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Development of Chitosan-Gelatin Nanofibers with Cellulose Nanocrystals for Skin Protection Applications

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Abstract. In this work, natural-based and biodegradable nanofibers were produced by electrospinning for drug delivery and wound dressing applications, using gelatin (Gel), chitosan (CS), cellulose nanocrystals (CNC) and natural propolis extract. The polymeric formulations and electrospinning parameters were optimized, resulting in the development of Gel/CS nanofibers with mean diameters of 97 nm. CNC were successfully introduced into the optimized Gel/CS solution and the viscosity and conductivity values were recorded. The developed nanofibers were characterized using FESEM, ATR-FTIR, TGA and WCA. The incorporation of different CNC concentrations improved the solutions' electrospinnability and the membranes' physical integrity. Defect-free and uniform Gel/CS/CNC nanofibers were observed by FESEM images, and the fibers' diameters slight increased. The hydrophilic character was maintained after the CNC incorporation. Finally, Gel/CS/CNC/Propolis nanofibers demonstrated antibacterial activity against both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria.

Introduction

In recent years, the increasing exposure to biological and chemical hazards not only by the military personnel but also the civilians highlight the importance for the search and development of novel protective clothing materials. Electrospun nanofibrous membranes have emerged as a promising alternative due to their simple production method, lightness, flexibility, breathability and the possibility to be applied directly onto textile substrates [1,2]. Due to their highly interconnected porosity with nano pore size, nanofibers can act as a physical barrier to block the penetration of biological and chemical agents. Additionally, the ability to incorporate different bioactive agents can not only provide new properties to the nanofibers, such as antimicrobial activity, but also to control the therapeutic agent's release and prolong its action. All these properties make nanofibrous mats very attractive for skin protection applications, including for drug delivery and wound dressing systems [3–5].

Nowadays, considering the environment concerns, the use of sustainable materials is acquiring more and more attention. Hence, the polymers derived from natural sources are preferable, like gelatin (Gel) and chitosan (CS), which are obtained from collagen and chitin, respectively. Besides their renewability, these polymers present great properties, such as biocompatibility, biodegradability and bioactivity. Moreover, CS, with its antimicrobial properties against a wide range of bacteria and fungi, and Gel, with its cell adhesion and proliferation properties, offer a unique combination with a lot of potential to be used for wound dressing and drug delivery systems [6–8].

Nevertheless, the poor mechanical properties and the higher difficulty to process when compared to synthetic polymers, are some of the drawbacks that limit the use of natural ones [9]. The incorporation of nanomaterials, like cellulose nanocrystals (CNC), is a promising approach to

overcome these limitations and to improve nanofibers' performance. In fact, CNC exhibit high strength and stiffness, low weight, biocompatibility and biodegradability. Furthermore, these nanofillers seem to have a positive impact in the morphology and diameters of electrospun nanofibers. Therefore, the introduction of CNC will allow the development of nanofibers with improved properties, without compromising their natural-based origin and biodegradability [10,11].

At the same time, the incorporation of natural extracts can create new properties into electrospun nanofibers. Propolis is a natural extract derived from the action of the bees in the resins of the plants, which presents several interesting properties, including antibacterial, antifungal, anti-inflammatory and antioxidant activities [12]. In addition, the development of nanofibers with natural extracts allows a continuous localized delivery of the bioactive agent, making it an efficient route of administration, with lower adverse effects [3].

Therefore, the main objective of this study was the development of fully natural nanofibers based on Gel/CS/CNC/Propolis via electrospinning for drug delivery and wound dressing applications. Firstly, the polymeric formulations were optimized as well as the electrospinning parameters. Afterwards, different CNC concentrations were incorporated into the optimized Gel/CS solution (0.1, 0.5, 1 and 2% (w/v)) and their effect was evaluated. The viscosity and conductivity of the polymeric solutions were analyzed. The developed nanofibers were characterized by Field Emission Scanning Microscopy (FESEM), Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR), Thermogravimetric analysis (TGA) and Water Contact Angle (WCA). Finally, the propolis natural extract, well known for its antibacterial activity, was added and its influence was studied. The antibacterial effect of the developed nanofibers was evaluated against Gram-negative and Gram-positive bacteria.

Materials and Methods

Materials

Gelatin (Gel, from porcine skin Type A) and chitosan (CS, high molecular weight) powders were obtained from Sigma-Aldrich as well as Dimethyl Sulfoxide (DMSO) anhydrous ≥ 99.9. Acetic acid glacial (AcOH) 99-100% a.r. and propolis natural extract were purchased from Chem-Lab NV and laboratories ORTIS sprl, respectively. Two bacterial strains: Gram-negative (*Escherichia coli*: ATCC 11303), and Gram-positive (*Staphylococcus aureus*: ATCC 6538) were used for the antibacterial tests.

