# Nanoparticle-stabilized Pickering emulsion for oral vaccine delivery

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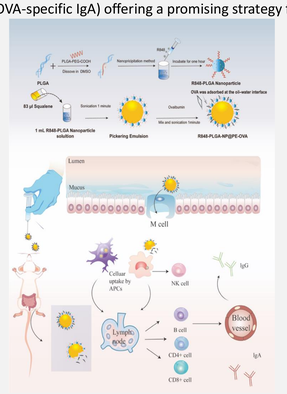
## Introduction

Vaccines have significant contribution to global public health by preventing and controlling infectious diseases such as influenza and polio. Injectable vaccines, delivered via intramuscular (IM), subcutaneous (SC), and intradermal (ID) routes, have been widely adopted due to their ability to elicit robust immune responses. However, injection-based delivery methods present certain limitations, including the need for a trained healthcare personnel, cold chain storage, and injection-associated discomfort, which may limit patient compliance and accessibility. However, oral vaccines, offering needle-free administration, present a promising alternative by improving patient compliance and facilitating a quick and convenient distribution, especially in resource-limited areas. Despite these advantages, oral vaccines face challenges such as antigen degradation by gastric acid and digestive enzymes, and poor mucosal uptake due to the intestinal epithelial barrier [1]. Pickering emulsions have gained attention as a promising platform for vaccine delivery due to their structural and physicochemical properties. Stabilized by solid particles, Pickering emulsions exhibit high flexibility and deformability [2]. Nanoparticles encapsulating the immunostimulant R848 (R848-NP) further improve efficacy by acting as stabilizers and adjuvants[3]. Therefore, we designed and fabricated a Pickering emulsion-based oral vaccine platform using squalene as the oil phase and R848-loaded nanoparticles (R848-NP) as both stabilizer and adjuvant. This platform was designed to efficiently co-deliver the antigen and adjuvant, to enhance APC activation, and subsequently elicit cellular immunity (e.g., T cell and NK cells), as well as humoral immunity (e.g., serum OVA-specific IgG, fecal OVA-specific IgA) offering a promising strategy for effective oral vaccination (scheme 1).




Scheme 1. Nanoparticle-stabilized Pickering emulsion for oral vaccine delivery

## Methods

Ovalbumin (OVA) loaded Pickering emulsion (R848-PLGA-NP@PE-OVA) was optimized by varying PLGA molecular weight, PLGA to PLGA-PEG ratio, and R848 concentration during nanoparticle fabrication. The effects of water-to-oil ratios (8:1 to 16:1) and nanoparticle concentrations on emulsion stability, OVA encapsulation efficiency, particle size, and zeta potential were evaluated. Particle size and zeta potential were measured using dynamic light scattering (Zetasizer Nano-ZS, Malvern). R848 loading was quantified by HPLC. OVA encapsulation efficiency was determined using an Amicon® Ultra centrifugal filter (50 kDa MWCO). The formulation was centrifuged at 4000 rpm for 15 minutes to separate free OVA. The amount of unencapsulated OVA in the filtrate was then quantified using a BCA protein assay kit (Thermo Scientific). The stability of R848-NP@PE-OVA in biological fluids was assessed by dispersing the samples in Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) and incubating them in a shaking incubator (100 rpm, 37°C). The in vitro release profiles of OVA and R848 from R848-PLGA-NP@PE-OVA were evaluated using a dialysis-based method adapted from previous reports. Uptake of R848-NP@PE-OVA by BMDCs: BMDCs were incubated with Free OVA-647 or R848-PLGA-NP@PE-OVA647 (5 µg/mL OVA) for 2 h, followed by CD11c staining and flow cytometry analysis. Cellular internalization was further visualized using Hoechst 33342 staining and imaged with a Cytation 7 system. Female C57BL/6 mice were randomly assigned to three groups: PBS, free OVA + R848, and R848-PLGA-NP@PE-OVA. On Days 1 and 7, mice received 100 µL of the respective formulations by oral gavage (100 µg OVA + 20 µg R848 per dose). On Day 11, blood, feces, and spleens were collected. Serum IgG and fecal IgA levels were measured using modified ELISA protocols.

**References**

1. Ramirez, J. E. V., et al. Advanced drug delivery reviews, 2017: 116-131.
2. Du, Y., et al. Biomaterials, 2022: 121313.
3. Toy, Randall, et al. Journal of Controlled Release. 2021: 866-877.

