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Manufacturing nanostructured chitosan-based 2D sheets with prolonged antimicrobial activity

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Abstract

The purpose of this study was to synthesize, characterize and test the antimicrobial and antifungal activity of chitosan-based hydrogels containing metal (silver – Ag) and oxide (zinc oxide – ZnO) nanoparticles (NPs) but also natural compounds such as usnic acid (UA). The two-dimensional (2D) sheets were obtained by electrospinning technique, with the aim to produce multifunctional wound dressing with regenerative and even anti-infective roles. The most important advantages of the electrospinning technique are related to the possibility of obtaining fibers with controlled morphology, usually having high specific surface and water and air penetration and the possibility of functionalizing these fibers and nets depending on the desired application. These advantages make it possible to use electrospinning for a wide range of biomedical applications, such as tissue engineering, controlled release, implantology, wound healing, and more. The obtained composite materials were characterized by infrared (IR) spectroscopy and scanning electron microscopy (SEM) and tested against common pathogens: *Pseudomonas aeruginosa* (Gram-negative staining), *Staphylococcus aureus* (Gram-positive staining) and *Candida albicans* (fungus).

Keywords: chitosan, hydrogels, multifunctional wound dressing, composite materials, biomedical applications.

Introduction

Wound healing is a significant global problem for the health care system [1]. Furthermore, an untreated plague can lead to a large area of necrosis and systemic infection [2]. Such complications can be avoided by using antibacterial biomaterials to treat or prevent tissue infection.

Several products, such as gauzes, hydrogels, foams, hydrocolloids, were tested as passive dressings for wounds and burns because of their effect on the local cellular response [1, 3], but also for them useful properties such as protection of the peri-wound skin, maintenance of suitable moisture at the wound level, prevention and maintenance of microbial biofilms, cleansing of injured tissues, elimination or minimization of pain, removal of dead spaces and nonviable tissues and control of odors [2, 4–8]. Over the time, numerous studies have been conducted for developing improved wound dressing using biocompatible materials with antibacterial and antifungal activity [9–12], such as collagen chitin, chitosan and their derivatives. These materials are capable to accelerate the healing processes at molecular, cellular, and systemic levels, in order to accelerate the wound repair by systematically designed dressing materials and the use of the same method in the treatment of the disease [5].

Due to the hemostatic stimulation of healing, antimicrobial, non-toxic, biocompatible and biodegradable properties, chitosan has been widely used as a local dressing in wound dressing [13]. Also, chitosan has been used in many application as antimicrobial and antifungal agent, for the prevention and treatment of infections [13].

It has been shown that the antimicrobial and antifungal properties of chitosan may be influenced by a number of factors, such as molecular weight, degree of deacetylation (DDA), ion resistance and pH of the dissolution medium. Dai *et al.*, have shown that depending on the physical state (films, coatings, hydrogels), chitosan shows very varied antimicrobial activity [13].

The mechanisms of action by which chitosan manifests its antimicrobial and antifungal activity have been studied [14–17]. Although the exact mechanism has not yet been elucidated, the intracellular leakage hypothesis is widely accepted [14, 16, 17]. In this mechanism, interactions between positively charged chitosan molecules and negatively charged bacterial surface take place. These interactions lead to modified membrane permeability, interruption of the microbial membrane, and subsequently leakage of proteins and other intracellular constituents [14, 17–21] that cause cell death [14, 20, 22]. At concentrations lower than 0.2 mg/mL, the positive charges of chitosan decreases, and interact with the negatively charged

surface of the bacteria thus causing agglutination. At concentrations higher than 0.2 mg/mL, because of the higher positive loaging of the chitosan, as well as the stronger interaction with the bacterial surface, these are maintained in suspension [14].

It has also been experimentally demonstrated that antimicrobial activity strongly depends on pH. In the case of a neutral pH, the loss of positive charges of the amino groups in the chitosan structure leads to the loss of antimicrobial activity, while in the conditions of an acidic pH the antimicrobial activity of chitosan is limited [14, 22].

Du *et al.* [23] demonstrated that antimicrobial and antifungic properties of chitosan were enhanced by loading chitosan with various metals [silver (Ag), copper (Cu)], usnic acid (UA), and oxide metals [zinc oxide (ZnO), titanium dioxide (TiO_2)]. Among all antimicrobial metals, Ag is the most used, especially because of the strong activity over a wide range of microorganisms [24].

ZnO nanoparticles (NPs) are biocompatible, nontoxic but noxious to microorganisms, inhibiting the growth of microorganisms by disintegrating the cell membrane [25]. Several studies reveals that ZnO NPs are stable under harsh processing, present photo-oxidizing activity against a wide range of biological species but also chemicals, exert self-sterilization and antimicrobial activity against microorganisms and also minimal effect on mammalian cells, at proper concentrations [26]. Thus, ZnO is considered to possess good antimicrobial properties and is trying to be exploited as an alternate for the antibiotics [27].

The most important advantage of the electrospinning technique is the possibility of obtaining very thin nanofibrilar sheets. Possessing tunable high specific surface these sheets/membranes can be functionalized depending on the desired application, the density of the functional groups being really high. These advantages make it possible to use electrospinning for a wide range of biomedical applications, such as: tissue engineering, wound healing, filtrating elements, controlled release, and more.