Extraction of Cellulose Nanocrystals (CNC)

CNC were extracted from a commercial bleached Stipa Tenacissima pulp via acid hydrolysis. This set was conducted at 45 ºC under continuous mechanical stirring using 64 wt% diluted sulfuric acid solution for 120 minutes. The cellulose suspension was then sonicated and centrifuged before being diluted with distilled water to stop the acid effect and left to settle overnight. The water was then decanted off and the suspension was centrifuged. The supernatant after the first centrifugation was decanted off and replaced by an equivalent volume of distilled water. The resulting suspension was centrifuged again. The centrifugation/supernatant decanting/distilled water addition was repeated 3 times in order to remove the free acid in the suspension as well as the soluble materials. The cellulose suspension was placed in a dialysis membrane and dialyzed until neutral pH. The subsequent suspension was freeze dried and stored in a refrigerator at 4 ºC before characterization.

Preparation of Gel/CS blends incorporated with CNC and propolis natural extract

Firstly, 15% (w/v) of Gel was dissolved in 50% (v/v) acetic acid under constant stirring at room temperature. After that, CS powder was added, and the solution was kept under stirring until complete dissolution of both polymers. Three different CS concentrations were studied (1, 2 and 3 % (w/v)). Consequently, different CNC concentrations (0.1, 0.5, 1 and 2 % (w/v)) were incorporated into the optimized polymeric solution. The addition of CNC powder was slowly to avoid CNC clusters formation and the polymer blend was left overnight under vigorous stirring, in order to obtain a total

homogenization. Finally, propolis natural extract was dissolved in a minimal amount of DMSO and introduced in Gel/CS/CNC solution to obtain final concentration of 2 mg/mL.

Electrospinning Conditions

All the solutions were electrospun in an electrospinning NF-103 equipment from MECC. Each solution was fed into a 12 mL syringe and a needle gauge 22 GP. 0.16X1.0. The produced nanofibers were collected in a static collector covered by aluminum foil. The environment temperature and relative humidity were controlled, ranging from 19 to 21 ºC and from 59 to 62 %, respectively. All the electrospinning parameters were optimized, namely the applied voltage, the distance between the needle and collector and the feed-rate. The best nanofiber's morphology was obtained with 22 kV of voltage, 160 mm of distance between the needle and collector and 0.2 mL/h of feed-rate. Figure 1 summarizes the optimization process by showing the different polymeric formulations and electrospinning parameters evaluated. The optimized conditions are highlighted in bold.

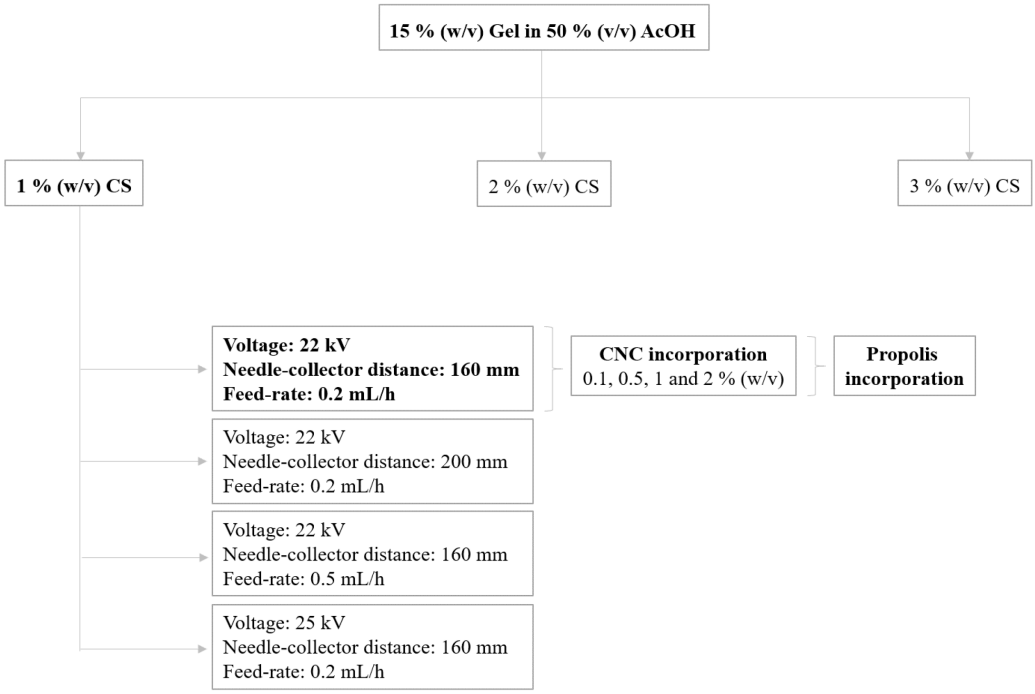

Fig. 1. Optimization process of the polymeric formulations and electrospinning parameters for the development of Gel/CS nanofibers.

