Preparation and Characterization of Chitosan/mPEG-PCL Blended Membranes for Wound Dressing and Controlled Gentamicin Release

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In this paper, a novel wound dressing membrane for controlled release of gentamicin (GE), while covering and protecting the wound was investigated. Chitosan (CHI) was associated with methoxy polyethylene glycol - polycaprolactone copolymer (mPEG-PCL) to prepare the blended wound dressing membranes. The use of copolymer mPEG-PCL was necessary to improve the compatibility between CHI and PCL. The association of CHI and PCL was required to control the water retention and release rate of encapsulated GE. In vitro release studies were performed with the mPEG-PCL/CHI-GE membranes in order to evaluate the effect of copolymer concentration on the kinetics of GE release and water uptake. Reduced burst release rates and swelling ratios were observed for the 1/2 and 1/4 mPEG-PCL/CHI-GE membranes. In addition, all gentamicin-loaded membranes inhibited S. aureus and E. coli growth, and demonstrated color, moisture and thermal stability. Therefore, mPEG-PCL/CHI-GE membranes showed important features for potential wound dressing and drug delivery applications.

Keywords: Copolymer, blend, methoxy polyethylene glycol, polycaprolactone, antibiotic, drug delivery.

1. Introduction

Skin is the largest organ of the body and protect it against infections, injuries, traumas and sunrays. Skin also is important in the control of body temperature. Skin lesions affect a large number of people in Brazil and in the world and can be caused by burns, chronic infections, vascular insufficiency, diabetes and hypertension, which can lead to death due to the loss of large areas of the skin. When skin is lost or lesioned, the wound should be covered and treated to avoid contamination of microorganisms. Gentamicin (GE) is one of the most commonly used antibiotics due to its low cost, wide antibacterial action, low rate of pathogenic resistance and allergy, good stability and water solubility. Based on the wound type, suitable dressing material must be used. Many researches can be found in the Literature regarding to the development of wound dressings, drug delivery systems and skin substitutes. Collagen chitosan, alginate, cotton, hyaluronic acid, gelatin, poly lactic acid, cellulose, polycaprolactone and other polymers can be used to prepare to prepared hydrogels, films and membranes for wound dressing application. The ideal dressing should provide or maintain moist environment; enhance epidermal migration; promote angiogenesis and connective tissue synthesis; allow gas exchange between wounded tissue and environment; maintain appropriate tissue temperature to improve the blood flow to the wound bed and enhances epidermal migration; provide protection against bacterial infection and be non-adherent to the wound and easy to remove after healing. Besides, it must provide debridement action to enhance leucocytes migration and support the accumulation of enzyme and must be sterile, non-toxic and non-allergic. It is not possible for a unique material to meet all the requirements for an ideal wound dressing. Thus, materials association can be an alternative to improve the performance of skin substitutes and to meet most of the desired requirements.

Chitosan (CHI) is a hydrophilic and cationic polymer obtained by chemical deacetylation of chitin, a natural polysaccharide found in shrimp and crab shells. It has been extensively studied in biomedical area due to its interesting biological properties such as biodegradability, biocompatibility, bioactivity, antibacterial, antitumor, antifungal and hemostatic activities. Disadvantage property of chitosan membrane that is unappropriated for controlled drug delivery application, is its high water uptake/swelling because of its large number of hydrogen bonds. This can compromise the sustained release of hydrophilic/water-soluble drugs, such as gentamicin. Therefore, an appropriate association to a hydrophobic...
polymer could achieve the controlled retention and release of a drug from chitosan.

Polycaprolactone (PCL) is a synthetic polymer, derived from petroleum and classified as semi-crystalline polyester. Due to its composition/structure, PCL has hydrophobic character and can be slowly degraded by the body. It has found several biomedical applications, such as controlled release of drugs, sutures and scaffolds for tissue engineering, due to its compatible properties and biodegradability\(^9\). Thus, combination of CHI and PCL can be used to prepare a blended membrane with unique properties for wound dressing and gentamicin controlled release.

