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MEMBRANES OF BIOPOLYMER BLENDS FOR WOUND HEALING APPLICATIONS

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Abstract. Silk fibroin (SF) and sodium alginate (SA) offer promising properties for application as biomaterials, especially as artificial skin and wound dressing. By blending SF and SA, it is possible to conjugate their properties, producing a material with suitable mechanical properties and good efficiency for wound healing applications. Degree of swelling in water, water contact angle, water vapor transmission, tensile strength and in vitro cytotoxicity tests were performed on blend membranes and in the pure component membranes. The blend membrane presented intermediate values for all tests when compared to pure SF and pure SA. SA membrane presented the highest degree of swelling (310%), while SF presented 59.5% of swelling. The water vapor transmission was also higher for SA membrane. SA presented the lowest water contact angle. It is known that the cellular adhesion and growth is enhanced in hydrophilic surfaces. The tensile strength of SA, blend and SF membranes were 95.70 MPa, 73.00 MPa and 52.62 MPa, respectively. All membranes presented good cell viability (90-100%), indicated by in vitro cytotoxicity test. SF presented the highest cell viability in all extract concentrations analyzed, similar to the negative control. The blend membrane obtained in this study is a non-cytotoxic material, suitable for applications in the biomaterial field. In addition, the presence of SF and SA in the blend is essential for the formation of a material with good physical properties and cell viability.

Keywords: *Silk fibroin, Sodium alginate, Cytotoxicity test, Wound healing*

1. INTRODUCTION

The use of polymers from renewable sources has been widely studied in the biomedical field as much as in the packaging industries. Natural polymers are, in general, biodegradable, biocompatible and can be obtained with low costs. However, in some cases, the biopolymers present undesired properties, as high degradation rate or unsatisfactory mechanical properties. A way to improve the properties of these materials is combining them with other polymers, in order to conjugate its properties.

Silk fibroin (SF) and sodium alginate (SA) are natural polymers obtained from silkworm cocoon and brown algae, respectively. Although the potential of application in the biomedical area (Vepari and Kaplan; Rinaudo et al., 2008), few studies of blend membranes of fibroin and alginate are found in literature.

SF has been studied in recent years for application in wound healing and tissue engineering due to its affinity with cell of bones, cartilages and ligaments tissues (Altman et al., 2003; Vepari and Kaplan, 2007). It possesses good water vapor and oxygen permeability, blood compatibility and improves the collagen formation and fibroblasts proliferation (Kweon et al., 2008). SA possess haemostatic action and is capable to keep an environment with adequate humidity for healing of wounds and burnings (Roh et al., 2006), it is also commercialized as dressing for wounds, as the AlgiDERM® and Sorbsan® (Rinaudo, 2006).

The aim of this study was to form blend membranes of SF and SA, in order to conjugate the properties of those biopolymers, obtaining a material with good physical properties and cell viability, aiming biomedical applications, especially in wound healing.

2. MATERIALS AND METHODS

2.1 Blend preparation

Silk cocoons of *Bombyx mori* silkworm (Bratac-Brazil) were degummed three times by soaking in 1 g/L of Na_2CO_3 solution at 85 °C for 30 min, to remove the sericin of the cocoons, and then rinsing in distilled water. The fibroin fibers were dried at room temperature and dissolved in a ternary solvent of $CaCl:CH_3CH_2OH:H_2O$ (1:2:8 molar) at 85°C to a concentration of 5 wt % (Um et al., 2001). The SF salt solution was dialyzed in distilled water for three days at *ca.* 10 ºC, to remove the salts of the solvent. The dialysis bath water was replaced every 24 h. The final SF aqueous solution was 2.5 wt %, and was diluted in distilled water to 2 wt %.

Sodium alginate (Vetec-Brazil) was dissolved in 0.1 M NaOH to a 2 wt % SA concentration. Glycerin was added in SA solution to act as plasticizer.

