UV-initiated crosslinking of electrospun chitosan/poly(ethylene oxide) nanofibers doped with ZnO-nanoparticles: development of antibacterial nanofibrous hydrogel

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Abstract

UV-initiated crosslinking of electrospun poly(ethylene) oxide (PEO)/chitosan (CS) nanofibers doped with zinc oxide nanoparticles (ZnO-NPs) was performed using pentaerythritol triacrylate (PETA) as the photoinitiator and crosslinker agent. The influence of the addition of PETA to the PEO/CS diameter and crosslinking of nanofibers was evaluated. The effect of irradiation time on the morphology and swelling properties of the crosslinked nanofibers were investigated. For ZnO-NPs, the minimum inhibitory concentrations were found at 1 mg/mL, and the minimum bactericidal concentrations at 2 mg/mL for all the strains tested. The nanofibrous hydrogel antibacterial effect was tested. This material enters the realm of fibrous hydrogels which have potential use in several applications as in the biomedical area.

Introduction

Electrospinning is a technique for micro and nanofibers fabrication that is currently being explored for purposes of fabricating polymeric biomaterials. Though the technique makes it possible to work with a plethora of materials in almost any area, it has found novel possibilities in tissue engineering.^[1] This technique allows for the incorporation of several nanocompounds on the surface or in the bulk of the fiber in one step. Many authors have incorporated metallic nanoparticles to obtain specific properties, such as an electric or bactericidal effect.^[2] Nanoparticles are being widely explored due to the different properties that arise thanks to their small size. Among them, zinc oxide (ZnO) presents multiple properties, having good photocatalytic activity, high stability, antibacterial activity, and nontoxicity.^[3] Nanogels are polymer nanoparticles with three-dimensional networks made by chemical and/or physical crosslinking of the polymer chains. Various nanogels have been designed with a focus on biomedical applications for drug-delivery systems (DDSs), regenerative medicine, and bio-imaging, among others.^[4] Despite the various nanogels that have been developed, the concept of fibrous nanogels in the biomedical field has not been extensively explored.

Nanofibers obtained by electrospinning are currently the best way to fabricate scaffolds, wound healing patches, or DDS. However, they are nonwoven fibers, which means they are susceptible to dissolution and have poor mechanical properties. Some authors have reported using a crosslinking process

to improve mechanical properties and reduce the water solubility of the nanofibers. That process was carried out by chemical crosslinking of carboxymethyl cellulose to obtain better food packaging properties. Ferreira et al. obtained crosslinked fibers of polycaprolactone and modified gelatin for applications in tissue engineering, thereby obtaining a biocompatible material resistant to bodily fluids.^[5] Meanwhile, Vashisth et al. carried out the crosslinking of polyvinyl alcohol (PVA) and gellan by different physical methods (heat and UV) and chemical crosslinking agents (methanol, glutaraldehyde, and calcium chloride), obtaining high degrees of insolubility.^[6] The efforts made to fabricate a fibrous nanogel are focused not only on the improvement of its properties but also on the development of electrospun crosslinked hydrogel membranes for their potential applications in different fields of knowledge.

Among natural polymers, chitosan (CS) is the second most readily available natural polymer, just behind cellulose.^[7] CS is a biocompatible, biodegradable, and nontoxic polymer that has generated great interest in biomedical applications such as tissue engineering and drug delivery. Many authors have fabricated CS nanofibers^[8]; nevertheless, to achieve this, the addition of easily spinnable polymers such as PVA or poly(ethylene) oxide (PEO) influences the successful formation of fibers.^[9] The latter one is a widely used nontoxic, hydrophilic, and biocompatible polymer. It is an attractive material for use in the biomedical field, with applications in the fabrication of scaffolds for tissue engineering or controlled drug release

