Development of Porous Lamellar Chitosan-Alginate Membranes: Effect of Different Surfactants on Biomaterial Properties

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ABSTRACT: In this work, porous lamellar chitosan-alginate membranes were developed without the use of freeze-drying methods or other vacuum-based approaches. The effects of two different surfactants, Tween 80 and Pluronic F68, on the properties of the membranes were evaluated, aiming at the production of stable consistent foams with improved polysaccharide dispersion. The membranes prepared with Tween 80 had a tensile strength around 1.5 MPa, elongation at break of 2.1% and liquid uptake from 590 to 1370% in distinct solutions, increasing their thickness in up to 3.9 times when immersed in water. The membranes obtained with Pluronic F68 had a tensile strength of 1.0 MPa, elongation at break of 2.0% and liquid uptake from 774 to 1380%, showing an increase in thickness around 3.2 times after exposure to water. The antimicrobial properties of both membranes were also evaluated, showing that despite being porous, the membranes can provide some protection against bacterial permeation. Therefore, membranes produced with Tween 80 and Pluronic F68 were considered to have high potential for use in the production of wound dressings and scaffolds for tissue engineering. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 624–631, 2011

Key words: membranes; biopolymers; biomaterials; chitosan; alginate; surfactants; wound dressing

INTRODUCTION

The use of polysaccharides in the biomedical field has been increasing due to properties as biocompatibility and biodegradability presented by many compounds in this category. Limitations as inflammatory response associated to synthetic materials can be suppressed by the use of these natural substances.[1] Examples of natural polymers frequently used in the constitution of wound dressings and scaffolds are chitosan and alginate.

Chitosan is a derivative of chitin, which is the second most abundant polysaccharide after cellulose, resulting from its deacetylation under alkaline conditions.[2] It is a copolymer of β-1-4-2-acetamido-2-deoxy-D-glucose and 2-amino-2-deoxy-D-glucose.[3] Chitosan has been receiving a great deal of interest because it has interesting properties such as biocompatibility and biodegradability, besides presenting analgesic, antitumor, hemostatic, hypocholesterolemic, antimicrobial, and antioxidant activity.[4] Therefore, chitosan has been investigated for application in various fields such as drug and gene delivery, tissue engineering, wound healing, functional foods, food preservation, biocatalyst immobilization, wastewater treatment, molecular imprinting, metal nanocomposites and for use in antimicrobial, antiviral and immunoadjuvant strategies.[3,4]

Alginate, on the other hand, is a polysaccharide found in brown algae.[2] It is composed of alternating blocks of 1-4-α-L-guluronic and β-D-mannuronic acid residues.[5] This biopolymer has been found to be extremely absorbent and when used in skin lesions, is able to maintain a moist microenvironment that promotes wound healing,[6] being able to form gels by reaction with divalent cations such as calcium.[5] The interactions between the polymer and the cations results in a tridimensional web of alginate fibers joined by ionic bonds. This hydrogel has gelifying properties, increasing the comfort provided by the wound dressing and relieving pain when the dressing is removed.[5,7,8] Alginate can also be absorbed by physiological fluids, due to its solubility when in the form of sodium salt.[9]

The carboxyl residues of the mannuronic and guluronic acids in alginate can interact with the amino groups of chitosan to form a polyelectrolyte complex (PEC).[3] The main interaction normally

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verified in this process is electrostatic attraction, but hydrogen bonds and hydrophobic interactions can also be involved.[10] The chitosan-alginate composite formed by complexation is still biodegradable and biocompatible and, in addition, it is more mechanically stable[3] and presents reduced swelling tendency,[11] being also resistant to pH conditions in which isolated chitosan and alginate dissolve.[3,12] and more effective as controlled-release systems than either the chitosan or alginate separately. Because of these properties, the chitosan-alginate PEC has been intensively investigated for wound dressings and scaffolds.