In this work, the synthesis of chitosan-based antimicrobial composite sheets is presented by using electrospinning. The antimicrobial activity was enhanced by the addition of ZnO and UA. Because of the incompatibility of chitosan solution (stable only in acidic conditions) and ZnO (unstable in acidic conditions), the manufacturing methodology of those composite sheets involved the *in situ* synthesis of ZnO by transforming Zn salts after the electrospinning step. This is also suitable because the exposure of the films to basic conditions consolidate the chitosan structure. The obtained composite materials were characterized by infrared (IR) spectroscopy and scanning electron microscopy (SEM). The as obtained films were tested against common pathogens: Gram-negative (*Pseudomonas aeruginosa* – PAO1) and Gram-positive (*Staphylococcus aureus* – ATCC 25923) bacteria, and fungi (*Candida albicans* – ATCC 10231). These films were designed to be used in regenerative and even anti-infectious applications but also can be used as antimicrobial packaging in food industry.

Materials and Methods

Materials and testing

All chemicals: chitosan (Aldrich Chemistry), glacial acetic acid (Silal Trading), usnic acid – $C_{18}H_{16}O_7$ (Roth), zinc acetate dihydrate – $C_4H_6O_4Zn \cdot 2H_2O$ (Sigma Aldrich) and silver nitrate – $AgNO_3$ (Sigma Aldrich) were used without purification.

The Fourier-transform infrared spectroscopy (FTIR) was performed on a Nicolet iS 50 FTIR spectrometer, equipped with a deuterated triglycine sulfate (DTGS) detector, which provides information with a high sensitivity in the range of 4000 cm^{-1} to 100 cm^{-1}. The spectra were obtained in attenuated total reflection (ATR) mode (diamond crystal) over the range of 400–4000 cm^{-1}, with a resolution of 4 cm^{-1} and 32 spectra were co-added to obtain high quality spectra with limited necessity of smoothing.

SEM was performed using the QUANTA 250 FEI microscope (FEI, Japan) equipped with the energy dispersive spectroscopy (EDS) module.

Manufacturing of chitosan-based films

The chitosan solution was prepared by dissolving 1.049 g of chitosan in acetic acid (100 mL acetic acid diluted with 50 mL distilled water), under continuous magnetic stirring for 12 hours, at room temperature.

The obtained chitosan solution was electrospun by using a TonghiTech Nanofibre Electrospining Unit equipped with a TonghiTech-TL-FG injectomat system. The experiments were carried out by using a 20 mL syringe and a G21 needle (inner diameter of 0.514 mm).

In order to determine the optimum deposit conditions, several attempts were made during which different deposition parameters were varied: deposition rate, voltages, working temperature and deposition time. The electrospinning conditions, suitable for our purposes, were: acceleration voltages -17.27 kV and +5.02 kV, heating power a sample of 0.506 kW, deposition time of 60 minutes, solution flow rate of 10 mL/h, the width of the film was 80 mm, the drum rotation speed of 140 rpm, and a needle scanning speed of 40 mm/s. The obtained materials were then dried in vacuum, at 60°C, overnight, to remove acetic acid and water.

Manufacturing of CS/ZnONPs composite films

Antimicrobial films based on chitosan (CS) and ZnO NPs were prepared by dissolving 1.049 g of chitosan and 0.0234 g of $Zn(CH_3COO)_2 \cdot 2H_2O$ in 150 mL acetic acid (100 mL acetic acid diluted with 50 mL distilled water), under continuous stirring for 12 hours, at room temperature.

This solution was electrospun under the same conditions as the reference chitosan solution. After deposition, the obtained film was introduced into 5 N NaOH solution to transform Zn^{2+} into ZnO NPs, along with the precipitation of chitosan and subsequently washed with water to remove NaOH and salts. The obtained materials were dried under vacuum, at 60°C, overnight.

Manufacturing of CS/UA films

Chitosan–usnic acid (CS/UA) films were prepared by dissolving 1.049 g of chitosan and 0.01 g of usnic acid

in acetic acid (100 mL acetic acid diluted with 50 mL distilled water), under continuous magnetic stirring for 12 hours, at room temperature. This solution was electrospun under the same conditions as the reference chitosan solution. The obtained materials were dried under vacuum, at 60°C, overnight.

Manufacturing of CS/ZnONPs/UA composite antimicrobial films

Complex antimicrobial films based on chitosan, zinc oxide and usnic acid (CS/ZnONPs/UA) was obtained by electrospinning the solution containing 1.049 g of chitosan, 0.0236 g $Zn(CH_3COO)_2 \cdot 2H_2O$ and 0.01 g of usnic acid. The dissolution was made as presented above. After electrospinning, the obtained film was immersed in 5 N NaOH solution to precipitate ZnO NPs and to consolidate the chitosan structure and subsequently washed with water to remove the NaOH and salts. This solution was electrospun under the same conditions as the reference chitosan solution. The obtained materials were dried under vacuum, at 60°C, overnight.

The content of the active agents of the four films prepared by electrospinning are presented in Table 1.

Table 1 – ***The composition of the antimicrobial films***

Sample code	Content of usnic acid [%]	Content of ZnONPs [%]
CS	–	–
CS/ZnO	–	1
CS/UA	1	–
CS/ZnONPs/UA	1	1

CS: Chitosan; ZnO: Zinc oxide; NPs: Nanoparticles; UA: Usnic acid.