systems.^[10] Various methods have been used to crosslink PEO and improve its resistance to dissolution in aqueous mediums. These methods include gamma radiation, silane-based crosslinking, and UV irradiation. UV-induced crosslinking has become the most common way to crosslink PEO. This method presents certain advantages, such as easy manipulation, high effectivity, and controllability, and the fact that it doubles as a sterilization method.^[11,12] Pentaerythritol triacrylate (PETA) has been researched as an initiator for the crosslinking process, as it can act in both solution or solid-state, and crosslinked products maintain a good grade of biocompatibility.^[13] PETA has been widely used to crosslink PEO. Şimşek et al. crosslinked PEO with the UV-initiating/crosslinking agent PETA and UV irradiation to obtain insoluble PEO nanofibrous mats.^[12] They found that PEO-PETA nanofibers became insoluble in the absence of UV irradiation. However, the application of UV irradiation is necessary to achieve effective crosslinking.^[14] Forbey et al. crosslinked PEO using PETA to generate a structured polymer-gel electrolyte for Li-polymer battery applications; they found that gel fraction and porosity data indicate that PETA is an effective crosslinking agent for the PEO mats, yielding gel fractions higher than 80%.^[15]

One potential use of the nanocomposite could be the prevention of wound infection. If bacteria colonize the wound, they could interfere with the healing process and lead to complications. Commonly, in extensive skin injury, a bacterial infection leads to serious consequences.^[16] Among them, *Staphylococcus aureus* can produce disease by toxins or superantigens, invade any organ or tissue, and cause suppuration, tissue necrosis, vascular thrombosis, and bacteremia. Commonly, these bacteria can colonize certain areas of the skin and mucous membranes. *Staphylococcus epidermidis* is another strain frequently isolated from infections of the skin and emerging as common nosocomial pathogens infecting immunocompromised patients carrying medical devices.^[17] Meanwhile, *Pseudomonas aeruginosa* has exhibited a high potential to generate multidrug resistance and has been associated with chronic wound infections in association with *S. aureus*.

One of the approaches for avoiding or treating wound infection is the use of dressings impregnated with antibacterial agents for eliminating bacterial in situ.^[18] This strategy decreases the need for prescribing systemic antimicrobials to prevent wound infection and generates a lower cost in medical attention.

In wound healing, electrospun nanofiber is the treatment of large wounds such as burns and abrasions.^[19] It is found that these types of wounds heal particularly rapidly and without complications if they are covered by a thin web of nanofibers of biodegradable polymers, such as CS. The mats have suitable pore size to assure the exchange of liquids and gases with the environment but have dimensions that prevent bacteria from entering. Mats of electrospun nanofibers generally show very good adhesion to moist wounds. Furthermore, the large specific surface area of up to 100 m²/g is very favorable for the adsorption of liquids and the local release of drugs on the skin, making

these materials suitable for application in hemostatic wound closure.^[20] Compared with conventional wound treatment, the advantage is also that scarring is prevented using nanofibers. The nanofibrillar structure of the nanoweb promotes skin growth, and if a suitable drug or antibacterial nanoparticles are integrated into the fibers, it can be released into the healing wound in a homogeneous and controlled manner.^[21,22]

This work aims to obtain CS/PEO fibrous nanogel by electrospinning-UV/crosslink and its functionalization with zinc oxide nanoparticles (ZnO-NPs). Our hypothesis was based on the nanofibers' crosslinking induces nanofiber gel formation, improving their non-solubilization, while the addition of ZnO-NPs confers the antibacterial effect.

Methods

Materials

Zinc acetate, Zn(Ac)₂•2H₂O, 97% pure, purchased from Meyer® (Mexico City, Mexico), and sodium hydroxide (NaOH), 98%, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Pentaerythritol triacrylate (PETA), PEO [molecular weight (M_v) 600,000], and CS medium (molecular weight with 75–85% deacetylation) were bought from Sigma-Aldrich (St. Louis, MO, USA). 99% glacial acetic acid used as a solvent was purchased from Meyer® (Mexico City, Mexico). All reagents were used without further treatment.