An ideal wound dressing should, among many characteristics, be easily conformed to body contours, maintain the humidity at the wound surface, provide a high level of comfort and admit long-term use. In this context, the chitosan-alginate PEC is quite attractive for the production of wound dressings.[7,13]

From an ideal scaffold, characteristics as excellent biocompatibility, biodegradability (corresponding to the rate of new tissue formation), cytocompatibility, and suitable microstructure (pore size and porosity) for transporting of cells, gases, metabolites, nutrients, and signal molecules are expected. The scaffold must also present adequate mechanical properties, being able to bear loads to provide stability to the tissues as it forms and to fulfill its volume. Additionally, it must be capable of promoting cell adhesion and of allowing the retention of the attached cells metabolic functions.[14–16] The number of studies involving the use of chitosan-alginate scaffolds are increasing in the literature as it can provide the required structural integrity and biodegradability.[17]

Most of the literature referent to the preparation of porous biodegradable scaffolds and membranes mentions the use of freeze-drying methods,[18–25] but this technique is expensive, time consuming and not easily up-scalable. The use of porogenic agents, such as NaCl or glucose is also frequently mentioned, but the scaffolds must be washed successively with large amounts of water to remove these compounds and their degradation products.[24,26,27] Other ways to introduce pores into scaffolds are phase inversion and high-pressure gaseification. Alternatively, the porous scaffolds can also be formed by polymer electrospinning processes, which provide a deposit of microfibers that can guide cell growth and differentiation.[28]

A number of techniques for the preparation of chitosan-alginate membranes is described in the literature.[10,29–31] A fairly reproducible method, based on adaptations of previously described procedures[29–31] was developed by Rodrigues et al.[10] This methodology, of particular interest if the aim is large-scale production, involves the preparation of coacervates from the raw materials under controlled conditions to allow the reaction between the polymers, resulting in a suspension of fine fibers of chitosan and alginate. The addition of a low polarity organic solvent to the chitosan solution, such as acetone, induces a less extended conformation in the polymer chains, therefore controlling the rate of reaction between the polyelectrolytes.[29] Also, pH should be appropriately adjusted before membrane drying to improve the electrostatic attraction between the oppositely charged polysaccharides. The fibers obtained can be further crosslinked with calcium ions. The final coacervate suspension is then dried, producing flexible membranes with thickness from 106 to 633 μm when wet, tensile strength from 6.86 to 31.14 MPa, elongation at break from 3.97 to 8.42% and an uptake of 19 g of water per gram of membrane.

The addition of biocompatible surfactants seems to be a promising alternative to improve polymeric distribution of chitosan-alginate membranes obtained by the aforementioned method. The combination between polymers and surfactants is well known and is frequently performed to attain, for example, colloidal stability, emulsification, suspension, and reology control.[32] Nonionic surfactants, as Tween 80 and Pluronic F68, are more adequate than ionic ones in case of direct skin contact due to their better biocompatibility.[33] Also, since these compounds do not present electrical charges, their potential to compete with the polymers during complexation should be low. Several studies focus the use of these two nonionic surfactants in combination with polymers for the production of microcapsules,[34–37] but no reports regarding their use for the production of membranes were identified so far. Therefore, the purpose of this work was to study the effects of the use of the surfactants Tween 80 and Pluronic F68 during chitosan-alginate membrane preparation on final product properties.

MATERIALS AND METHODS

Materials

Low-viscosity sodium alginate, 85% deacetylated chitosan and Pluronic F68 were obtained from Sigma-Aldrich Inc. (St Louis, MO), while Tween 80 was purchased from Synth (São Paulo, Brazil). All other reagents used were also of analytical grade. The water used in this work was distilled and deionized in a Millipore MilliQ system.

Membrane preparation

The membranes were prepared based on adaptations of the procedure described by Rodrigues et al.,[10] which enabled the production of two membranes per batch, and involved the use of surfactants to improve the polysaccharides dispersion in the

