

Biocomposite Membranes of Sodium Alginate and Silk Fibroin Fibers for Biomedical Applications

Mariana Agostini de Moraes, Marisa Masumi Beppu

School of Chemical Engineering, University of Campinas, 13083-852 Campinas-SP, Brazil

Correspondence to: M. M. Beppu (E-mail: beppu@feq.unicamp.br)

ABSTRACT: Biocomposite membranes from biodegradable and biocompatible natural polymers were prepared from sodium alginate solution reinforced with silk fibroin fibers in several fiber content by casting and solvent evaporation. The properties of these biocomposites were investigated by scanning electron microscopy, swelling test, water vapor transmission, mechanical and thermal analyses, and cytotoxicity test. A biocomposite with uniform fiber dispersion and good fiber–matrix interaction was obtained through the incorporation of fibroin fibers in the alginate membrane, even though the fibers were used without any surface treatment to enhance the interfacial adhesion. The incorporation of fibroin fibers improved the tensile strength and also provided a new property to the alginate, that is, the resistance to tear. Moreover, the use of silk fibroin fibers in polymeric composites can result in a material with adequate characteristics for application in the biomaterial field, especially as wound dressings, because of its nontoxic effect to cells, flexibility, and resistance to tear. © 2013 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 130: 3451–3457, 2013

KEYWORDS: biocompatibility; biopolymers and renewable polymers; composites; properties and characterization

Received 15 April 2013; accepted 30 May 2013; Published online 23 June 2013

DOI: 10.1002/app.39598

INTRODUCTION

Biocomposites are defined as composite materials that are composed of a biodegradable polymeric matrix, synthetic or natural, and biodegradable natural fibers or particles as reinforcement.^{1–3} Although biocomposites have been more recently studied, they are extensively found in nature, and present final structures with distinct mechanical properties to those that would be presented by their precursors alone. Examples of such structures are bamboo, bone, nacre, and wood among others.

Silk fibroin (SF) is a protein fiber spun by *Bombyx mori* silkworm. SF fibers are about 10–25 μm in diameter and a single cocoon may provide over 1000 m of SF fibers.^{4,5} SF fibers are classified as animal-based natural fibers, are biodegradable and highly crystalline, with an organized β -sheet structure. They exhibit high resistance to tension, good elasticity, and resilience. They are also highly stable to temperature, with degradation being initiated at temperatures above 150°C.¹

Several research groups have investigated SF as a promising resource for biotechnological and biomedical applications because of its good biocompatibility, good oxygen and water vapor permeability, biodegradability, minimal inflammatory reaction, sterilizability, possibility of preparation in different morphologies, high mechanical strength, high thermal stability, and microbial resistance.^{6–8}

All these properties make SF fibers a promising material to be used as a reinforcement in biocomposite materials and also to aggregate their inherent properties that are adequate for biomedical applications. Despite the excellent properties of SF fibers, there are just few studies in literature comprising the use of SF fibers in biocomposites and all of them use synthetic polymers as matrix, which can be processed by injection, extrusion, and compression molding.^{1,9–11} One of the biggest challenges for the future of biocomposites containing SF is the use of a natural polymer as matrix, producing a completely natural and biodegradable composite material. The interest in environmentally friendly materials is increasing significantly worldwide, making SF-reinforced biocomposites an ecologically friendly material.

Sodium alginate (SA) is a natural polymer presented in brown algae that can be used as matrix in a completely natural biocomposite reinforced with SF fibers. Alginates are linear block copolymers composed of 1,4-linked β -D-mannuronic acid (M) and α -L-guluronic acid (G). Alginates extracted from different species of seaweeds differ in M and G contents, resulting in differences in physical properties of the alginate products.¹² Because of the linear structure and high molecular weight, SA form strong membranes and good fibers in the solid state.¹³ SA is known to be nontoxic and biocompatible with a variety of cells. Because of these properties, SA has been studied for

application as biomaterials, mainly as matrix for drug delivery¹⁴ and as wound dressings.^{12,15}

For wound-healing applications, the wound dressing should keep the moisture in the wound, absorb the excess of exudate, allow fluids to exchange with environment, promote thermal insulation, be biocompatible, and should not cause any allergic or immune response. The dressing should act as barrier against bacteria, be flexible, easy to handle, and easily removed from the wound, among other characteristics.¹⁶

Natural polymers can be used as wound dressings but the main limitations on their use are the heterogeneity and batch variation, the poor stability, and mechanical performance. However, these limitations can be overcome by blending with different materials or by preparing composites with reinforced properties. A comprehensive literature review about synthetic and natural polymers (including SA and SF) used as wound dressings (especially for diabetic foot ulcer treatment) is presented by Moura and coauthors.¹⁶

B. mori SF fibers have been used in biomedical applications as sutures since the end of the nineteenth century.¹⁷ There are reports about the use of fibroin fibers for wound closure by surgeons for at least 3000 years.¹⁸ Complementarily, SA is one of the most applied natural polymers in wound healing because of its hemostatic action and moisture regulation.¹⁵ Nowadays, SA is presented in more than 10 commercial dressings, being a material with recognized healing properties.¹⁶

The aim of this study is to prepare biocomposite membranes of SA as matrix and SF fibers as reinforcement and to evaluate the effect of SF fiber content on the biocomposites properties. The possibility of application of these biocomposites as wound dressings is also evaluated by cytotoxicity test.