Nanoparticle synthesis

ZnO-NPs were prepared by a modified hydrothermal method described by Yuvaraja et al.^[23] NaOH (1 mol/L) solution was fully added for 5 min drop by drop to the Zn(Ac)₂ (0.4 mol/L) solution at a constant stirring of 350 RPM at 40 °C. After 10 min of reaction, the precipitate was separated by filtration and then washed six times with alternating washes of distilled water and absolute ethanol to remove any impurity. The ZnO-NPs were then dried in a desiccator for 48 h. Finally, the nanoparticles were stored in a desiccator to keep them dry.

Electrospinning process

Preparation of electrospinning solutions

The solvent used for all solutions consisted of glacial acetic acid at 70%. A 3% PEO solution was created by adding 0.21 g to 6.5 mL of solvent, while a 3% CS solution was created by adding 0.9 g to 2.5 mL of solvent. Each solution was stirred vigorously until it was completely dissolved. Afterward, the PEO solution was added to the CS solution and stirred for 24 h to create a 3% PEO/CS solution in a ratio of 70:3. Then, 0.009 g (3%) of ZnO-NPs was dispersed by sonication in 1 mL of distilled water, as the acidic medium could harm the ZnO-NPs if it were stored for a long time. Afterward, the ZnO-NPs were added to 17% and 25% PETA (w/w) (35.7 and 52.5 µL) PEO/CS solutions. A concentration of 25% was chosen as indicated by the work carried out by Zhou et al. A 30% lower concentration corresponding to 17% PETA with respect to the total weight of the polymers was also chosen,

to observe the effect of crosslinking and swelling on the initiator concentration. The solutions were stirred for another 3 h to guarantee homogeneity.

Electrospinning parameters

The solution was loaded into a 10 mL BD™ plastic syringe with an 18 G (1.02 mm) needle. A nanospinner 24 system (Inovenso®, Turkey) was used for the electrospinning process. The electrospinning device was configured to operate with 25 kV and a distance of 20 cm from the tip of the needle to the collector, which is equivalent to an electric field strength of 1.25 kV/cm. A flow rate of 0.4 mL/h provided by an Inovenso® pump (Inovenso®, Turkey) was used along with a controlled atmosphere, maintaining a temperature of 32 °C and relative humidity of between 30% and 45%. The samples were collected in aluminum foil in a rotating collector with a speed of 200 RPM. After that, the electrospun meshes were purified in the oven vacuum for 24 h to extract the residual solvent.

UV irradiation

A minimax® UV lamp (Spectroline®, USA) was used to irradiate the samples. The radiant flux of the UV lamp was 4.77 W/cm², with a wavelength of 365 nm at room temperature. Samples were cut into 5 cm × 5 cm mats and irradiated for 0, 60, 80, 100, and 120 min in the air. The samples were placed 3.53 cm under the head of the lamp, where the 4.77 W/cm² irradiance was achieved. The resulting samples were designated as PEO/CS-ZnO-X, with X being the time (in minutes) exposed to UV radiation.

Measurements and characterizations

The Fourier transform infrared spectra (FTIR/ATR) of the electrospun samples were measured with an FTIR Nicolet™ iS™ 5 (ThermoScientific®, USA). Each spectrum was obtained in transmittance mode by the accumulation of 32 scans and a spectral range of 4000–600 cm⁻¹. Samples were also observed via scanning electron microscopy with a JCM-6000Plus® Versatile Benchtop SEM (JEOL, USA). Samples were prepared by coating them with a gold/palladium alloy. The resulting images were analyzed with the ruler tool of ImageJ® software to determine the average diameter of the fibers. The maximum swelling ratios (ES, %) of the UV crosslinked samples were measured in distilled water at room temperature and 37 °C, respectively. Dry square samples measuring 5 cm × 5 cm and initial weight (W_1) were immersed in a vial with 15 mL of distilled water for 12 h. Afterward, the samples were removed from the vial, excess surface water was wiped away with a paper towel, and the gained weight (W_2) was recorded. The swelling ratio (ES) is calculated using the following equation:

