrical conductivity of the solutions. Likewise, Shalumon et al. [20] prepared sodium alginate nanofibers blended with poly(vinyl alcohol) adding zinc oxide nanoparticles. The nanoparticles increased the electrical conductivity improving the electrospinning process to produce 190–240 nm fibers with smooth and uniform surface. **Table 2** shows the diameter of the nanofibers prepared by electrospinning with various polymers.

5.2. Porosity and pore distribution

The porosity of biomaterials such as nanofibers is advantageous for their application in wound healing, because they provide more structural space to enhance cell seeding, in addition to facilitating cell proliferation and migration [61]. It also improves oxygen exchange, nutrient delivery, and absorption of exudates. In addition, small pores in dressing nanofibers reduce wound infections and dehydration during the healing process [12].

The porosity is analyzed with water vapor transmission rate (WVTR). Archana et al. [62] mentioned that an ideal dressing should maintain water loss from the skin at an adequate rate, between 2000 and 2500 g^{-2} day^{-1}, indicating that higher values dry wounds quickly and retard healing, finding that for a chitosan dressing with pectin and nanosized titanium dioxide (TiO_2) particles the WVTR was from 1950 to 2050 g^{-2} day^{-1}.

However, Ziabari et al. [63] mentioned that there is no specific literature to measure the size of the pores and their distribution along the fiber. The techniques currently used are indirect and not very precise. Therefore, image analysis techniques are used, and these tools are more precise and direct. Coimbra et al. [64] used scanning electron microscopy (SEM) to observe the morphology of the chitosan/pectin wound dressing, which formed porous shapes from sheetlike to fibrous-like structures. Additionally, Ninan et al. [65] used micro computed

Polymeric solution	Average diameter (nm)	References
CS/PVA	279.843	[14]
CS/HA	227.8 ± 154.3	[15]
PVA/SA	500–50	[16]
CS/PVA	75–400	[17]
SA/PEO	130 ± 51	[18]
CS/PEO	60–120	[19]
SA/PVA	190–240	[20]
SA/PVA	250 ± 30	[21]
CS/PEO	40	[53]
SA/PEO	250	[54]
CMCTS/CS/PEO	50–300	[56]
COL/CS	434–691	[57]
CMC/HA	15–35	[58]
CA/PVA	98.1–191.5	[59]
PEO/CS/PC/O	86	[60]

PVA, poly(vinyl alcohol); CS, chitosan; HA, hydroxiapatitha; SA, sodium alginate; PEO, poly(ethylene oxide); PL, polycaprolactone; PC, poly(ε-caprolactone); O, olive oil; CA, calcium alginate; CMCTS, carboxymethyl chitosan; COL, collagen.

Table 2. Diameter of nanofibers by electrospinning.

tomography (micro-CT) in pectin/carboxymethylcellulose/microfibrillated cellulose dressings (15–280 μm) obtaining 3D images and the quantity of the number of pixels in the pore image. Kumar et al. [66] measured the porosity of the chitosan/pectin/calcium carbonate ($CaCO_3$) dressings based on the empty spaces present (41.8%) as a fraction of the total volume by liquid displacement method. Also, Liuyun et al. [67] used liquid displacement method to measure porosity (77.8%) and pore diameter, between 100 and 500 μm, in nanohydroxyapatite/chitosan/carboxymethylcellulose dressings. Besides, these authors reported the presence of interconnected pores that favor the administration of nutrients.

Recently, Sarhan et al. [68] reported the use of an automatic analyzer called mercury porosimetry to measure the size of the pores; however, it only measures sizes in the range of 0.0018–400 μm, finding that in chitosan nanofibers/honey/PVA the diameter of the pores is 140 μm.

5.3. Water absorption capacity

One of the properties of dressings is the ability to absorb exudates in the wound and provide a moist environment. However, it affects the efficiency of oxygen and nutrient transfer [62].

According to Ninan et al. [65], the swelling analysis and the water uptake provide information about the ability of the dressing to transport the nutrients inside the pores, in addition to avoiding dehydration of the wound.

Choi et al. [69] mentioned that the hydrophilicity of chitosan is due to a modification in its structure, which improves its solubility in water at physiological pH. Archana et al. [62] evaluated the swelling of the dressings of chitosan/pectin and TiO_2 in phosphate-buffered sodium (PBS), finding the highest values (1215%) at pH 2.0 and the lowest (900%) at pH 7.0, due to the osmotic effect of the dressing due to the absence of ionized amino groups.

Liuyun et al. [67] reported the loss in weight, 30% in 30 days, by degradation of the dressings of chitosan/nanohydroxyapatite/carboxymethylcellulose, caused by intermolecular interaction between the components of the system and the microstructure, which favors the growth of the cells for the regeneration of the bone tissue.

Zarghami et al. [60] reported that cross-linking agents modify nanofibers and their swelling behavior. For chitosan nanofibers with poly(ethylene oxide) cross-linked with glutaraldehyde vapors, the swelling decreases from 300 to 75%, due to the aldimine bonds (—CH═N—) formed between the free amino groups of chitosan and the aldehydes of glutaraldehyde. This increases the diffusivity resistance of water and decreases its water absorption capacity.

Duan et al. [70] also used glutaraldehyde vapors as a cross-linking agent, causing a decrease in swelling from 328.7 to 146.2% and shrinkage of poly(lactide-co-glycolide)/chitosan/PVA dressings. Similarly, Chen et al. [71] used glutaraldehyde vapors to reduce the water solubility of chitosan nanofibers with collagen and poly(ethylene oxide).

5.4. Mechanical properties

The mechanical properties are evaluated through Young's modulus, tensile strength, and elongation at break by tensile tests with a universal mechanical testing machine [8]. Parameters such as the composition of the polymer solution, the interaction between its components, and the cross-linking agents affect the mechanical properties of fibrous materials during electrospinning [55].

