Porcine intestinal organoids as a model to study nanoparticle-based oral vaccine delivery across the intestinal barrier

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Abstract: Oral vaccines would result in significantly lower costs, and distress to animals. There are currently **no** commercial oral vaccines in the pig industry. Here, we will test nanoparticle-based vaccine delivery through the **(1)** development of multicellular porcine intestinal organoids, and **(2)** nanoparticle vehicles for transepithelial transport, while **(3)** reducing the need for animal use in research. We will use a complex porcine intestinal epithelial model, including microfold cells. Nanoparticles will be developed with varying size and surface charge to optimise intestinal transepithelial transport in vitro. Transferrin receptor (TfR) targeting will be employed if necessary to improve uptake. These data will improve our knowledge of porcine intestinal transepithelial transport and promote the development of oral vaccines for the pig industry. *Overall, we expect this project to set-up and test a new method for optimising oral vaccines, which could have huge impact on modern farming.*

Research Proposal

Background: The intestinal epithelium is an essential physical and biochemical barrier of the body, and the mucosal immune system in the intestines is also a critical defensive mechanism against pathogen invasion. Mucosal exposure of vaccines gives rise to specific immune responses characterized by the production of sIgA (secretory IgA). Some oral vaccines based on recombinant bacterial delivery systems and nanoparticles (NPs) or microparticles (MPs) have been developed and tested (1-3). Intestinal microfold cells (M cells) are the primary transepithelial transport site (4). M cells deliver luminal particles to the immune cells in lamina propria and initiate mucosal immune responses (5). Transferrin (Tf) transports iron to the cell with the endocytosis mediated by binding of Tf and Tf receptor (TfR) (6). TfR is expressed on the apical membrane of epithelial cells on the duodenum, ileum, and colon (7). Tf transport pathway is also a potential approach for delivering NPs on the intestinal epithelium (8). Non-targeted approaches, using e.g. variation surface charge and diameter will be tested first, as these are cheaper and easier to implement in practice.

There are still no commercially available oral vaccines for the pig industry. M cells-targeted oral vaccines against some porcine enteric viruses have been studied (9, 10). Most of them are designed based on a recombinant bacterial delivery system (11). However, poor bioavailability limits the development and the clinical application of oral vaccines. The detailed mechanism behind transepithelial transport of antigen on porcine intestines is unclear.

Factors, including surface charge and the size of NPs, affect the internalization of NPs (12). Internalization and transepithelial transport have been studied in cell lines (12, 13). However, data regarding porcine intestinal transepithelial transport is sparse. In a previous study, we established multicellular porcine intestinal epithelial models, intestinal organoids monolayer and apical-out 3D intestinal organoids and used them to investigate enteric virus infection (14). These physiological models allow us to observe intestinal transepithelial transport of antigenic particles on porcine intestinal epithelial in vitro study, and promote the development of oral M cells-targeted vaccines for the pig industry.

Aims:

(1) To investigate two main transepithelial transport pathways of NPs (M cell-mediated transepithelial transport and Tf transport pathway) on porcine 3D apical-out intestinal organoids or 2D organoids monolayer.

(2) To study the diameter- and charge-dependent efficiency of intestinal transepithelial transport of NPs on porcine intestines.

(3) To establish the intestinal organoid model in oral vaccine delivery studies, to reduce animal use.

Objectives:

(1) Establish a stable model of porcine intestinal M cells in 2D and 3D intestinal organoids.

(2) Design vaccine vehicles based on polymeric NPs, and optimise the rate of endocytosis in porcine intestinal organoids models (with the presence or absence of M cells).

(3) Design and test TfR-targeted NPs, to study TfR-mediated transcytosis in 2D and 3D organoids.

Methods:

1) The establishment of porcine intestinal M cells model

Porcine intestinal organoids cultures are developed with fresh tissue samples from the ileum of an adult pig. Apical-out 3D organoids and 2D organoids monolayer have already been established in our group (14). Recombinant receptor activator of nuclear factor kappa-B ligand (RANKL) will be used to induce M cell differentiation in organoids models. After 3-day RANKL stimulation, the M cells differentiation on organoids models are identified through immunostaining (anti-CK18) and RT-qPCR (for detecting M cell-specific genes, such as *Spib, Gp2, Marcksl1, Ccl9, Tnfaip2*).

2) The development of NP-based vaccine delivery vehicle with BSA-FITC.

NPs (e.g. PLGA or latex) will be loaded with FITC-labeled BSA, as a mock antigen (15, 16). FITC-BSA loaded NPs will be identified using microscopy. Diameters of 50-1000nm, and surface charge of roughly -100 to +30 mV will be tested.

3) Identification of TfR-expression and Tf-uptake on porcine intestinal organoids.

The expression of TfR on apical-out 3D organoids and 2D organoids monolayer will be studied by immunostaining. The uptake of fluorescently-labeled Tf on porcine intestinal organoids will be observed through a confocal microscope.

4) Development of TfR-targeted NPs.

TfR-targeting peptide will be covalently linked to the NP surface using copper-free click chemistry.

5) The transcytosis of NPs on 2D and 3D intestinal organoids models with the presence or absence of M cells.

NPs will be added to 2D and 3D organoids culture systems (with the presence or absence of M cells) at 50 μg/mL and incubated at 37 °C for two hours. The organoids will be collected for transcytosis

analysis after medium-removing and repeated washing with PBS. The uptake of Tf-NPs and FITC-BSA-NPs in epithelial cells in organoids will detected through immunostaining and confocal microscopy.

6) **Basolateral release of FITC-BSA-NPs and fluorescently-labeled Tf-NPs.**

The basolateral release of fluorescently-labeled NPs in apical-out intestinal organoids will be observed through a laser scanning confocal microscope. Release of NPs from the organoid monolayer will be measured by sampling the basolateral solution from the transwell culture system and direct fluorescence quantitation of the NPs.

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