Evaluation of the degree of swelling

For the determination of the degree of swelling, we used samples of 2 cm diameter per 2 cm of each sample of polymer membranes previously obtained. Distilled water was used as solvent. Each sample was immersed in 25 mL of distilled water at room temperature, reading from time to time the mass of the sample of polymer introduced into the solvent to determine the degree of swelling.

Antimicrobial activity

The strains used for this study were obtained from the strain collection of the Laboratory of Microbiology, Faculty of Biology, University of Bucharest, Romania.

Qualitative test methods

Disk-diffusion method adapted

The test uses the adapted diffusometric method [according to Clinical & Laboratory Standards Institute (CLSI) recommendations, 2015]. On the surface of Müller–Hinton (without glucose) 2% (pH 7.2–7.4) agarified medium (for bacterial species) and Sabouraud agarified with Chloramphenicol (for yeast species), 4 mm thick in Petri dishes Ø 10 cm), a standard inoculum of a microbial suspension of sterile saline water (SSA) from 18–24 hours cultures with a standard density of 1.5×10^8 colony-forming unit (CFU)/mL nephelometric with 0.5 McFarland Standard (for bacterial cells) and 3×10^8 CFU/mL density nephelometric with 1 McFarland (for fungi cells). Microbial cultures of 18–24 hours are obtained by sowing the studied strains on trypticase soy agar (TSA) and yeast peptone glucose (YPG), respectively. Subsequently, on the surface of the environment sown in the canvas are placed samples to test the states (cut in the form of discs with a diameter of 6 mm). The plates are incubated for 16–18 hours, at 37°C, to allow the growth of microorganisms and free diffusion of the active compounds in the test materials. The results are interpreted by assessing the diameter of the growth inhibition zones.

Quantitative methods

Growth of planktonic microorganisms in the presence of antimicrobial films

To test the effect of the films over the growth of microorganisms in liquid medium (planktonic culture), the obtained films were cut to 1×1 cm and sterilized by exposure to UV radiation for 20 minutes on each side. One fragment of sterile material was individually deposited in a well of a 6-well sterile plate. Over the deposited materials, 2 mL of liquid medium (simple bullion for bacteria and liquid YPG for yeasts) was added to the wells and then 50 µL of microbial suspension of 0.5 McFarland density (bacteria) or 1 McFarland (yeasts). The 6-well plates thus prepared were incubated at 37°C for 24 hours. After the expiration of the incubation time, 200 µL of the obtained microbial suspensions were transferred to 96 sterile dosing plates and the turbidity of the microbial cultures was measured spectrophotometrically, at 600 nm.

Evaluation of biofilm formation – solidified functional material

To test the effect of surfaces obtained on biofilm production, the materials obtained were cut to 1 cm/1 cm and sterilized by exposure to UV radiation for 20 minutes on each side. One fragment of sterile material was individually deposited in a well of a 6-well sterile plate. Over the deposited materials, 2 mL of liquid medium (plain bullion) and then 50 µL of 0.5 McFarland microbial suspension were added to the wells. The 6-well plates thus prepared were incubated at 37°C for 24 hours. After incubation, the materials were washed with SSA and the medium was changed to develop biofilms developed on them. The plates were incubated for different time periods (24, 48 and 72 hours, respectively). After the expiration of each incubation period taken into account, the sample on which the biofilm was developed was washed with SSA and deposited in a sterile tube in one mL of SSA. The tube was vigorously vortexed for 30 seconds and sonicated for 10 seconds, to separate the cells from the biofilm. The cell suspension obtained was diluted and various dilutions were seeded on solid culture media plates to obtain and quantify the number of CFUs.

Results

The antimicrobial films were characterized from the point of view of composition, phase interactions and morphology by using FTIR and SEM.

Infrared spectroscopy

The FTIR spectra of chitosan-based materials (Figure 1)

reveal the characteristic peaks of pure chitosan but, because of the low content of usnic acid and zinc oxide these cannot be clearly identified [28]. Thus, in all spectra of chitosan-based films, the specific bands of chitosan can be identified.

It exhibited a broadband at 3281 cm^{-1}, which can be assigned to the stretching vibrations of pendant groups, such as $–NH_2$ and –OH on the chitosan. The band at 2874 cm^{-1} corresponds to the asymmetric stretching vibrations of $–CH_2–$ groups of chitosan polymer. The bands cantered at 1552 cm^{-1} and 1378 cm^{-1} corresponds to the stretching vibration of C–O and C–N groups in chitosan. The band obtained at 1060 cm^{-1} arises due to the stretching vibrations of –C–O–C– linkages. From the IR spectra (Figure 1) recorded on composite materials based on CS and CS/ZnONPs it is observed that broad band attributed to the valence vibration of alcoholic OH groups moved to lower values, indicating the interaction between this group and ZnO [29, 30]. Also, a decrease in bandwidth around 1151 cm^{-1}, and 1060 cm^{-1}, corresponding to vibrational υ(O–H) is observed, suggesting the existence of interactions between chitosan and zinc oxide. In the case of composite materials based on CS, CS/ZnONPs/UA and CS/UA (Figure 1), changes in the intensity and shape of the peaks may be observed at 1643 cm^{-1} and 1250 cm^{-1}, indicating that they have interactions between UA, chitosan and ZnO NPs occurred. This conclusion is supported by moving the bands at 1643 cm^{-1}, at lower values. Also, for composite materials based on CS/ZnONPs and CS/ZnONPs/UA (Figure 1), there is a decrease in the bandwidth of about 1151 cm^{-1} and 1060 cm^{-1}, corresponding to O–H vibration, suggesting that there could be an interaction between chitosan and zinc oxide.