In some studies, it has been reported that obtaining nanofibers has great advantages for the development of medical prototypes, and the precise measurement of their mechanical properties is very important, especially in dressings which must be able to withstand the forces exerted by the growing tissue or during physiological and biomechanical activities [8].

The composition of the mixture influences the mechanical behavior of the fiber. Chitosan is a fragile and rigid polymer, while poly(lactic acid) (PLA) is resistant; it is possible to blend it to form nanofibers, which show greater tensile strength at rupture with decreases in elongation at break.

Archana et al. [62] studied the chitosan and pectin blend with nanoparticles of titanium dioxide (TiO_2). They found that by increasing the pectin content and the presence of the nanoparticles, the resistance increases significantly compared to those with a lower proportion of pectin and without the presence of TiO_2. Cui et al. [24] reported that pectin nanofibers with poly(ethylene oxide) (PEO) were rigid with values of Young's modulus (192.3 MPa) and tensile strength (14.6 MPa); this is attributed to the orientation of the polymer chains of pectin during electrospinning.

Cross-linking agents have been used with the intention of improving or adding properties; some of the most used cross-linking agents in electrospinning systems are glutaraldehyde vapors [60, 70, 71] and genipin [15].

Shalumon et al. [20] proposed cross-linking agents with glutaraldehyde vapors because it has less or no toxic effect. Chen et al. [71] elaborated by electrospinning nanofibers of chitosan/collagen/poly(ethylene oxide) cross-linked with glutaraldehyde vapors reported Young's modulus increase from 0.29 to 0.65 MPa and the decrease in tensile stress and strain.

Nanofibers of poly(lactic-co-glycolic acid)/chitosan/PVA cross-linked with vapors of glutaraldehyde succeeded to increase the tensile strength up to 3.8 MPa and Young's modulus tension up to 106.2 MPa; this is due to the union between the components of the structure [70].

Another cross-linking agent is genipin, which is extracted from the gardenia fruit (*Gardenia jasminoides*). This has been used in hydroxyapatite fibers with chitosan and was found to increase up to four to five times the stiffness with respect to non-cross-linked fibers [15].

5.5. Antimicrobial prophylaxis

Microbiological assays are evaluated through the inhibition provided by a biomaterial in the presence of microorganisms [61]. **Table 3** shows the microorganisms studied in microbiological assays with nanofibers. According to Heunis et al. [72], *Staphylococcus aureus* is the most prevalent microorganism in skin infections.

Infections in wounds are common, because the microorganisms damage the injured tissues causing adverse reactions to the immune system such as inflammation and tissue damage, retarding the healing process [65]. The main pathogenic bacteria that set the wound healing process at risk are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, some *Proteus*, *Clostridium*, and coliform species [48].

Study	Microorganism	Inhibitory effect	References
Fabrication of PEO/chitosan/PCL/olive oil nanofibrous scaffolds for wound dressing applications	*Escherichia coli*, *Staphylococcus aureus*	Superior against *E. coli*	[61]
Evaluation of chitosan nano-dressing for wound healing: characterization, in vitro and in vivo studies	*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus niger*	Superior against *B. subtilis*	[63]
The effect of increasing honey concentration on the properties of the honey/poly(vinyl alcohol)/chitosan nanofibers	*Staphylococcus aureus* *Escherichia coli*	Enhanced against *S. aureus* and *E. coli*	[68]
Chitosan-/polyurethane-blended fiber sheets containing silver sulfadiazine for use as an antimicrobial wound dressing	*Pseudomonas aeruginosa*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA)	Superior against MRSA	[73]
Fabrication of an antibacterial non-woven mat of a poly(lactic acid)/chitosan blend by electrospinning	*Escherichia coli*, *Staphylococcus aureus*	Enhanced incorporating Ag nanoparticles	[74]

Table 3. Antimicrobial assay that involves chitosan nanofibers.

The antimicrobial activity of chitosan is of broad spectrum and has been shown to be effective against Gram-positive and Gram-negative bacteria and many filamentous fungi and yeasts [40]. According to Kumar et al. [75], the cationic amino groups of chitosan have the ability to bind to the anionic groups of the microorganisms by inhibiting the presence of *Escherichia coli*, *Fusarium*, *Alternaria*, and *Helminthosporium*. Sarhan et al. [68] attributed this to the polycationic nature of chitosan, which allows it to interact with bacterial membranes that are negatively charged, favoring the loss of permeability, causing cell disruption and subsequently death.

The inhibitory effect in dressings is evaluated by several techniques. Au et al. [74] used the optical density technique in a chitosan/polylactic acid/silver nanofiber blend, mentioning that bacterial cells are opaque and when they propagate in solutions they become turbid; therefore, lower optical density indicates greater antibacterial activity.

Other authors used the bacterial disk inhibition method. Archana et al. [62] reported that in nanofiber dressings of chitosan/pectin/TiO_2 the antibacterial activity is excellent; in addition large surface areas facilitate microbial adsorption and accelerate the antimicrobial activity rate. Sarhan et al. [68] report efficiency against *E. coli* in nanofibers of chitosan and honey; the inhibitory effect due to the polycationic nature of chitosan is potentiated with honey due to its acidity, high sugar content, and hydrogen peroxide production capacity. Additionally, Zarghami et al. [60] found greater efficiency against *E. coli*, Gram-negative in nanofibers of chitosan with poly(ethylene oxide).

The use of metal nanoparticles had been shown to have high antimicrobial activity against bacteria, viruses, and other microorganisms [76]. Lee et al. [73] reported higher efficiency against *S. aureus* in chitosan/polyurethane nanofibers with silver sulfadiazine. Silver is known for its great antimicrobial properties and its use for pharmaceutical applications. Its antimicrobial activity is due to the interaction between silver particles with the bacterial cell, penetrating its wall and causing damage and cell death to components (DNA) [77].