$$ES = \frac{W_2 - W_1}{W_1} (100) \quad (1)$$

Antibacterial tests

Broth dilution test

The bacterial strains used in this study were obtained from the stock-culture collection of the Facultad de Odontalgia, Universidad Autónoma Benito Juárez de Oaxaca. For the tests, the strains used were *S. aureus* (ATCC 29123), *Escherichia coli* (ATCC 35218), *S. epidermidis*, and *P. aeruginosa*. The last two strains used are endemic to the region of southwest Mexico; each was characterized by a battery of cultural and biochemical tests. The experiments involving antimicrobial activity were carried out as described by the Clinical and Laboratory Standards Institute. For detailed procedure, see Ref. [24]. The starting suspension of each organism was matched to the optical density of 0.5 McFarland standard. Afterward, 1:20 dilutions of each bacterial suspension were done. The maximum concentration of the ZnO-NPs, previously suspended in Mueller-Hinton broth, was 2000 µg/mL, and serial dilutions (two-fold) were performed. Lastly, 5 µL of the respective strain was inoculated per well. To ensure the validity of this test, sterility controls and bacterial growth controls were used. Tests were performed three times for each strain. The inoculated microplates were incubated at 37 °C for 24 h. The presence or absence of turbidity in each well was recorded. The supernatant was transferred to a new microplate, and the bacterial readings were carried out. Optical absorbance was determined at 595 nm using a microplate reader. The results were expressed as a percentage of the viable bacteria compared with the untreated controls. The lowest concentration of ZnO-NPs that resulted in no turbidity (to the naked eye) was recorded as the minimum inhibitory concentration (MIC). The minimum bactericidal concentration (MBC) was identified by determining the lowest concentration of the antibacterial agent that reduced the viability of the initial bacterial inoculum by ≥99.9% or ≥3 log reduction of the inoculum count. MBCs were determined from the subcultures of the wells that showed no turbidity.

Disk diffusion method

The crosslinked CS/PEO and CS/PEO/ZnO 10% antibacterial activity was determined by a disk diffusion technique that conformed to the recommended standards of the National Committee for Clinical Laboratory Standards. [25] Mueller-Hinton agar plates were prepared and inoculated with 200 µL of bacterial culture. The culture was adjusted with the sterile saline solution to achieve turbidity equivalent to a 0.5 McFarland standard ($1-2 \times 10^8$ CFU/mL). Disks made of filter paper were impregnated with either 20 µL of chlorhexidine or saline solution, for positive and negative controls, respectively. Also, disks of CS/PEO and CS/PEO/ZnO 10% were prepared specifically for this test. The size of all the disks used was standardized at 6 mm in diameter. The disks were firmly placed on the inoculated agar plates. The disk diffusion method was performed against the same strains used for the broth dilution test mentioned previously. The

tests were performed in triplicate. The agar plates were incubated at 37 °C for 24 h, and the halos of inhibition were measured.

Results and discussion

Characterization

XRD analysis

ZnO-NPs [Fig. 1(a)] exhibit characteristic peaks at (100), (002), (101), (102), (110), (103), (200), and (112). This structure corresponds to the wurtzite structure that according to Sirelkhatim presents a greater antimicrobial activity than its cubic structural counterpart.^[3,26] PEO displays two planes in $2\theta = 19^\circ$ and $2\theta = 24^\circ$ [Fig. 1(b)], which defines a semicrystalline part [Fig. 1(c)]. CS has a broad peak at around $2\theta = 20^\circ$. This peak shows mainly an amorphous structure that is due to the high level of deacetylation of the compound. CS exhibited only a slight ordering of the chains and is in accordance with the reports by Kumar.^[27] CS/PEO/ZnO [Fig. 1(d)] exhibits two main peaks at $2\theta = 20^\circ$ and $2\theta = 25^\circ$. Both polymers present nearby peaks, in $2\theta = 19^\circ$ from PEO and $2\theta = 20^\circ$ from CS. However, the peak that remains visible in the composite material is from PEO in $2\theta = 19^\circ$. This may be due to

