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A bioactive film based on cashew gum polysaccharide for wound dressing applications

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ABSTRACT

This work presents the development of a new bioactive material for wound therapeutics which may play a dual role of modulate metallo proteinases activity while prevents infection blocking out pathogenic microorganisms and foreign materials. A CGP/PVA film was activated by covalent immobilization of trypsin. Results from biocompatibility test revealed that PDL fibroblasts grown on the surface of CGP/PVA and the high amount of viable cells proved absence of cytotoxicity. Trypsin immobilized onto CGP/PVA film remained 100% active after 28 days stored dried at room temperature. In addition, CGP/PVA-trypsin film could be used for 9 cycles of storage/use without loss of activity. After immobilization, trypsin retained its collagenolytic activity, indicating this material as a promising material for wound dressing applications.

1. Introduction

The wound healing stands as very complex and dynamic process, aiming the re-establishment of the damaged tissue's integrity and functionality. During the wound repair, the content of metallo proteinases (MMP) and risks of infection are two factors that can retard the progression toward closure a (Adhirajan, Shanmugasundaram, Shanmuganathan, & Babu, 2009; Bertoncelj et al., 2014). Hence the developing of biopolymer-based materials capable of positively regulate MMP balance, as well as provide a physical protection is considered vital in achieving faster healing of wounds (Chikazu et al., 2010; Vachon & Yager, 2006).

In the recent years, there was an increasing effort in the development of biomaterials that possess functionality providing physical protection and an optimal moisture environment for the wound (Vachon & Yager, 2006). A biomaterial can be defined as a material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body (Nair & Laurencin, 2007). The essential prerequisite to qualify a material as a biomaterial is biocompatibility, which is the ability of a material to perform with an appropriate host response in a specific application. Recently, researches on biomaterials for medical and related applications have moved from biostable biomaterials to biodegradable (hydrolytically or enzymatically degradable). This occurred mainly because of the increasing demand for eco-friendly materials leading to the search for alternative products obtained from renewable sources (Nair & Laurencin, 2007). One of the many approaches to produce eco-friendly materials is to blend biodegradable plastics with natural polymers (Batista, Lopes, Yamashita, & Fernandes, 2013; Lopez-Rubio, Gavara, & Lagaron, 2006; Silva et al., 2012; Silva, Ulhoa, Batista, Yamashita, & Fernandes, 2011; Yamashita, Nakagawa, Veiga, Mali, & Grossmann, 2005).

Deepening of knowledge in biomaterials for medical application has resulted in development of novel materials for wide variety of pathological conditions. Current researches have been shown that the covalent attachment of enzyme inhibitors and complexing agents to the carrier matrix display strong inhibition of metallo proteinases (Adhirajan et al., 2009; Schönfelder et al., 2005). Furthermore, the immobilization of proteolytic enzymes can display important role in the modulation of MMP activity as well as in the remodeling process during wound healing (Monteiro et al., 2007; Nikolic et al., 2010).

In this work, a biodegradable, biocompatible and bioactive film with promising biomedical application was produced by blending cashew gum polysaccharide (CGP) and polyvinyl alcohol (PVA).

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The CGP/PVA film was functionalized to allow its bio-activation by covalent attachment of trypsin.

2. Materials and methods

2.1. Materials

Samples of the cashew gum were collected from trees of *Anacardium occidentale* at CIALNE farm, Pacajus, Ceará, Brazil. Trypsin from bovine pancreas (EC 3.4.21.4) in a powder form, *N*-benzoyl-DL-arginine *p*-nitroanilide hydrochloride (BApNA), and polyvinyl alcohol were purchased from Sigma (St. Louis, MO, USA). All other chemicals used were of analytical grade.

2.2. Purification of cashew gum polysaccharides (CGP)

Nodules from cashew gum were milled, immersed in distilled water in a proportion of 20% (w/v), and kept under stirring at room temperature (25 °C), for 24 h. The solution was filtered to remove fragments of bark and then precipitated with ethanol, in the proportion of 1:3 (v/v), for 24 h. The precipitated cashew gum polysaccharide (CGP) was separated by filtration, washed with ethanol, dried and stored at room temperature (25 °C) in hermetically closed vials.

2.3. Production of the biodegradable film

The biodegradable film was composed of a blend of CGP and polyvinyl alcohol (PVA) as follows. The film was prepared by casting on glass molds a blend of 100 mL of 6% (w/v) PVA aqueous solution (pH 2.0), 100 mL of 6% (w/v) CGP aqueous solution, 1 mL of 1.0 mol L^{-1} sodium metaperiodate solution, 20 mL of 1.0 mol L^{-1} phosphoric acid solution and 200 mg of mannitol. The solvent evaporation was left to occur at room temperature and the dried film was exhaustively washed with distilled water until complete removal of the excess of iodine. The films were then dried at room temperature and stored in plastic vials.

