Materials Research Express

PAPER

A novel antibacterial and biocompatible wound cover made of gelatin/chitosan with silver nanoparticles of green synthesis

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Keywords: membrane, gelatin, chitosan, silver nanoparticles, wound care

Abstract

A dressing material based on the combination of gelatin, chitosan and silver nanoparticles with a suitable proportion has been developed and can be successfully applied in biomedical fields. The new gelatin/chitosan membranes were prepared using the chitosan suspension mixed with gelatin and silver nanoparticles (AgNPs), resulting in a biocompatible and antibacterial product. AgNPs were obtained by the reduction of silver nitrate with chitosan solution and added to chitosan/gelatin (GCs) blend solutions to obtain membranes by the casting method. Thus, membranes with three different AgNPs concentrations were produced: 30 mM, 20 mM and 10 mM AgNPs. To evaluate the characteristics of the membranes, physicochemical and morphological tests were carried out, such as infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and transmission (TEM), and in vitro cytotoxicity and bacterial assays. The formation of AgNPs was confirmed by Visible Ultraviolet to Ultraviolet to Visible (UV–vis) and TEM, where the nanoparticles were observed by the formation of the peak spectrum at a wavelength at 560 nm. According to the TEM images, polymorphic nanoparticles with an average size of 30 nm were obtained. Furthermore, the results of scanning electron microscopy (SEM) with energy dispersive spectroscopy (EDS) indicated the presence of silver evenly distributed within the membranes. The results obtained by (FTIR) showed spectral peaks characteristic of the membrane materials, that is, typical spectra of gelatin, chitosan and silver. These results could be explained by addition of free −OH, −NH₂ and −NHOCOCH₃ groups of the amorphous chitosan in the blends and a network structure through electrostatic interactions between the ammonium ions (−NH₃⁺) of the chitosan and the carboxylate ions (−COO−) of the gelatin. The concentrations of AgNPs 30 mM and 20 mM in the membranes attributed to them a high hydration rate and high water vapor permeability (WVP). Membranes with 30 mM AgNPs showed bacterial effect against Staphylococcus aureus and Pseudomonas aeruginosa, concentrations of 20 mM AgNPs and 10 mM AgNPs, bacteriostatic effect against Staphylococcus aureus and bacterial effect against Pseudomonas aeruginosa. In the results of the in vitro assays, 10 mM AgNPs membranes were not cytotoxic. With the results obtained, GC membranes with up to 10 mM AgNPs are candidates for use in the fields of biomaterials and biomedicine.

1. Section heading

The skin is the largest organ of our body and has as main functions to cover and ensure protection between the internal and external environment, acting in defense and collaborating with other organs for the proper functioning of the body, by controlling the temperature, homeostasis of fluids and sensory capacity [1].

However, these functions may be disrupted by damage or loss of skin integrity due to injury. The wound arises when the discontinuity of the corporeal tissue caused by some type of physical, chemical, mechanical or illness trauma occurs. For the treatment of wounds, in addition to cleaning, occlusion with coating is often

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necessary. These coatings can help the healing process by protecting the injured area by absorbing moisture, controlling the accumulation of exudates, and preventing exogenous contamination [2, 3].

The request for coating to help the tissue repair, especially for people with metabolic, vascular, arterial, and immunosuppressive diseases, is a constant search for the development of biomaterials. A biomaterial is a substance that has been designed to take a form which, alone or as part of a complex system, is used to through control of interactions with components of living systems, the course of any therapeutic or diagnostic procedure necessary. These coatings can help the healing process by protecting the injured area by absorbing moisture, controlling the accumulation of exudates, and preventing exogenous contamination [2, 3].

The synthesis of the silver nanoparticles was performed according to modified methodology of Vercik, Vercik and Rigo (2017) [18]. Using a solution of chitosan of low molar mass, as reducing agent and stabilizer, with concentration of 6.92 mg ml⁻¹ solubilized in acetic acid 1% (volume/volume), followed by the addition of 60 mM AgNO₃ solution. After mixing the solution was transferred into glass tubes and placed in a water bath with constant stirring for 21 h at 90 °C. To confirm the formation of AgNPs, analysis was performed by UV–vis spectroscopy. The equipment used was a spectrophotometer in the wave range of 800 nm to 190 nm (BEL model M51) and for visualize the AgNPs, it was used the TEM.