Table 2 joins together the main characteristic absorption bands of the CS, CS/ZnONPs, CS/UA, CS/ZnONPs/UA films. One can see that minor shifts can be observed along with relative intensity changes. The shifts can be explained based on the week interactions between the phases while the intensity changes can be assigned to the changes of concentration. Generally said, the most important shifts are observed for the OH, NH and COOH groups, which means that these groups are involved in the interactions between the components.

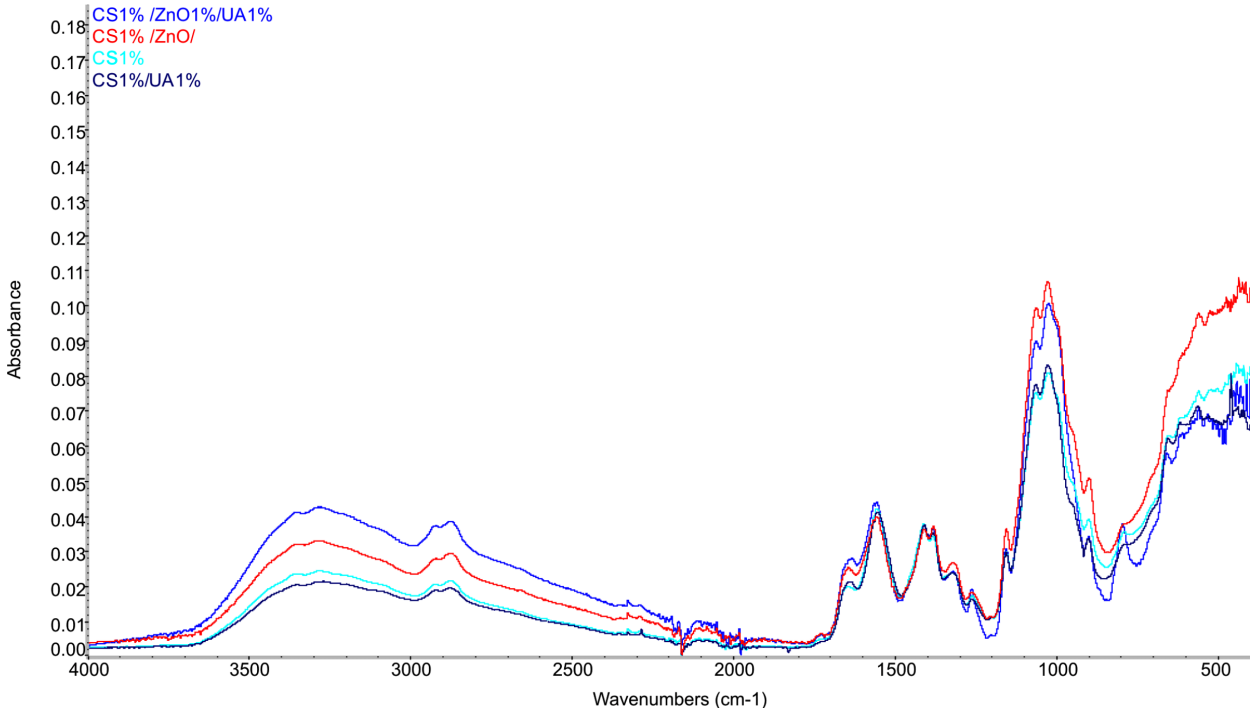

Figure 1 – ***FTIR spectra of CS, CS/ZnONPs, CS/UA, CS/ZnONPs/UA materials. FTIR: Fourier-transform infrared spectroscopy; CS: Chitosan; ZnO: Zinc oxide; NPs: Nanoparticles; UA: Usnic acid.***

Table 2 – ***FTIR main absorption bands (cm^{-1}) of the CS, CS/ZnONPs, CS/UA and CS/ZnONPs/UA films***

Composite materials	υ(O–H) υ_s(N–H)	υ(–C=O)	υ(–C=O) $\delta(NH_2)$	δ(OH)	$\delta(–CH_3)$	$\upsilon_s(–CH_3)$ $\omega(–CH_2)$ OH	υ_{as}(–C=O)	υ(–C=O) C–O–H, C–O–C, CH_2CO	ω(C–H)
CS	3281	2874	1643 1552	1408	1378	1318	1258 1152	1060 1019	896
CS/ZnONPs	3358	2875	1637 1582	1406	1370	1319	1261 1150	1058 1023	895
CS/UA	3266	2874	1633 1552	1406	1377	1317	1252 1152	1061 1023	897
CS/ZnONPs/UA	3273	2871	1630 1560	1406	1377	1317	1252 1152	1059 1022	897

FTIR: Fourier-transform infrared spectroscopy; CS: Chitosan; ZnO: Zinc oxide; NPs: Nanoparticles; UA: Usnic acid; s: Symmetric; as: Asymmetric.

