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Fabrication of mucoadhesive chitosan coated polyvinylpyrrolidone/cyclodextrin/clotrimazole sandwich patches for oral candidiasis

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ABSTRACT

This study aims to fabricate clotrimazole (CZ)-composite sandwich nanofibers using electrospinning. The CZ-loaded polyvinylpyrrolidone (PVP)/hydroxypropyl-β-cyclodextrin (HPβCD) fiber was coated with chitosan-cysteine (CS-SH)/polyvinyl alcohol (PVA) to increase the mucoadhesive properties and to achieve a sustained release of the drug from the nanofibers. The nanofibers were characterized using scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) spectroscopy and X-ray diffractometry (XRD). The nanofibers mechanical and mucoadhesive properties, drug release, antifungal activity and cytotoxicity were also assessed. The fibers were in the nanoscale with good mucoadhesive properties. The XRPD revealed a molecular dispersion of amorphous CZ in the nanofibers. The initial fast release of CZ from the nanofibers was achieved. Moreover, the sandwich nanofibers coated for longer times resulted in slower release rates compared with the shorter coating times. The CZ-loaded nanofibers killed the *Candida* significantly faster than the commercial CZ lozenges at 5, 15 and 30 min and were safe for a 2-h incubation. Therefore, these nanofibers may be promising candidates for the treatment of oral candidiasis.

1. Introduction

Candidiasis of the mouth and throat, also known as oropharyngeal candidiasis (OPC), is an opportunistic fungal infection that occurs when there is an overgrowth of yeast called Candida. Among the various Candida species, *Candida albicans* is still considered the most important fungal pathogen responsible for this disease (Budtz-Jorgensen, 1990; Wady et al., 2012). Many factors, including immunodeficiency and poor oral hygiene, can predispose a patient to this fungal infection (Park et al., 2015). The antifungal drugs including polyenes and azole antimycotics can be used either topically or systemically (Pallasch, 2002; Tonglairoum, Ngawhirunpat, Rojanarata, Kaomongkolgit, & Opanasopit, 2015).

Clotrimazole (CZ) is a wide spectrum triazole-based antifungal agent. CZ inhibits the enzyme cytochrome P450 14a-demethylase, which is required in fungal cell membrane synthesis. CZ has antimycotic activity against Candida spp., Cryptococcus spp., dermatophytes and Aspergillus spp. (Paradkar, Thakkar, Soni, Gandhi, & Gohel, 2015). However, CZ exhibits poor aqueous solubility (0.49 μg/ml) (Hoogerheide & Wyka, 1982) that may affect its antimycotic activity. Thus, increasing the solubility of CZ is necessary to increase its antimycotic activity. Cyclodextrins (CDs) are cyclic oligosaccharides composed of 1,4-linked glucopyranoside units that can form inclusion complexes with a broad range of drug molecules. These inclusion complexes have altered physicochemical properties from the individual drug molecules (Del Valle, 2004). Complexation of poorly soluble drugs with cyclodextrins can improve the dissolution and stability of the drugs (Albers & Muller, 1995; Bandi, Wei, Roberts, Kotra, & Kompella, 2004).

In OPC therapy, topical treatments are becoming increasingly popular due to greater patient compliance and reduced side effects (Pallasch, 2002; Mason et al., 2012). Topical dosage form for oral candidiasis are administered *via* suspensions, mouth rinses, gels and troches (Akpan & Morgan, 2002; Ellepola & Samaranayake, 2000; Ramesh & Reddy, 2010). However, salivary clearance of the drug can rapidly decrease the drug level in the affected area. An ideal dosage form for the treatment of oral candidiasis should provide sustained drug release and produce an antifungal effect

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for a prolonged period of time. These characteristics are achievable if the drug delivery system demonstrates sustained release, and mucoadhesive properties (Collins, Cookinham, & Smith, 2011; Gajra, Pandya, Singh, & Rabari, 2014).

Mucoadhesive controlled release systems have attracted great interest in recent years. Mucoadhesive polymers would be a promising candidate for these systems. Chitosan is an excellent natural mucoadhesive polymer that has been widely used in many fields because of its marvelous properties such as biocompatibility, biodegradability, renewability, and non-toxicity (Denkbaş & Odabaşi, 2000; Guibal, 2005). Chitosan has been shown to possess mucoadhesive properties owing to electrostatic interactions between positively charged chitosan and negatively charged mucous membrane (Bernkop-Schnürch, Guggi, & Pinter, 2004). However, chitosan has been modified in many research studies in order to have better mucoadhesive properties. One modification was done with the immobilization of thiol groups to chitosan to generate thiolated chitosan so as to form disulfide bonds with cysteine-rich subdomains of mucus glycoproteins (Bernkop-Schnürch, 2005). The improvement of mucoadhesive property of chitosan is expected to increase the contact time of the drug with the suitable areas.