6. Cross-linked copolymers for electrospinned chitosan biocomposites/nanofibers

6.1. Poly(vinyl alcohol)

Poly(vinyl alcohol) (PVA) is a synthetic polymer soluble in water and with excellent chemical resistance. It is recognized mainly for its non-toxicity, biodegradability, and biocompatibility, and it is used in the biomedical area. Since the 1950s it has been commercialized for the formation of highly hydrophilic fibers [17]. It is known that PVA nanofibers dissolve instantaneously in water. That is the main reason why cross-linking is recommended. Destaye et al. [13] cross-linked PVA nanofibers with glutaraldehyde vapors and reported that the concentration of glutaraldehyde increases water retention capacity and the swelling of nanofibers improves their mechanical properties.

For biomedicine, the chitosan nanofibers with PVA have been elaborated by electrospinning because they are more favorable for cell culture compared with only PVA [17]. Recently, chitosan

nanofibers blended with PVA are being studied as dressings for the treatment of diabetic foot ulcers. Ahmed et al. [30] demonstrated that nanosized pores protect damaged tissue from bacteria, while high porosity increases fluid absorption and promoting wound healing.

6.2. Sodium alginate

Sodium alginate is a nontoxic polysaccharide with applications in the food and pharmaceutical products. The natural sources are all species of brown seaweed. It is a water-soluble salt of alginic acid. It consists of two uronic acids, β-D-mannuronic acid (M) and α-L-guluronic acid (G), in β-(1–4) union. These acids form homopolymeric blocks M-M or G-G and blocks with an alternating sequence of M-G blocks [78].

The alginate is used in medicine to prepare wound dressings, Tehrani et al. [79]. Fan et al. [80] reported that calcium alginate fibers interact with wound exudates forming a gel, resulting on ion exchange between fiber calcium and sodium ions from exudates. Furthermore, this polymer has been used for the treatment of different wounds due to its biocompatibility properties [81].

Pure sodium alginate solutions cannot be processed by electrospinning because of their high viscosity. But it is possible using organic solvents or with water-soluble synthetic polymer blend such as poly(vinyl alcohol) (PVA) and poly(ethylene oxide) [82]. Islam et al. [16] reported that blend of sodium alginate with PVA can be processed by electrospinning and forms ultrafine nanofibers with uniform surface.

Yu et al. [83] elaborated wound dressings with chitosan/alginate/collagen and hydroxyapatite with potential application in bone tissue engineering due to a suitable structure for cell development. Similarly, Jeong et al. [84] from a polyelectrolyte blend with chitosan/PEO obtained nanofibers for wound dressings, demonstrating their cell promotion and potential use as a dressing.

6.3. Carboxymethylcellulose

Carboxymethylcellulose is a semisynthetic natural polymer obtained by carboxymethylation of cellulose with properties such as biocompatibility, low toxicity, and low degradation rate [85]. Likewise, Ninan et al. [65] attribute their water solubility to their composition from the β-(1 → 4) glucopyranose residues. Its use in biomedicine as a biocompatible material has been proven [86]. Besides, Chen and Fan [87] studied their efficiency in clinical studies with humans and animals, finding that postoperative damages such as abdominal adhesions are reduced.

Chitosan blends with carboxymethylcellulose are possible because it is an anionic polymer with similar structure to chitosan allowing strong ionic cross-linking between them [67].

Fouda et al. [56] elaborated antimicrobial dressings for biological use from chitosan and carboxymethylcellulose blend with silver nanoparticles. Also, Ninan et al. [65] prepared wound dressings from a mixture of pectin, carboxymethylcellulose, and microfibrillated cellulose for skin wound treatment.

6.4. Collagen

Collagen is one of the proteins of the extracellular matrix more abundant in mammals. It is recognized for being reabsorbable with excellent biocompatibility and the ability to promote tissue regeneration [83]. The extracellular matrices in tissues are nanofiber structures that act as wound dressing to attach cells in the tissue, control tissue structure, and regulate the cell phenotype [88].

The main challenge for tissue engineering dressings is to design and create biodegradable matrices that can mimic the composition and structure of extracellular matrices [89]. Cross-linking and blending with biomolecules or synthetic materials have been used to improve the stability of collagen dressings [83]. According to Chen et al. [87], collagen is widely used as a biomaterial in the medical and pharmaceutical field. Collagen and chitosan are blended mimic the components of the extracellular matrix. Chen et al. [90] mentioned that electrospinning using a suitable solvent such as the mixture of 1,1,1,3,3,3 hexafluoro-2-propanol with trifluoroacetic acid to make dressings with application in tissue engineering is possible. Also, Yin et al. [91] used these solvents in electrospinning of collagen/chitosan/poly(L-lactide-co-ε-caprolactone) blend to produce dressings with application in vascular graft.

7. In vivo and in vitro assays

In vivo and in vitro assays evaluate the response of a biomaterial in experimental models or simulations. Biodegradable biomaterials have the characteristic of being completely degraded by the body's enzymes when the support is no longer necessary [55].

Table 4 shows the different in vitro and in vivo tests performed with biomaterials. Archana et al. [62] indicated that the dressings of chitosan/pectin/TiO_2 induce 1.14% of hemolysis of erythrocytes; for that reason they are highly hemocompatible when accepting values up to 5%.

Gautam et al. [92] reported high proliferation, adhesion, and cell morphology in polycaprolactone/elastin/chitosan dressings. This is due to the synergistic effect between the carboxyl group of polycaprolactone, gelatin amino group, and hydroxyl group of chitosan. In addition, in vitro degradation was almost complete after 8 weeks.

Likewise, Kumar et al. [75] in chitosan/pectin/calcium carbonate nanofibers reported that the dressings are biodegradable, because the pectin-chitin matrix gradually degraded up to 60% in 21 days.

Duan et al. [70] reported high cellular viability, because the poly(lactic acid-co-glycolic acid)/chitosan/PVA nanofibers promote attachment and proliferation of fibroblasts; in addition their morphology changed from round to elongated with little cellular activity. Likewise, Coimbra et al. [64] reported cellular growth and proliferation with a typical cellular form of pectin/chitosan dressings, thus demonstrating their biocompatibility and non-toxicity. On the other hand, Mendes et al. [93] evaluated the cellular metabolism and integrity of the membrane in fibroblasts in chitosan/phospholipid nanofibers by reporting biocompatible dressings.