MATERIALS AND METHODS

Biocomposite Membranes Preparation

Biodegradable biocomposites were prepared using SF fibers as reinforcement in SA membranes. Continuous SF fibers (type Gregia 20/22 6A—Figure 1), spun from *B. mori* silkworm, were gently supplied by Bratac S/A (Bastos/SP/Brazil). These fibers were previously degummed with hot water at Bratac S/A to remove the sericin layer of the fibers. Each SF fiber used in our study had an average diameter of $\sim 50 \mu\text{m}$, which is higher than the average diameter reported in the literature for SF fibers ($10\text{--}25 \mu\text{m}$)^{4,5} because we used SF fibers processed in the fiber industry, composed by the physical combination of at least seven fibroin filaments, extracted from seven different cocoons. The as-received fibers were cut into ~ 1 cm length (established according to the literature^{10,11}) and dispersed with a polypropylene rod for ~ 10 min in a 2 wt % SA (Vetec/Brazil) alkaline solution in fiber contents of 20, 40, and 60 wt %. Prior to the biocomposite preparation, glycerin was added into SA solution to act as a plasticizer. Sixty weight percent of SF fibers was observed to be the maximum content of fibers that could be physically mixed in the SA matrix. The biocomposite membranes were prepared by casting the SA solution with dispersed SF fibers in polystyrene dishes and drying at room temperature (25°C), until no mass variation was

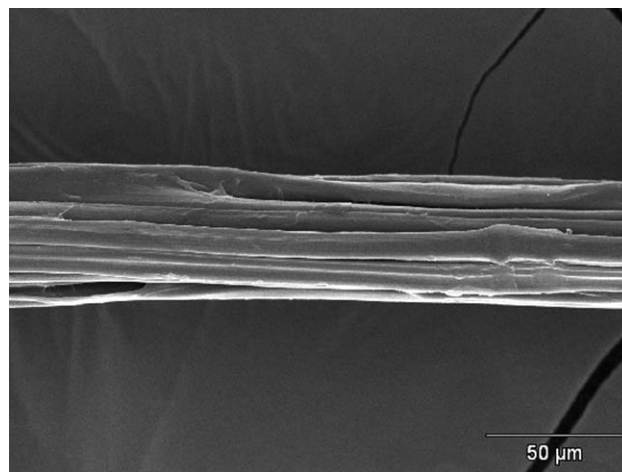


Figure 1. SEM micrograph of SF fibers used in this study.

observed. Pure SA membrane was prepared by casting SA solution in a polystyrene dish, followed by solvent evaporation, to be used as a control. After drying, the membranes were treated in 0.1 M sulfuric acid solution in 50 vol % ethanol, for 24 h, in order to stabilize the SA functional groups and prepare water-stable membranes, and then the membranes were rinsed with distilled water to remove any sulfuric acid residues. All the membranes were characterized after drying at room temperature.

Characterization

Scanning Electron Microscopy (SEM). Scanning electron microscope (SEM) was conducted on the surface of the as-prepared biocomposites and also on fracture surface of the samples after mechanical tests to examine the failure behavior induced by the tensile test. A LEO 440i (LEO, Cambridge, UK) SEM was used, with an acceleration voltage of 20 kV.

Swelling. The degree of swelling was determined gravimetrically. Pieces of 2.5 cm in diameter of pure SA and biocomposite membranes were weighed in the initial state (w_i), after being equilibrated at 50% relative humidity for 48 h. The samples were immediately immersed in 100 mL of distilled water (swelling medium) and weighed until reaching constant weight (w_f). The swelling of pure SF fibers was also measured. The swelling capacity of each sample was performed in triplicate and calculated as follows:

$$\text{Swelling (\%)} = \frac{(w_f - w_i)}{w_i} \times 100 \quad (1)$$

Water Vapor Transmission. The water vapor transmission (WVT) was determined according to ASTM (American Society for Testing and Materials) E96/E 96M (2005)—Standard Test Methods for Water Vapor Transmission of Materials. Briefly, the membranes were placed in a recipient containing anhydrous calcium chloride as a desiccant and this recipient was placed on a desiccator containing saturated aqueous NaCl solution, maintaining the ambient at 75% of relative humidity. The WVT through the membranes was determined gravimetrically by weighing the recipient every 12 h, for a period of 5 days. The

rate of WVT was determined from the slope of the weight change versus time line. The WVT of each sample was measured in triplicate. Equation (2) presents the calculus of WVT.