SF and SA were blended at blending ratios of 100/0, 75/25, 50/50, 25/75 and 0/100 wt %, respectively. Therefore, a membrane without macrophase separation could just be obtained in ratios of SF/SA 100/0, 25/75 and 0/100 wt %. After being stirred for 15 minutes at room temperature, the blends were casting at Petri dishes and dried at room temperature for solvent evaporation.

2.2 Characterization

Degree of swelling. The degree of swelling was determined gravimetrically. Pieces of 2.5 cm in diameter of SF, SA and SF/SA blend were weighed in the dry state (m_i), after being equilibrated at 50 % relative humidity for 48 hours. The samples were immediately introduced in 100 mL of distilled water as swelling medium and weighed at predetermined times, until reach constant weigh (m_f). The degree of swelling was calculated as follows:

$$Swelling\ (\%) = \frac{(m_f - m_i)}{m_i} \cdot 100 \quad (1)$$

Water contact angle. The contact angle, using distilled water droplet was measured to determine surface hydrophilicity. The water droplet was applied using a syringe and the static contact angle was measured using a goniometer (Tantec).

Water vapor transmission. The water vapor transmission was determined according to ASTM E 96M (2005). Briefly, the membranes were placed in a recipient containing anidrous calcium chloride as desiccant and this recipient was placed on a dessicator containing saturated aqueous NaCl solution, maintaining the ambient with 75% of relative humidity. The water vapor transmission through the membranes was determined gravimetrically by weighting the recipient each 12 hours, for a period of 5 days. The rate of water vapor transmission was determined from the slope of the weight change *versus* time line. Equation 2 presents the calculus of water vapor transmission.

$$WVT = \frac{\left(G/t\right)}{A} \quad (2)$$

Where G/t is the mass variation rate (slope of the straight line), in g/day, and A is the test area, in m².

Mechanical test. The mechanical tensile strength test was accomplished in a texture analyzer TA.XT2 (Stable Microsystems SMD), according to ASTM D882 (2002). Briefly, the membranes were cut into 7 x 2.5 cm pieces and placed in environment with 50% of relative humidity for 48 h. The thickness of the membranes was measured using digital micrometer (MDC-25S, Mitutoyo). The pieces were subjected to a tensile with speed of 10 mm/s and with initial distance of 50 mm.

Cytotoxicity test. *In vitro* biocompatibility was performed according to ISO 10993-5 (1999) using Chinese hamster ovary cell line (CHO-k1). The cells were maintained in RPMI medium supplemented with antibiotics and antimicotic (100 units/mL penicillin, 100 µg/mL streptomycin and 0.025 µg/mL amphotericin), 2 mM glutamine, and 10% calf serum, at 37 ºC in a humidified 5% CO_2 atmosphere until they reached confluence. For subculturing and for experiments, cells were harvested using 0.05% trypsin and 0.02% EDTA in phosphate-buffered saline at pH 7.4. The membranes were sterilized by UV irradiation for 30 minutes on each side of the membrane. The membranes were immersed in RPMI medium in a proportion of 1 cm²/mL, at 37 °C for 48 h. Cytotoxicity test was performed in a 96 wells microplates seeded with 3000 cells per well and extracts dilutions from 100 to 6.25%. The microplates were incubated for 72 h at 37 °C in a humidified 5% CO_2 atmosphere. The cell viability was measured by adding MTS/PMS (20:1) solution and incubated for more 2 h. The microplates were analyzed in a spectrophotometer ELISA at 490 nm. The test was compared with a negative control of high-density-polyethylene (HDPE) and a positive control of phenol 0.5 vol % in culture medium. The Cytotoxicity Index for 50% of cell viability (CI_{50}) was graphically estimated.

3. RESULTS

3.1 Degree of swelling

The results for the degree of swelling of the membranes are shown in Figure 1. Each measure was done in triplicate.