Nanofibers Characterization

Field Emission Scanning Microscopy (FESEM)

The morphological analysis of the electrospun mats was performed in an Ultra-high-resolution Field Emission Scanning Electron Microscopy (FESEM), NOVA 200 Nano SEM, FEI Company (Hillsboro, OR, USA). Before the analysis, the samples were covered with a very thin film (20 nm) of Au-Pd (80–20 weight %), using a high-resolution sputter coater, 208 HR Cressington Company (Watford, UK), coupled to a MTM-20 Cressington High Resolution Thickness Controller. Secondary electron images, i.e., topographic images, were performed at an acceleration voltage of 10 kV.

Nanofibers diameters

The diameters of electrospun nanofibers were determined using Image J software. The FESEM images of the samples were analysed, and the diameters were measured in 100 different locations.

Viscosity and Conductivity

The viscosity and electrical conductivity of the solutions were measured using a Rotary Viscometer serie VB 3000 and a 4510 Conductivity/TDS Meter, respectively. The viscosity

measurement of the solutions was performed using a R5 spindle and three different speeds (60, 100 and 200 rpm).

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR)

ATR-FTIR spectroscopy was used to study the chemical composition of electrospun nanofibers. This analysis was performed with an IRAffinity-1S, SHIMADZU equipment (Kyoto, Japan). Each spectrum was acquired in transmittance mode on a diamond ATR crystal cell by accumulation of 45 scans with a resolution of 4 cm^{-1} from 400 to 4000 cm^{-1}. The samples were analysed in different sites to ensure homogeneity.

Thermogravimetric analysis (TGA)

Thermal analysis was carried out on a STA 700 SCANSCI. The TGA trace was obtained in the range of 30–600 ºC, under nitrogen atmosphere, with a constant heating rate of 10 ºC/min.

Water Contact Angle (WCA)

WCA measurements were performed using a Contact Angle System with high-resolution camera attached. A volume of 5 µL of distilled water was dropped onto sample's surface, and the WCA was measured through the shape of the drop. The samples were analysed in ten different sites in order to ensure homogeneity.

Antibacterial activity

The antibacterial activity of the electrospun nanofibers was assessed according to the standard shake flask method (ASTM-E2149-01). For this test, two bacteria were used: Gram-negative, *Escherichia coli* (*E. coli*) and Gram-positive, *Staphylococcus aureus* (*S. aureus*). Bacterial suspensions were put in contact with the samples (1x2 cm) at 37 ºC during 24 h and 48 h. Serial dilutions were performed and spread on agar plates to determine the number of surviving bacteria. Three replicates of each system were evaluated in order to have reliable and reproducible results. The antibacterial activity, defined by Equation 1, is reported in terms of percentage of bacteria reduction.

$$\text{Bacteria reduction (\%)} = [A\text{-}B/A] \times 100\% \quad (1)$$

where A and B are the average number of bacteria before and after the contact with the samples, respectively.

Results

Morphology and diameters analyses

To produce defect-free Gel/CS nanofibers different parameters were optimized, including solution and electrospinning conditions. Firstly, several CS concentrations were tested in order to evaluate its effect on the nanofibers' diameter and morphology, as shown in Figure 2.