mixture. First, each surfactant was added, at a final concentration of 1% (v/v), to 180 mL of 0.5% (w/v) alginate solution. Pluronic F68, which is a powder, was added at a 1% (w/v) concentration. Then 90 mL of 1% chitosan in 2% aqueous acetic acid solution were mixed with 90 mL of acetone and the resulting solution was added at a 160 mL/h rate, with the aid of an infusion pump (model ST 670T, Samtronic), to the alginate/surfactant solution under a 500 rpm stirring rate by using a mechanical stirrer (model Q-251 D, Quimis) with a 4 cm in diameter three tilted-blade propeller. The final suspension was homogenized at 1000 rpm during 10 min. A 1*M* NaOH aqueous solution was added to the suspension to elevate the pH to 5.28 and the same stirring rate was maintained for 10 min. Then 3.6 mL of 2% (w/v) $CaCl_2$ aqueous solution were added to crosslink the alginate carboxyl groups that were not bound to the chitosan amines and the stirring followed for 10 min more. The temperature was maintained at 25°C in a stainless steel tank with internal diameter of 10 cm and height of 20 cm during all the aforementioned steps of preparation. The mixture was then transferred to two polystyrene Petri dishes (15 cm in diameter) and dried in an oven with air circulation (model 410D, Nova Ética) at 37°C for 24 h. For further crosslinking, the membranes were immersed in 150 mL of 2% (w/v) $CaCl_2$ aqueous solution for 1 h and washed twice in 150 mL of deionized water for 1 h. Finally, the membranes were dried at room temperature for 24 h.

Before characterization, the membranes were cut in appropriate sizes and sterilized with ethylene oxide at Acecil Central de Esterilização Comércio Indústria Ltda (Campinas, SP, Brazil), a specialized EO sterilization company. The process of sterilization employed an initial vacuum of 0.4 to 0.6 kgf/cm^2, temperature of 50°C and relative humidity of 30 to 80%. Oxyfume-30 (30% EO and 70% carbon dioxide) was added until the pressure in the chamber reached 0.5 kgf/cm^2. After an exposure period of 3.5 h, the vacuum was reestablished (0.4 to 0.6 kgf/cm^2) and the samples were aired with filtered air for 10 min. The residual EO was removed by keeping the samples under aeration for 48 h.

Membrane characterization

The membranes were basically characterized according to the methodology employed by Rodrigues et al.[10]

The morphology of the membranes was analyzed using a scanning electronic microscope (model LEO 440i, Leica). Before the analysis, 2 × 1 cm^2 samples were kept in a desiccator for 24 h and then fixed in adequate stubs to be coated with an ultra-thin layer of gold (92 Å) in a mini sputter coater (SC 7620).

The mechanical properties were evaluated using a universal testing machine (model H5K-S, Tinius Olsen), based on the ASTM D882 method (ASTM, 2005).[38] A cell load of 200*N*, a gauge length of 45 mm and a crosshead speed of 10 mm/min were employed for 8 × 1 cm^2 samples. The results were expressed as the averages of 10 samples per membrane type.

The uptake of different physiological solutions was evaluated in water, 0.9% NaCl aqueous solution, simulated body fluid (SBF) prepared as described by Kokubo et al.[39] and fetal bovine serum (FBS) (Nutricell, Brazil). Three 6 × 1 cm^2 samples per membrane type were used for each tested solution. The dry samples had their weight (W_d) determined and were then immersed in 10 mL of each of the physiological solutions during 24 h at 37°C. After this period, the excess of solvent was removed by gently pressing the sample between two sheets of absorbent paper for 10 s. The weight of the wet samples (W_w) was then determined. The uptake capacity (U) was calculated according to eq. (1).

$$U = \frac{(W_w - W_d)}{W_d} \times 100 \quad (1)$$

Membrane mass loss was also determined in the same solutions using three 6 × 1 cm^2 samples per membrane type. First, the samples had their initial dry weight (W_d) determined and were then immersed in 10 mL of each of the physiological solutions during 1 week at 37°C. After this period, the samples were removed from the solutions and dried at 37°C until a constant weight value (W_f) was reached. The percentage of mass loss (L) was calculated according eq. (2).

$$L = \frac{(W_d - W_f)}{W_d} \times 100 \quad (2)$$

Thicknesses of dry and wet membranes (after hydration for 24 h in deionized water, 0.9% NaCl, SBF or FBS at 37°C) were measured using a micrometer (Digimess) at four different positions close to the membrane border and at 90° angles from each other. The results were expressed as averages.

