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Membranes from latex with propolis for biomedical applications

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ABSTRACT

Recent findings have confirmed the suitability of NR derivated systems for regenerative medicine, in which the angiogenic properties of the NR latex are exploited, and also in drug delivery systems. This work aimed to prepare and characterize a system formed by NR matrix and propolis extract, which was incorporated into the NR matrix. We have investigated the surface morphology, wettability, and antimicrobial activity as a function of the propolis incorporation. We have demonstrated the possibility of obtaining membranes flexible, translucent and non-adhesive. This kind of system can be more effective and less toxic for clinical applications such a burn dressing.

1. Introduction

Due to the constant pursuit for the use of materials from renewable sources, there is a growing interest in the use of materials derived from natural rubber latex for many applications, including biomedical ones [1]. The suitability of natural rubber (NR) for regenerative medicine is evaluated from the viewpoint of angiogenic properties [2–4]. In addition, NR has high flexibility and ability for incorporation of drugs into its volume, which can be interesting for use in systems of drug delivery.

We are interested in the investigation of a material which combines the angiogenic properties of NR with the antimicrobial activity of natural products. This kind of system can be more effective and less toxic for clinical applications such a burn dressing. Propolis is a resinous substance collected and transformed by bees from parts of plants [5]. It has flavonoids as the main active ingredient [6,7]. Flavonois in propolis can be responsible for biological activity such as antibacterial, antiviral, fungicidal and immunestimulating [8]. Of special importance is the antimicrobial property of propolis which was found be effective against *Candida albicans* and several others microorganisms [9]. *C. albicans* is the major fungus isolated in burn wounds. Isolation of fungi increases over time in hospitalized patients and is associated with prolonged use of antimicrobials and extensive burns not covered by grafts [10].

In this work, we report on the characterization of the system composed of propolis and natural rubber. The surface morphology, wettability, and antimicrobial activity as a function of the incorporation of propolis into the NR matrix were investigated.

2. Materials and methods

Cis-1,4-polyisopreno (natural rubber – NR) was extracted from different rubber trees of the RRIM 600 clone (*Hevea brasiliensis*) in Nova Xavantina, State of Mato Grosso, Brazil. It was stabilized (pH 10.5) using a NH_4OH solution made with 4.7 mL of NH_4OH in 100 mL of latex [1]. In Brazil, besides wide variety of flora there are also several bees species among which stand out those belonging to Meliponinae subfamily, known as indigenous stingless bees. In this group are found the bees of the *Scaptotrigona genus*. Propolis samples were collected directly from beehives of *Scaptotrigona* sp. in a meliponary of Barra do Garças, State of Mato Grosso, Brazil. Propolis had resinous aspect, balsamic, with dark brown color, typical vegetable fragrance, and free of solid impurities [11] which exhibits antimicrobial, immunemodulatory and anti-inflammatory activity. The preparation of crude extract from propolis was basically the same as described by Possamai et al. [11]. The extract was solubilized in ammonia solution (pH 11.3). NR membranes were prepared by casting the liquid solution of stabilized latex in Petri dishes, at 65 °C for 12 h. We have prepared pure NR membranes and membranes with propolis in three different proportions: 10%, 20% and 30%. The final film thickness was about 1 mm.

The studies of the surface morphology of the films were performed using an atomic force microscope (Nanosurf EasyScan II) in intermittent mode (512 × 512 pixel). The images were acquired in scanning windows of 10 μm × 10μm. The roughnesses of membranes were determined using the Nanosurf Instruments software and the spectrophotometer Perkin-Elmer Spectrum 100 FT-IR was used to obtain spectra of the propolis extract, NR and NR+propolis membranes.

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The contact angle was measured with a homemade instrument in ambient conditions. Deionized water droplets (volume of 3 μL) were gently placed onto the film surfaces and the average values measured at six different locations of each sample were taken.

Evaluation of antifungal activity was performed covering half the surface of the cultures of *Candida albicans* (ATCC 14053) seeded onto a Sabouraud agar with NR membranes. The microorganism was seeded in Petri dishes at a concentration of 1.5×10^{8} cells/ml, and the membranes were deposited on the fungal culture after 5 min of seeding. The Petri dishes were incubated at 37 °C for 24 h and after that, the membranes were aseptically removed and observed the growth of fungus occurred on the plate.

3. Results and discussion

Fig. 1 shows the FT-IR spectra of NR, NR+propolis 10% and propolis extract, respectively.

When two materials are mixed together, physical blends versus chemical interactions are reflected by shifts in the characteristic spectra peaks [12]. The spectrum of NR membrane shows the characteristic bands of cis 1,4-polyisoprene: 835, 1127, 1375, 1448, 1663, 2912, 2926 and 2961 cm^{-1} [13]. For propolis extract the bands are observed in the range of 1000–1800 cm^{-1} [14]. In special, the 1250 cm^{-1} band arise from the C–O group of polyols [15] such as hydroxyflavonoids [7,15]. As shown in Fig. 1, differences in NR+propolis membrane were found in FT-IR spectra in the region 1325–1135 cm^{-1} assigned to CH_2 twisting of cis 1,4-polyisoprene [15]. The results of FT-IR suggested that the propolis is incorporated by NR membrane. It is important to note that the incorporation of propolis occurs for the all three concentrations studied and the variations of intensity or band shift have similar behavior.