the overlap of the CS band or to the loss of its crystallinity in the electrospinning process, with the ordering of its chains decreasing. It is difficult to differentiate the presence of the main peaks of zinc oxide nanoparticles in $2\theta = 31^\circ$, $2\theta = 34^\circ$, and $2\theta = 37^\circ$; however, it is possible to see a small region of peaks with a little more intensity between $2\theta = 31^\circ$ and $2\theta = 40^\circ$. In Fig. 1(d), some peaks at low intensity can correspond to the ZnO-NPs immersed into polymer nanofiber. Well-defined peaks are difficult to observe due to interference from nanofiber signals (CS and PEO). The presence of ZnO-NPs was confirmed with the antibacterial test.

Fourier transform infrared

Figure 2 shows the IR spectra of materials. PEO spectrum [Fig. 2(a)] showed at 2875 cm^{-1} the stretching of the C–H bond inside the group $-\text{OCH}_2-$. A band at 1185 cm^{-1} corresponds to the bending of the C–H bond. The small bands at 1460 and 1345 cm^{-1} are due to the symmetrical flexion of CH_2 and the stretching of CH_2 , respectively, and the broadband at 1050 cm^{-1} corresponds to the stretching of the C–O–C bond.^[28] CS spectrum [Fig. 2(b)] showed at 3420 cm^{-1} the corresponds to the N–H bond of the amino group present in CS and O–H interactions. The stretching at 2850 cm^{-1} can be attributed to the C–H symmetric bond. The small peak at 1640 cm^{-1} corresponds to the C=O amide bond. At 1150 cm^{-1} corresponds to the band of C–O–C. The band around 1370 cm^{-1} is due to the stretching vibration band of the group $-\text{NHCO}$ present in the amide.^[29] Figure 2(c) shows the PETA initiator spectrum. The broadband observed at 1720 cm^{-1} represents the stretching vibration of the carbonyl group present in the molecule, and the band at 1620 cm^{-1} corresponds to the stretching vibration of C=C. This last bond is of great interest because of its changes during the crosslinking process by radical polymerization. In the IR spectra CS/PEO [Fig. 3(d)], CS/PEO-ZnO [Fig. 3(e)], and CS/PEO doped with ZnO-PETA [Fig. 3(f)], a widening of the main band is observed around 1100 cm^{-1} . The size of this band is due mainly to the symmetric stretching vibration C–O–C present in the PEO chains. However, its widening can be attributed to the contribution of the band in 1050 cm^{-1} , which corresponds to the stretching vibration of group C–O–C present in CS. The broadband around 3420 cm^{-1} corresponds to the contribution of the N–H and O–H bonds the CS. The increase in the peak area of the C–H bond stretching at 2875 cm^{-1} resulted from the addition of C–H bonds from PEO and CS and that confirms the incorporation of both polymers on the fibers. At 1245 and 1260 cm^{-1} correspond to the symmetric torsion of CH_2 and the band around 1350 cm^{-1} corresponds to the flexing of CH_2 in PEO. Two bands around 1315 cm^{-1} are associated with the stretching vibration of the C–N group and the vibration of the $-\text{NHCO}$ group present in the amide. The small band showed around 1240 cm^{-1} indicates that the torsional vibration of the CH_2 bonds remains. The band around 1260 cm^{-1} and the appearance of a band around 1320 cm^{-1} could be due to a

Figure 1. XRD of (a) ZnO-NPs, (b) PEO, (c) CS, and (d) CS/PEO fibers doped with ZnO-NPs.

Figure 2: IR spectra of (a) PEO, (b) CS, (c) PETA initiator, (d) PEO/CS, (e) PEO/CS-ZnO3%, and (f) PEO/CS/PETA-ZnO3%.

displacement of the CS bands by the interactions of the C–N and –NHCO bonds with the CH₂ bonds present in the PEO chains.

