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Chitosan-based mucoadhesive films containing 5-aminolevulinic acid for buccal cancer's treatment

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ABSTRACT

Photodynamic therapy (PDT) is a relatively new method to treat various kinds of tumors, including those of the oral cavity. The topical 5-ALA-PDT treatment for tumors of the oral mucosa is preferred, since when administered systemically, there is a general photosensitization drawback in the patient. However, 5-ALA is a hydrophilic molecule and its penetration and retention is limited by topical route, including oral mucosa. We propose a topical delivery system of chitosan-based mucoadhesive film, aiming to promote greater retention of 5-ALA in tissue. The chitosan (CHT) films (4% w/w) were prepared using the solvent evaporation/casting technique. They were tested without 5-ALA resulting in permeability to water vapor (W.V.P = 2.15–8.54 g mm/(h cm^2 Pa) swelling ~300.0% (±10.5) at 4 h or 24 h and *in vitro* residence time >24 h for all tests. CHT films containing 10.0% (w/w) 5-ALA have resulted in average weight of 0.22 g and thickness of 0.608 mm as suitable characteristics for oral application. In the presence of CHT films both *in vitro* permeation and retention of 5-ALA (1.0% or 10.0%) were increased. However, 10.0% 5-ALA presented highest values of permeation and retention (~4 and 17 times respectively, compared to propylene glycol vehicle). On the other hand, *in vitro* mucoadhesion of CHT films was decreased (18.2-fold and 3.1-fold) by 5-ALA addition (1.0% or 10.0% respectively). However, CHT film containing 10.0% of 5-ALA can be a potential delivery system for topical use in the treatment of tumors of the oral cavity using PDT because it favored the retention of 5-ALA in this tissue and has shown convenient mucoadhesion.

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1. Introduction

Oral cancer is the fifth most common cancer in the world and which is usually treated with radical surgical excision, chemotherapy, and radiotherapy, separately or in combination. Although various treatment modalities are used, the survival rate for oral cancer patients remains low [1,2].

Photodynamic therapy (PDT) is an effective treatment for human premalignant and malignant lesions because it is noninvasive, well tolerated by patients, can be used repeatedly without cumulative side effects, and results in little scar formation [3,4]. It involves the use of a class of molecules accumulated in certain abnormal tissues (photosensitizers, PS) and activated by light at specific wavelengths. At these conditions, several ensuing photochemical reactions cause cell damage and death in the cancerous tissue.

5-Aminolevulinic acid (ALA) itself is not a PS but serves as a biological precursor for it, the protoporphyrin IX (PpIX) molecule in the heme biosynthesis pathway [5]. This PS can be activated intracellularly by red light at ~630 nm, forming reactive oxygen species (ROS) that accumulate in cancerous tissue, leading to cell destruction. 5-ALA is unique so far as it can produce reliable photosensitization when administered orally (30–60 mg/kg) [6] intravenously or topically [7]. By either route of administration, maximum tissue concentrations of PpIX are obtained within 4–6 h, followed by an almost equally rapid decline. Within 48 h, tissue levels of PpIX are back to background levels [8,9]. The lack of long-lasting photosensitization is the great advantage of 5-ALA over all other photosensitizers [10]. The greatest advantage for treatment of skin and oral cancers and precancers is that topical route restricts phototoxicity to the application site [4,7,11].

The topical 5-ALA-PDT has been explored in the three most common type of precancerous lesions of the oral cavity: (i) oral leukoplakia [1,10] (ii) oral erythroleukoplakia [1] and (iii) hyperplasia oral verrucosa [1,5,12] which can develop into squamous cell carcinoma or carcinoma oral verrucous. In these cases, PDT

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with topically applied 5-ALA has been used with relatively good clinical outcomes [1,7,12–14]. All these formulations contain 20% of 5-ALA in gel [1,5,12] in cream [10] or in ointment [15]. However, these are not ideal for application to mucosa, since it is necessary to use the mucoadhesive systems to enhance retention of the drug in the tissue for a sufficient time for carrying out the PDT. Moreover, 5-ALA is strongly hydrophilic and therefore not able to enter cells easily, which causes recurrence and a negative response to treatment. In addition, a penetration depth of 2 mm is expected [7,8] but is limited after topical application because 5-ALA cannot penetrate this depth, especially in thicker tumors. Besides the limited permeability of the buccal membrane and the small surface area [16] the continuous secretion of saliva are the major limitations in the development of delivery systems in this region and consequently not a suitable retention of 5-ALA. Compared to gels and ointments for oral application, patches [17–19] and films [20,21] are also more convenient and have received more attention. The polymeric films can cover a large area of the mucosa, playing the function of the drug release as the physical protection of the site. Additionally, an increase in the retention time and reduced frequency of applications (due to the decrease in the amount of drug administered to the oral mucosa) were found when bioadhesive polymer films are used [22,23]. Among the bioadhesive polymers, chitosan (CHT) has been explored due to its good biocompatibility, biodegradability, favorable toxicological and film forming properties [17,24]. Its mechanical properties can support the mechanical action on the application site, allowing their exploration as drug delivery system. CHT films are reported to be associated with several drugs in the literature for oral mucosal administration [25] in many types of cancer [26] including skin [27].

