Cellulose Acetate Phthalate Microencapsulation and Delivery of Plasmid DNA to the Intestines

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ABSTRACT: Cellulose acetate phthalate (CAP) microcapsules were formulated to deliver plasmid DNA (pDNA) to the intestines. The microcapsules were characterized and were found to have an average diameter of $44.33 \pm 30.22\ \mu\text{m}$, and were observed to be spherical with smooth surface. The method to extract pDNA from CAP was modified to study the release profile of the pDNA. The encapsulated pDNA was found to be stable. Exposure to the acidic and basic pH conditions, which simulates the pH environment in the stomach and the intestines, showed that the release occurred in a stable manner in the former, whereas it was robust in the latter. The loading capacity and encapsulation efficiency of the microcapsules were low but the CAP recovery yield was high which indicates that the microcapsules were efficiently formed but the loading of pDNA can be improved. *In vitro* transfection study in 293FT cells showed that there was a significant percentage of green-fluorescent-protein-positive cells as a result of efficient transfection from CAP-encapsulated pDNA. Biodistribution studies in BALB/c mice indicate that DNA was released at the stomach and intestinal regions. CAP microcapsules loaded with pDNA, as described in this study, may be useful for potential gene delivery to the intestines for prophylactic or therapeutic measures for gastrointestinal diseases. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:617–626, 2013
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INTRODUCTION

Genetic materials were shown to be delivered through oral route by adenovirus,[1] liposomes,[2] and biodegradable polymers.[3,4] The potential applications of oral gene delivery are gene therapy and genetic vaccines, which could benefit the management of gastrointestinal disorders and diseases.[5]

Oral delivery of substances may benefit from coating core materials with microparticulate sizes.[6] Encapsulation allows controlled release, which protects the encapsulated material from rapid degradation and prolongs its bioactivity.[7] The rate of release is gradual and depends on the rate of erosion of the encapsulation material.[8] Biodegradable polymers such as poly(D,L-lactic-*co*-glycolic) acid (PLGA)[3] and chitosan[4] are often used as microparticulate carriers in pharmaceuticals. They are designed to encapsulate, carry, and release pharmaceutical agents at targeted sites, while protecting the pharmaceutics

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against the biological environments such as the acidic gastric juice.[8]

Cellulose acetate phthalate (CAP) has been used as a microparticulate carrier for drugs,[6,9] vitamins,[10] hormones,[11] and probiotics.[12] CAP is a synthetic polymer, a derivative of cellulose, which is the most abundant organic polymer. As vaccine carriers, in particular, a vaccine against *Mycoplasma hyopneumoniae*,[13] as well as an oral hepatitis B vaccine, was developed with CAP encapsulation.[14] However, CAP is yet to be developed as a gene carrier.

Cellulose acetate phthalate functions as an enteric coating because of the presence of ionizable phthalate groups.[13] CAP is enteric and its solubility depends on the pH. It was characterized as being insoluble at pH less than 6 and soluble at pH greater than 6,[15] and a more recent study reported it as being insoluble at pH less than 3 and dissolves at pH more than 5.[16] It is able to protect encapsulated materials against the gastric acid in stomach and gradually releases the encapsulated materials in the slightly neutral to alkaline conditions in intestines.

Mucoadhesive CAP microcapsules[17] will randomly bind onto mucosal epithelial layer of the gut and degrade gradually to release the core material. In terms of safety, CAP is generally nontoxic for use in human biological system.[18] Furthermore, CAP formulation was found to release the core material in a sustained-release pattern.[7,19] Because of these properties, CAP has been in use in the pharmaceutical industry as an enteric coating or excipient for drugs. CAP-coated drugs are available in the form of tablets or capsules.

The study aimed to formulate and characterize CAP microcapsules for plasmid DNA (pDNA) encapsulation and subsequent oral administration.

MATERIALS AND METHODS

Materials

Cellulose acetate phthalate was purchased from Fluka (Buchs, Switzerland); phosphate-buffered saline (PBS) and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma–Aldrich (St. Louis, Missouri); acetone and hydrochloric acid (HCl) were purchased from J.T. Baker (Phillipsburg, New Jersey); sodium carbonate (Na_2CO_3) was purchased from Promega (Madison, Wisconsin); liquid paraffin, Span 80, and n-hexane were purchased from Merck (Hohenbrunn, Germany), Tris was purchased from Bio-RAD (Richmond, California), sodium acetate was purchased from Amresco (Solon, Ohio), and 95% ethanol was purchased from SYSTERM ChemAR® (Kielce, Poland).

Plasmid pVAX1© vector (Invitrogen, Carlsbad, California) was encapsulated in the CAP microcapsules for characterization of properties of resultant microcapsules. The plasmid vector carrying hMGFP (green fluorescent protein) was encapsulated in CAP microcapsules for *in vitro* gene expression assay. The pVAX–GFP reporter gene plasmid vector was constructed by Nograles et al.[20] DNA molecular weight marker of 1 kb was purchased from Thermo Scientific (Waltham, Massachusetts).

293FT cell line, Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), and Lipofectamine™ 2000 transfection agent were purchased from Invitrogen.

Female, 6–8-week-old BALB/c mice were purchased from Institute for Medical Research, Kuala Lumpur, Malaysia. All the animal studies were approved by the Animal House and Use Committee, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (ACUC approval no.: UPM/FPSK/PADS/BR-UUH/00443).

Formulation of CAP Microcapsules Loaded with pDNA

Cellulose acetate phthalate microcapsules were produced using the oil/water solvent evaporation method. This method, which includes pDNA into the solution, was modified from a formulation designed to encapsulate proteins.[21] One percent (w/v) of CAP was dissolved in 9:1 acetone to form 95% ethanol. Known amount of pVAX was added to this polymer solution. The polymer–pDNA solution was dropped into oily phase consisting of liquid paraffin and 1% (v/v) of Span 80. The emulsion was agitated using magnetic stirrer at 600 rpm to allow evaporation of acetone until microspheres have been formed and hardened. The microspheres were then collected by filtration and washed with n-hexane to remove excess liquid paraffin.[13,21] The microcapsules were subsequently stored at -20°C until use.

Size and Morphology

The morphology of CAP microcapsules was analyzed through optical and electron microscopy. The average size of microcapsules was determined by measuring the diameter of the microcapsules. The sizes of the microcapsules were determined using the inverted light microscope (Eclipse TS100; Nikon, Tokyo, Japan) set up with a digital camera (DS-Fi1; Nikon) and imaging software (NIS-Elements D 3.0; Nikon). The mean size was determined for approximately 100 randomly selected microcapsules of each set of preparation. The average size was calculated from the means of triplicate independent sets. CAP microcapsules that were empty (not loaded with pDNA) and loaded with pDNA were dried at 60°C overnight. The microcapsules were then metallized by gold coating (BAL-TEC SCD 005 Sputter Coater) and analyzed by scanning electron microscopy (LEO 1455 variable-pressure scanning electron microscope; Carl Zeiss, Oberkochen, Germany).