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Self-assembling bubble carriers for oral protein delivery

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ABSTRACT

Successful oral delivery of therapeutic proteins such as insulin can greatly improve the quality of life of patients. This study develops a bubble carrier system by loading diethylene triamine pentaacetic acid (DTPA) dianhydride, a foaming agent (sodium bicarbonate; SBC), a surfactant (sodium dodecyl sulfate; SDS), and a protein drug (insulin) in an enteric-coated gelatin capsule. Following oral administration to diabetic rats, the intestinal fluid that has passed through the gelatin capsule saturates the mixture; concomitantly, DTPA dianhydride produces an acidic environment, while SBC decomposes to form CO_2 bubbles at acidic pH. The gas bubbles grow among the surfactant molecules (SDS) owing to the expansion of the generated CO_2. The walls of the CO_2 bubbles consist of a self-assembled film of water that is in nanoscale and may serve as a colloidal carrier to transport insulin and DTPA. The grown gas bubbles continue to expand until they bump into the wall and burst, releasing their transported insulin, DTPA, and SDS into the mucosal layer. The released DTPA and SDS function as protease inhibitors to protect the insulin molecules as well as absorption enhancers to augment their epithelial permeability and eventual absorption into systemic circulation, exerting their hypoglycemic effects.

1. Introduction

Oral delivery of therapeutic proteins such as insulin can avoid the pain and discomfort associated with injections and greatly improve the quality of life of patients. Nevertheless, oral absorption of insulin is limited by hostile gastric and intestinal environments and by the poor epithelial permeability [1]. Experiments have shown that enteric-coated gelatin capsules containing insulin with protease inhibitors and absorption enhancers orally administered to rats can achieve increased plasma insulin levels and corresponding reductions in blood glucose [2]. The enteric coating is a pH-responsive polymer barrier, so the capsule remains intact in the highly acidic pH environment of the stomach; conversely, as the body fluid from the intestinal tract (neutral or slightly basic pH) comes into contact with the enteric-coated capsule, the water soluble polymer and gelatin dissolve and the drug load diffuses through the resulting pores.

However, the enteric polymer coated on the gelatin capsule does not dissolve instantly or completely in the small intestine because of its partial contact with the body fluid. Thus, a certain amount of the encapsulated protein drugs within the capsule might remain stuck within the partially dissolved capsule (Fig. 1). Another problem is that protein molecules may aggregate during their exposure to body fluid, which can limit their interaction with and entrance into epithelial cells and reduce their oral bioavailability. Therefore, increasing the bioavailability of oral protein drugs to a therapeutically acceptable level is still challenging.

To provide quick and effective delivery of a drug load from enteric-coated gelatin capsules, this study develops a novel carrier system that involves a foaming agent (sodium bicarbonate; SBC) that can generate CO_2 bubbles upon exposure to an acidic aqueous environment. The walls of these CO_2 bubbles consist of a nanoscale self-assembled film of water that can be sandwiched between two

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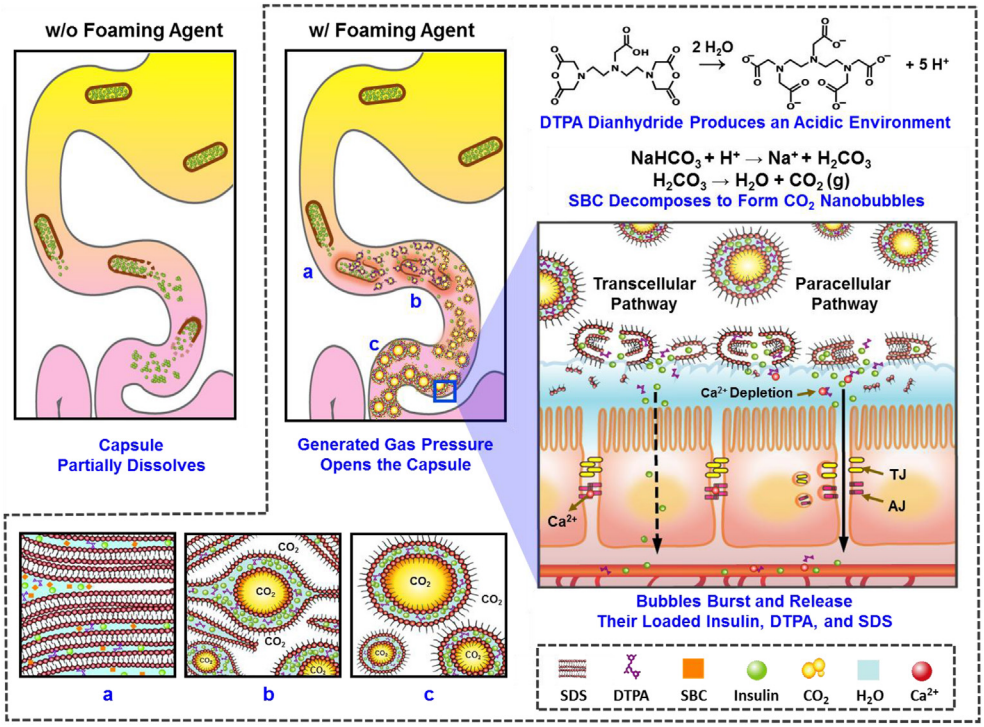

