Research Article

Chitosan Associated with the Extract of Unripe Banana Peel for Potential Wound Dressing Application

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The authors report the preparation of chitosan membranes associated with the extract of unripe banana peel. Extracts were prepared by decoction, using boiling distilled water. The extraction process was repeated three times. First and third extracts served as solvent to prepare chitosan membranes by solvent evaporation technique. The chitosan membranes associated with the first and third extracts of unripe banana peel exhibit good flexibility, transparency, and uniformity. Scanning Electron Microscopy images showed dense membranes. Brownish color of membranes was observed due to the presence of tannins, which was confirmed by Infrared Spectroscopy analysis. Thermal properties of the membranes were evaluated by Differential Scanning Calorimetry (DSC), which exhibited good thermal stability at physiological temperature (37°C). At this temperature and pH 7.2, membranes were also able to absorb fluids. The hydrophilic character of the membranes was determined by contact angle measurements. The membranes demonstrated their capacity for gaseous exchange and exhibited water-vapor permeability (WVP) rates comparable to injured skin one. Finally, high number of viable dermal fibroblasts was observed by indirect cytotoxicity assay suggesting potential application of these membranes as skin wound dressing.

1. Introduction

Trauma, vascular insufficiency, and diseases, such as diabetes, hypertension, rheumatism, and inflammatory disease, may cause wounds [1–3]. They are generally classified according to the thickness in which the subcutaneous layer is compromised as acute or chronic wounds [4]. Acute wounds are superficial while chronic ones can result by destroying all the layers in skin [5]. The tissue damage stimulates a coordinated physiological response to provide hemostasis and initiate the processes of inflammation, proliferation, and remodeling [1, 5]. This healing process is well known and occurs within 3 weeks for acute wounds but usually persist for a minimum of 12 weeks since the time of injury for chronic wounds [5, 6]. The delayed healing or nonhealing of chronic wounds is due to the prolonged pathological inflammation and/or disruption of the healing process [1, 5]. Moreover, they can heal without restoring normal anatomy and function of the damage tissue [1]. Chronic wounds are considered a public health problem and lead to serious morbidity and mortality [4]. They have been considerably increasing since one-third of the adult population is currently living with diabetes and it is estimated that between 10 and 25% of patients with diabetes will develop a foot ulcer in their lifetime [7]. Wound treatments are costly and involve several medical areas. The estimated annual expenditure on wound related treatments in the USA is over one billion dollars [5]. The current standard of care for chronic wounds consists of swabbing for infection, cleaning, and dressing [4]. The choice of dressing depends on several factors, such as the cause, size, depth, location, and
degree of exudation and level of contamination of the wound [6]. An ideal dressing should protect the wound from further trauma and against contamination, ensure a moist environment by absorbing or removing excess exudate, and be flexible, biodegradable, and cost-effective [6, 8]. Currently, there are hundreds of products, devices, and dressing materials on the market to aid in wound management. Nevertheless, the most currently available wound dressings are made using chitosan, hyaluronic acid, collagen, and silicon [4].

Chitosan based dressings have mostly been investigated to due to chitosan’s favorable properties suitable for wound healing, such as intrinsic antimicrobial properties and hemostatic activity, besides being biodegradable, biocompatible, bioadherent, and nontoxic [4, 8, 9]. Chitosan is derived from chitin, which can be found as the primary structural polymer in the exoskeleton of crustacean [9]. As a byproduct of fishing industry, it is generating large notice as biomaterial because of not only its interesting biological properties, but also its low-cost when compared to collagen and hyaluronic acid [4, 9].

Banana peel is another low-cost byproduct of food industry that exhibits attractive biological properties for wound healing management [9]. It is antioxidant and antimicrobial (bacterial, fungal, and yeasts activities) and demonstrates higher concentration of phenolic compounds and mineral content [9–11]. The healing and antimicrobial properties of banana peel are associated with the presence of tannin, which are found in higher concentrations in the unripe banana peel [12, 13]. Thus, association of chitosan and unripe banana peel might be a great alternative of a low-cost biomaterial for wound dressing application.


In this present work, we investigated morphological, thermal, chemical, hydrophilicity, and barrier properties of chitosan membranes associated with the extract of unripe banana peel by Scanning Electron Microscopy (SEM). The obtained membranes were previously dried in a desiccator with anhydrous calcium chloride for a week. Samples were coated with a 16nm thickness gold in a Sputter Coater (Baltec, model SCD050) for 60 seconds at 2\(^\text{−1}\). SEM was evaluated by Scanning Electron Microscopy (SEM). The membranes morphology and thermal analysis were recorded on Thermo Scientific Nicolet iS50 F Field Emission Scanning Electron Microscope.