In Fig. 3(f), when compared the PETA, two additional characteristic absorbance peaks, i.e., the C=O stretching at 1720 cm^{−1}, and the C=C stretching spectrum at 1620 cm^{−1}, are present and as we commented previously, the change in the intensity of the last bands indicate the formation of the covalent bond between PETA and PEO as describes mechanisms proposed by Zhou et al.^[30] When the PEO/CS/PETA-ZnO is exposed to UV irradiation at certain time, the intensities of the C=C and C=O peaks decreased gradually, suggesting the UV photo-initiating and photocrosslinking ability of PETA through the photo-initiation of the C=O bond and then the polymerization of the C=C bond. The band at 1550 cm^{−1} corresponds to the N–H bond present in the CS. It was not possible to find the presence

of ZnO-NPs by FTIR/ATR; however, according to Xiong et al.,^[31] the characteristic bands of zinc oxide nanoparticles are observed around 436 cm^{−1}. A further analysis was conducted to find it by using other techniques.

SEM analysis

From the images in Figs. 3(a)–3(f), size measurements were made with the ImageJ® software to determine the particle and/or fiber size and its dispersion through the corresponding histogram.

Figure 3(a) shows that most nanoparticles have an average size between 60 and 100 nm. In a hydrothermal synthesis process, such as the one used to synthesize these nanoparticles, the size of the particle is determined by the reaction time. With a longer reaction time, nanoparticles tend to agglomerate, forming a larger particle size. In this case, a total reaction time of

Figure 3: SEM of: (a) ZnO-NPs, (b) PEO/CS fibers, (c) PEO/CS fibers with 3% ZnO-NPs, (d) PEO/CS/ZnO with 17% PETA initiator, (e) PEO/CS/ZnO with 25% PETA initiator, and (f) PEO/CS/ZnO-100 with 17% of PETA initiator.

10 min allowed particle sizes with the relatively homogeneous distribution. The particle size is important because the particles should be embedded into the fiber structure to do not block the pores.

Figure 3(b) shows the PEO/CS fibers with their respective histogram. Most of the fibers in this sample have a diameter of between 150 and 400 nm. PEO/CS fibers with 3% ZnO-NPs are shown in Fig. 3(c). It is observed that the fiber diameter is around 180 nm, which is consistent with Fig. 3(b). The inclusion of ZnO-NPs did not significantly affect the average fiber diameter. It is possible to observe the nanoparticles distributed along the fibers. In general, the nanoparticles

are both on the surface and inside the fibers and their size ranges from between 20 and 80 nm, consistent with the size found in Fig. 3(a). The energy dispersive spectra of the samples obtained from punctual SEM-EDS analysis are also shown in Fig. 3(c). EDS elucidates the surface atomic distribution and chemical composition of nanoparticles present in nanofibers. The spectrum reflects the presence of Zn and O peaks indicated the presence of ZnO. The other signals of Au and C are due to the use of Au coating and the polymer backbone.

PEO/CS/ZnO with 17% PETA initiator and its respective histogram is shown in Fig. 3(d). The influence of PETA on the morphology and size of the fibers can be observed by the

formation of beads. The rheological and chemical changes in the composition of the solution can contribute to morphology as well. An increase in the diameter of the fibers is observed upon an increase in the concentration of PETA to 25% [Fig. 3(f)]. However, the size does not differ significantly with respect to fibers without initiator and nanoparticles (between 100 and 350 nm), i.e., an increase in the amount of PETA does not cause significant differences in the morphology of the fibers because both concentrations show the same quantity and quality of defects. Figure 3(f) shows PEO/CS/ZnO-100 with 17% of PETA. The fibers' morphology does not present significant changes in the radiation process.

