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Targeted oral delivery of BmpB vaccine using porous PLGA microparticles coated with M cell homing peptide-coupled chitosan

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ABSTRACT

M cells, the key players of the mucosal immunity induction, are one of the intestinal barriers for the efficient delivery of vaccines to mucosal immune tissues. To overcome the barrier, we have developed an efficient oral vaccine carrier that constitutes poly (lactic-co-glycolic acid) (PLGA) microparticle coated with M cell targeting peptide. In this study, a membrane protein B of *Brachyspira hyodysenteriae* (BmpB) as a model vaccine against swine dysentery was loaded into porous PLGA microparticles (MPs). The PLGA MPs were further coated with the water-soluble chitosan (WSC) conjugated with M cell homing peptide (CKS9) to prepare BmpB-CKS9-WSC-PLGA MPs. Oral immunization of BmpB vaccine with CKS9-WSC-PLGA MPs in mice showed elevated secretory IgA responses in the mucosal tissues and systemic IgG antibody responses, providing a complete immune response. Specifically, the immunization with these MPs demonstrated to induce both Th1- and Th2-type responses based on elevated IgG1 and IgG2a titers. The elevated immune responses were attributed to the enhanced M cell targeting and transcytosis ability of CKS9-WSC-PLGA MPs to Peyer's patch regions. The high binding affinity of CKS9-WSC-PLGA MPs with the M cells to enter into the Peyer's patch regions of mouse small intestine was investigated by closed ileal loop assay and it was further confirmed by confocal laser scanning microscopy. These results suggest that the M cell targeting approach used in this study is a promising tool for targeted oral vaccine delivery.

1. Introduction

Oral vaccines offer significant advantages over needle-based vaccines, such as easy handling, high patient compliance, low cost of production and induction of mucosal immunity [1], yet oral vaccination has several defects, such as degradation of the vaccine in the gastrointestinal (GI) tract due to the low pH and enzymes in the stomach, the impermeable GI epithelium as a physical barrier and inefficient targeting to the action site, resulting in a low bioavailability [2]. Therefore, the physicochemical properties and biological restrictions of the vaccine need to be considered for the development of an effective delivery system for oral vaccines [3].

Moreover, the vaccine should reach M cells to stimulate adequate immune responses after oral delivery. The M cells, specifically located on the follicle-associated epithelium (FAE) in Peyer's patch (PP) of the gut, possess a unique property to take up and deliver vaccines from the enteric environment into the PP via transcytosis [4]. Vaccines delivered through the M cells in the PP, referred to as gut-associated lymphoid tissues (GALT), facilitate the induction of vaccine-specific immune responses by activating antigen-presenting cells and lymphocytes [5,6].

Undoubtedly, the receptor-mediated targeting of M cells would be an approach of oral vaccination [7], but the lack of differentiation of M cell receptors from the adjacent enterocytes hindered the M cell targeting oral delivery [8]. Recently, we identified a CKSTHPLSC (CKS9) peptide by phage display which showed high affinity towards M cells [4]. The peptide facilitated the transport of chitosan nanoparticles across the M cell to enter the FAE in the PP demonstrating CKS9 as a potential M celltargeting ligand.

In recent years, the use of porous materials has been greatly extended in bio-related fields [9–11]. Particularly in drug delivery

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system, the porous biodegradable polymeric microparticles (MPs) are applied as protein and peptide carriers for oral delivery [12–15]. These porous MPs have attracted much attention because of their protection of proteins from degradation and their satisfactory releasing behavior [16]. While the pore sizes in MPs can be adjusted to control the release of the molecules, chemical modifications of the surfaces may indeed offer targeted delivery.

Swine dysentery (SD) is a contagious mucohemorrhagic disease of pigs that is caused by *pathogenic* intestinal spirochete *Brachyspira hyodysenteriae* [17]. Several attempts have been made to use either attenuated or genetically modified live avirulent vaccines for SD [18]. But, a recent approach to prevent pigs from SD used an outer envelope lipoprotein of *B. hyodysenteriae* (BmpB) and confirmed the protection of pigs from SD by vaccination with the recombinant BmpB [19].

In this study, we loaded BmpB into porous poly(lactic-co-glycolic acid) (PLGA) MPs coated with M cell homing peptide-coupled chitosan and investigated the *in vitro* and *in vivo* efficacy of the MPs for targeted oral delivery of BmpB as a SD vaccine.

2. Materials and methods

2.1. Materials

Carboxylic acid-terminated PLGA (lactic acid/glycolic acid: 50/50; MW = 17 kDa) was purchased from Purac Biochem (Gorinchem, Holland). Sodium oleate was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). CKS9 (CKSTHPLSC) was chemically synthesized by Anygen (Gwangju, Korea). Water-soluble chitosan (WSC, MW = 9600; degree of deacetylation = 91.8%) was kindly provided by Prof. Nah (Sunchon National University, Korea). Difco™ LB broth was purchased from BD (Sparks, MD, USA). Rabbit anti-BmpB antibody was purchased from AbClon (Seoul, Korea). 3,3′,5,5′-Tetramethyl benzidine (TMB); horseradish peroxidase (HRP)-conjugated goat anti-mouse IgA, IgG, IgG1 and IgG2a; and HRP-conjugated goat anti-rabbit IgG antibody were purchased from Santa Cruz Biotechnology(Dallas, TX, USA). Bovine serum albumin (BSA), coumarin 6, poly(vinyl alcohol) (PVA), *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI), tetramethylrhodamine isothiocyanate (TRITC)-conjugated Ulex Europaeus Agglutinin I lectin (UEA-I), and other chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Preparation of BmpB protein