2.2. Methods

2.2.1. Preparation of the Extract of Unripe Banana Peel. Extraction of the active compounds of the unripe banana peels was performed by the decoction method, in order to extract the water-soluble compounds. Firstly, the fruits were disinfected by immersion in a sodium hypochlorite solution (1% v/v) for 15 minutes. Then, fruits were washed with water and dried with absorbent paper. Pulps were discarded and the peels were cut and weighted. The disinfected peels (250 g) were added to 1 L of boiling distilled water for two hours, using a heating pad. The product was stored under refrigeration for 24 hours and then filtered to obtain the first extract (liquid portion) that was reserved for membrane preparation purpose and the extracted peels (solid portion), which was reused. In order to maximize the extraction of metabolites of the peels, the decoction process was repeated twice using the same extracted peels and the third extract (liquid portion of the third filtration) was also used to prepare the membranes.

2.2.2. Preparation of Membranes. The membranes were obtained by solvent evaporation technique. Three solutions were prepared containing 2% (w/v) of chitosan dissolved in three different solvents: distilled water (CB0), first extract (CB1), and third extract (CB3). The pH of all solvents was previously adjusted to 6.0 using acetic acid 1 M in order to promote the complete dissolution of chitosan. The solutions were then cast in plastic molds of 14 cm diameter and dried in an oven at 60\(^\circ\)C for 4 hours.

2.2.3. Morphological Analysis. The membranes morphology was evaluated by Scanning Electron Microscopy (SEM). The obtained membranes were previously dried in a desiccator with anhydrous calcium chloride for a week. Samples were coated with a 16nm thickness gold in a Sputter Coater (Bal-Tec, model SCD050) for 60 seconds at 2\times10^{-2} \text{ Pa} and 24\(^\circ\)C. Morphological analysis was performed using JEOL JSM-7500 F Field Emission Scanning Electron Microscope.

2.2.4. Infrared Spectroscopy (FTIR). The FTIR technique was used to characterize the functional groups present in the CB1, CB3, and unripe banana peel samples. FTIR spectra were recorded on Thermo Scientific Nicolet iS50 Spectrum ATR Pike Technologies, in the range between 4000 cm\(^{-1}\) and 525 cm\(^{-1}\).

2.2.5. Thermal Analysis. Differential Scanning Calorimetry (DSC) analysis was performed on the Calorimeter Q100 (TA Instruments) from 0\(^\circ\)C to 150\(^\circ\)C at a heating rate of 10\(^\circ\)C/min, under nitrogen atmosphere. Average of duplicates was used to determine the melting temperature and the enthalpy of fusion of each membrane. Crystallinity degree (Xc) of membranes was calculated by (1) [16]:

\[ X_c (\%) = \frac{\Delta H_m}{\Delta H_m^0} \times 100\%, \]  
(1)
where $X_c$ is crystallinity degree, $\Delta H_m$ is enthalpy of fusion of membrane, and $\Delta H^0_m$ is enthalpy of fusion of pure crystalline chitosan.

2.2.6. Moisture Content. The percentage of moisture of the membranes was determined by the gravimetric method. The membranes were weighted before (initial mass) and after (final mass) being placed in an oven with air circulation at 105°C for 24 hours. The percentage of moisture was calculated by (2). The average of triplicates was used to calculate the moisture content of the samples:

$$M(\%) = \left(\frac{m_i - m_f}{m_i}\right) \times 100\%, \quad (2)$$

where $M(\%)$ is percentage of moisture, $m_i$ is initial mass, and $m_f$ is final mass.

2.2.7. Contact Angle. Contact angle measurements were performed to verify the affinity of the membranes with water. The test was carried out in a contact angle System OCA-DataPhysics Software SCA20 instrument using deionized water at 24°C. Contact angle measurement was performed after a static time of 30 s. A tangent method and “Sessile Drop Fitting” in the software were applied to fit the drop shape and calculate the contact angle. For each membrane, three samples were used and six data points were taken for mean and standard deviation calculation.

2.2.8. Swelling Behavior. Samples were cut into pieces of 1 cm² and placed in a desiccator with silica for 24 h and then weighed to determine dry mass. They were immersed in phosphate buffered solution (PBS) pH 7.2 at 37°C for 24 h and 72 hours in order to simulate physiological condition. The soaked samples were then blotted with filter paper to remove nonabsorbed surface water and then weighed again to determine the wet mass. The percentage of swollen was calculated by (3). The average of three replicates was used.

$$S(\%) = \left(\frac{m_w - m_d}{m_w}\right) \times 100\%, \quad (3)$$

where $S(\%)$ is percentage of swelling, $m_w$ is wet mass, and $m_d$ is dry mass.