2. Materials and methods

2.1. Synthesis of AgNPs

The synthesis of the silver nanoparticles was performed according to modified methodology of Vercik, Vercik and Rigo (2017) [18]. Using a solution of chitosan of low molar mass, as reducing agent and stabilizer, with concentration of 6.92 mg ml⁻¹ solubilized in acetic acid 1% (volume/volume), followed by the addition of 60 mM AgNO₃ solution. After mixing the solution was transferred into glass tubes and placed in a water bath with constant stirring for 21 h at 90 °C. To confirm the formation of AgNPs, analysis was performed by UV–vis spectroscopy. The equipment used was a spectrophotometer in the wave range of 800 nm to 190 nm (BEL model M51) and for visualize the AgNPs, it was used the TEM.

2.2. Manufacture of membranes

2.2.1. Membrane control

To produce the membranes, the following compounds were used: medium molar mass chitosan (Sigma-Aldrich), 260 bloom gelatins (Gelita do Brasil™), glacial acetic acid, silver nitrate, glycerin, sodium hydroxide. Mechanical stirrer bench, polystyrene petri dishes 90 mm × 15 mm and drying oven with renovation/circulation of air.

Membranes with only gelatin and chitosan in the ratio of 1:1 were obtained by mixing the two precursor solutions: chitosan solution 2% (mass/volume) in 1% (volume/volume) acetic acid and an aqueous solution of 4% gelatin (mass/volume). This mixture was kept under constant stirring for 1 h, after was added 0.5% (volume/volume) of glycerin; the mixture was poured into sterile Petri, dried in an air circulation oven for 48 h at ± 35 °C.

After drying the membranes were immersed in 0.25 M NaOH solution for 2 h followed by serial washes, until they reached neutral pH and dried at room temperature for 24 h, as shown in figure 1.
2.2.2. AgNPs membrane
For the preparation of the chitosan solution 2% (mass/volume), the AgNPs solution of 60 mM was used to dissolve the chitosan, obtaining a concentration solution of 2% (mass/volume). After the 4% (mass/volume) gelatin solution was added. Membranes with concentrations of 30 mM, 20 mM and 10 mM of AgNPs were obtained by adjusting the mixtures of the chitosan solutions with AgNPs. All precursor mixtures of the membranes were kept under constant stirring for 1 h, followed by the addition of glycerin 0.5% (volume/volume), used as a plasticizer. The mixtures were poured into sterile petri dishes of 90 mm × 15 mm, dried in an air circulation oven for 48 h at ± 35 °C.

The membranes were dried by immersion in 0.25 M NaOH solution for 2 h, followed by serial washes with distilled water until they reached neutral pH and dried at room temperature for 24 h, as shown in figure 1.

2.3. Fourier-transform infrared (FTIR)
Analyzes with 30 mM, 20 mM and 10 mM AgNPs and the control membrane were performed on a Spectrum One (Perkin Elmer) spectrometer, using the Total Attenuated Reflectance technique. With a wave range of 6000 to 550 cm⁻¹, with a resolution of 2 cm⁻¹ and with 32 scans.

2.4. Transmission electron microscopy (TEM)
The transmission electron microscopy technique is important for material analysis because it allows the visualization of the morphology, the shape and the structure of the nanoparticles. For this, 100 μl of the nanoparticle’s solution was dripped on the carbon face of a grid for MET, kept in a greenhouse at 37 °C for 48 h. This characterization was made with MET JEOL JEM 2100, at the University of São Paulo at the Microscopy Laboratory at the Institute of Chemistry of São Carlos-SP.
2.5. Scanning electron microscopy (SEM)
The morphology of membranes was characterized by a scanning electron microscope, in which x-ray dispersive energy (EDS) spectroscopy analysis was performed to identify the chemical elements present in the membranes, especially the silver element. For this analysis, SEM (HITACHI, model TM 3000) was used; all images were made with a 15 kV electron beam.