The membranes antimicrobial properties regarding formation of inhibition zones of was evatuated using 1.5 × 1.5 cm^2 samples. Inside a laminar flow hood, the samples were hydrated for 1 min in sterilized water and then put on the surface of 90 mm diameter Petri dishes containing 20 to 25 mL of Tryptic Soy Agar (TSA, Difco Laboratories Inc., Detroit) culture medium (at 40 g/L in water) previously inoculated with 0.1% (v/v) of a suspension of *Staphylococcus aureus* (at a concentration of 4.3 × 10^8

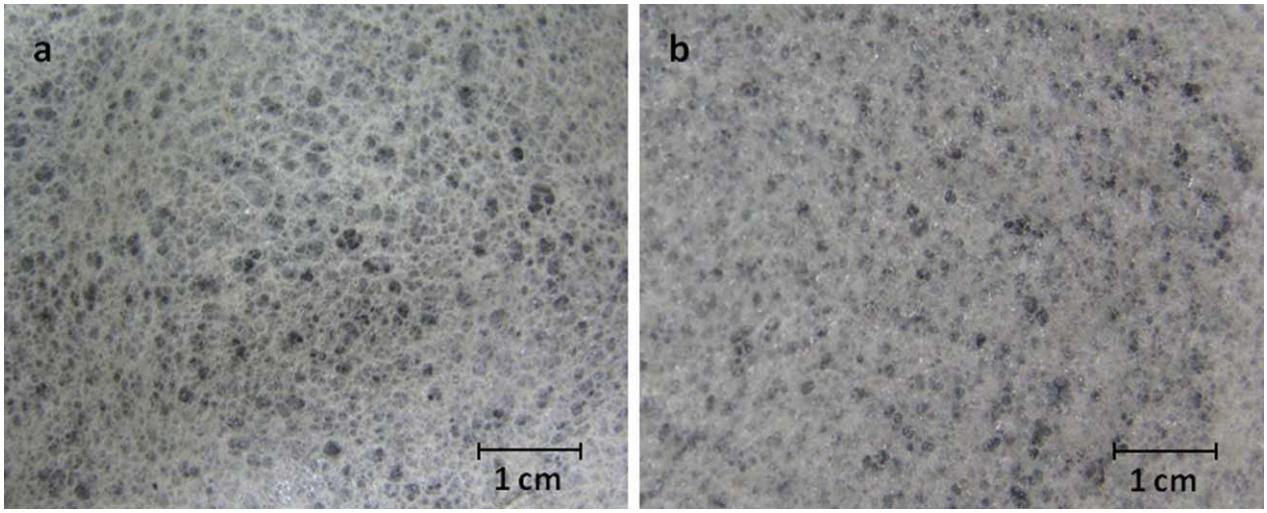

Figure 1 Visual aspect of membranes prepared with Pluronic F68 (a) and Tween 80 (b). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

colony forming units per milliliter CFU/mL) or *Pseudomonas aeruginosa* (at 5.0×10^8 CFU/mL). Then the Petri dishes were incubated at 35 ± 2°C in an incubator (model 002-CB, Fanem, Brazil) for 48 h. After this period, the formation of growth inhibitions zones around the membrane was evaluated.

Bacterial permeation through the membranes was evaluated in 2.5×2.5 cm^2 sterilized samples. First, inside a laminar flow unit, the samples were hydrated in sterilized water for 30 s and kept in the center of 90 mm diameter Petri dishes containing 20 to 25 mL of sterile solidified TSA culture medium. Then, 100 μL of an aqueous suspension of Pse*udomonas aeruginosa* (at 5.0×10^8 CFU/mL) or *Staphylococcus aureus* (at 4.3×10^8 CFU/mL) were added to the surface of the membranes and the Petri dishes were incubated at 35 ± 2°C for 48 h. The media around the membranes, the surface of the samples in contact with the atmosphere and also the side of the membranes in contact with the culture medium were evaluated concerning bacterial growth.

RESULTS AND DISCUSSION

Membranes aspect and morphology

The alginate-chitosan suspended PECs prepared in the presence of Tween 80 and Pluronic F68 had the aspect of fairly homogeneous white foams. After these foams dried, porous membranes were formed, with the typical aspect depicted in Figure 1.