2.2.9. Water-Vapor Permeability. The coefficient of permeability of water-vapor was determined gravimetrically according to the ASTM E96-95 [17]. Briefly, the thickness of the membranes was measured using a Digimatic Micrometer. Then, samples were cut to a size to cover the permeation cell orifice of 4.5 cm diameter, which resulted in a permeation area of 15.21 cm², and fasten to the cells containing anhydrous calcium chloride. The cells were placed in a hermetically sealed container containing a supersaturated sodium chloride solution in order to maintain 75% of relative humidity at 25°C. After two hours, cells were weighed and this procedure was repeated at regular intervals of time, for 72 hours. Water-vapor permeability coefficient was calculated by (4) and average of triplicates was used.

$$WVP = \frac{(G \cdot \delta)}{(A \cdot \Delta P_w)} \quad , \quad (4)$$

where $WVP$ is water-vapor permeability coefficient (g mm/m²-day-kPa), $G$ is mass gain per day (g/day), $\delta$ is average thickness of membranes (mm), $A$ is exposed area to mass transfer (m²), and $\Delta P_w$ is partial pressure difference across the film (kPa).

2.2.10. In Vitro Cytotoxicity. Indirect cytotoxicity was performed to evaluate the potential deleterious effects of substances released in the culture medium by the membranes. Thus, 10 mg of each sample was separately placed in 10 mL of high glucose DMEM medium and incubated at 37°C in order to potentially release deleterious substances for 24 hours. HDF cells (ATCC® PCS-201-010™), primary dermal fibroblasts derived from skin, were grown in high glucose DMEM medium (containing 10% heat inactivated fetal bovine serum, L-glutamine, NaHCO₃, pyridoxine-HCl, 4.5 g/L glucose, and 1% penicillin-streptomycin solution) as monolayer in a 75 cm² cell culture flasks in the 37°C incubator under CO₂. When up to 90% confluence, cells were harvested using 0.05% trypsin-EDTA, washed thrice with PBS (1200 rpm for 4 minutes), and resuspended in cell media. Then, cells were seeded in a 96-well plate at the density of 2 × 10⁴ cells/200 µL/well using the same medium and incubated overnight at 37°C in the presence of 5% CO₂. After overnight growth, the medium was removed from each well and replaced with 180 µL of fresh medium. Then, 20 µL of the previously prepared sample was added (triplicates). For the positive control wells 200 µL of fresh medium was added and 200 µL of D1 water was added to the negative control wells. The plate was placed back to the incubator and, after 24 h, the medium was removed from all the wells and replaced with 200 µL of fresh medium. Then, 20 µL of Alamar blue reagent solution (2 mL MTS + 100 µL PMS) was added to each of the wells. The plate was incubated for 3 hours and the fluorescence was read at 590 nm emission (550 nm of excitation). Average of the triplicates was taken to calculate cells viability.

3. Results and Discussion

3.1. Extract of Unripe Banana Peels. The extraction of water-soluble compounds present in the unripe banana peel was performed by the decocction method, which consisted of boiling the peel in distilled water. After filtering to remove the extracted peels, a brownish solution was obtained as first extract. The browning of the solution can be attributed to the enzymatic oxidation of the tannins, which are present in the peels [18]. The extraction procedure was repeated three times and a decrease of the brownish color of the extracts was observed when compared to the first extract. Both first and third extracts were used to prepare the membranes.
3.2. Preparation of the Membranes. The membranes were prepared by the solvent evaporation technique, by casting a solution in a circular mold. After drying, samples were easily unmolded. Flexible and uniform membranes were obtained and are shown in Figure 1. As expected, CB0 was transparent, while CB1 and CB3 were translucent due to the clear brown color. Kamel et al. (2016) also reported the brownish color of chitosan/banana peel powder nanocomposites after the filler addition [11]. Moreover, these authors reported an increase of the roughness of the nanocomposites due to the presence of the banana peel powder [11]. This increase of roughness was not observed for CB1 and CB3 membranes because of the different extraction method (decoction) used to obtain the extract of banana peel.

3.3. Morphological Analysis. SEM images of the membranes are shown in Figure 2. It can be observed that chitosan membrane (CB0) is dense, continuous, and homogeneous, while discontinuities and precipitates are found in CB1 and CB3. This may be attributed to the presence of the compounds extracted from the banana peel that structurally modified the chitosan matrix.