Study	Assessment	Results	References
Application of chitosan/PVA nanofiber as a potential wound dressing for streptozotocin-induced diabetic rats	In vivo (adult Wistar rats)	Good adherence and promote tissue bonding	[14]
Electrospinning of carboxymethyl chitin/poly(vinyl alcohol) nanofibrous scaffolds for tissue engineering applications	In vitro (human mesenchymal stem cells)	High cell adhesion and proliferation	[20]
Evaluation of chitosan nano-dressing for wound healing: characterization, in vitro and in vivo studies	In vitro (mouse fibroblast (NIH 3 T3 and L929)) In vivo (adult male albino rats)	Induces blood clotting Fast wound healing Surface of the wound was covered with new epithelium	[62]
Preparation and chemical and biological characterization of a pectin/chitosan polyelectrolyte complex scaffold for possible bone tissue engineering applications	In vitro (human osteoblast cells (CRL-11372))	Cells adhered and proliferated	[64]
Drug delivery and tissue engineering applications of biocompatible pectin-chitin/nano-$CaCO_3$ composite scaffolds	In vitro (NIH3T3, MG63, and L929 cells)	Efficient cell adhesion and proliferation	[66]
A nanofibrous composite membrane of PLGA-chitosan/PVA prepared by electrospinning	In vitro (rabbit dermal fibroblast from rabbit back skin)	High fibroblast viability	[70]
Fabrication and characterization of PCL/gelatin/chitosan ternary nanofibrous composite scaffold for tissue engineering applications	In vitro (mouse fibroblast cells (L929))	High fibroblast growth and proliferation	[92]
Hybrid electrospun chitosan-phospholipids nanofibers for transdermal drug delivery	In vitro (L929 cells)	Biocompatible nanofibers	[93]

Table 4. In vitro and in vivo assays with chitosan nanofibers.

In vivo assays with animal experimental models on chitosan/PVA nanofibers reported cases of completely healed wounds. This has been attributed to protection of the injured tissue against bacteria that can infect damaged scar tissue by the pores of dressing; in addition the process of fluid absorption and healing was increased after 10 days [14]. Archana et al. [62] achieved wound closure after 16 days with chitosan/pectin/TiO_2 dressings, which were adhered, did not dissolve upon contact with the wound, and had easy removal.

8. Conclusion

Many technological advances continue to exploit the properties offered by polymeric materials as biocomposites. One of the more innovative advances is electrospinning. This technique is simple, low cost, and effective for the development of nanofibers. These polymeric nanocomposites are used as biomaterials for the preparation of dressings.

The surface properties of the nanofibers show the influence of the electrospinning parameters, from their diameter, pore size, porosity, ability to absorb the liquids of the wounds, and their ability to allow gas exchange in the wound, while the mechanical properties are influenced by the composition of the polymer solution. All this provides information about the ability of dressings to adapt to wounds and their ease to manipulate. In addition, it is well known that blending polymers to produce biocomposites is an effective method to improve the performance of materials.

Chitosan is a polycationic polymer widely studied because it has biological properties that make it a good candidate to be used for wound dressings. Thus, the microbiological assays show the capacity of the chitosan dressings against the main microorganisms present in the skin and wounds, demonstrating their efficiency. In vitro tests, through the simulation of wound conditions, reveal their behavior in the body. In contrast, in vivo tests expose the response of the dressing in animal experimental models. However, clinical study reports in human models are required to learn the precise behavior of the biomaterial and their response in the wound.

This chapter exhaustively reviews the literature that discusses the preparation of chitosan dressings by electrospinning and its blending with other polymers, as well as the biological properties that determine its potential medical use in the healing of cutaneous wounds.

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Conflicts of interests

The authors declare no conflicts of interest.

Summary

As a summary, electrospinned chitosan nanocomposites are promising biomedical wound dressings.

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References

[1] Khor E, Lee YL. Implantable applications of chitin and chitosan. Biomaterials. 2003; **24**:2339-2349. DOI: 10.1016/S0142-9612(03)00026-7

[2] Dash M, Chiellini F, Ottenbrite RM, Chiellini E. Chitosan—A versatile semi-synthetic polymer in biomedical applications. Progress in Polymer Science. 2011;**36**:981-1014. DOI: 10.1016/j.progpolymsci.2011.02.001

[3] Geng X, Kwon O, Jang J. Electrospinning of chitosan dissolved in concentrated acetic acid solution. Biomaterials. 2005;**26**:5427-5432. DOI: 10.1016/j.biomaterials.2005.01.066

[4] Guyal R, Macri LK, Kaplan HM, Kohn J. Nanoparticles and nanofibers for topical drug delivery. Journal of Controlled Release. 2016;**240**:77-92. DOI: 10.1016/j.jconrel.2015.10.049

[5] Abdelrahman T, Newton H. Wound dressings: Principles and practice. Surgery (Oxford). 2011;**29**(10):491-495. DOI: 10.1016/j.mpsur.2011.06.007

[6] Nawalakhe R, Shi Q, Vitchuli N, Noar J, Caldwell JM, Breidt F, Bourham MA, Zhang X, McCord AG. Novel atmospheric plasma enhanced chitosan nanofiber/gauze composite wound dressings. Journal of Applied Polymer Science. 2013;**129**(2):916-923. DOI: 10.1002/APP.38804

[7] Smith IO, Liu XH, Smith LA, Ma PX. Nanostructured polymer scaffolds for tissue engineering and regenerative medicine. WIREs Nanomedicine and Nanobiotechnology. 2009;**1**:226-236. DOI: 10.1002/wnan.026

[8] Bhardwaj N, Kundu SC. Electrospinning: A fascinating fiber fabrication technique. Biotechnology Advances;**201, 28**:325-347. DOI: 10.1016/j.biotechadv.2010.01.004