Fig. 1. Schematic illustrations showing the formation and expansion of bubble carriers (see subfigures a, b, and c) developed from the orally administered gelatin capsule and their mechanism for delivering insulin across the epithelial barrier. TJ: tight junction; AJ: adherens junction.

layers of surfactant molecules to form a colloidal carrier for transporting protein drugs. Absorption can be increased by formulating the drug as a solubilizate within a colloidal dispersion [3]. This bubble carrier system is prepared by mixing diethylene triamine pentaacetic acid (DTPA) dianhydride, SBC, a surfactant (sodium dodecyl sulfate; SDS), and a protein drug (insulin). The DTPA dianhydride is an acidic oxide that dissolves in water to form an acid (DTPA) solution [4]. When reacting with the acid, SBC produces a bubbly CO_2 gas [5].

Fig. 1 shows a schematic of this bubble carrier system and how it works. When the water that passes through the gelatin capsule saturates the mixture, nanosized CO_2 bubbles are generated. As the gas pressure opens the capsule, the load is quickly released. The gas bubbles grow among the surfactant molecules, expanded by the generated CO_2. The reaction also proceeds outside the formed bubbles, which are therefore effectively suspended in a gas-rich environment. The SDS is an anionic surfactant consisting of a hydrophilic (water-loving) head and a hydrophobic (water-hating) tail. As the gas bubbles expand, the hydrophobic tail of each surfactant molecule is attracted to the gas-rich phase while their hydrophilic head is attracted to the water phase. Therefore, a bubble carrier system comprising a water film containing insulin and DTPA is self-assembled between double layers of surfactant molecules (SDS). The gas bubbles continue to expand until they come in contact with the mucosal layer covering the intestinal wall and eventually burst and release their loads of insulin, DTPA, and SDS. In the mucosal layer, DTPA serves a paracellular enhancer for delivering insulin molecules through the epithelial cells, and the amphiphilic surfactant (SDS) enhances their paracellular and transcellular permeability. Both DTPA and SDS also protect insulin molecules by functioning as protease inhibitors [2,6].

We hypothesize that the proposed bubble carrier system can mediate temporary alterations in the morphology of epithelial cell membranes and transient opening of their apical junctional complexes (AJCs), which facilitates the encapsulated insulin molecules in crossing the epithelial barrier and eventually being absorbed into systemic circulation, where they exert hypoglycemic effects.

2. Materials and methods

2.1. Materials

The SBC, DTPA dianhydride, SDS, and insulin (from bovine pancreas, 27.4 IU/mg) were obtained from Sigma–Aldrich (St. Louis, MO, USA). The fluorescein isothiocyanate (FITC)-insulin (bovine) and cyanine 3 (Cy3) were purchased from Invitrogen Corp. (Carlsbad, CA, USA) and Lumiprobe Corp. (Broward, FL, USA), respectively, and ^{123}I was acquired from Institute of Nuclear Energy Research (Taoyuan, Taiwan). All other chemicals and reagents used were of analytical grade.

2.2. Preparation of test capsules

Hard gelatin capsules (size 9; Torpac Inc., Fairfield, NJ, USA) were manually filled with a mixture of SBC (8.0 mg), DTPA dianhydride (8.0 mg), SDS (4.0 mg), and insulin (0.38 mg) according to manufacturer instructions. The capsules without SBC were used as a control. The prepared capsules were immersed in a methanol solution of Eudragit® L100-55 (15% w/v, Evonik Industries, Parsippany, NJ, USA) and then dried at room temperature using an air-blower; this procedure was repeated three times.

2.3. Characterization of test capsules

The *in vitro* dissolution of test capsules was performed in test tubes containing a phosphate buffered saline (PBS) solution at pH 2.0, pH 6.6, or pH 7.4 (adjusted by HCl); the test tubes were