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

where G/t is the mass variation rate (slope of the straight line), in g/day, and A is the test area, in m^2 .

Mechanical Tests. Mechanical tests of tensile strength and tear propagation force were performed in a texture analyzer TA.XT2 (Stable Microsystems SMD), according to ASTM D882–2002 (Standard Test Method for Tensile Properties of Thin Plastic Sheeting) and ASTM D1938–2008 [Standard Test Method for Tear-Propagation Resistance (Trouser Tear) of Plastic Film and Thin Sheeting by a Single-Tear Method], respectively. Briefly, the membranes were cut into pieces of 7 cm \times 2.5 cm and placed in an environment with 50% of relative humidity for 48 h. The thickness of the membranes was measured using digital micrometer (MDC-25S, Mitutoyo). For tensile test, the pieces were subjected to a tensile with speed of 10 mm/s and with initial distance of 50 mm. For measuring tear propagation force the test speed was 4.16 mm/s and the initial distance was 50 mm. A minimum of eight repetitions was performed for each sample.

Thermogravimetry. Thermogravimetric analysis (TGA) were performed in a TGA-50 (Shimadzu) in a temperature range of 25–600°C with a ramp rate of 10°C/min and a N_2 flow of 50 mL/min. The results were normalized as a function of the initial mass to a better evaluation.

Cytotoxicity. *In vitro* biocompatibility was performed according to ISO 10993-5:2009 (Biological evaluation of medical devices—Part 5: Tests for *in vitro* cytotoxicity) using a Chinese hamster ovary cell line (CHO-k1). The cells were maintained at a RPMI medium supplemented with antibiotics and antimicrobial (100 units/mL penicillin, 100 $\mu\text{g}/\text{mL}$ streptomycin, and 0.025 $\mu\text{g}/\text{mL}$ amphotericin), 2 mM glutamine, and 10% calf serum, at 37°C 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 (ethylenediamine tetraacetic acid) in phosphate-buffered saline at pH 7.4. The membranes were sterilized by UV irradiation for 30 min on each side of the membrane. The membranes were immersed in RPMI medium in a proportion of 1 cm^2 of membrane/mL of RPMI, at 37°C for 48 h. Cytotoxicity test was performed in 96-well microplates seeded with 3000 cells per well and extract 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 a MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)/PMS (phenazine methosulphate) (20:1) solution and incubating for 2 h, at 37°C. The microplates were analyzed in a spectrophotometer reader type ELISA at 490 nm. The test was compared with a negative control (that keeps the cell viability in 100%) of 0.2 g/mL high-density-polyethylene (HDPE) and a positive control (that kills the cells) of 0.5 vol % phenol in culture medium. Tukey–Kramer test was used to analyze the results and differences between samples were considered statistically significant for $P < 0.05$.

RESULTS AND DISCUSSION

Morphology

The morphology of the surface of the biocomposite membranes was investigated by SEM. The photographs and SEM micrographs of the biocomposite membranes are shown in Figure 2. The biocomposite with 20 wt % of SF fibers presented a continuous matrix of SA, with all the SF fibers well dispersed and incorporated in SA matrix; no voids were observed and it presents a relatively smooth surface. In the biocomposite with 40 wt % of SF fibers, SA still acts joining the SF fibers, however, it is verified that some fibers were not completely covered by the matrix. Because of the higher amount of SF fibers in biocomposites with 60 wt % of SF fibers, the amount of SA solution was not enough to cover all the fibers and some interconnected pores can be observed by SEM. The presence of pores in the membrane would be adequate for use as scaffold, because the pores possess the required size for scaffolds, usually in the range of 100 μm .

Swelling

The degree of swelling is an important parameter that determines the application of a material as wound dressing, once it is expected that the material have the capacity to absorb high amounts of exudates which are released from the wounds. Figure 3 shows the degree of swelling of the SF/SA biocomposites in water. The swelling of the membranes occurred mainly in the first minute of immersion in water, and the water uptake capacity was established in 15 min. The swelling in the biocomposites occurred mainly in SA matrix. SA is a highly hydrophilic biopolymer because of carboxyl and hydroxyl groups presented in its structure, which gives a significant swelling when SA membrane is immersed in water.¹⁹ On the other hand, SF fibers are highly crystalline and hydrophobic and do not absorb as much water as the SA. In fact, the presence of SF fibers in the biocomposite decreases the degree of swelling and the extent of this decrease is proportional to the mass quantity of SF in each biocomposite.

