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Manufacture and characterization of chitosan/PLGA nanoparticles nanocomposite buccal films

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ABSTRACT

Oral bioavailability of *C*-glycosyl flavonoid enriched fraction of *Cecropia glaziovii* (EFF-Cg) is limited due to its chemical complexity. The purpose of this study is the prospective evaluation of chitosan buccal films impregnated with EFF-Cg-loaded nanospheres as a drug delivery system for labial herpes treatment or for buccal administration. EFF-Cg-loaded PLGA nanospheres were prepared by double emulsion solvent evaporation technique. Nanoparticles were embedded into buccoadhesive chitosan films in different concentrations in order to obtain nanocomposite films. Films were characterized in term of morphology, mechanical properties and water absorption test. Furthermore a cytotoxicity assay was analyzed to evaluate the biocompatibility of systems. The results obtained from these analyses revealed that nanocomposite films present transparent appearance in all composition and Scanning Electron Microscopy (SEM) images show a continuous and compact section structure. Compared to the control film, mechanical responses of nanocomposites presented lower tensile strength values and no significant effect on the elongation at break. Dynamic Mechanical Analysis (DMA) tests indicated that increasing of NP concentration caused decreased stiffness and an increased of glass transition temperature values. Direct cytotoxicity test shows that nanoparticles and chitosan films not induce cytotoxic effect. Given the promising results, the study concludes that the developed buccal film impregnated with EFF-Cg-loaded nanospheres could be a promising approach for effective delivery of EFF-Cg.

1. Introduction

Some species from the genus *Cecropia* Loefl. have extensive popular use in Brazil. Those are native to Central and South America and are known as embaúba (Matos & Lorenzi, 2008). Leaves of *Cecropia glaziovii* Snethl are described in traditional medicine for control of high-blood pressure and as diuretic (Costa et al., 2011; Costa, Schenkel, & Reginatto, 2011). Conventionally, the leaves of the plant are boiled in water and the infusion is drunk throughout the day. Previous studies have reported some pharmacological activities of the *Cecropia glaziovii* extracts. The extractive solutions of the leaves exhibited hypotensive (Lima-Landman et al., 2007; Ninahuaman et al., 2007), antiasthmatic (Delarcina et al., 2007), antidepressant (Rocha et al., 2007), antacid/antiulcer (Souccar et al., 2008), hypoglycemic (Arend et al., 2015), anti-inflammatory and antioxidant effects (Muller et al., 2016). Beyond that, a preliminary studies conducted by Silva et al. (2010) evaluated the antiherpes effects of the enriched flavonoid fraction (EFF-Cg) obtained from *Cecropia glaziovii* Snethl aqueous crude extract. The results indicates that EFF-Cg can be regarded as a phytopharmaceutical candidate for the treatment of herpetic infections once that presented the more

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promising antiviral activity against human herpes virus types 1 and 2 compared the other samples.

Nanocomposites are functional materials composed of nanoparticles dispersed inside the polymeric matrix (Arockianathan et al., 2012; Arockianathan, Sekar, Kumaran, & Sastry, 2012). These materials form an interdisciplinary area that brings together biology, materials science, and nanotechnology (Hule and Pochan, 2007 Hule & Pochan, 2007). Many nanocomposites are found proper to be used as drug delivery carriers because they frequently, exhibit remarkably improved properties compared with the pure polymer. The introduction of nanoparticles can reduce the burst drug release effect, increase the stability of drug and provide a slower and more continuous release mode of drugs (Satarkar & Hilt, 2008).

For the production of nanocomposite in biomedical fields the biopolymers including chitosan have frequently been used as materials. This natural polymer presents excellent properties, such as non-toxicity, biocompatibility, biodegradability (Croisier & Jérôme, 2013; Muzzarelli & Muzzarelli, 2005; Wang, Du, Luo, Lin, & Kennedy, 2007; Wu, Wei, Wang, Su, & Ma, 2007). These properties combined with some of the polymeric properties of chitosan have made this polysaccharide as a promising candidate for tissue engineering (Croisier & Jérôme, 2013; Di Martino, Sittinger, & Risbud, 2005) and drug delivery applications (Bernkop-Schnürch & Dünnhaupt, 2012). Linear structure of the chitosan results in tough, flexible and transparent films (Tharanathan, 2003; Vieira, Da Silva, Dos Santos, & Beppu, 2011). There are several reports about the drug release behavior from the chitosan nanocomposites in the biomedical field with different applications and the vastly different functional requirements for each of these applications (Venkatesan et al., 2011).

The production of nanocomposites based on chitosan has been the focus of extensive studies in the literature for different applications. Chitosan/TiO_2 nanocomposites were synthesized and demonstrated a stronger inhibition in growth of rice bacterial pathogen *Xanthomonasoryzae*. The results suggested that nanocomposite is promising to be developed antibacterial materials (Li et al., 2016). Chitosan-silver nanocomposites were prepared and reported as a potential material as antimicrobial and anticancer agents in the field of nanomedicine. This nanocomposite was studied with lung cancer cell line (A549) and the synergistic effect of chitosan and silver nanoparticles demonstrated a potent anticancer activity (Arjunan et al., 2016; Arjunan, Kumari, Singaravelu, Kandasamy, & Kandasamy, 2016). Furthermore, Tada et al. (2010) developed a chitosan film containing PLGA nanoparticles for localized dual-drug release. Results showed that this platform might be used as implants for cancer therapy delivering the drugs precisely to the tissue under treatment.