2.6. Swelling
The membranes were cut in triplicate, obtaining samples with 2 cm of diameter, weighed, and immersed in 0.01 M phosphate buffered saline (PBS) solution at pH 7.4 and incubated at 37 °C. The samples were taken from the PBS for weighing in the times: 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h and 24 h. To obtain the swelling values (equation (1)), where MW is the mass of the sample after swelling, Md is the mass of the sample before swelling, S is the swelling rate.

\[ S(\%) = \frac{(M_w - M_d)}{M_d} \times 100 \]  

2.7. Water vapor permeability (WVP)
The water vapor permeation test was adapted from the American Society for Testing and Materials (ASTM) standard, which defines it as the rate of passage of water vapor between an area and a specific thickness, induced by differences in vapor pressure between two surfaces under controlled conditions of temperature and humidity.

The membranes were weighed and the thickness measurements. They were fixed onto the capsules containing 60 g of silica with 15.2 cm² of area, this set was weighed and placed in the desiccator containing distilled water. The desiccators were conditioned in the B.O.D. The parameters determined for this test were: temperature at 35 °C and relative humidity of 50%. The permeability of the membrane to WVP was determined by the increase of the mass of the system, due to the absorption of moisture by the silica. To analyze the data, it was determined collection of the masses of the capsules twice a day for 5 days.

2.8. Cytotoxicity assay
To verify that the membranes exhibit toxicity to cells, cytotoxicity of the indirect assay (IC 50) was followed, with Chinese Hamster Ovary (CHO) cells as provided for in ISO 10993-5: 2009. On which is based on the sample concentration that shows toxicity to 50% of the cells, i.e., the sample concentration which leads to half of apoptotic cells remained contact.

The membranes were cut 1 cm in diameter, sterilized for 2 h in ultraviolet light, and kept submerged in RPMI medium with 5% fetal bovine serum, as well as the positive control (0.2% phenol) and the negative control (100 mg ml⁻¹ alumina). After, as cells, membranes and controls were incubated separately for 24 h.

Followed by 24 h of incubation, the media where the membranes and controls were incubated, were serially diluted: 100%, 50%, 25%, 12.5% and 6.25%, in addition the medium of the assay plate was discarded. Thus, the dilutions were transferred to the wells of the plate, which was incubated again for 24 h.

Finally, after incubation, to highlight the living cells of the dead, a colorimetric method was used with Neutral Red (NR). In this way, the plate was incubated again for 3 h. After the wells were washed with PBS and added NR cell extraction solution. The result was analyzed by absorbance in a microplate reader, obtaining data to verify whether there was a cytotoxic effect.

2.9. Antibacterial activity
The membranes were tested on bacteria found on wounds such as Staphylococcus aureus ATCC 6538 and Pseudomonas aeruginosa ATCC 15442. The materials used were: Tryptic Soy Broth (TSB-Oxoid™) and Mueller Hinton Agar (Oxoid™) as positive control, the antibiotic gentamicin (Sensifar™), bacteriological incubator (Qualxtro™), agitation incubator (Tecnal®-TE-420), 150 mm petri dishes (Kasvi®) and the Beckman Coulter-Du® 800 Spectrophotometer.

First, the bacteria were cultured in TSB medium under constant stirring at 37 °C for 24 h, concomitantly, the membranes and gentamicin were sterilized in ultraviolet light for 2 h, and incubated under stirring on TSB medium at the same concentration as the cell assays, 100 mg ml⁻¹, 24 h at 37 °C. At the same time the bacteria were cultured for 24 h at 37 °C.

After this time, the membranes and gentamicin were removed from the media and then 1 × 10⁵ bacteria per ml were added in this media and, again incubated for 24 h at 37 °C. After, aliquots of 100 μl were plated out and plated on a solid agar medium under two conditions: one immediately after addition of the bacteria in the media, before being incubated, identified as time ‘0’, and one after 24 h incubation of the media with the bacteria, identified as time ‘24’. Finally, all plates, after completion of the respective incubation periods, were
photographed for comparison of growth of bacterial colonies on the plates ‘time 0’ and ‘time 24’, shown in figure 2.