The degree of swelling is directly related to the mobility of the polymeric chains. The molecules of the polymer retain water, increasing their 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.²⁰

Water Vapor Transmission

In Figure 4, a slight decrease in the WVT value is observed for the biocomposite containing 20 wt % of SF when compared to the pure alginate matrix. This occurs because of the presence of SF fibers that present lower permeability than the SA membrane. In this biocomposite, all SF fibers are incorporated in the SA matrix, without voids (confirmed by SEM; Figure 2), thus, the presence of SF fibers decreases the permeation area of SA matrix. In the biocomposites with 40 wt % of SF, a similar behavior is observed. However, at the same time, the permeation area is decreased by the increasing amount of fibers and the presence of small voids in the biocomposite increases the

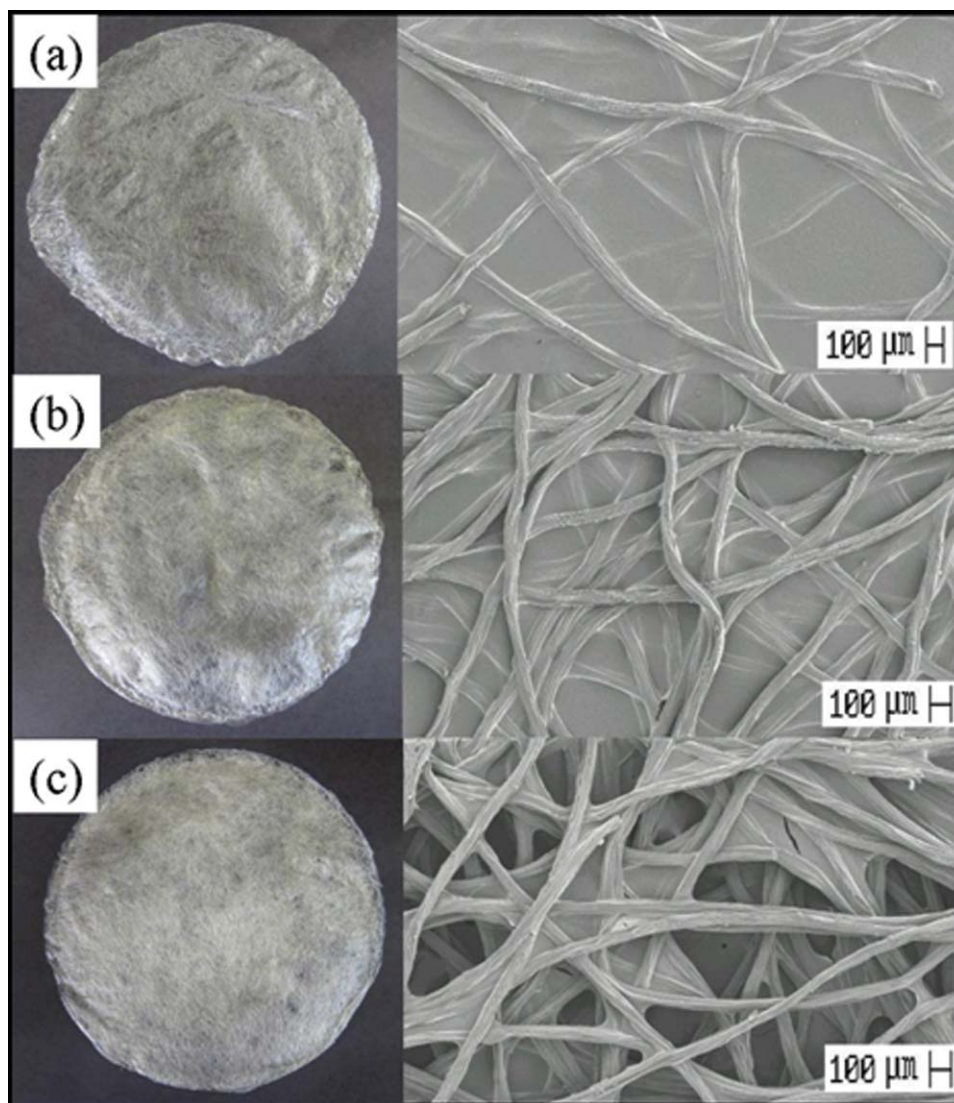


Figure 2. Photographs and SEM micrographs of surface of SF/SA biocomposites containing (a) 20 wt %, (b) 40 wt %, and (c) 60 wt % of SF fibers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

WVT. The high WVT for the biocomposite with 60 wt % of SF fibers is justified by the presence of large voids throughout the entire biocomposite. These voids allow the direct passage of

water through the membrane. The values of WVT of the SF/SA biocomposites are in accordance with the range of WVT values of commercial wound dressing.²¹

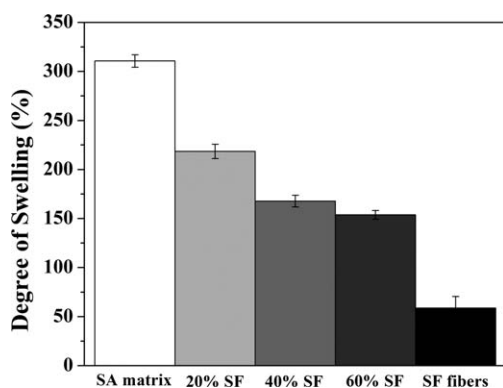


Figure 3. Swelling of SA matrix, SF fibers, and SF/SA biocomposite with several SF fibers content.