The morphology and wettability of the membranes are altered by the incorporation of propolis. In order to perform the wettability and microscopy experiments, the propolis extract was deposited on a glass slices. Fig. 2 shows AFM images in a scan window of 10 μm × 10 μm from pure and mixed propolis and NR membrane. For films of pure propolis, the surface is flat and smooth. The NR membrane presents pores on its surface. The amount and dimension of these pores diminishes with the incorporation of propolis in the membrane. The image with 30% of propolis showed small nodules. The roughness of the membranes is also affected by the incorporation of propolis. The results are shown in Table 1. The roughness decreases probably because the propolis fills the pores of the membrane.

The first image in Fig. 2 shows the optical image of a drop of water on the NR membrane. This experiment was carried out in order to estimate the wetting contact angle, θ_c, which is related to the spreading ability of a liquid on a surface. It should be noted that wettability increases as the contact angle decreases. From Table 1 it can be seen that the membranes are become more hydrophilic with increasing concentration of propolis. This result is best viewed in Fig. 3. It is interesting to note that the pure propolis is completely hydrophilic ($\theta_c=0°$).

Microbiological tests showed that contact with the pure NR membrane or mixed with propolis extract inhibit the growth of colonies of *C. albicans*, as showed in Fig. 4. It is possible to note that the membrane becomes completely opaque during the incubation period.

4. Conclusions

In this study, we have demonstrated the possibility of obtaining membranes highly flexible, translucent and non-adhesive from the mixture of propolis and natural rubber. Tests showed that the

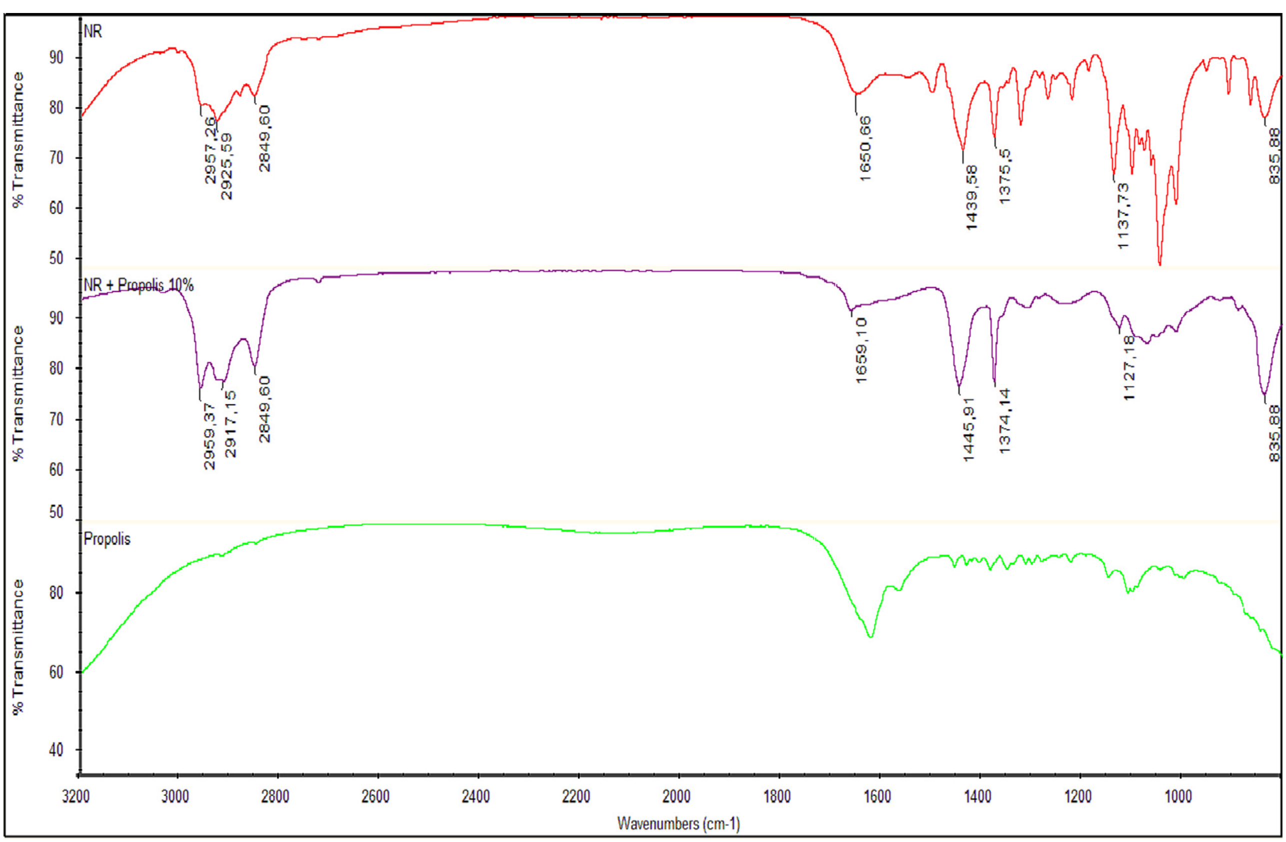

Fig. 1. FT-IR spectra of NR, NR+propolis 10% and propolis extract, from top to bottom.