3.4. FTIR Analysis. Infrared Spectroscopy analysis was carried out to identify the functional groups found in the compounds extracted from the unripe banana peel. Figure 3 shows the spectra obtained for CB1, CB3, and unripe banana peel (powder). The spectra were collected in the range 4000–500 cm\(^{-1}\). A broad band observed in all spectra from 3500 to 3000 cm\(^{-1}\) can be attributed to the sum of the –OH stretching, which is characteristic of polyphenolic extracts [19]. In addition, CH, CH\(_2\), and CH\(_3\) stretching vibrations derived from carbohydrates can be observed in the region of 2960–2925 cm\(^{-1}\) [20]. Nevertheless, these regions do not show conclusive evidences of the presence of tannins in unripe banana peel and membranes. The presence of phenols/tannins in CB1, CB3, and unripe banana peel spectra can be suggested by the presence of bands at 1600 cm\(^{-1}\) as characteristic bands of the aromatic skeleton chain, which can be attributed to the C=C bond of the ring. The stretching of the C=C-C aromatic bond appears in the region of 1618–1449 cm\(^{-1}\) [19–21]. Kame et al. (2016) also reported a band at 1630 cm\(^{-1}\) in the banana powder spectrum [11]. The bands around 770 cm\(^{-1}\) found in CB1 and banana peel spectra can also indicate the presence of aromatic rings. Moreover, bands between 1320 and 1340 cm\(^{-1}\) can be attributed to angular deformations C-O-H of phenols, derived from the pirae ring of tannins [20]. The stretching in 1340 cm\(^{-1}\) is due to the C-O of typical of flavonoid C-rings [21]. Although FTIR analysis was not conclusive,
Table 1: Thermal properties of raw materials and membranes: melting temperature and enthalpy of fusion of chitosan, unripe banana peel powder, and membranes obtained by Differential Scanning Calorimetry. Crystallinity degree ($X_c$) of membranes calculated by (1).

<table>
<thead>
<tr>
<th>Sample/property</th>
<th>Chitosan</th>
<th>Banana peel</th>
<th>CB0</th>
<th>CB1</th>
<th>CB3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_m$ (°C)</td>
<td>85.08</td>
<td>77.74</td>
<td>82.21</td>
<td>82.56</td>
<td>82.27</td>
</tr>
<tr>
<td>$\Delta H_m$ (J/g)</td>
<td>473.0</td>
<td>344.7</td>
<td>493.6</td>
<td>383.7</td>
<td>490.8</td>
</tr>
<tr>
<td>$X_c$ (%)</td>
<td>—</td>
<td>—</td>
<td>52.18</td>
<td>40.56</td>
<td>51.88</td>
</tr>
</tbody>
</table>

Figure 3: FTIR spectra of unripe banana peel and chitosan membranes prepared with the extracts of banana peel: (dashed) banana peel; (grey) chitosan membrane prepared with the first extract, CB1; and (black) chitosan membrane prepared with the third extract, CB3. Banana peel data is shown in the secondary transmittance intensity axis.

3.5. Thermal Analysis. Differential scanning calorimetry (DSC) results are shown in Table 1. The melting points ($T_m$) found for the membranes were well above physiological temperature (37°C), suggesting good thermal stability. Moreover, similar melting temperatures were observed for CB0, CB1, and CB3, suggesting that the extracted compounds did not influence melting temperature. On the other hand, the fusion enthalpy of CB1 (383.7 J/g) was considerably lower than that found for CB0 and CB3 (493.6 J/g and 490.8 J/g, resp.). The reduced CB1 enthalpy of fusion indicates a lower crystallinity degree (Table 1) that can be explained by the presence of high concentration of extracted compounds (Figure 2). These compounds may act as plasticizers/impurities and make polymeric chains organization more difficult, reducing the crystallinity degree [16, 23]. Crystallinity is an important parameter and may considerably affect mechanical and physical properties of the membranes.

3.6. Contact Angle. The experiments for contact angle measurements were performed in order to evaluate water affinity (hydrophilicity), an important parameter for wound dressings [4, 6, 8]. The contact angle indicates interfacial interaction between a solid and a liquid. Cohesive force of the liquid tends to form a spherical drop, while adhesive force of the solid tends to spread the water-drop [24]. Contact angle lower than 90° indicates hydrophilic character of the solid whereas contact angle higher than 90° is found for hydrophobic materials [24]. Time is also a parameter to be considered since the angle of contact may vary due to solid-liquid interactions during the measurements [24]. Surface roughness also plays an important role in the contact angle [24].