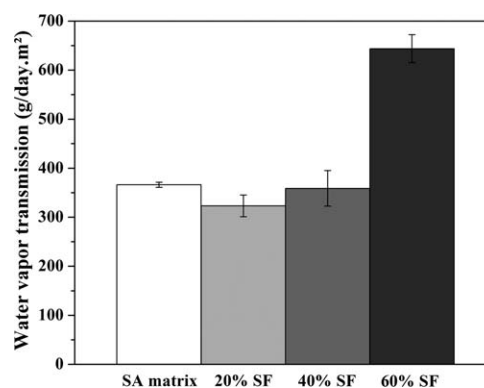


Figure 4. WVT of SA matrix and SF/SA biocomposite with several SF fibers content.

Table I. Results of Tensile and Tear Propagation Tests

	Strength at break (N)	Tear propagation force (N)
SA matrix	73.04 ± 9.83	Not detected by the equipment
Biocomposite 20 wt % SF/SA	53.28 ± 2.02	1.54 ± 0.32
Biocomposite 40 wt % SF/SA	118.29 ± 16.47	3.35 ± 0.60
Biocomposite 60 wt % SF/SA	179.94 ± 36.72	7.63 ± 1.08

Mechanical Properties

The results of strength at break (N) and tear propagation force (N) are shown in Table I. We could not evaluate the values of tensile at break (force/transversal area) because, for its calculation, it is necessary to take into account the thickness of the biocomposites. The main problem is that the thickness measured for the biocomposites is, in fact, the thickness of the SF fibers incorporated in the matrix and not the thickness of the SA matrix, where the rupture takes place. Because of this fact, incorrect values of tensile at break would lead to wrong conclusions about such test. Therefore, only the results comprising the strength at break will be evaluated in this study.

The tear propagation force of the biocomposites was highly increased by the increase of SF fiber content. An interesting thing is the fact that because of the incorporation of SF fibers in the SA matrix, a material with new properties was obtained. In this case, the membranes were much more flexible and also presented an additional property, namely, the resistance to tear. Dense membranes of biopolymers are easily ripped when they have a small irregularity on its edges but with the incorporation of fibers this property is changed. The resistance to tear of pure SA membrane was so low that it was not detected by the equipment. The good flexibility and the good resistance to tear presented by the biocomposites are interesting for applications as wound dressings.

From the tensile test, the strength necessary to fracture the SA matrix in the biocomposites increases with increasing SF fibers content (except for the biocomposite containing 20 wt % of SF fibers), characterizing the effect of reinforcement of the fibers in the matrix.

In order to verify the adhesion among SF fibers and SA matrix, SEM micrographs of the fracture surface were taken immediately after the tensile test, as shown in Figure 5. SF fibers were well adhered to the matrix [Figure 5(b,c)]. The fibers, instead of being pulled out from the matrix, were fractured along with it, which indicates a strong adhesion between SF fibers and SA matrix.⁹ The pull out of fibers was only observed in the biocomposite with 20 wt % of SF fibers [Figure 5(a)]. This phenomenon indicates a poor interfacial adhesion between the fibers and the matrix in this biocomposite.

It is known that the fiber orientation, fiber length distribution, fiber dispersion, and fiber–matrix adhesion play a key role on biocomposites properties.⁹ In our study, good fiber dispersion and a narrow range of fiber length distribution was achieved for