One way to blend hydrophilic and hydrophobic molecules is to add chemicals that have both hydrophilic and hydrophobic moieties, such as surfactants\(^10\). Another alternative is to chemically modify the polymeric backbone to attach hydrophilic/hydrophobic molecules in order improve the compatibility. Copolymerization is also a strategy for polymeric materials. Copolymerization of PCL with methoxy polyethylene glycol (mPEG) forms block copolymers that are biocompatible and biodegradable polymers for potential use as drug delivery systems\(^11\). In addition, the miscibility between hydrophilic CHI and hydrophobic PCL may be increased by the use of the mPEG-PCL copolymer\(^12\).

Therefore, the aim of this paper was to prepare and characterize chitosan-mPEG-PCL membranes loaded with gentamicin to be later used as wound dressing for sustained antibiotic release. The blended membranes were characterized for chemical composition, morphology, color, thermal stability, moisture content and water uptake. Moreover, the in vitro gentamicin controlled release was studied over an extended period of 1 week and the effect of copolymer concentration on the kinetics of gentamicin release was also evaluated. The antibacterial activity of the CHI/mPEG-PCL membranes was tested in vitro against E. coli and S. aureus, most common bacteria found in skin wounds.

2. Materials and Methods

2.1 Materials

High molecular weight chitosan (CAS# 419419), ε-caprolactone monomer (molecular weight 114.14 g/mol), methoxy polyethylene glycol (molecular weight 550 g/mol) and ninhydrin (CAS# N4876) were purchased from Sigma/Aldrich - USA. Gentamicin sulfate injectable solution (280 mg/2mL) was purchased from Neo Química - Brazil. Glacial acetic acid (60.05 g/mol and density 1.05 g/cm\(^3\)) and acetone were purchased from Proquímios - Brazil.

2.2 Copolymerization of PCL and mPEG

The poly (ethyleneglycol) methyl-ether-poly(ε-caprolactone) (mPEG-PCL) copolymer was synthetized by Dr. Marques’ research group, using the aforesaid monomers, as previously described\(^9\).

2.3 Preparation of CHI/mPEG-PCL membranes

Eight membranes were prepared by varying the concentration of mPEG-PCL and GE in the formulation, according to the Table 1. For all compositions, 9mL of 1M acetic acid was used to dissolve CHI, and 5mL of acetone was used to dissolve mPEG-PCL. Acetone was also added to the formulations free of mPEG-PCL. For GE loaded membranes, 1mL of 5.6 mg/mL gentamicin solution was added to the polymeric solution. The antibiotic solution was replaced by Milli-Q water for GE free formulations. Therefore, the final solution volume for all formulations was 15mL. The membranes were obtained by casting 1.5 mL of each solution in a circular mold (diameter 2.5 cm), followed by the evaporation of solvent at room temperature.

3. Characterization of the Membranes

3.1 Thickness

A bench micrometer (Outside Micrometer, Vonder) was used to measure the thickness in 10 different points of the membrane. The average thickness was calculated by the arithmetic mean of the 10 values.

3.2 Color and opacity

The color and opacity of the membranes were analyzed in a Colorimeter Minolta CR-300. The luminance parameters L* were determined, ranging from 0 (black) to 100 (white); a*, green (-60) to red (+60); and b*, from blue (-60) to

| Membrane | Methoxy Polyethylene Glycol – Polycaprolactone | Chitosan | Gentamicin |
|-----------|-----------------------------------------------|----------|------------|
| CHI       | -                                             | 100 mg   | -          |
| CHI-GE    | -                                             | 100 mg   | 5.6 mg     |
| 1/8 mPEG-PCL/CHI | 12.5 mg                                    | 100 mg   | -          |
| 1/8 mPEG-PCL/CHI-GE | 12.5 mg                                | 100 mg   | 5.6 mg     |
| 1/4 mPEG-PCL/CHI   | 25 mg                                        | 100 mg   | -          |
| 1/4 mPEG-PCL/CHI-GE | 25 mg                                    | 100 mg   | 5.6 mg     |
| 1/2 mPEG-PCL/CHI   | 50 mg                                        | 100 mg   | -          |
| 1/2 mPEG-PCL/CHI-GE | 50 mg                                    | 100 mg   | 5.6 mg     |