[9] Li M, Mondrinos MJ, Ghandi MR, Ko FK, Weiss AS, Lelkes PI. Electrospun protein fibers as matrices for tissue engineering. Biomaterials. 2005;**26**:5999-6008. DOI: 10.1016/j.biomaterials.2005.03.030

[10] Hamori M, Nagano K, Kakimoto S, Naruhashi K, Kiriyama A, Nishimura A, Shibata N. Preparation and pharmaceutical evaluation of acetaminophen nano-fiber tablets: Application of a solvent-based electrospinning method for tableting. Biomedicine & Pharmacotherapy. 2016;**78**:14-22. DOI: 10.1016/j.biopha.2015.12.023

[11] Sill TJ, von Recum HA. Electrospinning: Applications in drug delivery and tissue engineering. Biomaterials. 2008;**29**(13):1989-2006. DOI: 10.1016/j.biomaterials.2008.01.011

[12] Kai D, Liow SS, Loh XJ. Biodegradable polymers for electrospinning: Towards biomedical applications. Materials Science and Engineering: C. 2014;**45**:659-670. DOI: 10.1016/j.msec.2014.04.051

[13] Destaye AG, Lin CK, Lee CK. Glutaraldehyde vapor cross-linked nanofibrous PVA mat with in situ formed silver nanoparticles. ACS Applied Materials & Interfaces. 2013;**5**(11):4745-4752. DOI: 10.1021/am401730x

[14] Majd SA, Khorasgani MR, Moshtaghian SJ, Talebi A, Khezri M. Application of chitosan/PVA nano fiber as a potential wound dressing for streptozotocin-induced diabetic rats.

International Journal of Biological Macromolecules. 2016;**92**:1162-1168. DOI: 10.1016/j.ijbiomac.2016.06.035

[15] Frohbergh ME, Katsman A, Botta GP, Lazarovici P, Schauer CL, Wegst GK, Lelkes IP. Electrospun hydroxyapatite-containing chitosan nanofibers crosslinked with genipin for bone tissue engineering. Biomaterials. 2012;**33**:9167-9178. DOI: 10.1016/j.biomaterials.2012.09.009

[16] Islam MS, Karim MR. Fabrication and characterization of poly(vinyl alcohol)/alginate blend nanofibers by electrospinning method. Colloids and Surfaces, A: Physicochemical and Engineering Aspects. 2010;**366**(1):135-140. DOI: 10.1016/j.colsurfa.2010.05.038

[17] Jia YT, Gong J, Gu XH, Kim HY, Dong J, Shen XY. Fabrication and characterization of poly(vinyl alcohol)/chitosan blend nanofibers produced by electrospinning method. Carbohydrate Research. 2007;**67**(3):403-409. DOI: 10.1016/j.carbpol.2006.06.010

[18] Leung V, Hartwell R, Elizei SS, Yang H, Ghahary A, Ko F. Postelectrospinning modifications for alginate nanofiber-based wound dressings. Journal of Biomedical Materials Research Part B. 2014;**102**(3):508-515. DOI: 0.1002/jbm.b.33028

[19] Pakravan M, Heuzey MC, Ajji A. A fundamental study of chitosan/PEO electrospinning. Polymer. 2011;**52**(21):4813-4824. DOI: 10.1016/j.polymer.2011.08.034

[20] Shalumon KT, Annulekha KH, Nair SV, Nair SV, Chennazhi KP, Jayakumar R. Sodium alginate/poly(vinyl alcohol)/nano ZnO composite nanofibers for antibacterial wound dressings. International Journal of Biological Macromolecules. 2011;**49**:247-254. DOI: 10.1016/j.ijbiomac.2011.04.005

[21] Stone SA, Gosavi P, Athauda TJ, Ozer RR. In situ citric acid crosslinking of alginate/polyvinyl alcohol electrospun nanofibers. Materials Letters. 2013;**112**:32-35. DOI: 10.1016/j.matlet.2013.08.100

[22] Tonsonmboon K, Oyen M. Composite electrospun gelatin fiber-alginate gel scaffolds for mechanically robust tissue engineered cornea. Journal of the Mechanical Behavior of Biomedical Materials. 2013;**21**:185-194. DOI: 10.1016/j.jmbbm.2013.03.001

[23] Van der Schueren L, Steyaert I, De Schoenmaker B, De Clerck K. Polycaprolactone/chitosan blend nanofibres electrospun from an acetic acid/formic acid solvent system. Carbohydrate Polymers. 2012;**88**(4):1221-1226. DOI: 10.1016/j.carbpol.2012.01.085

[24] Cui S, Yao B, Sun X, Hu J, Zhou Y, Liu Y. Reducing the content of carrier polymer in pectin nanofibers by electrospinning at low loading followed with selective washing. Materials Science & Engineering. C, Materials for Biological Applications. 2016;**59**:885-893. DOI: 10.1016/j.msec.2015.10.086

[25] Haider A, Haider S, Kang IK. A comprehensive review summarizing the effect of electrospinning parameters and potential applications of nanofibers in biomedical and biotechnology. Arabian Journal of Chemistry. 2015. DOI: 10.1016/j.arabjc.201

[26] Rutledge GC, Fridrikh SV. Formation of fibers by electrospinning. Advanced Drug Delivery Reviews. 2007;**59**(14):1384-1391. DOI: 10.1016/j.addr.2007.04.020

[27] Huang ZM, Zhang YZ, Kotaki M, Ramakrishna S. A review on polymer nanofibers by electrospinning and their applications in nanocomposites. Composites Science and Technology. 2003;**63**(15):2223-2253. DOI: 10.1016/S0266-3538(03)00178-7

[28] Dabirian F, Ravandi SH, Sanatgar RH, Hinestroza JP. Manufacturing of twisted continuous PAN nanofiber yarn by electrospinning process. Fibers and Polymers. 2011;**12**(5):610. DOI: 10.1007/s12221-011-0610-6