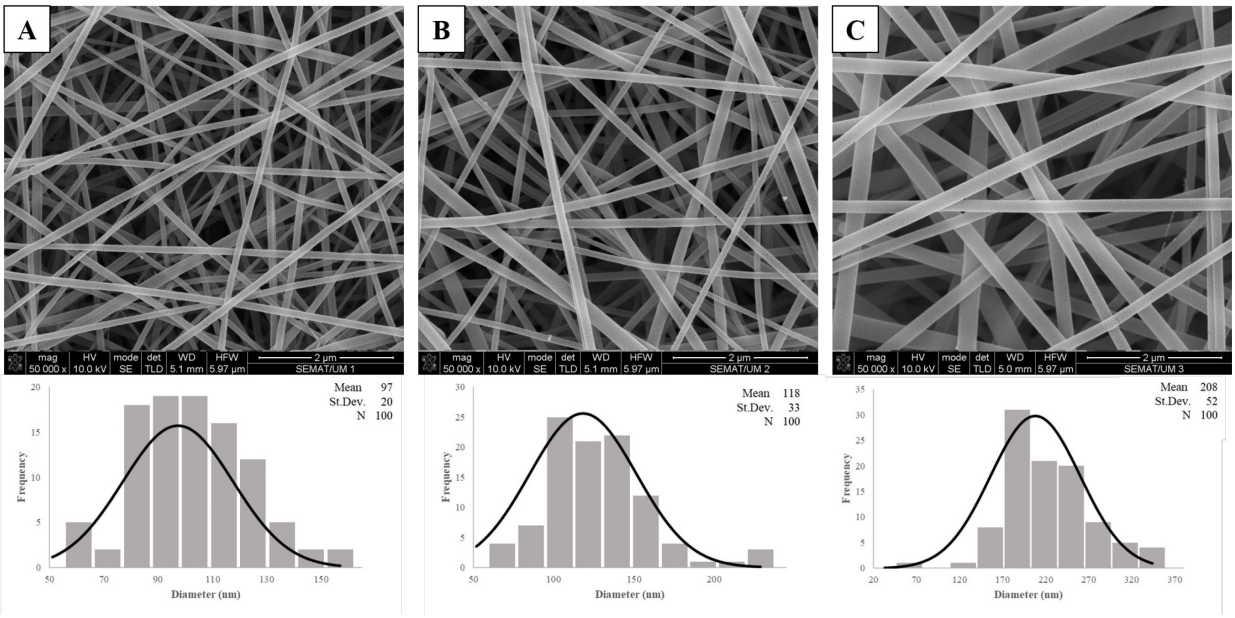

Fig. 2. FESEM images of Gel/CS fibers produced via electrospinning using 15% (w/v) of Gel and different CS concentrations: **A)** 1, **B)** 2 and **C)** 3 % (w/v), with magnifications of 2 μm, and their respective diameter distribution histograms.

As demonstrated in Figure 2, Gel/CS electrospun fibers without any defects were successfully produced with all the CS concentrations under study, ranging from 1 to 3 %. Nevertheless, an increase in fiber's size was observed according to CS concentration. In fact, using 1, 2 and 3 % of CS, fibers with mean diameters of 97, 108 and 208 nm were obtained, respectively. Therefore, the system with smaller diameters (1% of CS) was chosen and different electrospinning parameters were evaluated in order to verify if it was possible to obtain electrospun membranes with better morphology. The results are shown in Figure 3.

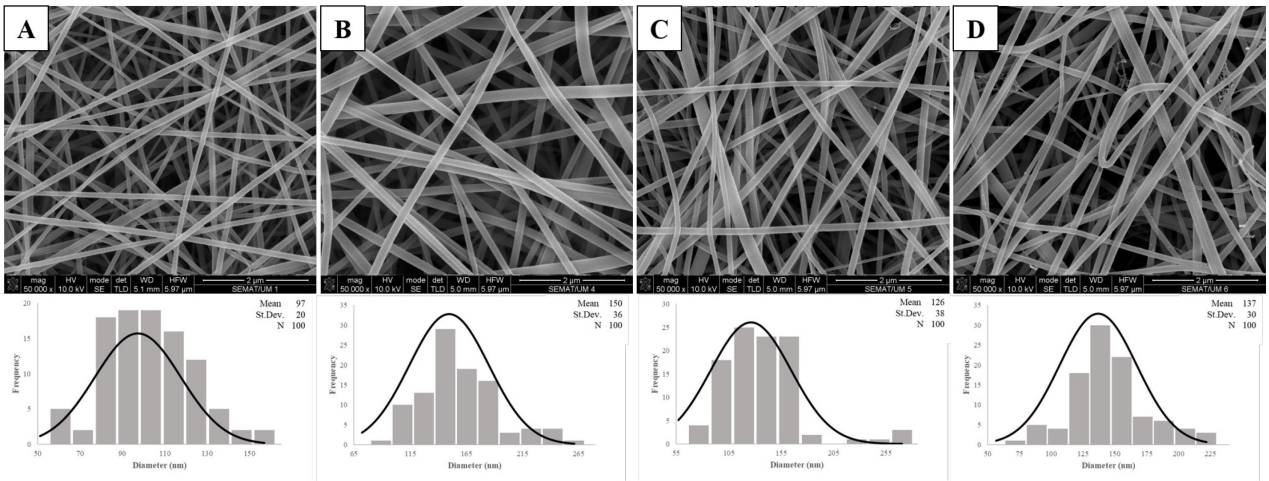

Fig. 3. FESEM images of Gel/CS fibers produced via electrospinning with different electrospinning parameters: **A)** 22 kV, 160 mm, 0.2 mL/h, **B)** 22 kV, 200 mm, 0.2 mL/h, **C)** 22 kV, 160 mm, 0.5 mL/h and **D)** 25 kV, 160 mm, 0.2 mL/h, with magnifications of 2 μm, and their respective diameter distribution histograms.