Rodrigues et al.[10] employed a deaeration step by vacuum to remove air bubbles from the polymeric mixture before drying in the oven, obtaining lamellar membranes. In this work, membranes were also prepared in the absence of surfactants and not exposed to the deaeration step to compare the pores formed in this situation with the structure obtained in the presence of the tested surfactants. The membranes obtained in this condition were not homogeneous regarding both pore size and spatial distribution. Samples prepared with surfactants show a much greater number of pores and a more homogeneous structure.

The foam formation is believed to be due to the surfactants hydrophilic-lipophilic balance (HLB) values. Tween 80 and Pluronic F68 have high HLB values, of 15 and 29, respectively,[40,41] meaning that these surfactants form clearer solutions in water and have the ability of forming foams.

Typical scanning electron microscopy images of the obtained membranes are shown in Figures 2 and 3.

The membranes prepared in the presence of both surfactants present a high number of fibers and pores [Figs. 2(a,b) and 3(a,b)], as a consequence of the presence of the coacervates and of many air bubbles inside the membranes, respectively. The cross sections [Figs. 2(c) and 3(c)] show the presence of intercommunicating pores distributed in the lamellar structure. Apparently, the pores did not go across the entire membrane thickness, what would be quite relevant regarding avoidance of direct microorganism penetration in case the aim is to use the membranes as wound dressings.

If the goal is to use the membranes as scaffolds for tissue engineering applications, it is important that pores are sufficiently large to accommodate a growing cell population. Also, pore interconnectivity facilitates nutrient and waste exchange by cells deep within the construct.[14] The membranes obtained in this work present pore diameters ranging from 90 to 300 μm, approximately, which can be considered as adequate for mammalian cell growth.

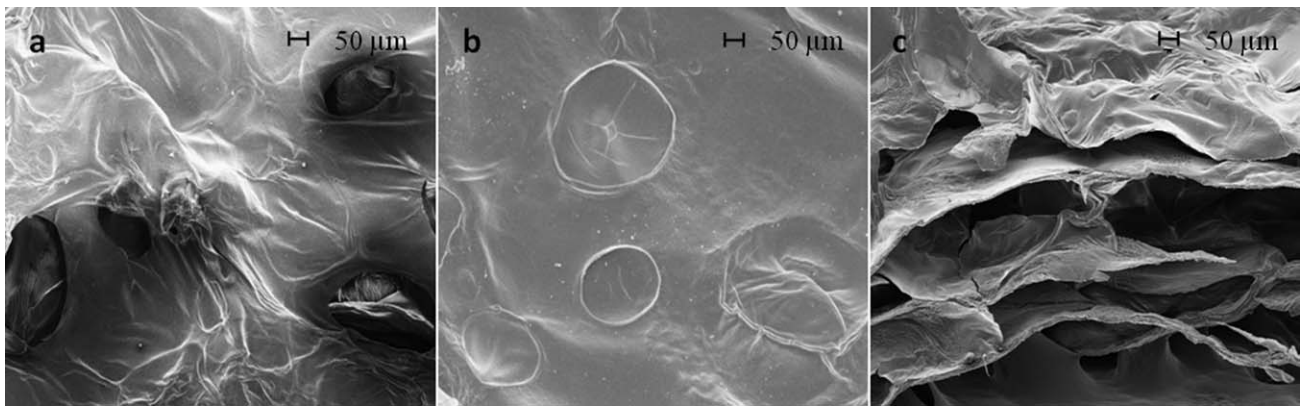

Figure 2 Electron scanning micrographs of the surface (a,b) and cross section (c) of membranes prepared in the presence of Pluronic F68.

Influence of Tween 80 and Pluronic F68 on membranes mechanical properties

The adequacy of mechanical properties of a scaffold or a wound dressing (stiffness and strength) is an important requirement in tissue engineering to inhibit collapse during the patient's routine activities.[14] The results obtained for membranes mechanical properties are shown in Table I.

The tensile strength is slightly higher for the membranes prepared with Tween 80, while the elongation at break did not differ significantly between the two types of membrane. The differences between the two membrane types may be due to the different molecular structure of the surfactants. Short polyoxyethylene chains have a more efficient molecular organization at the air/liquid interface.[32] The polyoxyethylene chains of Tween 80 are smaller than Pluronic F68 molecules, therefore, the use of Tween 80 may result in more coherent packing at the air/liquid interface, improving the organization of the polysaccharidic chains and leading, as a consequence, to stronger membranes.