Escherichia coli harboring the gene encoding for the BmpB protein was inoculated into LB broth and incubated at 37 °C with shaking at 100 rpm for 4 h. The culture was induced with IPTG (1 mM) and incubated for an additional 12 h. *E. coli* cells were collected by centrifugation and washed twice with phosphate buffered saline (PBS). The cells were suspended in His-binding buffer for cell lysis by sonication to collect the expressed His-tagged protein. The protein was separated from the cell debris by centrifugation at 12,000 rpm for 30 min. The protein was finally purified according to the manufacturer's instructions (Clontech). The purity of the protein was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The collected protein was dialyzed overnight against water (pH 7.9) at 4 °C. The purified protein was freeze dried and maintained at −20 °C until use.

2.3. Preparation of CKS9-conjugated chitosan

CKS9-conjugated water-soluble chitosan (CKS9-WSC) was prepared by the conjugation of WSC with CKS9 using NHS/EDC coupling agents following a previously described method [4]. The synthetic scheme is shown in Fig. 1. The composition of the CKS9-WSC was determined by ^{1}H NMR spectroscopy (Avance600, Bruker, Germany) and Fourier transform infrared spectrometer (FT-IR; Nicolet 6700, Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.4. Preparation of porous PLGA MPs

2.4.1. Preparation of BmpB-loaded porous PLGA MPs (BmpB-PLGA MPs)

The BmpB-PLGA MPs were prepared using a water-in-oil-in-water ($W_1/O/W_2$) double-emulsion solvent evaporation method, as previously described [20] with some modifications. First, 7.5 mg of BmpB was dissolved in 100 μl of distilled water, and a sodium oleate solution was prepared in 200 μl of distilled water, which was used as the W_1 phase. PLGA (0.12 g) was dissolved in 2 ml methylene chloride (O phase) and emulsified with the W_1 phase by sonication on ice to form the stable initial emulsion (W_1/O). Second, the resultant emulsion was added drop wise into 40 ml of a 5% (w/v) PVA solution and emulsified for 2 h at a predetermined speed using a direct driven digital stirrer, resulting in the formation of the $W_1/O/W_2$ emulsion. Third, 50 ml of a 6% (v/v) isopropanol solution was poured into the double emulsion to extract the organic solvent and then stirred for approximately 4 h in a

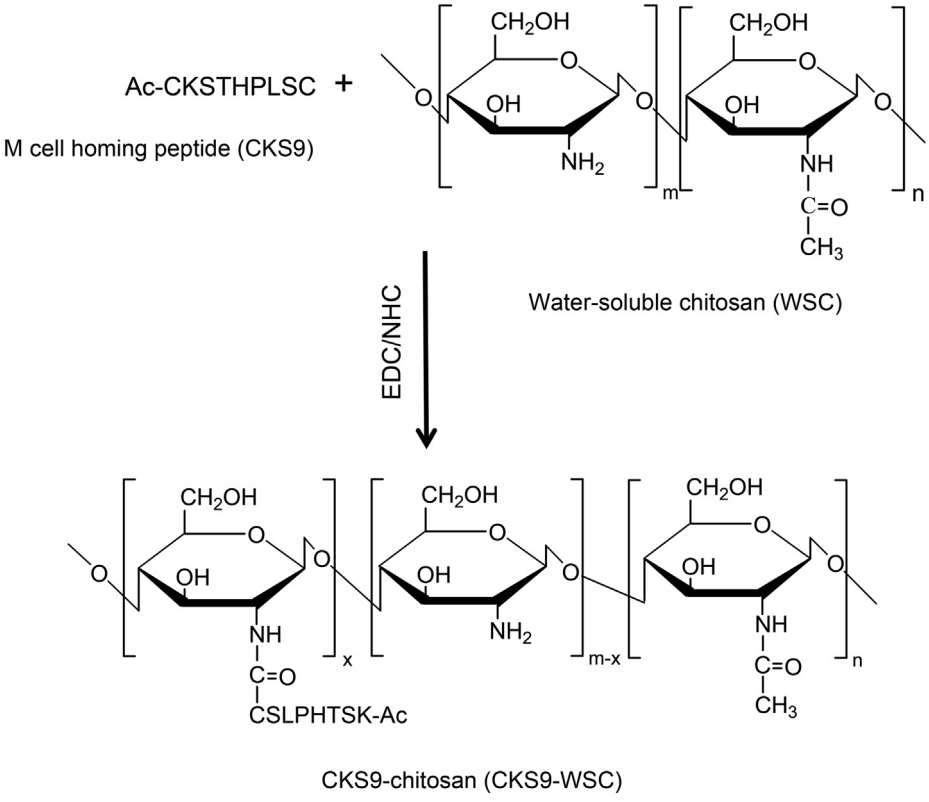

Fig. 1. Schematic diagram for the synthesis of CKS9-WSC.