Scanning electron microscopy

Surface morphology of chitosan-based materials were evaluated using SEM. The SEM images of chitosan (Figure 2) highlight the fibrillar nature of the film. These fibers, usually measure hundreds of microns in length and tens of microns in diameter, the fibers being randomly oriented. At higher magnification – 5000× (Figure 2c), some micrometric and submicrometric beads can be identified which means that during the film formation, along with the electrospinning of the chitosan solution also electrospraying occurs. In the case of CS/ZnONPs (Figure 3), the presence of ZnO lead to an important change of the morphology of the film, the surface aspect being rather granular than fibrillar. In the case of CS/UA film (Figure 4), the surface morphology is similar to that of pure chitosan. The surface is smooth, compact and homogeneous but, again, microbeads can be identified on the surface of the films.

The SEM images (Figure 5) highlight the fibrillar nature of the composite CS/ZnONPs/UA films, as well as the presence of micrometric agglomerates, which are mainly due to the presence of ZnO NPs, the surface becoming rough and heterogeneous. These agglomerates are uniformly distributed onto the surface, their size distribution being narrow as visible from Figure 5b (at higher magnification – 5000×).

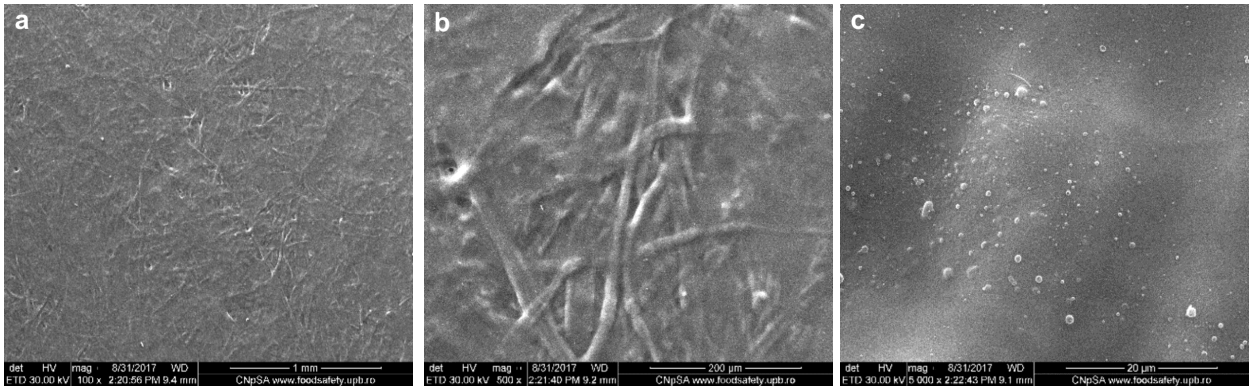

Figure 2 – ***Representative SEM images of the surface of CS film. SEM: Scanning electron microscopy; CS: Chitosan.***

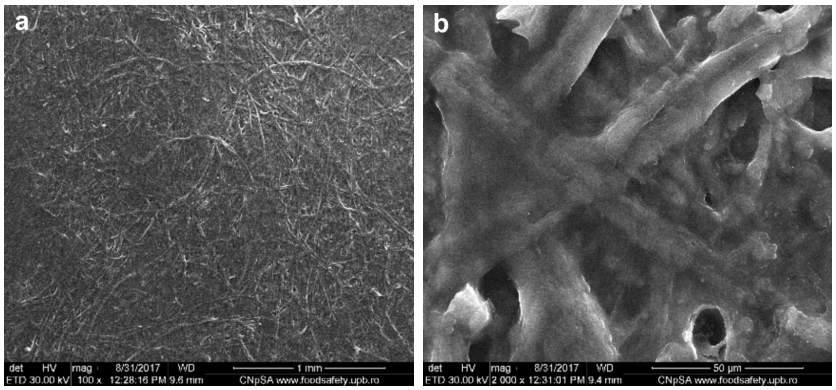

Figure 3 – ***Representative SEM images of the CS/ZnONPs film. SEM: Scanning electron microscopy; CS: Chitosan; ZnO: Zinc oxide; NPs: Nanoparticles.***

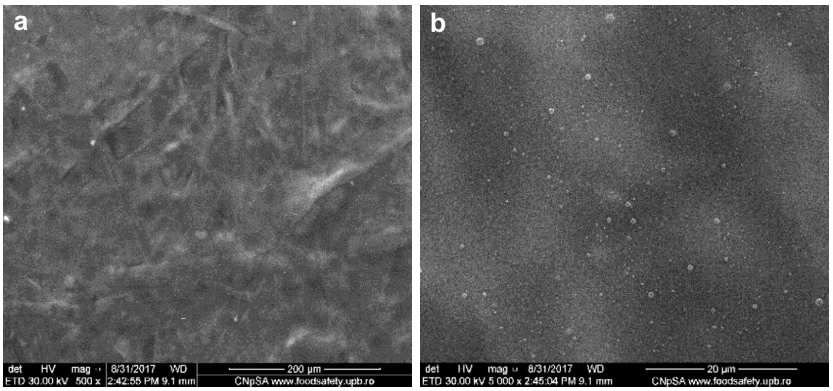

Figure 4 – ***Representative SEM images of the CS/UA NPs films. SEM: Scanning electron microscopy; CS: Chitosan; UA: Usnic acid; NPs: Nanoparticles.***