The membranes prepared with surfactants are more fragile than the ones obtained by Rodrigues et al.[10] This was already expected due to the surfactants foam formation effect. Tensile testing of hydrated chitosan-based scaffolds shows that porous membranes have greatly reduced elastic moduli (0.1–0.5 MPa) compared with nonporous chitosan membranes (5–7 MPa).[15]

Porous chitosan-alginate scaffolds prepared through freeze-drying procedures[18] apparently show lower tensile strength (around 0.6 MPa, calculated from the tenacity values provided by the authors) than the membranes obtained in this work. On the other hand, elongation at break values from 3 to 10% are reported for chitosan-alginate sponges obtained through freeze-drying presented.[18,20] However, since there is no standardization of the conditions under which the mechanical properties of polysaccharide films are normally determined, direct comparisons of results from distinct authors may be somehow misleading.

Membranes behavior in aqueous media

The values obtained for the uptake of water, 0.9% NaCl aqueous solution, SBF and FBS at 37°C after 24

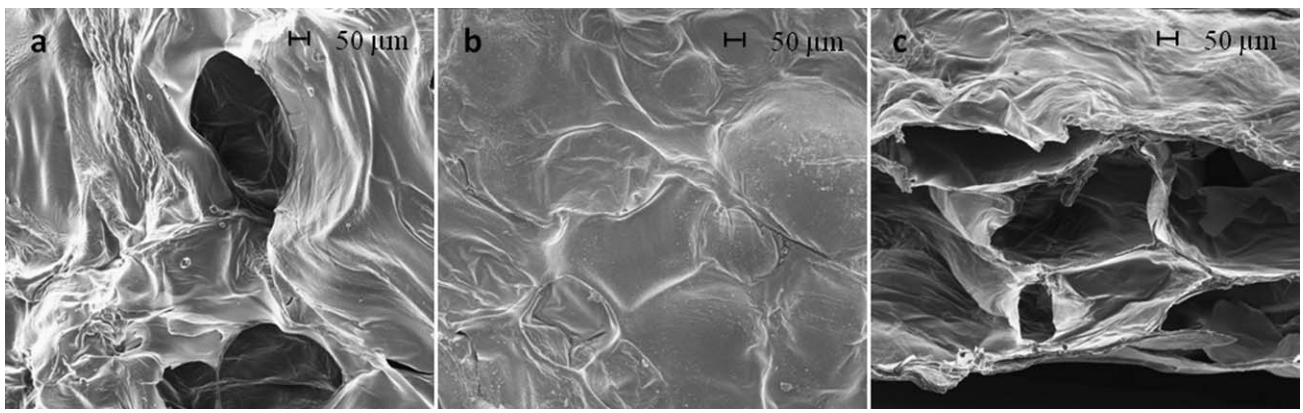

Figure 3 Electron scanning micrographs of the surface (a,b) and cross section (c) of membranes prepared in the presence of Tween 80.

TABLE I
Mechanical Properties of the Membranes

Property	Tween 80	Pluronic F68
Tensile strength (Mpa)	1.54 ± 0.16^{a}	0.98 ± 0.07^{b}
Elongation at break (%)	2.06 ± 0.13^{c}	1.96 ± 0.14^{c}

Different letters in the same line indicate significant difference at 95% confidence limits (Tukey test).

h, and also the membranes mass loss when exposed to the same solvents for one week at 37°C are shown in Table II.

According to the Tukey test, the liquid uptake capacity did not differ significantly for membranes prepared with the distinct surfactants, but varied within the used aqueous media. The membranes absorbed more water than any of the remaining solutions. In comparison to the membranes prepared by Rodrigues et al.,[10] the material herein described present similar behavior regarding fluid absorption. The obtained uptake values are also comparable to previously reported data for chitosan-alginate sponges prepared through lyophilization procedures.[18,19] The membranes obtained by Kucharska et al.[18] absorbed around 1750% of water after 180 min, whereas the membranes produced by Öztürk et al.[19] were capable of absorbing from 651 to 1204% of phosphate buffer solution at pH 7.4 after 10 min. Yu et al.,[21] on the other hand, reported higher uptake values for porous chitosan-alginate membranes, ranging from 900 to 7300% after immersion in physiological buffer saline (PBS) for 24 h. The higher absorption reported in the last mentioned work may be attributed, in addition to differences in membrane structure due to the lyophilization procedure, to the different pH conditions employed during membrane preparation, as well as to distinct alginate-chitosan blend ratios and to the use of $CaSO_4$ as the reticulating agent.