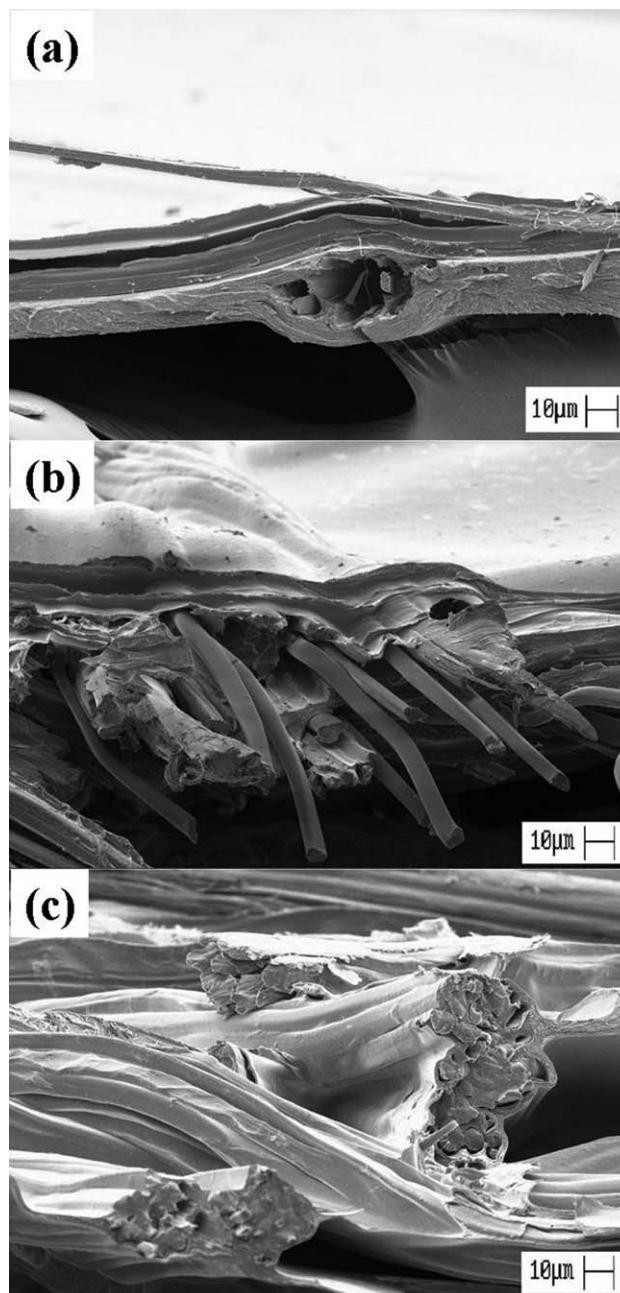


Figure 5. SEM micrographs of fracture surface of SF/SA biocomposites containing (a) 20 wt %, (b) 40 wt %, and (c) 60 wt % of SF fibers after the tensile test.

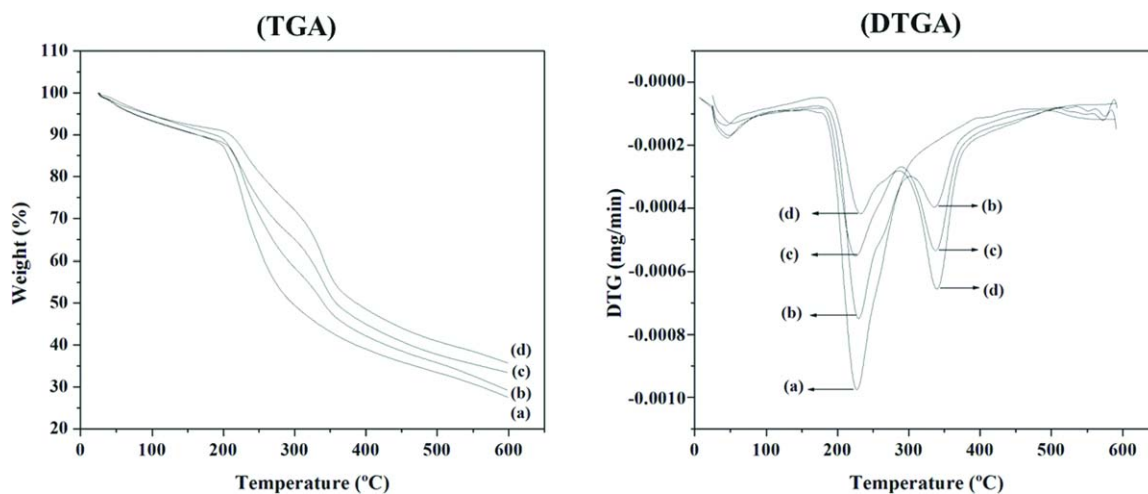


Figure 6. TGA and DTGA thermograms of (a) SA matrix and SF/SA biocomposite containing (b) 20 wt %, (c) 40 wt %, and (d) 60 wt % of SF fibers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

all SF fibers content. However, the fibers were randomly oriented in SA matrix. In biocomposites with aligned fibers, the mechanical stress is transferred directly from the matrix to the fibers during loading condition, characterizing the reinforcing effect of the fibers. Despite being a negative parameter, the randomly oriented SF fibers in our study lead to entanglements among the fibers in the biocomposites with 40 and 60 wt % of SF fibers. The fibers when entangled exhibit better reinforcement effect and, besides the fiber–matrix adhesion, there is also the fiber–fiber entanglement that acts as reinforcing agent for the biocomposite. Therefore, in the biocomposite with 20 wt % of SF fibers, the amount of fibers is not enough for these entanglements, justifying the decrease in values of tensile strength and the occurrence of fibers pull out.

It is important to remember that the SF fibers used in this study did not undergo any surface treatment, usually done to increase the adhesion between the fibers and the matrix, resulting in better mechanical properties. This indicates the potential use of natural SF fibers in composite materials for the most diverse purposes.

Thermogravimetry

TGA is a useful analysis to determine the degradation behavior of the matrix and the fiber in a composite and also provide information about the mutual effect of the composite components.¹ Figure 6 presents the thermogravimetric and derivative thermogravimetric curves of SA matrix and SF/SA biocomposites. The TGA indicates the thermal stability of the biocomposites, whereas the derivative thermogravimetric curves, DTGA, indicate the decomposition temperature of the membranes.