Figure 4 shows contact angle measurements for CB0 at different times. As expected, the contact angle was decreasing with time, which can be attributed to water-membrane interaction and swelling. Table 2 shows the contact angle measurements for CB0, CB1, and CB3 at 30 s. All samples showed hydrophilic character (contact angle lower than 90°). Similar angles were measured for CB0 and CB3, while lower contact angle value was observed for CB1. This can be attributed to the presence of high concentration of extracted...
Table 2: Thickness, moisture content, contact angle, and water-vapor permeability coefficients of the membranes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Thickness (μm)</th>
<th>Percentage of moisture (%)</th>
<th>Contact angle (°)</th>
<th>WVP coefficient (g·mm/m²·day·kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB0</td>
<td>88.47 ± 6.85</td>
<td>13.26 ± 2.44</td>
<td>68.21 ± 1.42</td>
<td>22.07 ± 1.76</td>
</tr>
<tr>
<td>CB1</td>
<td>69.27 ± 8.01</td>
<td>13.84 ± 2.07</td>
<td>61.72 ± 0.28</td>
<td>14.08 ± 1.04</td>
</tr>
<tr>
<td>CB3</td>
<td>58.06 ± 6.20</td>
<td>12.31 ± 3.93</td>
<td>66.40 ± 1.45</td>
<td>15.75 ± 0.50</td>
</tr>
</tbody>
</table>

Figure 4: Contact angle images took at different times for the chitosan membrane, CB0. Total time: 2 minutes and 20 seconds.
compounds that modified the roughness of this membrane as it can be seen in Figure 2.

Contact angle and roughness play an important role on cell adhesion since cells attachment in hydrophilic and rough surfaces is favorable. Thus, all membranes demonstrate adequate properties for potential cellular attachment in the wound site.

3.7. Moisture Content. Oven drying at standardized temperature (105°C) is one of the methods to determine water content of a sample. The percentages of moisture of CB0, CB1, and CB3 are shown in Table 2. No significant difference was observed in the moisture content of the membranes, suggesting that the presence of extracted compounds did not influence their water content.

3.8. Swelling Behavior. On the other hand, the presence of the extract of unripe banana peel affected the water uptake of the membranes. It is important to mention that an ideal wound dressing should be able to keep the wound moist and absorb fluids and exudates [4, 6, 8]. The swelling ratios were calculated after 24 and 72 hours at physiological condition and the results are shown in Figure 5. All samples reached the swelling equilibrium at up to 24 hours since no significant changes in the swelling ratios were observed at 72 hours. Nevertheless, for both periods studied, considerable differences on swelling were observed. The water uptake was inversely proportional to the extract concentration. Thus, CB1 was swollen less due to the high concentration of extracted compounds. Similar behavior was observed for enthalpy of fusion, suggesting that these compounds interacted with the polymeric matrix, reducing crystallinity and water uptake due to chitosan-OH interactions. Kamel et al. (2016) also reported a decrease in the swelling of chitosan/banana peel powder nanocomposites, which may reduce their WVP [11]. Therefore, a wound dressing should have a WVP rate in the range of 8.33 to 10.42 mg/cm²·h [25].

3.9. Water-Vapor Permeability (WVP). In order to investigate the effects of the extract of unripe banana peels on the barrier properties of chitosan membranes, water-vapor permeability experiment was carried out for 3 days according to the ASTM E95-96 [17]. WVP coefficients of CB0, CB1, and CB3 are shown in Table 2. The presence of extracted compounds on CB1 and CB3 reduced the water-vapor permeability coefficients of these membranes when compared to CB0. This may be due to the structural changes on the polymeric matrix caused by the presence of the extract (Figure 2). In addition, WVP of hydrophilic films may also be affected by swelling, which can reduce the resistance to mass transfer as observed for CB0. Kamel et al. (2016) also reported an increase in water resistance of chitosan/banana peel powder nanocomposites, which may reduce their WVP [11].

4. Conclusion

The preparation of chitosan membranes associated with the extracts of unripe banana peel was successfully demonstrated. The presence of tannins in the membranes was
confirmed by the color change, SEM, and FTIR analyses. The membranes showed good thermal stability at physiological temperature and the CBI degree of crystallinity was reduced by the presence of the extracted compounds. Contact angle measurements (90°) indicated hydrophilic character of the membranes, which permits cellular attachment in the wound site. All membranes were able to absorb fluids at physiological conditions and permit gaseous exchange, demonstrating WVP rates in the desired range for skin substitutes. In conclusion, indirect cytotoxicity against dermal fibroblasts was not observed for the membranes associated with the unripe banana peel extracts, suggesting their potential use as skin wound dressing.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References