2.10. Statistical analysis
For the statistical analysis, the Origin Pro 8 software was used to confirm the normal distribution of the evaluated values, through the variance analysis (ANOVA) and the Tukey test, with a significance level of 5%.

3. Results and discussion

3.1. Synthesis of AgNPs
The silver nanoparticles were prepared by green reduction method, the appearance of brown color at the end of the synthesis indicated the formation of AgNPs. The formation of the nanoparticles can be confirmed by the absorbance at 427 nm, figure 3(A). The analysis obtained by TEM found AgNPs with sizes ranging from 3 nm to 30 nm, figures 3(B) and (C).

During the AgNPs synthesis, a colloidal solution went from colorless to dark brown. This color transition, as well as the pigment of the coloration, is related to the molar concentration of AgNO₃ and chitosan present in the colloidal system [19]. According to the AgNPs spectra obtained by UV–vis, it was possible to identify the formation of AgNPs. These data agree with data found in the literature, such as Murugadoss and Chattopadhyay (2008) [20], where the same profile of UV–vis spectra of silver nanoparticles was observed, so that observed in these studies’ absorption bands at between 410–471 nm.

3.2. Membranes
Membrane control and with different concentrations of AgNPs (30 mM, 20 mM, 10 mM) are shown in figure 4. The difference in staining is observed, the higher the concentration of AgNPs membrane will have a more intense yellow color. Hence, a strong yellow color is imparted to the 30 mM membrane, while the 20 mM membrane appears darker yellow, the lower concentration of AgNPs, the 10 mM, contains a very light-yellow shade and the control membrane did not show color, being transparent. As it was verified that the membrane with 30 mM presented greater rigidity than the others, so that when folding it, it splits in two, contrary to the other membranes, including the control, which are malleable. The combination of two or more polymers, through the physical mixing of different materials, has a wide applicability in biomaterials, because through this approach devices with better physicochemical, mechanical and biological characteristics can be acquired when compared with the compounds isolated [21]. Chitosan is a biopolymer capable of obtaining membranes and films suitable for the treatment of skin lesions, however, it presents a low mechanical resistance and low absorption capacity of body fluids [22].

The combination of this biopolymer with another, such as gelatin, can improve the above-mentioned characteristics. Gelatin blends with chitosan have a better affinity with the cells, and result in a more elastic and soft material, comparing chitosan isolated, in addition to providing a better water absorption and oxygen permeability [22]. Furthermore, it has been found in the literature that adding AgNPs in polymeric films, it is possible to develop increased mechanical strength and can infer the change in ductility due to the occupation of spaces in the polymer matrix the nanoparticles, which should be filled by the polymer chains [23].
3.3. Fourier-transform infrared
The analysis of FTIR, figure 5, was used to evaluate the profile of the compounds of the samples, obtaining information of the structures in relation to the bands of the molecular groups constituting the membranes. The most intense bands are: 3295 cm$^{-1}$, 3046 cm$^{-1}$, 1625 and 1540 cm$^{-1}$ and at 1237 cm$^{-1}$, relative to gelatin. While the relative spectra of chitosan where more intense bands were found at: 3322 cm$^{-1}$, 2893 cm$^{-1}$, 1634–1551 cm$^{-1}$, 1392–1308 cm$^{-1}$ and 1149–1024 cm$^{-1}$.

According to the FTIR analysis it is found in the membranes with AgNPs that although there is a small change in all bands, for example, gelatin spectrum, the band 3295 cm$^{-1}$ was shifted to 3300 cm$^{-1}$, all samples achieved the same profile, independent of the concentration of nanoparticles, as shown in figure 5. The membranes with AgNPs resembled the FTIR profile of gelatin and chitosan, it can be stated that the AgNPs added in the samples did not change the structure of the membranes. These results could be explained by the introduction of free $\text{−}\text{OH}$, $\text{−}\text{NH}_2$ and $\text{−}\text{NHOCOCH}_3$ groups of the amorphous chitosan in the blends and a network structure through electrostatic interactions between the ammonium ions ($\text{−}\text{NH}_3^+$) of the chitosan and the carboxylate ions ($\text{−}\text{COO}^−$) of the gelatin.