[29] Charernsriwilaiwat N, Opanasopit P, Rojanarata T, Ngawhirunpat T, Supaphol P. Preparation and characterization of chitosan-hydroxybenzotriazole/polyvinyl alcohol blend nanofibers by the electrospinning technique. Carbohydrate Polymers. 2010;**81**(3):675-680. DOI: 10.1016/j.carbpol.2010.03.031

[30] Ahmed S, Ikram S. Chitosan based scaffolds and their applications in wound healing. Achievements in the Life Sciences. 2016;**10**(1):27-37. DOI: 10.1016/j.als.2016.04.001

[31] Croisier F, Jérôme C. Chitosan-based biomaterials for tissue engineering. European Polymer Journal. 2013;**49**(4):780-792. DOI: 10.1016/j.eurpolymj.2012.12.009

[32] Berger J, Reist M, Mayer JM, Felt O, Peppas NA, Gurny R. Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. European Journal of Pharmaceutics and Biopharmaceutics. 2004;**57**(1):19-34. DOI: 10.1016/S0939-6411(03)00161-9

[33] Baldrick P. The safety of chitosan as a pharmaceutical excipient. Regulatory Toxicology and Pharmacology. 2010;**56**(3):290-299. DOI: 10.1016/j.yrtph.2009.09.015

[34] Mansouri S, Lavigne P, Corsi K, Benderdour M, Beaumont E, Fernandes JC. Chitosan-DNA nanoparticles as non-viral vectors in gene therapy: Strategies to improve transfection efficacy. European Journal of Pharmaceutics and Biopharmaceutics. 2004;**57**(1):1-8. DOI: 10.1016/S0939-6411(03)00155-3

[35] Kean T, Thanou M. Biodegradation, biodistribution and toxicity of chitosan. Advanced Drug Delivery Reviews. 2010;**62**(1):3-11. DOI: 10.1016/j.addr.2009.09.004

[36] Waibel KH, Haney B, Moore M, Whisman B, Gomez R. Safety of chitosan bandages in shellfish allergic patients. Military Medicine. 2011;**176**(10):1153-1156

[37] Ng WL, Yeong WY, Naing MW. Polyelectrolyte gelatin-chitosan hydrogel optimized for 3D bioprinting in skin tissue engineering. International Journal of Bioprinting. 2016;**2**(1):53-62. DOI: 10.18063/IJB.2016.01.009

[38] Sharp RG. A review of the applications of chitin and its derivatives in agriculture to modify plant-microbial interactions and improve crop yields. Agronomy. 2013;**3**(4):757-793

[39] Rao SB, Sharma CP. Use of chitosan as a biomaterial: Studies on its safety and hemostatic potential. Journal of Biomedical Materials Research. 1997;**34**(1):21-28. DOI: 0.1002/(SICI)1097-4636(199701)34:1<21::AID-JBM4>3.0.CO;2-P

[40] Kong M, Chen XG, Xing K, Park HJ. Antimicrobial properties of chitosan and mode of action: A state of the art review. International Journal of Food Microbiology. 2010;**144**(1):51-63. DOI: 10.1016/j.ijfoodmicro.2010.09.012

[41] Xia W, Liu P, Zhang J, Chen J. Biological activities of chitosan and chitooligosaccharides. Food Hydrocolloids. 2011;**25**(2):170-179. DOI: 10.1016/j.foodhyd.2010.03.003

[42] De Vrieze S, Westbroek P, Van Camp T, Van Langenhove L. Electrospinning of chitosan nanofibrous structures: Feasibility study. Journal of Materials Science. 2007;**42**(19):8029-8034. DOI: 10.1007/s10853-006-1485-6

[43] Kriegel C, Kit KM, McClements DJ, Weiss J. Influence of surfactant type and concentration on electrospinning of chitosan-poly(ethylene oxide) blend nanofibers. Food Biophysics. 2009;**4**(3):213-228. DOI: 10.1007/s11483-009-9119-6

[44] Lee SH, Wang S. Biodegradable polymers/bamboo fiber biocomposite with bio-based coupling agent. Composites. Part A, Applied Science and Manufacturing. 2006;**37**(1):80-91. DOI: 10.1016/j.compositesa.2005.04.015

[45] Vert M. Polymeric biomaterials: Strategies of the past vs. strategies of the future. Progress in Polymer Science. 2007;**32**(8):755-761. DOI: 10.1016/j.progpolymsci.2007.05.006

[46] Binyamin G, Shafi BM, Mery CM. Biomaterials: A primer for surgeons. Seminars in Pediatric Surgery. 2006;**15**(4):276-283. DOI: 10.1053/j.sempedsurg.2006.07.007

[47] Zhang Y, Lim CT, Ramakrishna S, Huang ZM. Recent development of polymer nanofibers for biomedical and biotechnological applications. Journal of Materials Science. Materials in Medicine. 2005;**16**(10):933-946. DOI: 10.1007/s10856-005-4428-x

[48] Boateng JS, Matthews KH, Stevens HN, Eccleston GM. Wound healing dressings and drug delivery systems: A review. Journal of Pharmaceutical Sciences. 2008;**97**(8):2892-2923. DOI: 10.1002/jps.21210

[49] Newman GR, Walker M, Hobot JA, Bowler PG. Visualisation of bacterial sequestration and bactericidal activity within hydrating Hydrofiber® wound dressings. Biomaterials. 2006;**27**(7):1129-1139. DOI: 0.1016/j.biomaterials.2005.07.046

[50] Caló E, Khutoryanskiy VV. Biomedical applications of hydrogels: A review of patents and commercial products. European Polymer Journal. 2015;**65**:252-267. DOI: 10.1016/j.eurpolymj.2014.11.024

[51] Zahedi P, Rezaeian I, Ranaei-Siadat SO, Jafari SH, Supaphol P. A review on wound dressings with an emphasis on electrospun nanofibrous polymeric bandages. Polymers for Advanced Technologies. 2010;**21**(2):77-95. DOI: 10.1002/pat.1625