2.4. Scanning electron microscopy (SEM)

The microstructure of CGP/PVA films before and after trypsin immobilization were observed using scanning electron microscopy (JEOL microscope, 6610). Before the analysis each film was fixed on a support using double side adhesive tape, placed horizontally with an angle of 90°. Then samples were gold coated with a sputter coater (BALTEC AG, Balzers, Liechtenstein) under 15 mA for 60 s. All samples were examined using an accelerating voltage of 20 kV.

2.5. Immobilization of trypsin

The covalent immobilization of trypsin onto CGP/PVA film was tested by adding 1 mL of trypsin solution (0.1 mg mL^{-1}) to a strip (1 cm^2) of CGP/PVA previously activated with 0.1 mol L^{-1} sodium metaperiodate solution (Silva et al., 2012). After immobilization the strip of CGP/PVA-Trypsin was exhaustively washed with 0.1 mol L^{-1} Tris–HCl buffer, pH 8.0 in order to remove unbounded enzyme.

The optimum immobilization conditions were determined using a Central Composite Rotatable Design (CCRD). The parameters and their levels selected for the study of trypsin immobilization onto CGP/PVA film were: pH (4.0, 6.0, 8.0), immobilization time (15, 30 and 60 min) and immobilization temperature (5 °C, 15 °C and 25 °C). In addition, a central point (pH 6.0, 30 min and 15 °C), with two replicates was also included for statistical evaluation (Table 1).

The CCRD were analyzed coupled to Response Surface Methodology (RSM) by using the software Statistica 6.0 (Statsoft Inc., Tulsa, USA, 1997). The adjustment of the experimental data for the independent variables in the RSM was represented by the second-order polynomial equation (Eq. (1)):

$$y = \beta_0 + \sum_j \beta_j x_j + \sum_{i<j} \beta_{ij} x_i x_j + \sum_j \beta_{jj} x_j^2 + e \quad (1)$$

where y is the dependent variable to be modeled; β_0, β_j, β_{ij} and β_{jj} are regression coefficients, x_i and x_j are independent variables and e is the error. The model was simplified by dropping terms that were not statistically significant by ANOVA ($p = 0.05$).

2.6. Trypsin activity assay

The activity of free and immobilized enzyme was determined according to methodology described by Erlanger, Kokorky, and Cohen (1961) with modifications. Briefly: 50 μL of trypsin solution (0.1 mg mL^{-1}), 800 μL of 0.1 mol L^{-1} Tris–HCl buffer, pH 8.0 and 150 μL of 4 mmol L^{-1} BApNa solution prepared in dimetilsulfoxide were incubated at 37 °C for 25 min. After reaction, the rate of hydrolysis was measured by the readings of the absorbance at 405 nm. One unit of activity (U) was expressed as the amount of enzyme required to release 1 μmol of *p*-nitroaniline per min by use of the molar extinction coefficient of *p*-nitroaniline at 405 nm of 9100 M^{-1} cm^{-1}. The activity of trypsin immobilized onto CGP/PVA films was measured as described, except that trypsin solution was replaced by 1 cm^2 of CGP/PVA-trypsin film suspended in 0.1 mL of 0.1 mol L^{-1} Tris–HCl buffer pH 8.0. In addition, the course of BApNa hydrolysis reaction for CGP/PVA-trypsin was also evaluated.

2.7. Effect of pH and temperature on the trypsin activity

The effect of temperature and pH on the activity of the CGP/PVA-trypsin was evaluated by using a Central Composite Rotatable Design (CCRD) 2^2, using alpha for rotatability (1.41). For the experimental design, the two independent variables were used, both represented in two levels: for temperature, it was used 18 °C (low level) to 38 °C (high level); for the pH, it was used 5 (low level) to 9 (high level). Two star points (13.9 °C and 42.1 °C for temperature; pH 4.2 and 9.8) and a central point (28 °C; pH 7) with two replicates, were included for statistical evaluation (Table 2).

Results from CCRD coupled to Response Surface Methodology (RSM) were analyzed using the software Statistica 6.0 (Statsoft Inc., Tulsa, USA, 1997). The adjustment of the experimental data for the independent variables in the RSM was represented by the second-order polynomial equation (Eq. (2)):

$$y = \beta_0 + \sum_j \beta_j x_j + \sum_{i<j} \beta_{ij} x_i x_j + \sum_j \beta_{jj} x_j^2 + e \quad (2)$$

2.8. Storage and operational stability

For storage stability studies, the CGP/PVA-trypsin was kept at room temperature (dried film) and 4 °C (CGP/PVA-trypsin in 0.1 mol L^{-1} glycine buffer pH 3.6 containing 0.6 mmol L^{-1} calcium chloride). The activity of immobilized enzyme was determined by BApNA hydrolysis, as described above.

Operational stability, defined as the combination of storage and repeated use, was also tested by storing PVA/CGP-trypsin in the conditions described above, followed by cycles of repeated use. The BapNa hydrolysis was assessed at regular intervals. After each cycle of BApNA hydrolysis reaction, the CGP/PVA-trypsin film was washed twice with 0.1 mol L^{-1} Tris–HCl buffer pH 8.0 and stored for further use.