The antibacterial activity of the CHI/mPEG-PCL membranes was tested in vitro against E. coli and S. aureus, most common bacteria found in skin wounds.
yellow (+60). The total color difference (ΔE*) was calculated according to Equation 1.

\[ \Delta E^* = \left[ (L^* - L_a^*)^2 + (a^* - a_a^*)^2 + (b^* - b_a^*)^2 \right]^{1/2} \]  

(1)

### 3.3 Morphological analysis

A Scanning Electron Microscopy (Leo 440i, England) was used to morphological characterization of the membranes before and after gentamicin in vitro release. Samples were previously coated with a 16 nm thick of gold film in a Sputter Coater (model SCD 050, Bal-Tec) for 60 seconds at an operating pressure of 2x10⁻² Pa at 24 ºC.

### 3.4 Infrared analysis (FTIR)

Samples were characterized by FTIR (ATR) in a Spectrometer (Cary 630, Agilent Technologies) from 650 to 4000 cm⁻¹. The Microlab software was used to treat the data.

### 3.5 Moisture content

Moisture content of the membranes was determined by the gravimetric method. The percentage of moisture was calculated according Equation 2.

\[ M(\%) = \frac{(M_i - M_f)}{M_i} \times 100 \]  

(2)

Where

- \( M(\%) \) = percentage of moisture;
- \( M_i \) = initial sample mass (g);
- \( M_f \) = final sample mass (g);
- \( W_s \) = mass of the swollen sample (g);
- \( W_d \) = mass of the dry sample (g);

### 3.6 Swelling behavior

Membranes were dried in a desiccator for 24 hours and weighed (Wd). After such procedure they were immersed in PBS, pH 7.4 and were kept at 37°C (to simulate body temperature) for 24 hours, when they were weighed again (Ws). The percentage of swelling, S(%), was obtained through Equation 3.

\[ S(\%) = \frac{(W_s - W_d)}{W_s} \times 100 \]  

(3)

### 4. Thermal Analyses

#### 4.1 Thermogravimetric analysis (TG) and derived thermogravimetry (DTG)

TG analysis was performed in a TG-DTA / DSC Apparatus (STA 449 F3 Jupiter, Netzsch). The analyzes were performed under a nitrogen atmosphere in the temperature range from 30°C to 900°C, at a heating rate of 10°C / min.

### 4.2 Differential scanning calorimetry (DSC)

Thermal analysis were performed in a Calorimeter (TA-Q100) by using the following conditions: temperatures range from -130°C to 200°C, at a heating rate of 2°C / min, under atmosphere of nitrogen.

### 5. In vitro Gentamicin Release and Antibacterial Activity

#### 5.1 Standard curve for gentamicin

In order to obtain the standard curve relating the gentamicin concentration to the absorbance, different samples of known concentrations of gentamicin were prepared from the dilution of the injectable gentamicin solution (280 mg/2mL). A colorimetric procedure, previously established was used for gentamicin quantification. The method is based on ninhydrin reaction with primary and secondary amines present in gentamicin. This reaction produces a purple color and the absorption of the gentamicin-ninhydrin mixtures at 400 nm has a linear relationship with the gentamicin concentration. The samples were previously reacted with ninhydrin for 15 min at 95°C and cooled in an ice bath, followed by determination of visible absorbance at 400 nm.