[52] Newsom EC, Connolly KL, Nehal KS. Facilitating healing of granulating wounds: Dressings, dermal substitutes, and other methods. Current Dermatology Reports. 2015;**4**(3):125-133. DOI: 10.1007/s13671-015-0108-3

[53] Bhattarai N, Li Z, Edmondson D, Zhang M. Alginate-based nanofibrous scaffolds: Structural, mechanical, and biological properties. Advanced Materials. 2006;**18**(11):1463-1467. DOI: 10.1002/adma.200502537

[54] Haider S, Al-Zeghayer Y, Ali FAA, Haider A, Mahmood A, Al-Masry WA, Aijaz MO. Highly aligned narrow diameter chitosan electrospun nanofibers. Journal of Polymer Research. 2013;**20**(4):105. DOI: 10.1007/s10965-013-0105-9

[55] Lu JW, Zhu YL, Guo ZX, Hu P, Yu J. Electrospinning of sodium alginate with poly(ethylene oxide). Polymer Journal. 2006;**47**(23):8026-8031. DOI: 10.1016/j.polymer.2006.09.027

[56] Fouda MM, El-Aassar MR, Al-Deyab SS. Antimicrobial activity of carboxymethyl chitosan/polyethylene oxide nanofibers embedded silver nanoparticles. Carbohydrate Polymers. 2013;**92**(2):1012-1017. DOI: 10.1016/j.carbpol.2012.10.047

[57] Chen ZG, Wang PW, Wei B, Mo XM, Cui FZ. Electrospun collagen–chitosan nanofiber: A biomimetic extracellular matrix for endothelial cell and smooth muscle cell. Acta Biomaterialia. 2010;**6**(2):372-382. DOI: 10.1016/j.actbio.2009.07.024

[58] Garai S, Sinha A. Biomimetic nanocomposites of carboxymethyl cellulose–hydroxyapatite: Novel three dimensional load bearing bone grafts. Colloids and Surfaces, B: Biointerfaces. 2014;**115**:182-190. DOI: 10.1016/j.colsurfb.2013.11.042

[59] Tarun K, Gobi N. Calcium alginate/PVA blended nano fibre matrix for wound dressing. Indian Journal of Fibre & Textile Research. 2012;**37**:127-132

[60] Zarghami A, Iran M, Mostafazadeh A, Golpour M, Heidarinasab A, Haririan I. Fabrication of PEO/chitosan/PCL/olive oil nanofibrous scaffolds for wound dressing applications. Fibers and Polymers. 2015;**16**(6):1201-1212. DOI 10.1007/s12221-015-1201-8

[61] Chan BP, Leong KW. Scaffolding in tissue engineering: General approaches and tissue-specific considerations. European Spine Journal. 2008;**17**(4):467-479. DOI: 10.1007/s00586-008-0745-3

[62] Archana D, Dutta J, Dutta PK. Evaluation of chitosan nano dressing for wound healing: Characterization, in vitro and in vivo studies. International Journal of Biological Macromolecules. 2013;**57**:193-203. DOI: 10.1016/j.ijbiomac.2013.03.002

[63] Ziabari M, Mottaghitalab V, Haghi AK. Evaluation of electrospun nanofiber pore structure parameters. Korean Journal of Chemical Engineering. 2008;**25**(4):923-932. DOI: 10.1007/s11814-008-0151-x

[64] Coimbra P, Ferreira P, De Sousa HC, Batista P, Rodrigues MA, Correia IJ, Gil MH. Preparation and chemical and biological characterization of a pectin/chitosan polyelectrolyte complex scaffold for possible bone tissue engineering applications. International Journal of Biological Macromolecules. 2011;**48**(1):112-118. DOI: 10.1016/j.ijbiomac.2010.10.006

[65] Ninan N, Muthiah M, Park IK, Kalarikkal N, Elain A, Wong TW, Grohens Y. Wound healing analysis of pectin/carboxymethyl cellulose/microfibrillated cellulose based composite scaffolds. Materials Letters. 2014;**132**:34-37. DOI: 10.1016/j.matlet.2014.06.056

[66] Kumar PS, Ramya C, Jayakumar R, Lakshmanan VK. Drug delivery and tissue engineering applications of biocompatible pectin–chitin/nano $CaCO_3$ composite scaffolds. Colloids and Surfaces, B: Biointerfaces. 2013;**106**:109-116. DOI: 10.1016/j.colsurfb.2013.01.048

[67] Liuyun J, Yubao L, Chengdong X. Preparation and biological properties of a novel composite scaffold of nano-hydroxyapatite/chitosan/carboxymethyl cellulose for bone tissue engineering. Journal of Biomedical Science. 2009;**16**(1):65. DOI: 10.1186/1423-0127-16-65

[68] Sarhan WA, Azzazy HM, El-Sherbiny IM. The effect of increasing honey concentration on the properties of the honey/polyvinyl alcohol/chitosan nanofibers. Materials Science and Engineering: C. 2016;**67**:276-284. DOI: 10.1016/j.msec.2016.05.006

[69] Choi C, Nam JP, Nah JW. Application of chitosan and chitosan derivatives as biomaterials. Industrial and Engineering Chemistry Research. 2016;**33**:1-10. DOI: 10.1016/j.jiec.2015.10.028

[70] Duan B, Yuan X, Zhu Y, Zhang Y, Li X, Zhang Y, Yao K. A nanofibrous composite membrane of PLGA–chitosan/PVA prepared by electrospinning. European Polymer Journal. 2006;**42**(9):2013-2022. DOI: 10.1016/j.eurpolymj.2006.04.021

[71] Chen JP, Chang GY, Chen JK. Electrospun collagen/chitosan nanofibrous membrane as wound dressing. Colloids and Surfaces, A: Physicochemical and Engineering Aspects. 2008;**313**:183-188. DOI: 10.1016/j.colsurfa.2007.04.129