All samples showed to be quite stable when in contact with the tested aqueous solutions, which simulated body fluids and solutions that could be used to hydrate the membranes previously to their use as wound dressings or scaffolds for cultivation of animal cells. The maximum mass losses observed were around 31%, and similarly to the results of liquid uptake, the difference between mass loss values was only significant when comparing the behavior of the same membrane type in distinct solutions. The highest mass loss was observed in water. FBS caused one of the samples to even discretely gain mass, probably because of the adsorption of proteins and other compounds present in the liquid.

The results referent to uptake capacity and mass loss could be explained by increased charge screening in solutions with high ionic strength.[42,43] The screening resulting from the formation of a counterion cloud (with ions provided by the tested aqueous media) may have reduced the electrostatic repulsion between the polysaccharides, increasing the chains hydrophobic character and flexibility.[44] Consequently, the increase in ionic strength led to a more packed and stable structure, impairing water from penetrating the membrane. As a way of verifying this theory, membrane thickness was measured in different conditions: when dry and after a contact period of 24 h with the tested aqueous solutions at 37°C. The results are shown in Table III.

The membranes prepared in the presence of Tween 80 are more compact than the ones prepared in the presence of Pluronic F68 when in dry state. Both membranes showed higher thicknesses when compared to the membranes obtained by Rodrigues et al.,[10] which was already expected due to the incorporation of air during foaming of the polymeric mixture. Membranes thickness increased, after exposure to water, around 390% in the case of those prepared with Tween 80 and 320% for those produced in the presence of Pluronic F68, corroborating the high water uptake capacity of both membranes.

Table III also shows that thickness varied according to the type of solution used. The highest thickness for wet membranes was obtained in water, followed by 0.9% NaCl, SBF, and FBS. These data agree with the results discussed earlier for liquid uptake and mass loss. The increase in ionic strength seems to have caused a reduction in membrane expansion or swelling, probably due to the screening

TABLE II
Liquid Uptake and Mass Loss in Physiological Solutions

	Liquid uptake (%)		Mass loss (%)	
Media	Tween 80	Pluronic F68	Tween 80	Pluronic F68
Water	1367 ± 49^{aA}	1383 ± 42^{aA}	31.38 ± 0.98^{eA}	30.47 ± 2.16^{eA}
0.9% NaCl	1127 ± 73^{bB}	1196 ± 53^{bA}	6.54 ± 0.83^{fBC}	8.33 ± 2.16^{fAB}
SBF	590 ± 22^{cC}	774 ± 69^{cB}	11.84 ± 1.60^{gB}	13.01 ± 3.26^{gAB}
FBS	676 ± 25^{dC}	795 ± 84^{dB}	-8.24 ± 6.86^{hC}*	3.04 ± 10.35^{iB}

Different lower case letters in the same line indicate significant difference at 95% confidence limits (Tukey test). Different capital letters in the same column indicate difference at 95% confidence limits (Tukey test).

* Mass gain instead of loss

TABLE III
Thickness of Membranes Before and After Exposure to Different Aqueous Media

System	Thickness (μm)	
	Tween 80	Pluronic F68
Dry membrane	380 ± 30^{aAE}	410 ± 60^{bA}
Membrane in water	1480 ± 170^{cBC}	1310 ± 80^{cB}
Membrane in 0.9% NaCl	1190 ± 50^{dBCD}	1290 ± 40^{dB}
Membrane in SBF	950 ± 50^{eCDE}	1130 ± 30^{fB}
Membrane in FBS	730 ± 90^{gADE}	900 ± 30^{gC}

Different lower case letters in the same line indicate significant difference at 95% confidence limits (Tukey test). Different capital letters in the same column indicate difference at 95% confidence limits (Tukey test).

of electrical charges. As a consequence of this increased packing, the membranes were less capable of absorbing liquid and further dissolving in it.