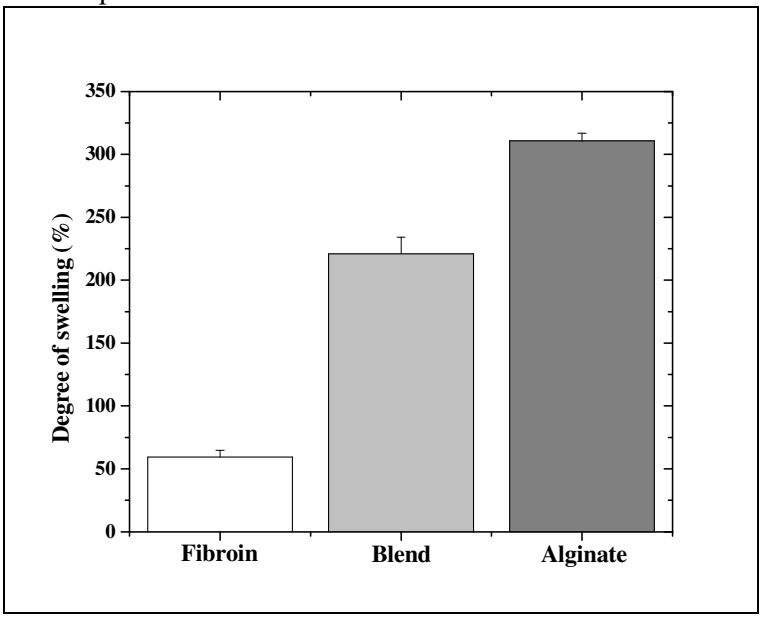

Figure 1: Degree of swelling for fibroin, blend and alginate membranes.

It is verified that the SF membrane presents low degree of swelling when compared to SA membrane and that the blend membrane presented intermediate behavior between them.

The degree of swelling is directly related with the mobility of the polymeric chains. The molecules of the polymer retain water, increasing its mobility. It is known that the water is an excellent plasticizer for some polymers, and this is related to the capacity of water retention. The more water the polymer is capable to retain (higher degree of swelling), the higher the plasticizer effect of the water (Olivas and Barbosa-Canovas, 2008).

SA is a highly hydrophilic biopolymer due to carboxyl and hydroxyl groups presented in its structure, that confer a significant swelling when SA membrane is immersed in water (Kalyani, Smitha et al., 2008). On the other hand, SF possesses hydrophobic amino acids in its structure and, after the treatment carried out with the H_2SO_4, it is on its highly organized form, called β-sheet. The β-sheet structure does not allow the swelling and water plasticization.

3.2 Water contact angle

The water contact angle test provides information about the hydrophilicity of the material. More hydrophilic surfaces present lower values of the contact angle (Cai et al., 2002). The hydrophilicity is one of the most important factors that affect the cytocompatibility of biomaterials. The cell adhesion and growth are directly influenced by the wettability of the surfaces, once a major part of the cells prefers to anchor in hydrophilic surfaces (Esposito et al., 2007).

Table 1: Water contact angle values for fibroin, blend and alginate membranes.

	Water contact angle* (θ)
Fibroin	81º ± 3º
Blend	72º ± 2º
Alginate	59º ± 3º

* Average ± standard deviation (n=10).

It can be verified in Table 1 that the SF membrane presents the highest value for the water contact angle. This value is in accordance with the literature for SF membranes treated by immersion in methanol, 81º ± 2º (Jin et al., 2004). For SA membrane, the water contact angle value is also in accordance with the literature (Xie et al., 2010). SA presents higher hydrophilicity when compared with SF that indicates a better cell attachment. For the water contact angle, the blend membrane presents antagonic behavior, with a higher influence of SF component.

3.3 Water vapor transmission

Figure 2 present the results for water vapor transmission. Each measure was done in triplicate.