In the TGA curve, two main transitions can be observed. The first mass loss is observed up to $\sim 200^{\circ}\text{C}$, related to SA matrix transitions, and the second mass loss takes place in the range of ~ 200 – 350°C , related to SF fibers transitions. The thermal stability of the biocomposites was enhanced by the SF fibers, which present better thermal stability than the SA matrix.

Those two peaks of mass loss are better visualized on the DTGA curve. SA matrix shows one peak of weight loss at 226°C , attributed to the thermal degradation of SA and to the formation of carbonaceous residues.²² SF thermal decomposition is influenced by the physical and morphological properties of the sample, with the molecular orientation being one of the fundamental parameters.²³ Well-oriented fibers decompose in temperatures above 300°C ; materials with crystalline structure of β -sheet decompose at 290 – 295°C ; and amorphous SF decomposes below 290°C . By incorporating SF fibers in the SA matrix, a new degradation peak appears, at 338°C , attributed to the well-oriented SF fibers. By increasing SF weight percentage in the biocomposites, the peak intensity related to SF fibers increases, whereas the peak intensity related to SA matrix gradually decreases. The changes in the peak intensity are directly

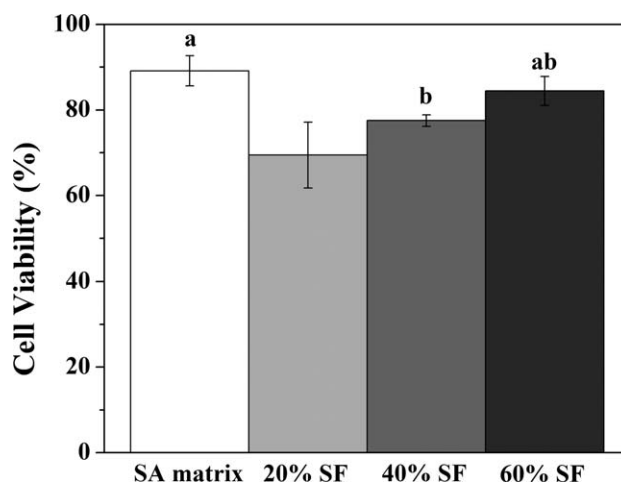


Figure 7. Viability of CHO cells in the extract of SA matrix and SF/SA biocomposites with several SF fibers content. Same letter indicates that there is no statistically significant difference ($P < 0.05$) between averages by Tukey–Kramer test.

related to the amount of SF in the biocomposite membranes. No significant changes were observed in the temperature at which the peaks are located, indicating that the biocomposites are formed by the physical combination of SF and SA, without changing the thermal behavior of the components.

Cytotoxicity

The results for the viability of CHO cells when in contact with the extract (100% concentration) of the SA matrix and SF/SA biocomposites are shown in Figure 7. SA matrix and SF/SA biocomposites with 40 and 60 wt % of SF fibers were not toxic to cells. A drop in cells viability for the biocomposite with 20 wt % of SF to ~70% is observed. This value was considered cytotoxic once it is in the boundary recommended by ISO 10993-5 (2009). For this reason, the biocomposite with 20 wt % SF was considered unsuitable for biomaterials applications and should not be used for further studies.

The reason for the drop in cell viability is not known until now, because SF fibers are recognized nontoxic materials and the SA matrix had cell viability of approximately 90%. Increasing SF content in the biocomposite leads to an increase in cell viability, which excludes the hypothesis of cytotoxicity of SF fibers. It is known that the surface topography and also the presence of different phases in the material will influence the cell behavior.^{24–26} The combined effect of different materials on cell behavior and inflammatory reaction in the body was observed in native silk and was attributed to the presence of sericin. Sericin and fibroin alone do not induce inflammatory reactions *in vivo*, however, when combined (native silk) the inflammatory reaction takes place.⁶ A similar trend is observed in our study, where the combined effect of SF and SA reduces the cell viability, especially in low contents of SF fibers. Thus, we can conclude that in terms of biocompatibility, an antagonist effect of SA and SF is observed, resulting in worse results than its components alone.

CONCLUSIONS

Biocomposite membranes of SF fibers and SA matrix were prepared with uniform dispersion of the fibers in the matrix. A good fiber/matrix adhesion was observed in all fibers content, with exception of the biocomposite with 20 wt % of SF fibers. The biocomposites presented interesting properties, such as high flexibility and resistance to tear. Increasing fiber content in the biocomposite membrane increased the mechanical properties.

The potential use of SF and SA alone in the biomedical field as wound dressing was already proved in literature, and the nontoxicity of these materials was also confirmed in this paper. This property combined with the good swelling capacity, WVT through the membrane, good thermal resistance, flexibility, and good mechanical properties presented by the biocomposites developed in our study emphasize the possibility of use of these membranes in wounds and burns healing.