According to Ma et al.,[27] dermis polymeric substitutes are generally thinner than the human dermis, whose thickness varies from 0.5 to 2 mm depending on age, sex, and body area. Taking this information into consideration, all membranes prepared in this work could be potentially employed as wound dressings. Porous membranes thicknesses reported by other authors are higher than the ones obtained in this work, varying from 2.5 to 8 mm[18,20,24,25] probably because of the higher mass of material per area used, as well as due to the larger size of the pores resulting from preparation methods involving vacuum. The consulted literature does not point an ideal thickness for porous scaffolds, because it depends on the body region where the cells should grow. However, the use of scaffolds with thickness below 1 mm is reported.[45] Hence, regarding this issue, the prepared membranes could potentially be used with success also in tissue engineering.

TABLE IV
Bacterial Growth in Contact with the Porous Chitosan-Alginate Membranes

Cell type	Response type	Tween 80	Pluronic F68
Pseudomonas aeruginosa	I.Z.	None	None
	G.S.	Moderate	Moderate
	G.A.	Weak	Weak
	G.M.	Weak	Weak
Staphylococcus aureus	I.Z.	None	None
	G.S.	None	None
	G.A	Moderate	Moderate
	G.M.	Moderate	Moderate

I.Z., inhibition zone; G.S., microbial growth around the membrane; G.A., microbial growth on membrane surface (in contact with the atmosphere); G.M., microbial growth bellow membrane surface (in contact with the culture medium).

Analysis of membranes antimicrobial properties

The results referent to the formation inhibition zones and bacterial permeation through the membranes are shown in Table IV.

The membranes prepared in the presence of the surfactants Tween 80 and Pluronic F68 had similar behaviors, presenting no formation of visible inhibition zones (I.Z.) and none to moderate microbial growth in the surrounding medium (G.S.). The absence of inhibition zone indicates that the chitosan-alginate membranes do not release bactericide and/or bacteriostatic substances into the culture medium.

Similar results were found by Rodrigues et al.[10] for chitosan-alginate lamellar membranes prepared in the absence of surfactants. A possible explanation given by the authors is that the formation of the polyelectrolyte complex (PEC) caused the screening of the groups responsible for the bactericide and/or bacteriostatic activity of chitosan. According to Yu et al.,[21] when *in vivo*, the chitosan-alginate PEC slowly dissociates, releasing free chitosan and alginate molecules. The free positive chitosan molecules may then interact with the negative components of bacteria cell walls, causing their destruction by damaging their membranes.

Microbial growth around the membranes (G.S.) was moderately intense for *Pseudomonas aeruginosa*, whereas microbial growth above and below (G.A. and G.M) the membranes surfaces was greater for *Staphylococcus aureus*. As the growth on the surface was not intense for both species of bacteria, it implies that the membranes may confer some protection against the proliferation of pathogens.

CONCLUSIONS

In this work, two surfactants were tested with the goal of producing stable foams with improved polysaccharide dispersion useful as wound dressings or as scaffolds applicable to tissue engineering. The results showed that membranes prepared in the presence of Tween 80 and Pluronic F68 showed to be quite promising for the desired purposes, despite showing relatively limited mechanical resistance, what could be circumvented, for instance, by changing the reticulation procedure or by the addition of a plastifying agent. Both membrane types presented a sponge-like porous structure, and the aqueous solutions uptake capacity of these membranes, varying from around 600 to 1400%, along with the swelling ratio concerning thickness, from approximately 190–390%, turn them adequate for the treatment of highly exuding wounds. The produced membranes also showed fairly good stability when maintained in physiological solutions for one week at 37°C. Moreover, this type of membrane may protect, to a

certain extent, skin wounds against bacterial growth and permeation, which was verified for Pseudomonas aeruginosa and *Staphylococcus aureus*. Finally, a quite positive aspect about this type of chitosan-alginate porous membrane is that it is of easy production and does not demand the use of expensive freeze-drying methods, which are frequently used.[14,18–25] Applications of the obtained membranes are possible not only in the treatment of wounds, but also as scaffolds in the tissue engineering field, in regions of the body that do not require appreciable mechanical effort.

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