Regarding Figure 3, fibers with different morphologies were obtained when several electrospinning parameters were changed, namely: applied voltage, distance from needle to collector and feed-rate. By increasing the distance between the needle and collector (from 160 to 200 mm), fibers with higher diameters were obtained, from 97 nm (Figure 3A) to 150 nm (Figure 3B). Using higher feed-rates, like 0.5 mL/h, besides the increase in fibers' diameters to 126 nm, the FESEM images and the respective histogram showed a less homogeneous distribution of the fibers, as it can be observed when comparing Figure 3 A) with C). Finally, when the applied voltage was changed from 22 kV (Figure 3A) to 25 kV (Figure 3D), the nanofibers mats exhibited higher number of defects as well as higher diameters (137 nm). Hence, the use of 22 kV, 160 mm of distance between needle

to collector and feed-rate of 0.2 mL/h allowed the production of more uniform and defect-free nanofibers with smaller diameters, as shown in Figure 3A.

After the optimization of Gel/CS formulation and electrospinning parameters, several CNC concentrations (0.1, 0.5, 1 and 2 % (w/v)) were introduced in the optimized polymeric blend formulation. Figure 4 shows the FESEM images and the diameter distribution histograms of Gel/CS/CNC nanofibers.

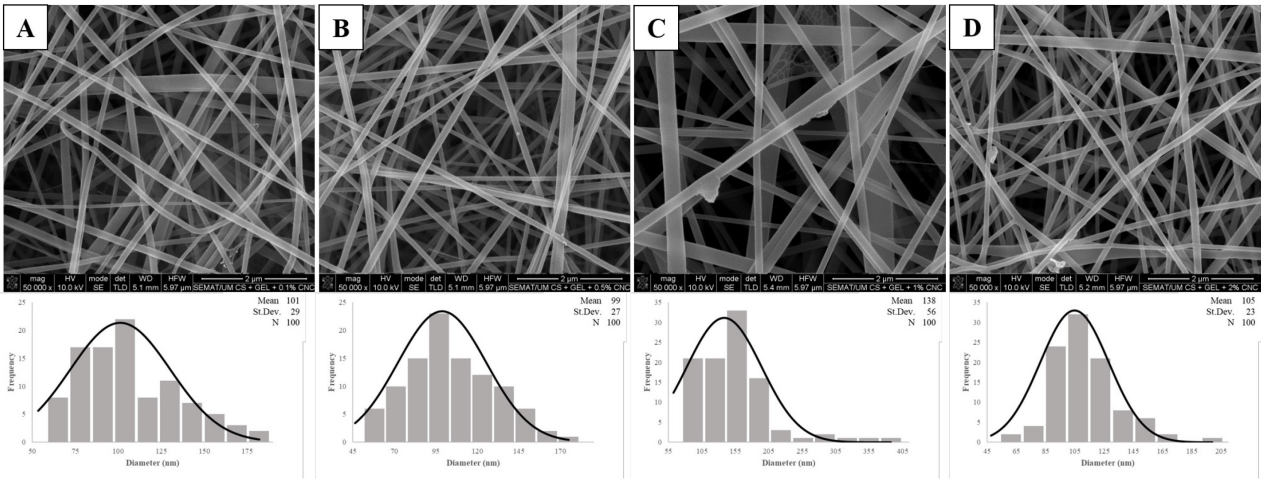

Fig. 4. FESEM images of Gel/CS nanofibers with different CNC concentrations: **A)** 0.1, **B)** 0.5, **C)** 1 and **D)** 2 % (w/v), with magnifications of 2 µm, and their respective diameter distribution histograms.

The introduction of different CNC concentrations into Gel/CS formulations resulted in defect-free nanofibers, although a slight increase in their diameters was observed from 97 nm to 101, 99, 138 and 105 nm according to CNC amount. Most importantly, the CNC incorporation not only favored the solution's electrospinnability but also improved the membrane's integrity, making them more resistant, which is essential for skin applications. Furthermore, the appearance of some CNC was visible using 1 and 2 % (Figure 4 C-D), confirming their successful incorporation onto Gel/CS nanofibers.

Viscosity and Conductivity

The solution's electrospinnability is highly associated with several parameters, including its viscosity and conductivity. Therefore, these properties were assessed for all the prepared solutions, and the results are shown in Table 1.

Table 1. Viscosity and electronic conductivity of several Gel/CS solutions.

CS/Gel solutions	Viscosity (mPa)			Conductivity
	200	**100**	**60**	**(µS)**
15 % Gel/1 % CS	580	870	1060	105.5
15 % Gel/1 % CS + **0.1 % CNC**	610	890	1150	98.4
15 % Gel/1 % CS + **0.5 % CNC**	850	980	1210	99.7
15 % Gel/1 %CS + **1 % CNC**	880	1120	1430	108.8
15 % Gel /1 % CS + **2 % CNC**	940	1280	1610	134.2

As it can be seen in Table 1, the introduction of CNC led to an increase in viscosity values of Gel/CS solutions, and this increase was proportional to CNC concentration. Similar results were also reported by Ridolfi *et al*., who observed a rise in viscosity values of polymeric solutions when CNC were incorporated. This finding could be due to the increase in entanglement of the polymer chains by the interaction between the CNC and the polymers [13]. The conductivity values slightly decreased with the incorporation of smaller amounts of CNC (0.1 and 0.5 %). Nevertheless, higher CNC concentrations (1 and 2 %) promoted an increase in conductivity, which can be related with the negative charges of sulfate groups present in CNC surface [13,14]. Higher values of viscosity promoted an increase in fibers' diameters, while higher values of conductivity are related with lower

dimeters, due to more electrostatic stretch during electrospinning process. Hence, the increase in conductivity is important to neutralized the viscosity effect on fibers' diameters [14].