#### 5.2 In vitro release assay

Experiments on in vitro release of gentamicin from the membranes were performed at 37°C in 0.2 M PBS, pH 7.4. Two independent experiments were performed in order to evaluate the initial release (one hour) and long-term release (one week). In the first experiment, the release medium was collected and replaced with fresh buffer after each 15 minutes until complete one hour (15, 30, 45 and 60 minutes). The second experiment consisted in collecting samples from the release medium after each 24 h and replaced with fresh buffer each time. This procedure was repeated for 7 days and the experiment was carried out in triplicate. For both experiments, the collected medium was previously reacted with ninhydrin for 15 min at 95°C, followed by determination of visible absorbance at 400 nm. Gentamicin concentration was taken from the standard curve.

#### 5.3 Antibacterial activity

Both bacteria, Staphylococcus aureus and Escherichia coli, were independently grown in nutrient broth for 24 hours at 37°C. The antibacterial activity study was carried out by agar diffusion technique: 30 ml of PCA (Plate Count Agar) were added to each Petri dish and then inoculated with 0.1 ml of bacterial solution (E. coli and S. aureus). The membranes were then placed in contact with the agar (1 membrane of 2.5 cm diameter/quadrant) and the plate was incubated for 24
hours at 37°C. After this, bacterial growth’s inhibition zones were measured around the membranes. This antibacterial activity study was carried out in triplicates.

6. Results and Discussion

6.1 Thickness

Thickness of membranes was measured in order to verify the distribution of the solution in the mold. Uniformity of thickness plays an important role in the barrier property of the membranes. The average thickness of the membranes is shown in Table 2. As expected, no significant difference was observed comparing the average thickness of the membranes.

6.2 Color and opacity

Color and opacity are properties of great relevance for membranes used as wound dressings, since it is of great importance that the wound can be visualized, to evaluate the presence of infection and the healing process evolution. As expected, all prepared membranes are translucent. Table 3 summarizes the results of the colorimetric analysis.

Table 2. Average thickness (µm) of membranes unloaded and loaded with gentamicin.

| Membrane       | Thickness (µm) | Membrane       | Thickness (µm) |
|----------------|----------------|----------------|----------------|
| CHI            | 2.2 ± 0.2      | CHI - GE       | 2.8 ± 0.7      |
| 1/8 mPEG-PCL/CHI | 2.1 ± 0.5    | 1/8 mPEG-PCL/CHI - GE | 3.4 ± 1.0    |
| 1/4 mPEG-PCL/CHI | 2.5 ± 0.4    | 1/4 mPEG-PCL/CHI - GE | 2.5 ± 0.7    |
| 1/2 mPEG-PCL/CHI | 3.0 ± 0.8    | 1/2 mPEG-PCL/CHI - GE | 3.1 ± 1.1    |

In relation to the luminescence parameter (that varies from 0 for black to 100 for white), the values of L* was around 70 for all samples, but CHI-GE showed the highest L*. Regarding to the a* parameter, the chromatic coordinate that varies from -60 (green) to +60 (red), all membranes showed results near to zero, indicating the absence of these colors. Similar behavior was observed for the b* parameter, which varies from -60 (blue) to +60 (yellow). Positive values were observed for all samples, suggesting a discrete yellowish of the samples with mPEG-PCL and GE.

The total color difference (ΔE*) was calculated by the Equation 1 and no significant difference was observed comparing the membranes, except for CHI-GE, which showed higher total color difference. Mei et al. (2013) investigated the properties of blended films incorporated with perilla oil. The authors also reported an increase in total color difference (ΔE*) after the oil incorporation. Thus, incorporation of yellow components, such as gentamicin sulfate solution and perilla oil, may cause an increase in total color difference.