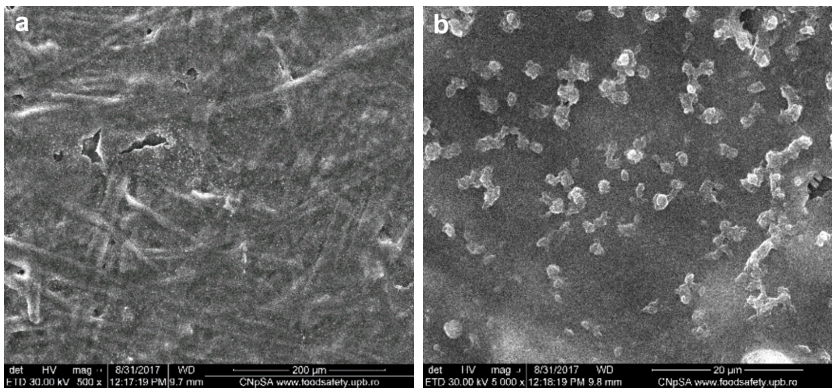

Figure 5 – ***Representative SEM images of CS/ZnONPs/UA films. SEM: Scanning electron microscopy; CS: Chitosan; ZnO: Zinc oxide; NPs: Nanoparticles; UA: Usnic acid.***

The swelling degree

The swelling degree of the films obtained *via* electrospinning was carried out using distilled water, at room temperature.

Figure 6 highlights a different swelling behavior of the four films, the highest swelling degree belonging to CS and CS/UA, while the lowest swelling degree belongs to the sample loaded with 1% UA and 1% ZnO. Even if, the one can expect that samples with usnic acid should highlight lowest swelling, most probably because of the limited content of UA the decrease is not so important. The samples containing ZnO NPs exhibit lower swelling, this being explained based on their more compact structuration because ZnO has a higher density (~5.61 g/cm^3) comparing with chitosan (apparent density of 0.15–0.3 g/cm^3).

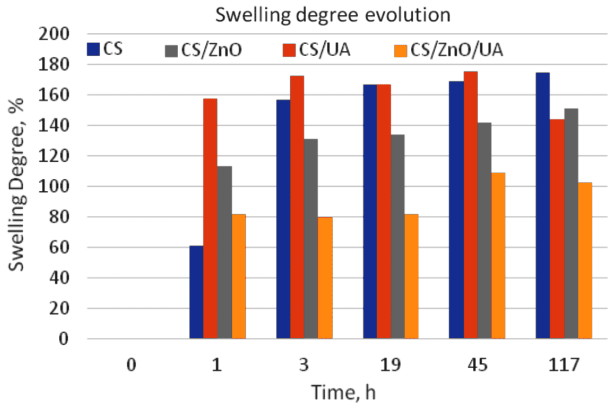

Figure 6 – ***The swelling behavior of CS, CS/ZnONPs, CS/UA and CS/ZnONPs/UA film. CS: Chitosan; ZnO: Zinc oxide; NPs: Nanoparticles; UA: Usnic acid.***

The evolution of water absorption is not continuous for all samples due to the slight degradation of the chitosan matrix. For samples CS and CS/ZnONPs, the evolution of water absorption shows a normal profile, the samples absorbing water up to 24 hours and increase their mass with 174% and 151%, respectively, while CS/UA and CS/ZnONPs/UA absorbed 172% and 108.5%, respectively. Moreover, CS/UA and CS/ZnONPs/UA start to lose mass after 45 hours, which can be assigned to the degradation of the samples (over 20% mass loss for CS/UA after 45 hours and only 6% for CS/ZnONPs/UA) (Table 3).

Table 3 – ***The swelling degree***

Antimicrobial film	Swelling degree [%]	Time of maximal swelling [hours]
CS	174.4	117
CS/ZnONPs	151	117
CS/UA	172.2	45
CS/ZnONPs/UA	108.5	45

CS: Chitosan; ZnO: Zinc oxide; NPs: Nanoparticles; UA: Usnic acid.

Antimicrobial activity

Antimicrobial activity was tested on cultures of *C. albicans*, *S. aureus* and *P. aeruginosa*, in planktonic regime or biofilm.

Growth of planktonic microorganisms in the presence of materials

The films based on CS and ZnO, the antimicrobial activity of the films depends from strain to strain. For the *S. aureus* strain, activity is virtually the same for all samples containing ZnO, which indicates that this activity is virtually induced by its presence, probably due to the interaction between the positive charged cell surface and the hydroxyl ions. In the case of *P. aeruginosa* Gram-negative bacteria and the beneficial *C. albicans* fungus, the complex mechanism makes the antimicrobial activity of the CS/UA/ZnONPs ternary systems even lowest than the binary systems involved, and further studies are needed to understand this evolution (Figure 7).

Assessment of biofilm formation

The microbiological assessment is essential because the behavior of the associated cells in the biofilm is clearly different from that of the planktonic cells. In this case, it can be seen that on the *S. aureus* strain all samples containing usnic acid exhibit a net improved antimicrobial activity compared to pure chitosan and the tendency is that after 24 hours, the viability of the strains decreases. In the case of *P. aeruginosa*, an extremely good antimicrobial activity is observed in the case of pure chitosan but in the case of other systems, the antimicrobial activity on the biofilm is lower. For the *C. albicans* strain, antimicrobial activity is low, which indicates that these systems are not very toxic to this bacterial strain. In fact, this result is promising because good anti-

microbial activity is highlighted for the undesired/non-desired/infections generating microorganisms but is safe for the beneficial microorganisms existing in the human body (Figures 8–10).