3.4. Scanning electron microscopy
Scanning electron microscopy allows the observation of membranes by the incidence of an electron beam that spreads in the sample, which are reflected forming an image that corresponds to the surface of the sample. The photomicrographs of the AgNPs membranes, shown in figure 6, all presented a smooth surface with no pores, independent of the concentration of nanoparticles. The nanoparticles were incorporated in the polymer matrix,
Figure 5. Spectra obtained by Infrared spectroscopy with attenuated reflection of the membranes.

Figure 6. SEM-EDS micrographs of membranes with AgNPs 30 mM, 20 mM and 10 mM.
as shown in figure 6, analyzed by means of SEM with dispersive energy detector (EDS), was able to identify the silver in the samples.

Scanning electron microscopy allows the observation of membranes by the incidence of an electron beam that spreads in the sample, which are reflected forming an image that corresponds to the surface of the sample. The photomicrographs of the AgNPs membranes, shown in figure 6, all presented a smooth surface with no pores, independent of the concentration of nanoparticles. The nanoparticles were incorporated in the polymer matrix, as shown in figure 7, analyzed by means of SEM with dispersive energy detector (EDS), was able to identify the silver in the samples.

As observed in figure 6, the membranes have a typical morphology of dense membranes, that is, there is no presence of pores in the samples. The difference between the dense and the porous membranes is related to the preparation of the samples, and in the porous membranes reagents such as inert polymers and rigid silica microparticles are added during the formation of membranes [24–26]. The porous membranes while allowing gas exchange with the larger environment that dense due to its covering pore structure, however they can also allow the microorganism will exceed, as they are able to break easily than dense membranes [27]. As observed in figure 6, the carbon atoms are predominant in all membranes, due to being a membrane made of biopolymers, which has many carbon atoms in its composition, as well as AgNPs, which were synthesized by the same biopolymer, chitosan. In view of this fact, the silver identification is low compared to the predominant atoms constituting the membranes. Through SEM-EDS analysis, it was possible to verify that the nanoparticles are indeed present in the membranes, and it was verified that the membranes have uniformity in their morphology.

3.5. Swelling test

The development of a membrane with the ability to remain moist, as well as absorbing fluids, is important to promote better healing, especially when applied to dry wounds or with little exudates. Through the swelling test, is possible observe the hydration capacity of the membranes. As observed in figure 7, all membranes swelled and had a high degree of hydration, the mean swelling rate being: 30 mM AgNPs membrane was 500%, 20 mM membrane was 450% and the 10 mM membrane was 420%. The control membrane, its rate of swelling was much lower than the others, on average 150%.

The hydration of wounds is the most important factor during a treatment, so when the wound is in a humid environment, it accelerates its healing. When comparing the healing of a wet wound with a dry one, it is observed that with the application of a wet cover in the cutaneous lesion, it will provide a protection of the external environment, remove the tissue debris, donate fluids to the injury, causing stimulation of epithelial cells. While in dry wounds, they will have excess proteases, which delay local epithelization, as well as are more susceptible to bacterial infections [28].

The development of a membrane with the ability to remain moist and absorbing fluids, is important to promote better healing, especially when applied to dry wounds or with little exudates. The swelling test is reported in several studies the high-capacity materials made with gelatin and chitosan absorb fluids [29–31]. As in the research by Fan et al. (2016) [32], obtained high swelling rates with hydrogels of gelatin/chitosan blends, assigning this result the chitosan macromolecules, which facilitate the expansion of the chains that form the hydrogel matrix. In addition to Nagahama et al. (2009) [30], which found a high swelling rate in

![Figure 7. Swelling rate of membrane with AgNPs 30 mM, 20 mM and 10 mM and control.](image-url)
gelatin/chitosan membranes due to the hydrogen bonds formed between these compounds. According to the research by Kanmani and Rhim (2014) [33] which, through a gel film with AgNPs, observed that the addition of the nanoparticles in the gelatin films, caused the reduction of the interactions among the polymer chains, resulting in increased availability of free hydroxyl groups, facilitating the absorption of water. Similarly, Yoksan and Chirachanchai (2010) [34] verified that in chitosan matrices with AgNPs, water penetration is more accessible, due to the reduction of the polymer interactions that occur in the matrix.