Chemical analysis

ATR-FTIR spectroscopy was used to characterize the obtained nanofibers and to study the possible interactions between the polymers. Figure 5 shows the ATR-FTIR spectra of Gel, CS and CNC powders as well as Gel/CS nanofibers and Gel/CS/2%CNC nanofibers (as an example). Some of the most important bands were discriminated in order to compare the different samples under study.

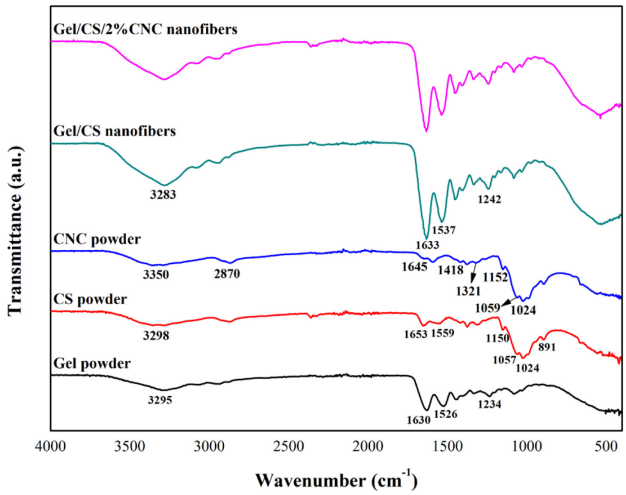

Fig. 5. ATR-FTIR spectra of Gel, CS and CNC powders and Gel/CS and Gel/CS/CNC nanofibers.

Gel and CS powders spectra exhibit a broad peak in the region of 3200-3500 cm^{-1}, which is due to N-H stretching and O-H stretching vibrations [8,15,16]. The characteristic bands of Gel powder are detected at 1630, 1526 and 1234 cm^{-1}, corresponding to C=O of amide I, N-H of amide II and N-H of amide III, respectively [8]. Because of the common functional groups found in Gel and CS, peaks with similar wavenumbers are observed in spectra of both powders. In fact, CS powder spectrum also displays typical bands at 1653 and 1559 cm^{-1}, which are attributed to C=O stretching (amide I) and N-H bending (amide II), respectively. Moreover, the bands at 1150, 1057, 1024 and 891 cm^{-1} are from CS saccharide region, where the peak at 1150 cm^{-1} is attributed to symmetric stretching of the C-O-C bond and the one at 1057 cm^{-1} corresponds to C-O stretching vibrations [6,13].

In Gel/CS nanofibers spectrum, the characteristic peaks of both polymers were detected. Bands at 1633 and 1537 cm^{-1} are assigned to the N-H stretching vibration of amide I and amide II, respectively, while the peak at 1242 cm^{-1} corresponds to amide III [15]. Moreover, the increase in intensity of the band around 3200-3500 cm^{-1} suggests good molecular compatibility resulting from an intermolecular interaction ascribed to hydrogen bonds between both polymers. Hence, this analysis allowed to confirm that after the blend of Gel and CS, the characteristic bands of the two components still existed on Gel/CS nanofibers spectrum, proving the possibility of miscibility of Gel/CS after electrospinning process [17].

Regarding the spectrum of CNC powder, the bands observed in the range of 3300-3400 cm^{-1} and 2800-2900 cm^{-1} are attributed to the stretching vibrations of the O-H and C-H groups, respectively. Furthermore, the peaks at 1645, 1418 and 1321 cm^{-1} correspond to the OH bending of the adsorbed water, the symmetric bending of CH_2 and the bending vibrations of the C-H and C-O groups of the rings in polysaccharides, respectively [18–20]. Finally, the peaks at 1024-1152 cm^{-1} represents the C-O stretching and C-H rocking vibrations of the pyranose ring [18]. In Gel/CS/CNC spectrum, the typical CNC peaks cannot be easily observed due to the equipment low sensitivity for small amounts of CNC and overlap of CNC peaks with the ones of CS.

Thermal analysis

TGA was performed in order to evaluate the thermal stability of the developed nanofibers. The curves of TGA and the first-order derivate (DTG) of Gel, CS and CNC powders as well as Gel/CS and Gel/CS/CNC nanofibers are shown in Figure 6.