CHT is a cationic polysaccharide produced by deacetylation of chitin, insoluble in water and soluble in acidic solutions, and presents electrostatic interactions with potential bioadhesive forces [28,29]. Mucoadhesion increases contact between polymer/mucus, improving drug release. Moreover, CHT also acts as a penetration enhancer for various hydrophilic drugs, increasing the absorption of these in the oral mucosa by neutralizing the anionic sites of mucosal cells [30,31]. Another interesting property of CHT is as immunoadjuvant, that is, it stimulates the hosťs immune response to the photodestructive effect on the tumor cells in experiments of PDT using CHT [32,33].

The association of 5-ALA and CHT is promising and has been reported in the literature, presenting cytotoxic effects on various tumor lines, both *in vivo* and *in vitro* [34,35]. Pharmacokinetics studies of PpIX [3] have demonstrated that i.p injection of association of 5-ALA and CHT followed by irradiation after 3 h, ensures high concentrations of the PS in the carcinosarcoma in rats, enhancing the PDT efficiency. They as well as other authors have also suggested an antioxidant effect of CHT [36,37].

The present study aims to develop and characterize of chitosan-based mucoadhesive film to release 5-ALA to enhance its retention in oral mucosa for optimization of PDT treatment in this tissue.

2. Material and methods

5-Aminolevulinic acid (purity 98%) was obtained from Dye Pharmaceuticals (Campinas, São Paulo, Brazil). Chitosan (MMW 190,000–310,000 Da) of 85.0% deacetylated and medium viscosity was purchased from a Sigma–Aldrich, U.S.A. Analytical reagent grade sodium phosphate dibasic anhydrous (P.A) and monobasic sodium phosphate anhydrous (P.A) were obtained from VETEC (Rio de Janeiro, Brazil); formaldehyde and propylene glycol from ISOFAR (Rio de Janeiro, Brazil) and Lactic acid (85–90% purity) from PROQUIMIOS (Rio de Janeiro, Brazil).

2.1. Methods

2.1.1. Pig buccal (Cheek) mucosa

The cheek mucosa obtained from the slaughterhouse shortly after the death of the animal (*UFRRJ*, *Seropédica*, *RJ*) was cleaned, separated from underlying tissue (muscle, fat, and skin), and frozen at −20 °C until use. They were obtained in a slaughterhouse, dissected, cleaned and stored according to descriptions in the literature [38,39] since they are very similar to human tissue in terms of barrier lipid composition, histology, ultrastructural organization and permeability [40,41].

2.1.2. Preparation of chitosan films

Chitosan (CHT) films were prepared by the solvent evaporation/casting technique [29] varying the final weights (0.4–1.0 g) and drying times (18.5–25.0 h) using a climatic chamber. Pure chitosan films (without drug) were obtained by dispersion of appropriate amounts of CHT in 2.0% v/v lactic acid aqueous solution stirred under mechanical agitation until homogenization, to yield 4.0% (w/w) of CHT films. Propylene glycol (PG) at 10% (w/w) was added as plasticizer. Films were loaded with weighed amounts of 5-ALA to a final concentration of 1.0% or 10% (w/w). These mixtures were subjected to tests of homogenization times in ultrasound bath (30 min to 5 h) for removing air bubbles and complete homogenization of the components. The solutions were individually cast in plastic circular bases (area = 1.77 cm^2) following drying in a climatic chamber at 37 °C (±2 °C) and relative humidity of 60–65% for a period of time (18.5–25 h) (in the absence or presence of 5-ALA). Each film added by different concentrations of 5-ALA had its respective control, that is, 5-ALA in PG vehicle.

2.1.3. Characterization of films

The film samples prepared with CHT and 5-ALA (1% or 10% w/w) were subjected to the tests of: weight, film thickness, pH measurements and mucoadhesion. Permeability to water vapor (P.W.V), water absorption (swelling), and *in vitro* residence time were carried out in the films without 5-ALA.

2.1.3.1. Weight, thickness and pH determination. The films of CHT (with or without 5-ALA, $n = 6$) were individually weighed on a digital analytical scale [24]. The thicknesses of CHT films (with or without 5-ALA, $n = 6$) was measured using a digital micrometer, in five different parts of the film area (center and four corners) and the average value was determined by five determinations [42].

The pH of the 4% (w/w) CHT solutions was determined before drying step (with or without 5-ALA) using PHS-3B PHTEK potentiometer ($n = 3$).

2.1.3.2. Permeability to water vapor (P.W.V). Permeability of moisture and gases through the film is important to keep it comfortable in the application region. The CHT films without ALA ($n = 6$) were tied on top of open bottles of the same type and size (volume ± 13 mL and test area of 1.77 cm^2) containing silica gel inside (5 g). The bottles were placed in a climatic chamber at temperature of 25 °C (±2 °C) and relative humidity of 50% (±2%) [27,43] at different times (1–48 h). The weights of bottles were recorded individually, before and after the session in the climatic chamber, and P.W.V was calculated in each time (1, 2, 4, 24 and 48 h) according to Eq. (1) [42].

$$\text{P.W.V. (g mm/h cm}^2\text{ pa)} = \frac{\text{WVT.L}}{\Delta P} \quad (1)$$

where W.V.T = water vapor transmission (g/h cm^2) = G/tA, where: G = variation in weight (g), t = time (h), A = test area (cm^2), L = mean thickness of the films (mm), ΔP = difference of partial pressure of

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