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Composite membranes of alginate and chitosan reinforced with cotton or linen fibers incorporating epidermal growth factor

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ABSTRACT

Suture threads of cotton or linen, in crossed and random orientation, were added to alginate-chitosan membranes intended to wound coatings application to improve the mechanical properties. The elongation at break increased to about 5 and 8 times for membranes with linen and cotton, respectively, both in the crossed orientation. The addition of the threads increased roughness and opacity of the membranes and reduced the liquid absorption capacity and water vapor transmission rate. The lowest toxicity to human fibroblasts was observed for extracts of membranes produced with linen, and incorporation in them of epidermal growth factor was able to slightly increase cell proliferation.

1. Introduction

Cell therapy has been explored as a new alternative for the treatment of chronic cutaneous wounds, such as pressure and diabetic ulcers. This approach comprises restoring the viability or function of deficient tissues by applying a suspension of autologous or allogeneic cells directly to the lesion site or culturing them on specific scaffolds that are subsequently transferred to the wounded region [1,2]. After cell application, membranes obtained from natural polymers can be used as wound coatings to provide protection and prevent dehydration associated or not with a biocompatible adhesive such as fibrin glue. Besides the protective function as physical barrier, these membranes can also aid the process of wound healing by releasing bioactive agents at the injury site, such as anti-inflammatory compounds, antibiotics, and growth factors, contributing to an efficient and fast physiological response [3,4].

Alginate and chitosan are among the most common natural polymers used for biomedical applications because of their biocompatibility, non-toxicity and biodegradability [5]. When these two polysaccharides are combined in appropriate pH conditions, the strong electrostatic interaction between the amino groups of chitosan and the carboxyl groups of alginate results in the formation of a polyelectrolyte complex (PEC), which is also biodegradable and biocompatible, but mechanically more stable than either of the individual components [6].

One of the biggest challenges associated with alginate and chitosan-based membranes, especially when porous structures are desired, is obtaining matrices with appropriate biomechanical properties. The tensile strength of the biomaterial should be compatible with that of the injured tissue, which, in the case of skin, is about 20 MPa [7]. Alginate-chitosan (1:1) dense membranes have shown tensile strength varying from 20.4 MPa [8] to 31.1 MPa [9], which are appropriate for wounds dressings. However, porous membranes produced with surfactants have tensile strength values ranging from 0.98 to 3.1 MPa [9,10]. Furthermore, the low elongation at break has been a major limitation of polysaccharide membranes and several studies have reported maximum values of 5% [10–14]. This value is much lower than that of the skin, which is about 60% [15], discouraging the application of these membranes to areas such as joints, which would demand membranes with higher elongation properties.

The properties of alginate-chitosan membranes may be improved by addition of fiber to the polymeric matrices, resulting in composites. Suture threads of cotton and linen, for example, are biocompatible, non-toxic, and their incorporation in the membranes might significantly improve their overall mechanical properties. Several factors such as the type of fiber, its ratio to the polysaccharide mixture, and orientation and size of fibers can influence the properties of the final material [16]. Many studies have shown the preparation of composites with natural fibers [17–20]. However, the properties and the cytotoxic behavior of polysaccharide-based membranes containing sutures are not known.

Growth factors can be added to the wound dressings to be released at the injury site, contributing to the differentiation and metabolism of cells, thus regulating the process of repair of the tissue [21]. The

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human epidermal growth factor (hEGF) is a single chain peptide composed of 53 amino acids. This factor is known for its broad biological activity, with the ability to stimulate the proliferation of epidermal cells and other cell types, such as fibroblasts and endothelial cells, and accelerate wound healing [22–24].

In this study, dense and porous membranes of alginate and chitosan (1:1 in mass) containing suture threads of linen or cotton were produced. The effects of the thread type and orientation on the physicochemical properties of the membranes were evaluated. The indirect cytotoxicity of the membranes, with and without epidermal growth factor (hEGF), to fibroblast cells from human eyelid was also determined.

2. Materials and methods

2.1. Materials

Chitosan from shrimp shells (C-3646, lot #109 K0043, deacetylation degree of 88%, average molar mass of 1260 kDa, intrinsic viscosity of 848 mL/g at 25 °C) and alginic acid sodium salt (A-2033, average molar mass of 90 kDa, intrinsic viscosity of 690 mL/g at 25 °C, composed of 61% mannuronic acid and 39% guluronic acid) were both obtained from Sigma-Aldrich (USA). Poloxamer 188, human epidermal growth factor (hEGF), phosphate-buffered saline (PBS), thiazolyl blue tetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were also obtained from Sigma-Aldrich (USA). Calcium chloride dihydrate and sodium hydroxide were obtained from Merck (Germany) and acetic acid was obtained from Synth (Brazil). Fetal bovine serum (FBS), α-MEM, and RPMI-1640 cell culture medium were obtained from Nutricell (Brazil). Non-absorbable multifilament sutures of cotton and linen with USP size 3-0 (diameter range 0.20–0.25 mm) were obtained from Polysuture (Brazil) and Bioline (Brazil), respectively.