Gel fibers properties: swelling and antibacterial activity

Swelling degree

The swelling analysis of samples with 17% and 25% (w/w) of PETA irradiated at different times was carried out. Non-irradiated samples were immersed in water, where they completely dissolved in approximately 15 min. The amount of PETA initiator strongly influences the swelling capacity of the material because fibers with a PETA content of 25% swell up to five times more than do fibers with a PETA content of 17%. The irradiation time also influences the swelling process, with maximum swelling in an irradiation time of 100 min for a content of 17% and 120 min for a content of 25%. According to these results, the PETA amount is the most important parameter for obtaining a maximum swelling due to higher fiber crosslinking. The crosslinking of PEO and cellulose nanocrystals (CNC) fibers by UV technique and PETA (10%) have been investigated by Zhou et al. in N_2 atmosphere^[32]; the finding was that nanofibers exhibited a similar fiber diameter to ours, though the swelling was almost higher (21 g/g maximum swelling). The fibers were prepared from PEO and a small percentage of CNCs (max 20%). Their results showed that swelling decreases when CNCs content is increased. Nevertheless, our experiments were carried out in O_2 atmosphere, and though it is an oxidative atmosphere, it was possible to obtain a great degree of swelling, increasing the quantity of natural polymer and simplifying the process. Similar work to obtain nanofibrous hydrogels, though using a chemical method, was carried out by Koosha et al.^[33] They produced a nanofibrous hydrogel of PVA/CS crosslinked with glyoxal (5%), obtaining a maximum swelling of 272% to PVA/CS nanofibers and 400% swelling with fibers reinforced with halloysite nanotubes (HNTs). The results showed an increase in hydrophilicity in HNT-reinforced nanofibers favoring the attachment of fibroblast cells. However, in this case, the swelling percentage was lower than CS/PEO/ZnO-3% and a toxic crosslinker was used. The SEM analysis of samples irradiated at 100 min after the swelling process (it is shown in Supplementary Fig. S1). PETA-17% shows a characteristic hydrogel structure. Almost all fibers disappear and become a continuous structure, and a certain amount of PEO fibers may have dissolved. On the other hand, the fibers retain their structure and the dissolution

phenomenon is smaller in contrast to the sample with PETA-17%. By maintaining its structure and resisting the dissolution process, PETA-25% reached a swelling of up to 770%. The analysis shows that 25% PETA content and a 100 min exposure to UV radiation are enough to obtain a nanofibrous hydrogel with a huge swelling capacity like a regular hydrogel. The SEM image of the sample with PETA-25% shows a membrane morphology with a homogeneous pore size distribution, with an average size of between 200 and 400 nm (it is shown in Supplementary Fig. S1).

Antibacterial test

The antibacterial activity test of ZnO-NPs was performed using the broth microdilution test. For all the strains tested, the MIC was found at 1000 $\mu\text{g/mL}$ and the MBC was found at 2000 $\mu\text{g/mL}$ [Fig. 4(a)]. The percentages of bacterial inhibition at 2000 mg/mL were 97.28 ± 0.62 for *S. aureus*, 96.89 ± 0.71 for *E. coli*, 95.96 ± 0.90 for *S. epidermidis*, and 95.19 ± 0.27 for *P. aeruginosa*.

From Fig. 4(b), one can also observe the halos of inhibition from the agar plates inoculated with *S. aureus*, *E. coli*, *S. epidermidis*, and *P. aeruginosa* to the CS/PEO/ZnO-10%. The average sizes $\pm$ standard deviation of the inhibition zones were 11 ± 0.31 , 13 ± 0.29 , 12 ± 0.30 , and 13 ± 0.29 mm, respectively.

The disk diffusion test provides qualitative results by categorizing bacteria as susceptible, intermediate, or resistant for standardized antibiotic drugs, which for the present nanocomposite cannot be determined. Therefore, it is a typing tool based on the resistance phenotype of the microbial strain tested. Within the results of the disk diffusion test, well-defined halos were observed for all the strains studied. This finding is relevant because it means that there is a low risk of developing immediate bacterial resistance to the nanocomposite studied. However, because the bacterial growth inhibition does not mean bacterial death, this method cannot distinguish bactericidal and bacteriostatic effects. The size of the halo of inhibition is not a positive correlation of the antibacterial effect; it depends on the diffusion capacity of the agent tested in the agar medium.