6.3 Morphological analysis

Although all membranes were visually homogeneous, microscopic analysis was performed to evaluate the interaction between CHI and mPEG-PCL and the microstructural characteristics of the membranes. Figure 1 shows the images obtained by SEM for the CHI, CHI-GE, 1/8 mPEG-PCL/CHI and 1/2 mPEG-PCL/CHI-GE membranes. Figure 1a and 1c shows a dense and homogeneous chitosan film. By Figure 1, it was possible to observe the uniformly incorporation of the mPEG-PCL copolymer in the chitosan matrix. Figure 1b and 1d shows a dense and homogeneous chitosan film. According to the authors, separation of phases was not observed only for membranes drying at 55°C. Therefore, the copolymer mPEG-PCL used in our study promoted a better miscibility between of PCL-CHI, as observed in Figure 1b and 1d.

Cooper et al. (2013) prepared chitosan-polycaprolactone based membranes by Electrospinning technique. SEM images of the electrospun membranes showed better incorporation of PCL in the CHI matrix for 1/3 PCL/CHI composition, however, more than one phase was observed for all formulations. Sarasam and Madihaly (2005) reported the characterization of chitosan-polycaprolactone blends prepared by solvent evaporation in an oven at various temperatures followed by solvent annealing with chloroform vapors. According to the authors, separation of phases was not observed only for membranes drying at 55°C. Therefore, the copolymer mPEG-PCL used in our study promoted a better miscibility between of PCL-CHI, as observed in Figure 1b and 1d.

The incorporation of gentamicin in the 1/2 mPEG-PCL/CHI-GE membrane can be suggested by the presence of gentamicin sulfate crystals on the surface of the membranes in the Figure 1c and 1d. Nevertheless, antibiotic incorporation in the membranes was confirmed by FTIR analysis.

6.4 Infrared Spectroscopy analysis

This analysis was performed for the raw materials: chitosan powder, mPEG-PCL powder and gentamicin solution; and for the obtained membranes in order to identify the
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Figure 1. Scanning electron micrographs of (a) CHI; (b) 1/2 mPEG-PCL/CHI; (c) CHI - GE; (d) 1/2 mPEG-PCL/CHI - GE.

Figure 2. Infrared spectra of raw materials: (black) mPEG-PCL, (red) chitosan and (green) gentamicin solution.

characteristic groups of the raw materials in the membranes. The infrared spectra of raw materials are shown in Figure 2. According to Yao et al. (2013), the main characteristic peak of chitosan is found at 1655 cm\(^{-1}\) as it can be observed for this sample\(^{16}\). Ketones, which are functional groups characteristic of PCL (C=O) show as intense band between 1870 cm\(^{-1}\) and 1540 cm\(^{-1}\), more specifically absorb at 1715 cm\(^{-1}\)\(^{16,17}\). It is also verified that the band corresponding to the amine group of gentamicin is between 1650 cm\(^{-1}\) and 1500 cm\(^{-1}\), coinciding with the chitosan peak, but being of a slightly higher intensity. For the gentamicin solution there is an intense band corresponding to the OH group between 3500 cm\(^{-1}\) and 3000 cm\(^{-1}\). Moreover, there is a low intensity band at 3442 cm\(^{-1}\) that can be attributed to water and the hydroxyl group of PEG in the copolymer. Bands between 2946 cm\(^{-1}\) and 2888 cm\(^{-1}\) refer to the organic groups (CH and CH\(_2\)) of...
mPEG-PCL. The ester group (-OC=O) characteristic peak is also observed at 1725 cm\(^{-1}\) in the copolymer spectrum\(^{17}\).

Figure 3 shows the FTIR spectra of the membranes unloaded with gentamicin. As expected, the main groups of CHI and mPEG-PCL was found in all spectra. The band corresponding to chitosan (N-H) is of low intensity, while the band corresponding to PCL (C = O) appears intensely.