Recently, extensive research on drug delivery systems has been performed to improve therapeutic effects and to reduce the toxicity of conventional dosage forms. Electrospun nanofibers offer great flexibility in the selection of materials or drugs for drug delivery applications. Moreover, electrospun nanofibers exhibit marvelous characteristics such as high loading capacity, high encapsulation efficiency, they provide coincident delivery of various compounds, and are cost effective (Arthanari et al., 2014; Hu et al., 2014; Van Roey, Haxaire, Kamya, Lwanga, & Katabira, 2004). In our previous study, the fast dissolving CZ-loaded nanofiber mats were fabricated using PVP/HPβCD as the fiber forming polymer. The PVP/HPβCD nanofibers exhibited fast release and antifungal activity. However, for the treatment of oral candidiasis, not only is the fast antifungal activity required, but a sufficiently prolonged period where the drug concentration is above the minimum inhibitory concentration (MIC) would also be more beneficial than a system that only achieves a high CZ concentration for a short period (Tonglairoum, Ngawhirunpat, Rojanarata, Kaomongkolgit, & Opanasopit, 2014). Therefore, the new type of nanofibers (sandwich nanofibers) was developed in order to increase the mucoadhesive properties of the nanofiber mats to facilitate prolong contact with oral mucosa. Moreover, the CS/PVA nanofiber can swell and control the release of the drug with prolong period. In the present investigation, hydrophilic CZ-composite sandwich nanofibers were developed using electrospinning. The CZ-loaded polyvinylpyrrolidone (PVP)/hydroxypropyl-β-cyclodextrin (HPβCD) inner fiber was coated with chitosan-cysteine (CS-SH)/polyvinyl alcohol (PVA) to increase the mucoadhesive properties of the nanofiber mats, and to sustain the release of the drug from the nanofiber mats. The physicochemical properties, release characteristics, loading efficacy, antifungal activity, and cytotoxicity of the CZ-composite sandwich nanofibers were investigated through *in vitro* studies.

2. Materials and methods

2.1. Materials

Clotrimazole (CZ), 2-hydroxypropyl-β-cyclodextrin (HPβCD), polyvinylpyrrolidone (PVP, MW. ~1,300,000), chitosan (degree of deacetylation 0.85, MW 110 kDa) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Chemical Co. (St. Louis, MO, USA). L-Cysteine hydrochloride, monohydrate, was purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Polyvinyl alcohol (PVA) (degree of polymerization ≈1600, degree of hydrolysis ≈97.5–99.5 mol%) was purchased from Fluka. Sabouraud dextrose broth was purchased from Becton, Dickinson and Company (Franklin Lakes, NJ, USA). The human gingival fibroblasts (HGF) were obtained from the Faculty of Dentistry, Naresuan University, Thailand. Dimethyl sulfoxide (DMSO) was obtained from BDH Laboratories, UK. Dulbecco's modified Eagle's medium (DMEM), trypsin-EDTA, penicillin–streptomycin antibiotics and fetal bovine serum (FBS) were obtained from GIBCO-Invitrogen (Grand Island, NY, USA). All other reagents and solvents were of analytical grade and were used without further purification.

2.2. Synthesis of thiolated chitosan (CS-SH)

Thiolated chitosan was synthesized by reacting chitosan and cysteine, the thiol group carrier, using 1-ethyl-3-(3-dimethylamino propyl) carbodiimide (EDAC) as the catalyst. Briefly, CS and cysteine were dissolved in 1% v/v HCl and deionized water, respectively. The cysteine carboxylic acid group was activated using 150 mM EDAC for 20 min. The pH of the system was controlled within the range of 4–5 throughout the experiment. The solutions were stirred for 6 h, and then the CS-SH was isolated by dialyzing the mixture for 3 days. The sample was frozen and dried at −49 °C and 0.07–0.09 bar (Freezone 2.5, Labconco, UK). The amount of thiol was assessed using Ellman's reagent. Briefly, 250 μL of a standard cysteine and the sample were mixed with 50 μL of Ellman's reagent in Eppendorf tubes. All tubes were incubated for 15 min before the absorbance at 412 nm was measured using UV–visible spectrophotometer (PG Instrument, Oasis Scientific Inc., USA). The cysteine standards were used to estimate the amount of thiol immobilized on the CS-SH.

2.3. Fabrication of CZ-loaded PVP/HPβCD nanofibers

The CZ-loaded PVP/HPβCD nanofiber mats, used as the inner fiber, were fabricated by electrospinning as previously described (Tonglairoum et al., 2014). Briefly, 8% PVP and 70 mM HPβCD were dissolved in an ethanol:water:benzyl alcohol (EtOH:H_2O:BzOH) solvent mixture using a 70:20:10 volume ratio. Clotrimazole (20 wt% to polymer) was added into the mixture and stirred for 12 h at room temperature. The electrospinning process was performed at 25 °C with a fix applied voltage of 15 kV. The distance between the tip and the collector was 15 cm, and the feeding rate was 0.4 mL h^{-1}.

2.4. Fabrication of CZ-incorporated mucoadhesive sandwich nanofibers

CZ-loaded PVP/HPβCD nanofibers were used as the inner fiber membrane. The inner fiber membranes were subsequently coated by electrospinning with a 2% CS-SH/EDTA and 10% PVA mixture or a 2% CS/EDTA and 10% PVA mixture (with a weight ratio of 30:70) for 3 h and 6 h, respectively. The electrospinning process was conducted at 25 °C at a fixed applied voltage of 15 kV. The distance between the tip and the collector was 15 cm, and the feeding rate was 0.3 ml h^{-1}.

2.5. Characterization of the mucoadhesive sandwich nanofibers

2.5.1. Scanning electron microscope (SEM)

The morphological appearances of the nanofiber mats were observed using a scanning electron microscope (SEM, Camscan Mx2000, England). Each of the fiber mats samples was sputtered with a thin layer of gold prior to SEM observations. Based on these SEM images, the average diameter of the fibers was measured using image analysis software (JMicroVision V.1.2.7, Switzerland).