[72] Heunis TDJ, Dicks LMT. Nanofibers offer alternative ways to the treatment of skin infections. BioMed Research International. 2010;**2010**:1-10. DOI: 10.1155/2010/510682

[73] Lee SJ, Heo DN, Moon JH, Park HN, Ko WK, Bae MS, Jung BY. Chitosan/polyurethane blended fiber sheets containing silver sulfadiazine for use as an antimicrobial wound dressing. Journal of Nanoscience and Nanotechnology. 2014;**14**(10):7488-7494. DOI: 10.1166/jnn.2014.9581

[74] Au HT, Pham LN, Vu THT, Park JS. Fabrication of an antibacterial non-woven mat of a poly(lactic acid)/chitosan blend by electrospinning. Macromolecular Research. 2012;**20**(1):51-58. DOI: 10.1007/s13233-012-0010-9

[75] Kumar MNR. A review of chitin and chitosan applications. Reactive and Functional Polymers. 2000;**46**(1):1-27. DOI: 10.1016/S1381-5148(00)00038-9

[76] Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances. 2009;**27**(1):76-83. DOI:10.1016/j.biotechadv.2008.09.002;10.1016/j.biotechadv.2008.09.002

[77] Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: green synthesis and their antimicrobial activities. Advances in colloid and interface science. 2009;**145**(1-2):83-96. DOI: 10.1016/j.cis.2008.09.002

[78] Wang Q, Hu X, Du Y, Kennedy JF. Alginate/starch blend fibers and their properties for drug controlled release. Carbohydrate Polymers. 2010;**82**(3):842-847. DOI: 10.1016/j.carbpol.2010.06.004

[79] Tehrani NS, Faridi-Majidi R, Atar H, Rezayat M. In: 4th International Conference on Nanostructures (ICNS4). Kish Island, I.R. Iran. 12-14 March; 2012. p. 820-822

[80] Fan L, Du Y, Zhang B, Yang J, Zhou J, Kennedy JF. Preparation and properties of alginate/carboxymethyl chitosan blend fibers. Carbohydrate Polymers. 2006;**65**(4):447-452. DOI: 10.1016/j.carbpol.2006.01.031

[81] Pereira R, Mendes A, Bártolo P. Alginate/Aloe vera hydrogel films for biomedical applications. Procedia CIRP. 2013;**5**:210-215. DOI: 10.1016/j.procir.2013.01.042

[82] Shen W, Hsieh YL. Biocompatible sodium alginate fibers by aqueous processing and physical crosslinking. Carbohydrate Polymers. 2014;**102**:893-900. DOI: 10.1016/j.carbpol.2013.10.066

[83] Yu CC, Chang JJ, Lee YH, Lin YC, Wu MH, Yang MC, Chien CT. Electrospun scaffolds composing of alginate, chitosan, collagen and hydroxyapatite for applying in bone tissue engineering. Materials Letters. 2013;**93**:133-136. DOI: 10.1016/j.matlet.2012.11.040

[84] Jeong SI, Krebs MD, Bonino CA, Samorezov JE, Khan SA, Alsberg E. Electrospun chitosan–alginate nanofibers with in situ polyelectrolyte complexation for use as tissue engineering scaffolds. Tissue Engineering. 2010;**17**(1-2):59-70. DOI: 10.1089/ten.tea.2010.0086

[85] Pasqui D, Torricelli P, Cagna M, Fini M, Barbucci R. Carboxymethyl cellulose—Hydroxyapatite hybrid hydrogel as a composite material for bone tissue engineering applications. Journal of Biomedical Materials Research. Part A. 2014;**102**(5):1568-1579. DOI: 10.1002/jbm.a.34810

[86] Maver T, Kurečič M, Smrke DM, Kleinschek KS, Maver U. Electrospun nanofibrous CMC/PEO as a part of an effective pain-relieving wound dressing. Journal of Sol-Gel Science and Technology. 2016;**9**(3):475-486. DOI: 10.1007/s10971-015-3888-9

[87] Chen H, Fan M. Chitosan/carboxymethyl cellulose polyelectrolyte complex scaffolds for pulp cells regeneration. Journal of Bioactive and Compatible Polymers. 2007;**22**(5):475-491. DOI: 10.1177/0883911507081329

[88] Mo X, Chen Z, Weber HJ. Electrospun nanofibers of collagen-chitosan and P (LLA-CL) for tissue engineering. Front. Mater. Sci. China. 2007;**1**(1):20-23. DOI: 10.1007/s11706-007-0004-2

[89] Huang C, Chen R, Ke Q, Morsi Y, Zhang K, Mo X. Electrospun collagen–chitosan–TPU nanofibrous scaffolds for tissue engineered tubular grafts. Colloids and Surfaces, B: Biointerfaces. 2011;**82**(2):307-315. DOI: 10.1016/j.colsurfb.2010.09.002

[90] Chen Z, Mo X, Qing F. Electrospinning of collagen–chitosan complex. Materials Letters. 2007;**61**(16):3490-3494. DOI: 10.1016/j.matlet.2006.11.104

[91] Yin A, Zhang K, McClure MJ, Huang C, Wu J, Fang J, El-Newehy M. Electrospinning collagen/chitosan/poly(ʟ-lactic acid-co-ϵ-caprolactone) to form a vascular graft: Mechanical and biological characterization. Journal of Biomedical Materials Research. Part A. 2013;**101(**(5):1292-1301. DOI: 10.1002/jbm.a.34434

[92] Gautam S, Chou CF, Dinda AK, Potdar PD, Mishra NC. Fabrication and characterization of PCL/gelatin/chitosan ternary nanofibrous composite scaffold for tissue engineering applications. Journal of Materials Science. 2014;**49**(3):1076-1089

[93] Mendes AC, Gorzelanny C, Halter N, Schneider SW, Chronakis IS. Hybrid electrospun chitosan-phospholipids nanofibers for transdermal drug delivery. International Journal of Pharmaceutics. 2016;**510**(1):48-56. DOI: 10.1016/j.ijpharm.2016.06.016

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