For that reason, the addition of higher concentrations of AgNPs facilitated the penetration of the phosphate buffer solution into the membranes, because they were dispersed in the matrices, interfering in the bonds between the polymer networks of both gelatin and chitosan. This result corroborates with data found in the literature indicating that if the hydrogen bonds rupture between the chains forming the matrix, there will be an increase in the spaces in the polymer chain and that this may make possible the solution entry [29, 35–37].

The ideal coverage should be damp or maintain local humidity and promote gas exchange with the environment to prevent waterproofing of the injured site. Considering that, the waterproofing of the lesion makes it difficult to diffuse the water vapor, resulting in a deposit of fluid in the wound, which may cause tissue tearing [23]. Therefore, the water vapor permeation test is important to verify the ability of the membrane to allow breathing of injured skin. The 30 mM AgNPs membrane presented the highest thickness, as seen in table 2, and this parameter is directly proportional to the WVP rate. Therefore, this membrane will have more 'voids' in the polymer chain than the others, due to the concentration of AgNPs resulting in a higher WVP rate, since the diffusion of water vapor occurred more easily and presented the higher swelling rate, due to the disruption of the hydrogen bonds formed between chitosan and gelatin in a humid environment [23, 33].

3.6. Water vapor permeability

Table 1 shows the results obtained from the WVP assay of all membranes. The results presented the results by means of a general analysis, where the mass difference per day on the area of permeation is related [23, 38–40].

The membranes with higher concentration of AgNPs had a higher permeation rate of water vapor, specifically with 1011.58 g m⁻² day, while the other samples obtained very similar rates, the membrane of 20 mM showed a rate of swelling of 958.42 g m⁻² day and 10 mM with 1001.05 g m⁻² day, and the control 892.63 g m⁻² day, so that no significant difference (p < 0.05) by Tukey’s statistical method. Moreover, recalling that the membrane 30 mM, showed the greatest thickness, as seen in table 2, and this parameter is directly proportional to the WVP rate.

To define which types of wounds the membranes may be applied in, the normal WVP rate of the skin, which is in the range of 240 g m⁻² per day, should be considered. However, this permeation rate changes when the skin is injured, ranging from 3400 to 5200 g m⁻² day [39]. These results, as can be seen in table 1, suggest that these membranes, 30 mM, 20 mM and 10 mM of AgNPs, can be applied to wounds with high exudates, so that they allow the exchange of adequate water vapor with the external environment [41]. In addition, considering that the membranes have a high capacity of swelling and WVP, it would cause a control in the exudates of the wounds, so that they would absorb these fluids, also allowing the exchange of gases between the external environment and the lesion. Thus, the membranes may aid the healing process by controlling the number of exudates while maintaining the wet injury and allowing gas exchange wound [33, 39, 41].

| Sample (mM) | WVP (g m⁻² dia) | Standard deviation |
|-------------|----------------|--------------------|
| 30          | 1011.58        | 13.4               |
| 20          | 958.42         | 17.8               |
| 10          | 1001.05        | 1.1                |
| Control     | 892.63         | 2.2                |

| Samples (mM) | Thickness (mm) | Standard deviation |
|-------------|----------------|--------------------|
| 30          | 0.111          | 0.009              |
| 20          | 0.083          | 0.006              |
| 10          | 0.076          | 0.02               |
| Control     | 0.063          | 0.005              |
3.7. Cytotoxicity Assay

The cytotoxicity assay (IC 50), figure 8, shows the concentration, by means of serial dilutions, in which the samples present cytotoxic action in 50% of the cells, in other words, it will demonstrate which concentration of the sample affects half of the cells, leading to apoptosis. For this, the test was based on the ISO 10993-5: 2009, which is recommended to use CHO cells, positive controls (toxic effect) and negative (non-toxic) control, such as phenol and alumina, respectively.

As shown in figure 8, only the samples of higher concentrations of AgNPs, membranes with 30 mM and 20 mM, obtained a toxic effect for the cells, while the membranes with 10 mM of AgNPs and the control sample did not present cytotoxicity.