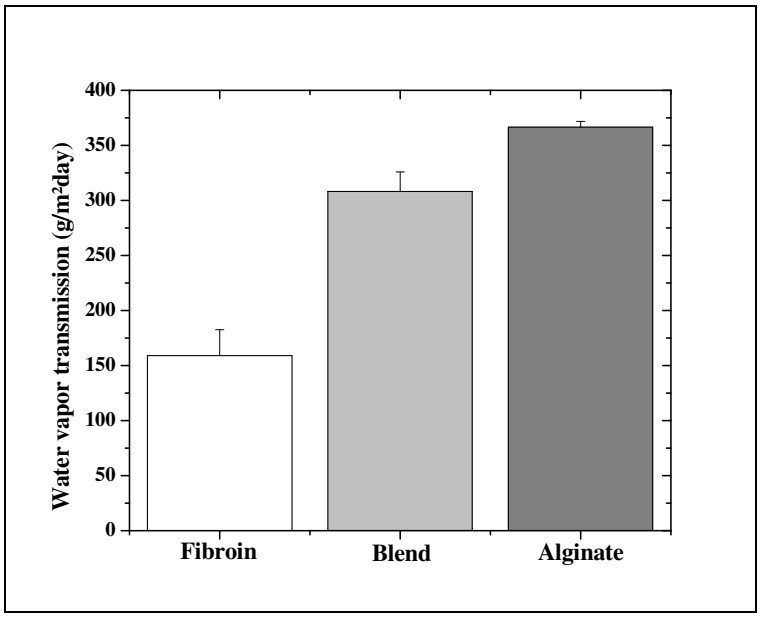

Figure 2: Water vapor transmission of fibroin, blend and alginate membranes.

SA membrane presented the highest value of water vapor transmission, in accordance with the literature (Remunanlopez and Bodmeier, 1997). The transmission can be controlled by altering the extent of alginate reticulation. It is also known that the addition of plasticizers decrease the intermolecular bond forces between the polymers chains, increasing its permeability (Olivas and Barbosa-Canovas, 2008). It is important to remember that SA and blend membranes possess glycerin on its structure, which could cause an increase in the values of WVT.

The WVT is an important factor to be observed in membranes for wound dressings applications. The dressings must prevent the high evaporation of corporeal fluids and at the same time they must keep the environment of the wound with adequate humidity. The values of water vapor transmission presented in this study are in accordance with the values of commercial dressings (of 76 the 9360 g/m².day) (Wu et al., 1995), what indicate its potential for use as dressings.

3.4 Mechanical test

Table 2 shows the results of the mechanical test, concerning about tensile strength and elongation for the studied membranes.

Table 2: Mechanical test results for fibroin, blend and alginate membranes.

	Tensile strength* (MPa)	Elongation* (%)
Fibroin	52.62 ± 10.79	1.81 ±0.61
Blend	73.00 ± 11.70	3.46 ± 0.92
Alginate	95.70 ± 6.14	7.73 ± 2.23

* Average ± standard deviation (n=8).

From the results of the mechanical test it is verified that the incorporation of SA in SF improved its tensile strength, due to the high resistance of alginate. Moreover, the elongation of the membranes had a significant increase, when compared with the membrane of pure SF.

It was verified that it is possible to improve the mechanical properties of SF membranes through the incorporation of SA and glycerin, obtaining biopolymeric blends. The glycerin addition has an important paper in the results of the mechanical test, therefore it allow the formation of a blend membrane more malleable, facilitating its manipulation.

3.5 Cytotoxicity test

Figure 3 and 4 present the cytotoxicity tests results for all extract concentrations and a histogram for 100% of extract, respectively.