The aim of this work is development and characterization of nanocomposites chitosan films containing PLGA nanoparticles loaded with enriched flavonoid fraction of *C. glaziovii*. The PLGA nanoparticles containing EFF-Cg were developed and characterized in our previous work. (Santos et al., 2016). While, this study focused on the characterization of morphological and mechanical properties, in order to evaluate the advantage of nanocomposites as a drug delivery system for labial herpes treatment. Furthermore the cytotoxic effect of chitosan nanocomposite films was also analyzed.

2. Material and method

2.1. Materials

Biodegradable polymer poly (lactic-co-glycolic) Resomer PLGA 503H (LA:GA 50:50, M_w 24000–38000; inherent viscosity: 0.32–0.44 dL g^{-1}; end group: free carboxylic acid) was purchased from Boehringer Ingelheim (Ingelheim am Rhein, Germany) and stored in a freezer prior to use. Freeze-dried enriched flavonoid fraction of *Cecropia glaziovii* Snethl (EFF-Cg) was used as a water-soluble drug. Poloxamer 188 (Kolliphor P188, BASF, Ludwigshafen, Germany) and sorbitan monooleate (SPAN 80, MERCK, Darmstadt, Germany) were used as surfactant in the aqueous and organic phase, respectively. Chitosan was purchased from Sigma Aldrich (St. Louis, USA)with low molecular weight (approximately 50 000–190 000 Da) and degree of deacetylation of 83%. Acetic acid was purchased from Merck (Darmstadt, Germany) and dichloromethane was purchased from Sigma Aldrich (St. Louis, USA) and were of analytical grade. Ultrapure water was obtained from a Milli-Q apparatus (Merck Millipore, Darmstadt, Germany).The cell line used was fibroblast of green monkey kidneys (VERO-ATCC:CCL81)(Department of Clinical Virology, Göteborg University, Sweden). Eagle's minimum essential medium (MEM), and penicillin G, streptomycin and amphotericin B were purchased from Cultilab (Campinas, Brazil); fetal bovine serum (FBS) was obtained from Gibco (Carlsbad, USA). MTT was obtained from Sigma Aldrich (St. Louis, USA) and dimethylsulfoxide (DMSO) was purchased from Merck (Darmstadt, Germany).

2.2. EFF-Cg –loaded PLGA nanoparticles preparation

Nanoparticles were prepared using a modified solvent emulsification-evaporation method based on a w/o/w double emulsion technique (Rescignano et al., 2015). Briefly, 40 mg of EFF-Cg was dissolved in 2 mL of ultrapure water (internal aqueous phase, w_1) and added to 4 mL of dichloromethane containing 100 mg of PLGA and 100 mg of sorbitan monooleate (organic phase, o). The water-in-oil (w_1/o) emulsion was obtained after 15 min, using a tip sonicator (VIBRA CELL Sonics mod. VC 750, Newtown, USA). This primary emulsion was mixed with 40 mL of 5% (w/v) poloxamer 188 solution (external aqueous phase, w_2) using the same sonication conditions describe above to obtain the double emulsion (w_1/o/w_2). For solvent extraction, the double emulsion was evaporated under reduced pressure at 35 °C. The obtained particles were collected by centrifugation at 11000 rpm for 10 min and washed out two times with ultrapure water and then lyophilized. The characterization of the nanoparticles in term of size, polydispersity index and zeta potential was performed immediately after synthesis. The morphological and structural characterization was evaluated after lyophilization step.

2.3. Chitosan nanocomposite film preparation

Chitosan film was prepared by the solvent evaporation/casting technique. Chitosan was dissolved in acetic acid aqueous solution 0.1 M (v/v) in order to obtain 2% (w/v) chitosan solution, under stirring until total homogenization of the mixture. The chitosan films were obtained by drying 20 mL solutions into Petri dishes (9 cm diameter) and dried at 37 °C for 48 h.

Nanocomposite films were obtained by adding EFF-Cg-loaded PLGA nanoparticles during the preparation of the chitosan solution in following concentrations: 3, 5 and 10% of nanoparticles with respect to the weight of chitosan (w/w).

2.3.1. Morphological characterization

The morphology of nanocomposites microstructure was observed by scanning electron microscope (SEM, XL30 Philips, Billerica, USA). Pieces were cut by cryofracture and mounted on specimen holders in such a way that the inner part was visualized. The dried samples were coated with an ultrathin coating of gold deposited on the sample by high-vacuum metallization.

Films thickness was measured using a digital micrometer (Mahr Millitast1085/Göttingen, Germany). Five thickness measurements