According to the IC 50 results it was found that 30 mM and 20 mM AgNPs membranes presented cytotoxicity to the cells, due to high concentrations of AgNPs [42]. As well as, according to the Tukey statistical test, with the hypothesis of equality being rejected for \( P < 0.05 \), there is a significant difference between the membranes with 30 mM AgNPs and the membrane with 10 mM AgNPs of q value 4.72. Oxidative stress is considered a major cause of cytotoxic effects, so it is related to the imbalance between the production of free radicals and the inability of the cells to react against them [43]. Intracellular oxidative stress could be accelerated by AgNPs by disrupting the balance between oxidant and antioxidant processes [44]. As well, the induction of generation of reactive species with oxygen (ROS), which leads to mitochondrial membrane permeability, causes damage to the respiratory chain, triggering apoptosis [45].

Therefore, the control membrane and the 10 mM AgNPs membrane, did not present cytotoxicity. The first one only contains biocompatible compounds, which aid the cellular processes, while the second one presents low concentration of AgNPs, which do not trigger a negative biological response.

3.8. Antibacterial activity

The analyzes of bacterial growth are based on the observation and comparison of the formation of colonies in the agar plates, where the bacteria were cultivated for 24 h. After incubation, the plates were photographed in sequence (time 0) and after 24 h of incubation (24). In addition, positive (antibiotic) and negative control plates (culture medium + bacteria) were compared with the plate’s membranes, as these were compared to each other.

To highlight the effects of the membranes on the bacteria, digital photographs of the plaques were made and arranged in decreasing order of nanoparticle concentration, figure 9. First, the bactericidal action was verified in bacteria Staphylococcus aureus, the membrane with the highest concentration of 30 mM of AgNPs, then on membranes with 20 mM and with 10 mM, there was reduction of the colonies. While the bactericidal effect was found in all membranes with AgNPs in the test made with the bacterium Pseudomonas aeruginosa, figure 10. As well as no inhibitory effect of bacterial growth was observed on the control membrane.

The antibacterial activity verified in the membrane of 30 mM, 20 mM and in 10 mM. The antibacterial effect observed in S. aureus occurred only in the membrane with AgNPs concentration, whereas in the membranes of 20 mM AgNPs and in the 10 mM AgNPs, there was a reduction of the bacterial colonies, but it was not effective in eradicating them, S. aureus showed higher resistance when in contact with TSB medium in which the membranes were incubated when compared to P. aeruginosa bacteria. The antibacterial effect was higher in P.
*P. aeruginosa* is an aerobic gram-negative, non-spore forming, very common in hospitals and intensive care units, and presents high resistance to antiseptic pathways used in health care units. Thus, contamination of patients with this bacterium may occur, in patients with lung or burn wound infection, especially in patients with compromised immune systems, causing complications in the patient’s setting, such as urinary infection, ulcers and pneumonia, which may increase the cases obtained in hospitals [46].

The mode of action of AgNPs on bacteria the interactions of the Ag$^+$ and AgNPs ions with the sulfur bound in the cell wall, breaking it, caused the inhibition of bacterial growth. In addition, the presence of Ag$^+$ and AgNPs ions, both in cells and in bacteria, may increase the activity of the mitochondria, generating ROS, resulting in damage to the protein, lipid and amino acid complexes [47, 48].

Therefore, it is evident the antibacterial activity in the presence of AgNPs, which can be applied in the treatment of tissue lesions due to its antibacterial action [49–52].

4. Conclusions

The route of obtaining nanoparticles using chitosan as a reducing agent was efficient. The AgNPs altered the swelling behavior and permeation to water vapor of the membranes, it was observed that the higher the concentration of AgNPs, the higher swelling and WVP values were obtained. With the obtained results it is possible to infer that the membrane of 10 mM, according to the test *in vitro*, is considered biocompatible. In addition, the membranes presented a bacterial growth-reducing effect against *P. aeruginosa*, being a great option for advancement in clinical trials, as a complementary cover to the treatment of chronic wounds.
Acknowledgments

The authors would like to thank the University of São Paulo de São Carlos and Pirassununga and the Interunit Graduate Program in Bioengineering of the Federal University of São Carlos for their contribution to the characterization of the material and to the facilitation of work facilities. Like CAPES and CNPq for their financial support.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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