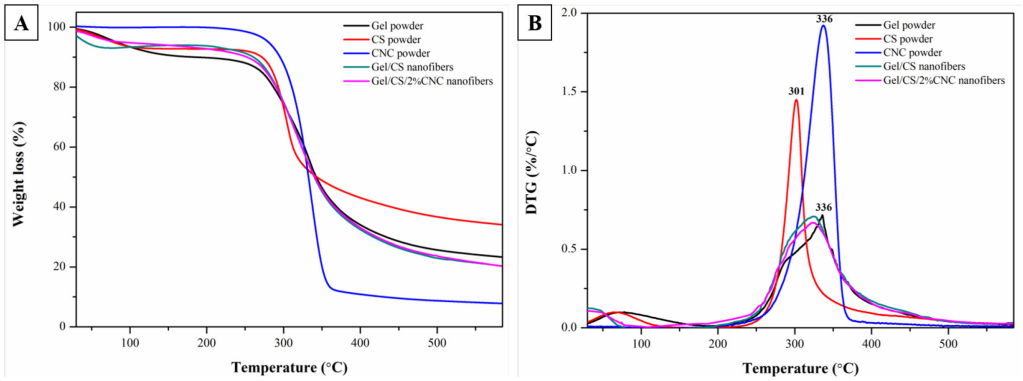

Fig. 6. TGA (**A**) and first-order derivate (DTG) (**B**) curves of Gel, CS and CNC powders and electrospun nanofibers of Gel/CS and Gel/CS/CNC.

The TGA and DTG curves showed that the main degradation stage of Gel and CS powders occurred between 200-450 ºC, reaching a maximum degradation peak at 336 ºC and 301 ºC, respectively. In Gel/CS nanofibers, two degradation stages can be observed: the first one (30-200 ºC) can be related to absorbed water and residual solvent (acetic acid) still present in nanofibers, while the second one (200-450 ºC) with a weight loss around 67 % corresponds to the thermal degradation of the polymeric chains of Gel and CS [13,15,21]. Due to the similarity of the Gel and CS degradation temperatures, only a broader single peak was observed in DTG curve of Gel/CS nanofibers. Regarding CNC powder thermographs, a main degradation step was detected between 250-400 ºC, with a maximum degradation peak at 336 ºC, which not only matches with the maximum degradation peak of Gel powder but is also identical to CS peak. Therefore, the curves of Gel/CS/CNC nanofibers were very similar to the nanofibers without CNC, where only one peak in DTG curve was observed, that corresponds to Gel, CS and CNC degradation.

Hydrophilic/hydrophobic character

The surface hydrophilicity plays an important role in skin applications, namely for wound dressing systems. Hydrophilic nanofibrous mats promote an increase in absorption efficiency of wound exudates when compared to hydrophobic ones [22]. To investigate the influence of CNC on the surface hydrophilicity of electrospun Gel/CS nanofibers, the WCA was measured. Gel/CS nanofibers showed a WCA of about 50°, which is less than 90°, indicating that the surface is hydrophilic. The incorporation of the different amounts of CNC led to a small increase ranging from 55-60°, which didn't change significantly the hydrophilicity of the nanofibers. Therefore, with the CNC incorporation, the electrospun nanofibers were able to maintain their hydrophilic character.

Antibacterial activity

To protect the skin against the action of microorganisms, and consequently, the development of infections, the nanofibrous mats should exhibit antibacterial effect. In this way, to evaluate the potentiality of propolis natural extract to produce antibacterial nanofibers, the antibacterial effect of Gel/CS, Gel/CS/1%CNC and Gel/CS/1%CNC/Propolis nanofibers was tested. Figure 7 presents the antibacterial results for the several nanofibrous mats against *S. aureus* and *E. coli* bacteria by shake flask method during 24 h and 48 h of contact