Figure 4 shows the FTIR spectra of gentamicin-loaded membranes and gentamicin solution. As expected, an increase on the intensity of the hydroxyl and amine groups peaks was observed for the gentamicin-loaded membranes, confirming the antibiotic incorporation in these membranes. Aquino et al. (2013) reported absorption bands between 1645 and 1550 cm\(^{-1}\) in gentamicin loaded microparticles, which were attributed to the bending of N-H bond of the primary and secondary amines\(^{18}\). Bajpai et al. (2017) also reported a characteristic peak of -NH2 functionality at the vibrational wavenumbers 3802-3462 cm\(^{-1}\) in the spectrum of gentamicin sulfate loaded alginate films, as well as observed in the Figure 4\(^{19}\).

6.5 Moisture content

The percentage of moisture of the membranes, calculated by the Equation 2, is summarized in Table 4. It was not possible to establish a trend of moisture as a function of the concentration of mPEG-PCL in the membranes, as well as of the presence of gentamicin. However, all membranes presented values similar to those reported for chitosan membranes in the Literature, suggesting that the presence of gentamicin and mPEG-PCL did not influence the water content\(^{3}\).

6.6 Swelling behavior

Water uptake by the membranes is an important feature for application as wound dressing, since in the healing process the wound site must be kept moist and the membrane should be able to absorb the exudate fluids\(^{6}\). On the other hand, for medicated wound dressings, the swelling percentage can affect the drug release\(^{8}\). The percentages of swelling of the membranes were calculated by the Equation 3 and are shown in Table 4. As expected, a reduction of water uptake was observed for the membranes prepared with the mPEG-PCL copolymer. However, no significant difference was observed comparing CHI and 1/8 mPEG-PCL/CHI membranes. This behavior can be attributed to the hydrophobic character of PCL. Moreover, the effect of the copolymer, and hence of the swelling rate on the gentamicin release was evaluated by in vitro test.

6.7 Thermal analyses

Thermal characterization of the membranes is important to evaluate their stability at different temperatures and the main thermal transitions that occur.

6.7.1 Thermogravimetric (TG) and thermogravimetric analysis (DTG)

The mass loss was determined by the thermogravimetric method. The percentage of mass loss of the raw materials and obtained membranes are shown in Table 5. Cooper et al. (2011) found a peak of chitosan degradation around 220ºC and another at 390ºC for PCL degradation\(^{20}\). These
peaks of mass loss was observed in the derivative curves of CHI and mPEG-PCL (Figure 5). According to Niamsa et al., (2008) the peak mass loss for mPEG was found at 418°C and the degradation of PCL was observed at 360°C, coinciding with the temperature range of mass loss observed for the membranes containing mPEG-PCL.10

6.7.2 Calorimetric analysis (DSC)

The melting temperatures (Tm) and enthalpy for the raw materials and CHI/mPEG-PCL membranes are shown in Table 5. As expected, Tm values of the membranes are well above the body temperature (37°C), suggesting good thermal stability for wound dressing application. Moreover, membranes showed intermediary values of Tm between the CHI and mPEG-PCL melting points. Cardoso et al. (2014) also investigated thermal properties of hybrid membrane of chitosan/poly (ε-caprolactone) for tissue engineering.21 The authors also reported an increase on the melting temperature of the PCL-CHI hybrid membrane in comparison to PCL.21

Table 5. Results of thermal analyses of raw materials and membranes unloaded with gentamicin: thermogravimetric and calorimetric parameters.

| Sample           | Temperature Range (°C) | Mass loss (%) | Tm(°C) | ΔH(J/g) |
|------------------|------------------------|---------------|--------|---------|
| Powder mPEG-PCL  | 300 a 400              | 93            | 44.14  | 65.8    |
| Powder Chitosan  | 250 a 400              | 70            | 63.00  | 575.0   |
| CHI              | 250 a 450              | 58            | 51.81  | 552.8   |
| 1/8 mPEG-PCL/CHI | 250 a 450              | 59            | 57.32  | 516.2   |
| 1/4 mPEG-PCL/CHI | 250 a 450              | 76            | 52.12  | 358.5   |
| 1/2 mPEG-PCL/CHI | 250 a 450              | 68            | 53.49  | 458.9   |

Figure 5. Derived derivative thermogravimetric curves of polymeric raw materials and membranes unloaded with gentamicin. (pink) chitosan, (light blue) mPEG-PCL, (blue) CHI, (green) 1/8 mPEG-PCL/CHI, (black) 1/4 mPEG-PCL/CHI and (red) 1/2 mPEG-PCL/CHI.