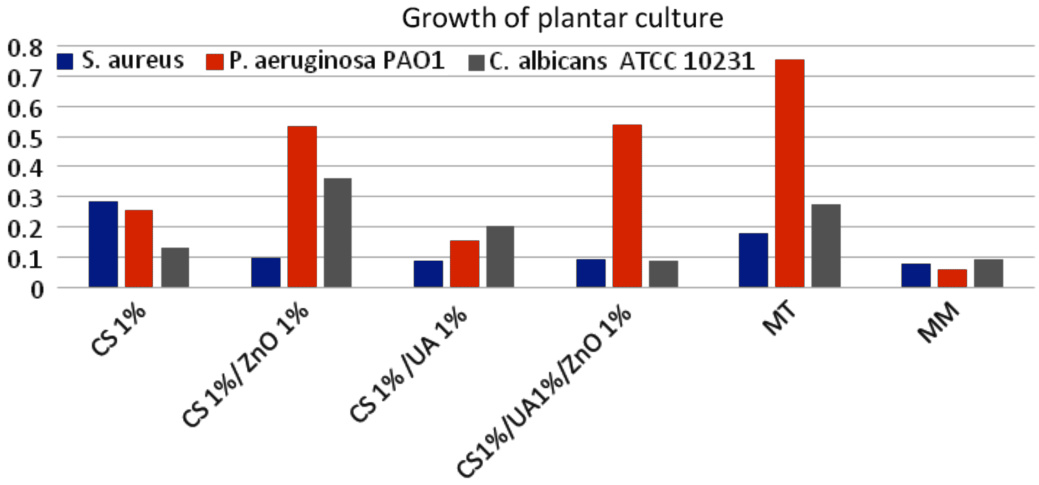

Figure 7 – ***Quantitative method for testing plantar cultures. CS: Chitosan; ZnO: Zinc oxide; UA: Usnic acid; MT: Reference of the strain; MM: Culture reference.***

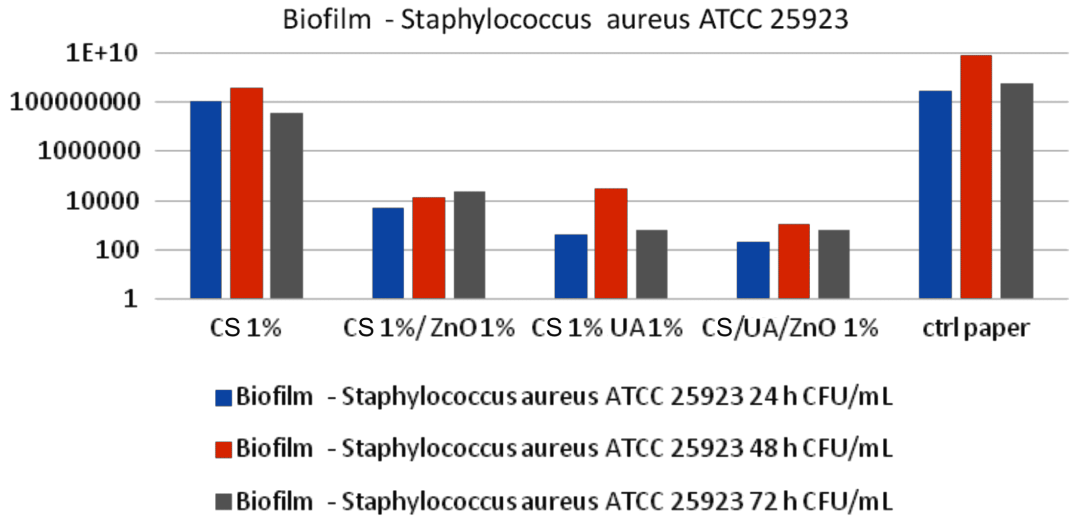

Figure 8 – ***Growth of planktonic microorganisms in the presence of materials tested on S. aureus ATCC25923. CS: Chitosan; ZnO: Zinc oxide; UA: Usnic acid.***

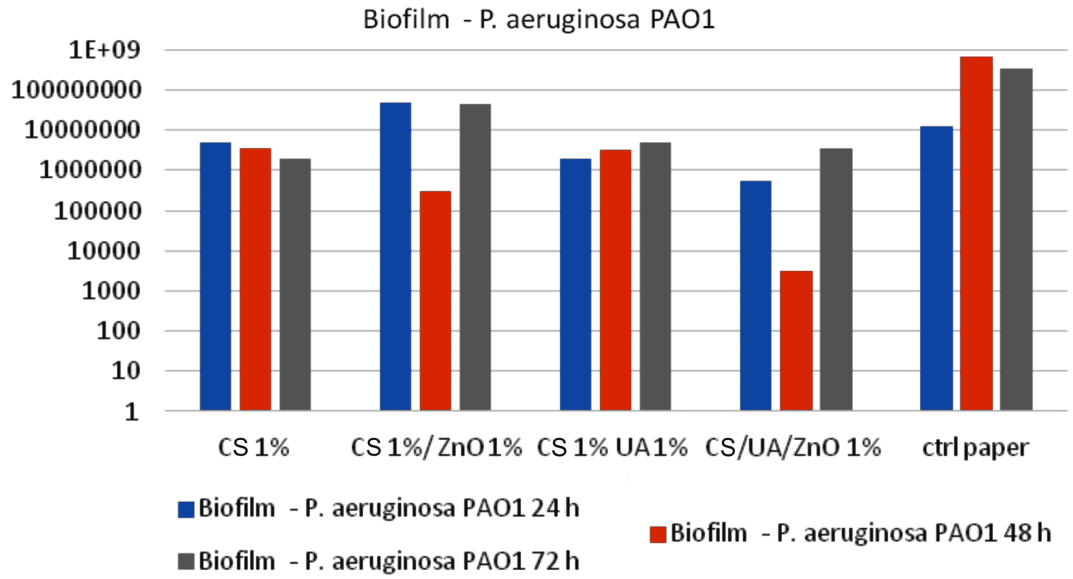

Figure 9 – ***Growth of planktonic microorganisms in the presence of materials tested on P. aeruginosa PAO1. CS: Chitosan; ZnO: Zinc oxide; UA: Usnic acid.***