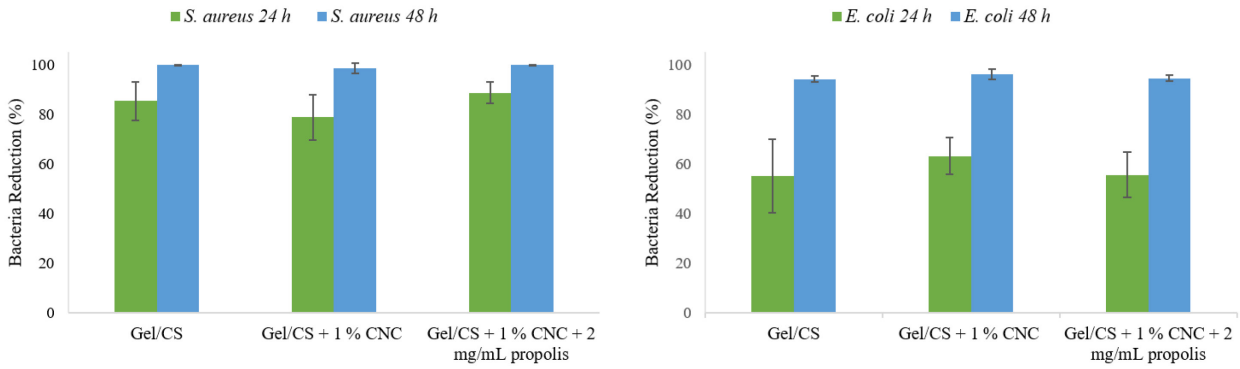

Fig. 7. Bacteria reduction of Gel/CS, Gel/CS/1%CNC and Gel/CS/1%CNC/Propolis nanofibrous membranes against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria during 24 h and 48 h of contact.

After 24 h of exposure, Gel/CS nanofibers already exhibited antibacterial activity against both bacteria, although this effect was more pronounced on Gram-positive. Increasing the contact time of nanofibers with bacteria for 48 h, an enhancement in bacteria reduction from 85.2 to 99.6 % for *S. aureus* and from 55.1 to 94.1 % for *E. coli* was observed. This result can be due to the strong antibacterial effect of CS, as demonstrated in several studies. In fact, the interaction between the positively charged of CS with the negatively charged bacterial cell surface can alter the permeability of bacteria membrane, leading to leakage of intracellular material, which can result in cell death. [8,17,23]. The incorporation of CNC into the Gel/CS nanofibers led to a bacteria reduction of 98.6 % and 96.04 % for *S. aureus* and *E. coli* bacteria after 48 h of contact, respectively, which means that CNC promoted a slight increase in antibacterial effect against *E. coli* in comparison to Gel/CS nanofibers. According to Tyagi *et al.* CNC particles can induce damage to bacteria cell membrane due to their rigid, narrow and rodlike shape, making the membranes more susceptible to CS entering [24].

In order to improve the antibacterial effect of Gel/CS/CNC nanofibers, propolis natural extract was incorporated. The addition of natural extract promoted an increase in the reduction of *S. aureus* bacteria from 78.7 to 88.5 % and from 98.6 to 99.7 % after 24 h and 48 h of contact, respectively. Besides presenting a higher antibacterial effect when the propolis extract was incorporated, the Gel/CS/CNC/Propolis nanofibers also showed a faster response, as observed by the higher bacteria reduction after 24 h of exposure when compared with Gel/CS/CNC nanofibers. On the other hand, the incorporation of propolis extract didn't show any significant reduction in *E. coli* bacteria. In fact, it has been reported that propolis is more efficient against Gram-positive in comparison to Gram-negative bacteria, which can be related to the outer membrane of the Gram-negative [12,25]. Hence, in order to enhance the effect against Gram-negative bacteria, higher concentrations of propolis extract need to be evaluated.

Conclusions

In this work, bio-based nanofibers were developed by electrospinning technique using natural and biodegradable materials such as Gel, CS, CNC and propolis natural extract. Different polymeric formulations (CS concentration) and electrospinning parameters (applied voltage, feed-rate and distance between needle and collector) were tested and optimized. The condition that allowed the development of nanofibers with the lowest mean diameters (97 nm) and more uniform size distribution was with 15 % (w/v) of Gel and 1 % (w/v) of CS with a voltage of 22 kV, feed-rate of 0.2 mL/h and a distance between the needle and collector of 160 mm. Several CNC concentrations (0.1, 0.5, 1 and 2% (w/v)) were incorporated into the optimized polymeric formulation and the developed nanofibrous mats were characterize using different techniques. The CNC incorporation promoted a great improvement in solution's electrospinnability and in membrane's physical integrity, making them more resistant and less brittle. FESEM images proved the successfully incorporation of CNC into the Gel/CS formulation, although the nanofibers' diameters slight increased from 97 to

101, 99, 138 and 105 nm, according to CNC amount. ATR-FTIR spectroscopy revealed a strong interaction between the polymers under use. TGA analysis demonstrated the thermal degradation of the three components of Gel/CS/CNC nanofibers. Furthermore, these membranes showed hydrophilic character, which was maintained after CNC incorporation. Finally, it was found that the incorporation of propolis natural extract in the Gel/CS/CNC system enhanced the antibacterial effect for *S. aureus* bacteria. Several studies still on going in order to verify the advantages of CNC incorporation, as reported by different works in Gel/CS systems. In addition, in order to improve the bacteria reduction for *E. coli*, higher extract concentrations are being studied. Overall, the developed nanofibers based on biopolymers and natural agents (Gel/CS/CNC/Propolis) are very promising scaffolds to be employed in drug delivery and wound dressing applications.

Acknowledgements

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