On the other hand, the fusion enthalpy of 1/4 mPEG-PCL/CHI and 1/2 mPEG-PCL/CHI blended membranes was considerably lower than that found for CHI. Reduced enthalpy of fusion suggests lower crystallinity degree that can be explained by the presence of mPEG-PCL in the membranes. This copolymer has lower fusion enthalpy and may reduce the crystallinity of the chitosan-based membranes.22 Crystallinity degree may also influence other properties, such as the water uptake by the membranes.

7. In vitro Gentamicin Release and Antibacterial Activity

7.1 Standard curve for gentamicin

The gentamicin standard curve was obtained using the colorimetric method, which is based on the reaction of ninhydrin with the primary and secondary amines of gentamicin. The linear model between absorbance and concentration is given by the equation $y = 0.00207x + 0.19376$ and $R^2 = 0.983$, where x is the gentamicin concentration and absorbance. The $R^2$ parameter is greater than 0.95, indicating a satisfactory fit of the equation to the data obtained. Thus, this curve was used to calculate the concentrations of gentamicin released in the in vitro release assays, which were performed in two experiments.

7.2 Initial Burst Release

Firstly, the release of gentamycin was measured for 15 minutes, 30 minutes, 45 minutes and 1 hour (Figure 6) in order to simulate the initial burst release. In the first 15 minutes, greater release of gentamicin from the membranes CHI+GE and 1/8 mPEG-PCL/CHI-GE was observed due to the higher percentage of swelling of these samples. The 1/4 and 1/2 mPEG-PCL/CHI-GE membranes released less gentamicin, indicating that the blend of CHI and mPEG-PCL copolymer can be effective in delaying the burst release process.

Figure 6. In vitro Gentamicin burst release from (black) CHI-GE, (red) 1/8 mPEG-PCL/CHI, (green) 1/4 mPEG-PCL/CHI and (blue) 1/2 mPEG-PCL/CHI at 15, 30, 45 and 60 minutes.
7.3 Long-term Release

The long-term release results are shown in the Figure 7. It was only possible to observe significant decrease on the gentamicin released from the membrane 1/2 mPEG-PCL/CHI-GE and 1/8 mPEG-PCL/CHI-GE when compared to CHI-GE. Again, the membrane with the highest concentration of mPEG-PCL released the lowest concentration of gentamicin in the first day. Nevertheless, from the 2nd to the 7th day, no significant difference was observed for the gentamicin released from all tested membranes. In addition, the minimal inhibitory concentration (MIC) of gentamicin against *Staphylococcus aureus* (MIC\textsubscript{50} 0.2 µg/mL) was delivered during the analyzed period, suggesting that membranes are capable to protect the wound from *S. aureus* infection for one week\textsuperscript{23}.

7.4 Antibacterial activity

Agar diffusion technique was used to evaluate the antibacterial activity of the CHI, mPEG-PCL/CHI and gentamicin-loaded membranes. The main bacteria found in infected wounds, *E. coli* and *S. aureus*, were tested and the inhibition zone diameter was measured around the samples. It was not possible to observe zone of inhibition of the bacterial growth around the membranes without the incorporation of the antibiotic in the plates inoculated with *E. coli*. According to Dutta et al. (2009), chitosan has high antimicrobial activity against pathogenic organisms including fungi and Gram-positive and Gram-negative bacteria\textsuperscript{24}. However, this property could not be verified by this test, probably due to the low concentration of chitosan used to prepare the membranes.