Ma et al. synthesized ZnO-NPs using the hydrothermal method and tested against *C. albicans*.^[34] The dose used for the test was 5000 $\mu\text{g/mL}$. The inhibition rates of the different morphological ZnOs were 90% (mulberry-like ZnO particles, size = 150 nm), 85% (sheet-like ZnO, size = no data), and 50% (flowerlike ZnO, composed of some tightly aggregated nanoneedles with an average diameter of 100 nm). In our study, using a lower dose (2000 $\mu\text{g/mL}$) with the same number of bacteria in the inoculum, we obtained a higher percentage of bacterial inhibition (95% or more) in all the strains under study. It is possible that this activity can be attributed to particle size, but it is not the only factor involved in the bacterial activity.

On another hand, Stanković et al. obtained diverse morphologies of ZnO-NPs using the hydrothermal method.^[35] After a 10-min treatment period for a dispersion concentration of

Figure 4: (a) Graph of the dilution broth test of ZnO-NPs. (b) The disk diffusion test, 0 = saline solution negative control, 1 = CS/PEO, 2 = CS/PEO/ZnO-NPS 10%, and 3 = chlorhexidine positive control.

5 mol/L ZnO, a commercial ZnO powder showed an 85% percentage of microbial cell reduction. Concerning the synthesized ZnO powders, the dispersion of ZnO particles (stabilized with PGA) showed the lowest antibacterial activity, 86%; meanwhile, the highest antibacterial activity was observed for the dispersion of ZnO particles (stabilized with PVA), i.e., a microbial cell reduction of 98%. The authors declared that different ZnO-NPs tested against *E. coli* were comparable to the antibacterial properties of the same powders on the *S. aureus* bacterial culture. This could mean that the ZnO-NPs act on both types of gram-positive and gram-negative bacteria, by another means that does not involve the internalization of the particles, and so, the effect is indifferent to the type of bacterial membrane. In the present study, we noticed a similar effect in *S. aureus* in comparison to *E. coli*.

It has been suggested that the production of reactive oxygen species is a mechanism for the bactericidal activity; such a mechanism explains cell wall damage due to ZnO-localized interaction, enhanced membrane permeability, and the internalization of NPs due to the loss of proton motive force and the uptake of toxic dissolved zinc ions.

Because this nanocomposite exhibits antibacterial properties and its handling is simple, the material's flexibility would allow it to be placed on a wound on the skin. However, more tests will be necessary, such as using an animal model at a later stage of this investigation to ensure its biocompatibility.

Conclusions

A novel crosslinked CS/PEO fibrous hydrogel was prepared by electrospinning/UV irradiation. The crosslinking of electrospun nanofibers was successfully achieved with the use of PETA as a crosslinker and UV irradiation, where PETA concentration was the most important parameter to crosslink CS/PEO nanofibers. According to SEM and swelling analysis, the UV-crosslinking treatment showed that nanofibers acquire resistance to solubility in water and maintained the fibrous structure. Crosslinking fibers showed the highest swelling degree of 770% at an irradiation time of 100 min and a PETA concentration of 25%. If applied in the biomedical area, the swelling would be expected less due to influence of some parameters (osmotic pressure, pH, temperature, biological serum, ionic strength, and so on). Fibers in biomedical areas are especially useful in deep wounds given their ability to be packed into deep concave spaces because of its expansion when the turn in gel form. Nanofiber reported in this work would be capable to pack in deep wounds because of fiber diameter sizes. Also, the samples modified with ZnO nanoparticles showed an antibacterial effect on *S. aureus*, *E. coli*, *S. epidermidis*, and *P. aeruginosa*. Nanofibers presented in this work enter the realm of fibrous hydrogels, which could have potential use in several applications, such as in the biomedical area. The crosslinking by UV-photocrosslinking is a very used technique in the photocuring industry, so these nanofibers will be easily scalable and sterilizable at the same time.

Supplementary material

The supplementary material for this article can be found at <https://doi.org/10.1557/mrc.2020.74>.

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