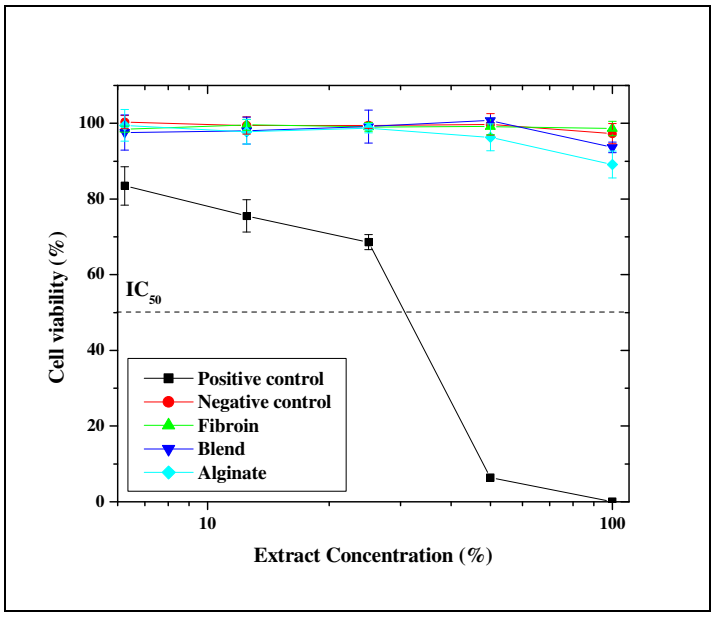

Figure 3: Cell viability of fibroin, blend and alginate membranes.

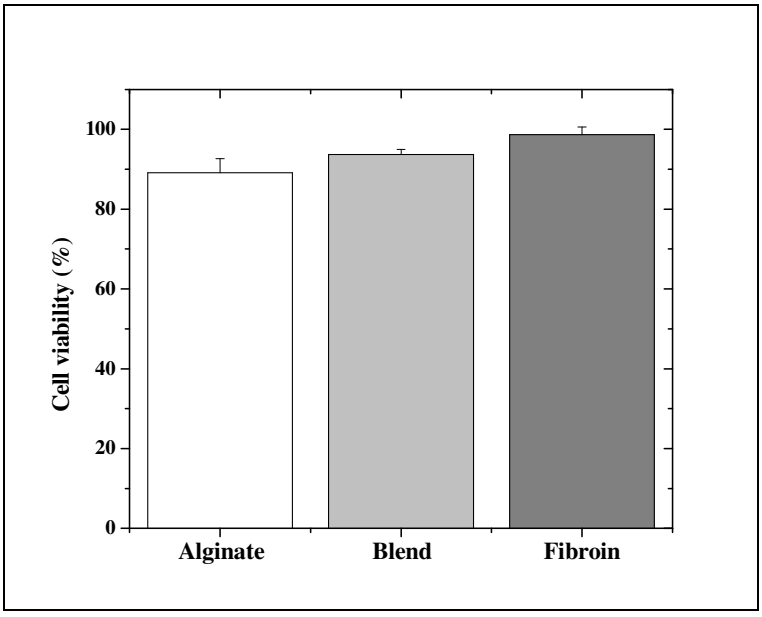

Figure 4: Cell viability of the membranes in 100% extract concentration.

None of the membranes presented cell viability inferior to the CI_{50}. The cell viability was remained between 90% and 100% for all the analyzed membranes, and according to ISO 10993-5 (2009), they can be considered non cytotoxic. The alginate membrane, in an extract concentration of 100%, presented a small fall in the cell viability, around 90%. The membrane of fibroin presented cell viability comparable to the negative control in all the analyzed extract concentrations. The blend membrane had intermediate behavior between fibroin and alginate.

4. CONCLUSIONS

A blend membrane of silk fibroin and sodium alginate was successfully obtained in a proportion of 25:75 SF:SA. The blends combined the better physical properties of SA and the good cell viability of SF, obtaining a material with conjugated properties of its components. The SF/SA blend membrane is a non-cytotoxic material, that present good hydrophilicity and water vapor transmission and mechanical strength values suitable for application as wound dressings.

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