ACKNOWLEDGMENTS

The authors thank Andrea Rodas and Olga Higa, from the Biotechnology Center of the Energy and Nuclear Research Institute (IPEN/USP) for performing the cytotoxicity tests and to CNPq for the financial support.

REFERENCES

1. Lee, S. M.; Cho, D.; Park, W. H.; Lee, S. G.; Han, S. O.; Drzal, L. T. *Compos. Sci. Technol.* **2005**, *65*, 647.
2. Bogoeva-Gaceva, G.; Avella, M.; Malinconico, M.; Buzarovska, A.; Grozdanov, A.; Gentile, G.; Errico, M. E. *Polym. Compos.* **2007**, *28*, 98.
3. Fowler, P. A.; Hughes, J. M.; Elias, R. M. *J. Sci. Food Agric.* **2006**, *86*, 1781.
4. Vepari, C.; Kaplan, D. L. *Prog. Polym. Sci.* **2007**, *32*, 991.
5. Plaza, G. R.; Corsini, P.; Perez-Rigueiro, J.; Marsano, E.; Guinea, G. V.; Elices, M. *J. Appl. Polym. Sci.* **2008**, *109*, 1793.
6. Altman, G. H.; Diaz, F.; Jakuba, C.; Calabro, T.; Horan, R. L.; Chen, J. S.; Lu, H.; Richmond, J.; Kaplan, D. L. *Biomaterials* **2003**, *24*, 401.
7. Li, M. Z.; Lu, S. Z.; Wu, Z. Y.; Tan, K.; Minoura, N.; Kuga, S. *Int. J. Biol. Macromol.* **2002**, *30*, 89.
8. Um, I. C.; Kweon, H. Y.; Park, Y. H.; Hudson, S. *Int. J. Biol. Macromol.* **2001**, *29*, 91.
9. Cheung, H. Y.; Lau, K. T.; Tao, X. M.; Hui, D. *Compos. Part B Eng.* **2008**, *39*, 1026.
10. Han, S. O.; Lee, S. M.; Park, W. H.; Cho, D. *J. Appl. Polym. Sci.* **2006**, *100*, 4972.
11. Li, W.; Qiao, X. Y.; Sun, K.; Chen, X. D. *J. Appl. Polym. Sci.* **2008**, *110*, 134.
12. Qin, Y. M. *Polym. Int.* **2008**, *57*, 171.
13. Rinaudo, M. *Polym. Int.* **2008**, *57*, 397.
14. Dong, Z. F.; Wang, Q.; Du, Y. M. *J. Membrane Sci.* **2006**, *280*, 37.
15. Roh, D. H.; Kang, S. Y.; Kim, J. Y.; Kwon, Y. B.; Kweon, H. Y.; Lee, K. G.; Park, Y. H.; Baek, R. M.; Heo, C. Y.; Choe, J.; Lee, J. H. *J. Mater. Sci. Mater. Med.* **2006**, *17*, 547.
16. Moura, L. I. F.; Dias, A. M. A.; Carvalho, E.; de Sousa, H. C. *Acta Biomater* **2013**, *9*, 7093.
17. MacIntosh, A. C.; Kearns, V. R.; Crawford, A.; Hatton, P. V. *J. Tissue Eng. Regen. Med.* **2008**, *2*, 71.
18. Ki, C. S.; Park, Y. H.; Jin, H. *J. Macromol. Res.* **2009**, *17*, 935.
19. Kalyani, S.; Smitha, B.; Sridhar, S.; Krishnalaha, A. *Desalination* **2008**, *229*, 68.
20. Olivás, G. I.; Barbosa-Canovas, G. V. *Lwt Food Sci. Technol.* **2008**, *41*, 359.
21. Wu, P.; Fisher, A. C.; Foo, P. P.; Queen, D.; Gaylor, J. D. S. *Biomaterials* **1995**, *16*, 171.
22. Siddaramaiah; Swamy, T. M. M.; Ramaraj, B.; Lee, J. H. *J. Appl. Polym. Sci.* **2008**, *109*, 4075.
23. Freddi, G.; Pessina, G.; Tsukada, M. *Int. J. Biol. Macromol.* **1999**, *24*, 251.
24. Gil, E. S.; Park, S.-H.; Marchant, J.; Omenetto, F.; Kaplan, D. L. *Macromol. Biosci.* **2010**, *10*, 664.
25. Liu, Y.; Li, X.; Qu, X.; Zhu, L.; He, J.; Zhao, Q.; Wu, W.; Li, D. *Biofabrication* **2012**, *4*, 1.
26. Roohani-Esfahani, S. I.; Lu, Z. F.; Li, J. J.; Ellis-Behnke, R.; Kaplan, D. L.; Zreiqat, H. *Acta Biomater.* **2012**, *8*, 302.