On the other hand, after 24 h, all gentamicin-loaded membranes inhibited *E. coli* growth. The inhibition zone diameters ranged between 1.0 and 1.5cm (Table 6), indicating the release of the antibiotic from the membranes to the culture medium. Bajpai et al. (2016) investigated the antibacterial activity of alginate films loaded with gentamicin\textsuperscript{19}. The authors reported a zone of inhibition of 3cm in the Plate inoculated with *E. coli*, suggesting the film has fair antibacterial activity\textsuperscript{19}.

In order to confirm gentamicin release from the membranes, scanning electron micrographs of the CHI-GE and 1/2 mPEG-PCL/CHI were taken before and after the Agar Diffusion test. Figure 8 illustrates the release of gentamicin from the 1/2 mPEG-PCL/CHI membrane, indicated by the presence of empty spaces in place of the gentamicin sulfate crystals.

Regarding to the antibacterial activity of the membranes against *S. aureus* (Figure 9), similar behavior observed for *E. coli* was found, i.e. only gentamicin-loaded membranes inhibited the *S. aureus* growth. Similar diameters of inhibition were measured for both bacteria.

Sorensen and Sorensen (1993) investigated the antibacterial activity of gentamicin against four strain of *S. aureus* using Mueller-Hinton (MH) broth and surgical wound fluid (WF) as test media\textsuperscript{25}. According to the authors, during the first hour of incubation, there was a marked concentration-dependent kill rate\textsuperscript{25}. Thus, it is important to have a high concentration of gentamicin released to the media in the first hour, as we demonstrated in the Figure 6, in order to eliminate all bacterial contamination. These authors also reported that after the first hour of incubation, the kill rate was independent of the gentamicin concentration in both media (MH and WF), suggesting that the gentamicin-loaded membranes can control the bacterial growth for at least one week (Figure 7)\textsuperscript{25}.

8. Conclusions

We prepared homogeneously blended CHI and mPEG-PCL membranes loaded with gentamicin for potential medicated wound dressing application. The obtained membranes are translucent, which would facilitate the visualization of the wound, and capable of absorbing fluids that would help keeping the wound moisture. The membranes are thermally stable at physiological temperature and able to release incorporated gentamicin at physiological pH and temperature conditions. Released antibiotic from the gentamicin-loaded membranes inhibited *E. coli* and *S. aureus* growth, suggesting their use as medicated wound dressings. Nevertheless, *in vivo* studies must be performed in order to evaluate the biocompatibility and efficiency of the membranes.

### Table 6. Diameter of the zone of inhibition (cm) around gentamicin loaded membranes against *E. coli* and *S. aureus*.

| Membrane                  | *E. coli* Diameter (cm) | *S. aureus* Diameter (cm) |
|---------------------------|-------------------------|---------------------------|
| CHI-GE                    | 1.4 ± 0.1               | 1.2 ± 0.1                 |
| 1/8 mPEG-PCL/CHI-GE       | 1.1 ± 0.1               | 1.0 ± 0.1                 |
| 1/4 mPEG-PCL/CHI-GE       | 1.5 ± 0.0               | 1.2 ± 0.1                 |
| 1/2 mPEG-PCL/CHI-GE       | 1.2 ± 0.1               | 1.1 ± 0.1                 |
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Figure 8. Scanning electron micrographs of (a) CHI-GE before antibacterial test (500x); (b) CHI-GE (1000x) after antibacterial test; (c) 1/2 mPEG-PCL/CHI-GE before antibacterial test (500x) and (d) 1/2 mPEG-PCL/CHI-GE after antibacterial test (1000x).

Figure 9. Antibacterial activity of (a) CHI; (b) CHI-GE; (c) 1/4 mPEG-PCL/CHI-GE; (d) 1/4 mPEG-PCL/CHI; (e) 1/2 mPEG-PCL/CHI; (f) 1/2 mPEG-PCL/CHI-GE; (g) 1/8 mPEG-PCL/CHI and (h) 1/8 mPEG-PCL/CHI - GE against *S. aureus*. 
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