2.2. Membrane preparation

Alginate and chitosan membranes (1:1 in mass) were obtained by the addition of 200 mL of chitosan solution at 1% w/v (in 1% v/v acetic acid aqueous solution) to 200 mL of alginate aqueous solution at 1% w/v. The addition was made with a peristaltic pump (Model Minipuls 3, Gilson, USA) at a flow rate of 200 mL/h, at 25 °C and stirring at 500 rpm in a stainless steel tank with internal diameter of 10 cm and height of 20 cm. The pH of the final mixture was elevated to 7.0 by adding sufficient 1 M NaOH, while stirring at 1000 rpm. The carboxyl groups of alginate that were not bound to the chitosan amino groups were crosslinked by the addition of 10 mL of 1% (w/v) $CaCl_2$ aqueous solution. The solution was degassed with a vacuum pump (model Q-355B, Quimis) for 2 h, transferred in aliquots of 85 g to polystyrene Petri dishes (15 cm in diameter) and dried in an oven with air circulation (model 410, Nova Etica) at 37 °C for 24 h. The dried membranes were subjected to a secondary crosslinking procedure by immersion in 150 mL of aqueous calcium chloride solution (2% w/v) for 30 min, followed by two washing steps of 30 min each in 200 mL of deionized water. A final drying step was carried out at room temperature for 24 h.

Porous membranes were prepared by adding Poloxamer 188 to the alginate solution at 0.2% (w/v). With the exception of deaeration under vacuum, which was not performed for these formulations, the remaining steps were the same as those described previously.

2.3. Preparation of membranes containing suture threads

For the production of dense and porous composites, linen and cotton suture threads were added in two different geometrical orientations: crossed (long fibers), and at random orientation (short fibers), according to the scheme illustrated in Fig. 1.

To obtain the crossed orientation represented in Fig. 1(a), the Petri dishes used as casts for drying the membranes were perforated laterally with spacing of 0.5 cm between adjacent holes. The threads were passed through the holes to attain the desired orientation and the thread ends were fixed with adhesive tape to the dish side. Previously prepared chitosan-alginate suspensions, with primary crosslinking (added or not with Poloxamer 188), were then poured over the casts and dried at 37 °C for 24 h. The dried composites membranes were removed from the Petri dishes and then subjected to the additional crosslinking procedure as previously described in Section 2.2. The amount of suture thread used was 0.4 g per gram of polymeric solution.

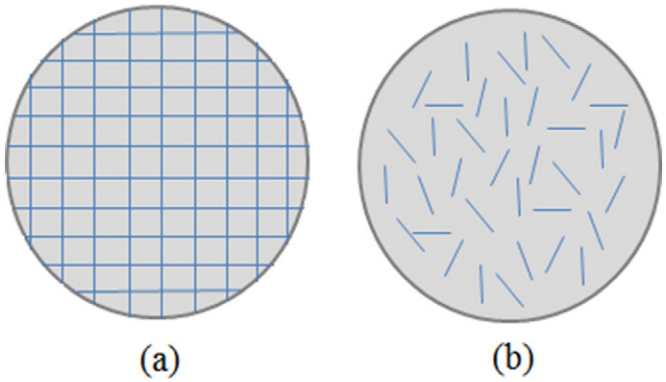

Fig. 1. Representation of the orientation of the suture threads in the polysaccharide matrices: crossed (a) and randomly distributed (b).

The thread for random orientation (Fig. 1b) was cut into 0.5-cm pieces and 0.4 g of each type of fiber were randomly distributed on the Petri dishes containing the previously prepared polymeric solution. The steps of drying and secondary crosslinking were the same as those described for membranes in Section 2.2.

Membranes containing 0.2 g of sutures/g polymeric solution were also produced, but the results obtained for this composition were not reproducible due to the large spaces devoid of fibers in the matrix and hence these composites were not further analyzed. Alternatively, two synthetic absorbable sutures based on polyglactin and polyglycolic acid were used to obtain composites with alginate-chitosan membranes. However, these threads remained weakly attached to the polymeric matrix and their easy detachment from the scaffolds resulted in properties with very low reproducibility. Therefore, their use was discontinued.

For identification purposes, functional membranes containing cotton sutures (C) in crossed (C) and random (R) orientation were designated C_C, and C_R, respectively. Similarly, membranes produced with linen sutures (L) with different orientations were designated L_C, and L_R. The dense membranes were identified by the letter D (C_i-D or L_i-D) and porous membranes were denoted by the letter P (C_i-P or L_i-P).

2.4. Growth factor hEGF incorporation in the membranes

Growth factor was incorporated in porous membranes produced with linen. All steps of membrane production were performed as previously mentioned, except for the ones described as follows. After primary crosslinking of the CA PEC with calcium ions, the lyophilized hEGF previously dissolved in 10 mM acid acetic solution to a concentration of 1 mg/mL was added to the polymers' suspension at the proportion of 30 μg per gram of total polysaccharide. To prevent hEGF degradation, drying of these membranes was performed at 25 °C. These membranes were designated EGF_1.

2.5. Membrane characterization

Membrane thickness, water vapor permeability (WVP), mechanical properties, opacity and morphological analysis (SEM) were performed using the procedures reported by Bierhalz et al. [11]. Briefly, the thickness of the membranes was measured at 10 random positions with a digital micrometer (MDC-25S; Mitutoyo, Japan). For water vapor