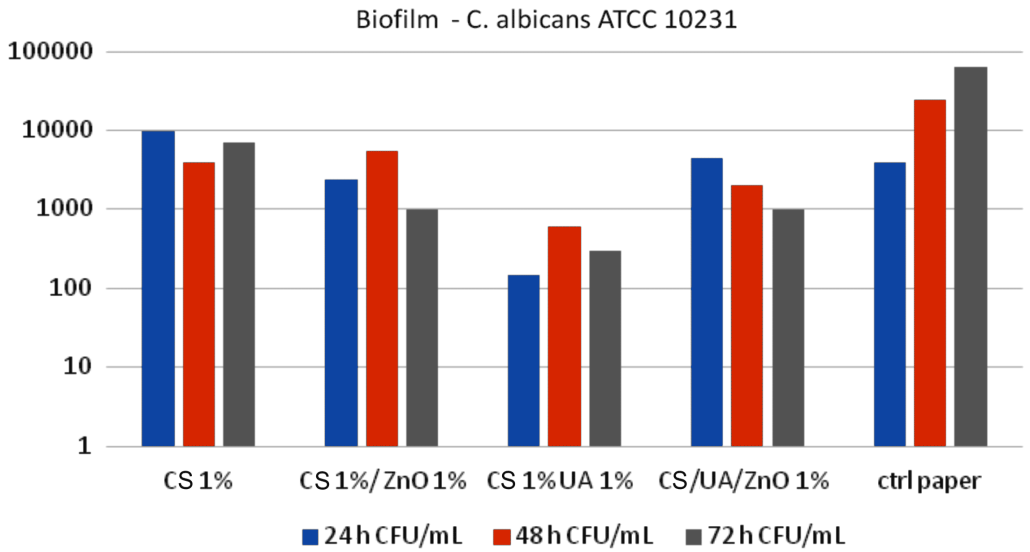

Figure 10 – ***Growth of planktonic microorganisms in the presence of materials tested on C. albicans ATCC 10231. CS: Chitosan; ZnO: Zinc oxide; UA: Usnic acid.***

Discussions

Many biopolymers are exploited in obtaining wound dressings, some of the most important biopolymers being collagen and chitosan [1, 2, 31, 32]. Chitosan has been widely used as a local dressing in wound dressing due to the hemostatic stimulation of healing, antimicrobial, non-toxic, biocompatible and biodegradable properties [13]. Over the time, chitosan has been widely studied as an antimicrobial and antifungal agent, in order to prevent and treat infections owing to its intrinsic anti-microbial properties [13]. The literature survey shown that the antimicrobial and antifungic properties of chitosan were also enhanced by loading chitosan based materials with various metals (silver, copper), biological agents (usnic acid), and oxide metals (ZnO), titanium dioxide (TiO_2) [23, 24].

ZnO is considered to possess good antimicrobial properties against Gram-positive bacteria (*Staphylococcus*, *Streptococcus*, *Pneumococcus*, *Mycobacterium tuberculosis*) and is trying to be exploited as an alternate for the anti-biotics [27]. It also demonstrated that the ZnO exhibits antiviral, antiprotozoal, antimitotic, anti-inflammatory and analgesic activity.

It is important to mention that chitosan can be dissolved only in acidic conditions so, it is impossible to be able to disperse ZnO in chitosan gel without dissolving it. In order to overcome this, the procedure proposed to start from chitosan gel and ZnO precursor and, after the electrospinning to transform Zn^{2+} into ZnO by alkaline treatment.

The presence of ZnO as well as usnic acid can be also exploited because of the proved, intrinsic antitumoral activity so, it is expected that these researches to be also continued in developing anticancer platforms [33–35].

Conclusions

In this study, a series of chitosan-based antimicrobial films were obtained by electrospinning technique. In the study, four types of CS, CS/ZnONPs, CS/UA and CS/ZnONPs/UA. The aim of the paper is still to improve the intrinsic antimicrobial activity of the chitosan by adding antimicrobial agents, such as ZnO NPs, as well as usnic acid have been selected and tested which according to the literature show very good antimicrobial properties on both bacteria and fungi. Although the mechanism of action of antimicrobial activity of these agents is not fully known, however, many studies have tried to elucidate this problem over time. Based on the physico-chemical characterization, it has been observed that the use of various antimicrobial agents influences the morphology of composite materials. The antimicrobial activity of these structures is promising, the films obtained being protective against the beneficial *C. albicans* fungus but active on *S. aureus* and *P. aeruginosa*, both in the planktonic regime and in the biofilm. Polymeric chitosan systems with low ZnO NPs and/or usnic acid can be used as biological membranes with antimicrobial activity.

Conflict of interests

The authors declare no conflict of interests.

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