## Results

### Fabrication and characterization of R848-PLGA-NP

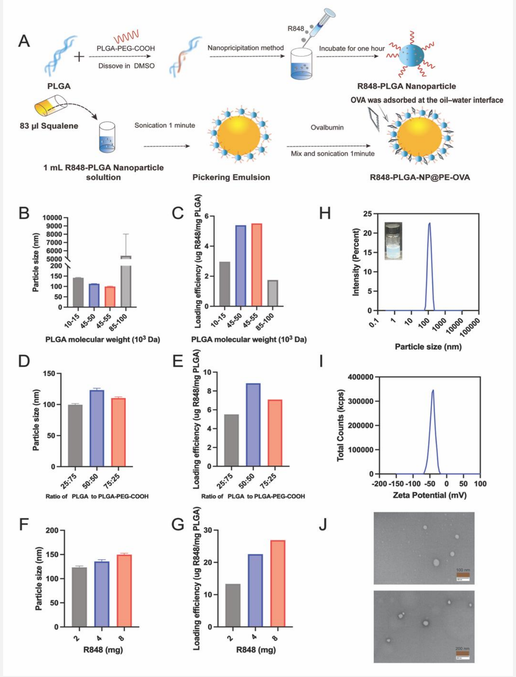



Figure 1. Optimization and Characterization of R848-PLGA-NP. (A) Schematic representation of the fabrication process of R848-PLGA-NP@PE-OVA. (B-G) Optimization of R848-PLGA-NP formulation. (B-C) Effects of different PLGA molecular weights on the particle size and loading efficiency of R848-PLGA-NP. (D-E) Influence of varying PLGA to PLGA-PEG-COOH ratios on the particle size and loading efficiency of R848-PLGA-NP. (F-G) Impact of different R848 concentrations on the particle size and loading efficiency of R848-PLGA-NP. (H-I) Particle size distribution and zeta potential of R848-PLGA-NP. (J) TEM morphology of R848-PLGA-NP. All data are represented as mean ± SD.

### Cellular uptake of R848-PLGA@PE-OVA

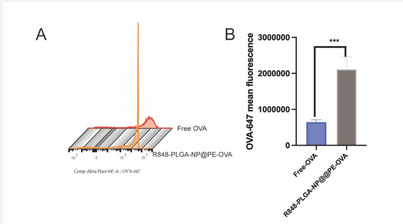



Figure 4 Uptake of R848-PLGA-NP@PE-OVA by BMDCs. (A) Histogram of OVA-647 signal intensities from BMDCs (B) Quantitative analysis of cellular uptake by flow cytometry (mean ± SD, n = 4), Student's t-test ***P < 0.001 (C) Fluorescence microscopy images of BMDCs incubated for 2 hours with free OVA, R848-PLGA-NP@PE-OVA, or PBS. Scale bar: 100 µm.

### Fabrication and characterization of R848-PLGA-NP@PE-OVA

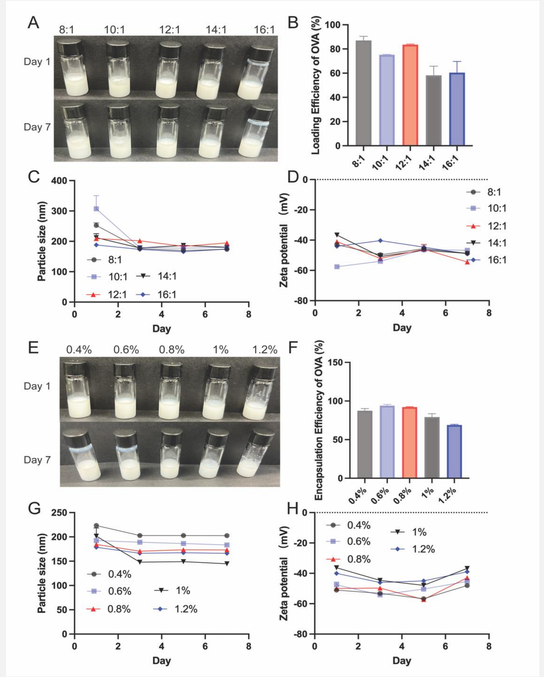



Figure 2. Optimization of oil-to-water ratio and R848-PLGA-NP concentration to fabricate R848-PLGA@PE-OVA. (A–D) Effects of varying oil-to-water ratios on the appearance, OVA encapsulation efficiency, particle size, and zeta potential of R848-PLGA-NP@PE-OVA. (E–H) Effects of different particle concentrations on the appearance, OVA encapsulation efficiency, particle size, and zeta potential of R848-PLGA-NP@PE-OVA. All data are represented as mean ± SD.

### Antigen-specific IgG and IgA responses

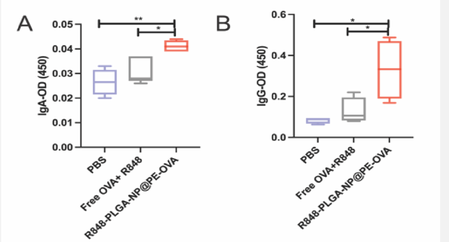



Figure 5 Humoral responses following oral immunization (A) OVA-specific IgA levels in fecal samples. (B) OVA-specific IgG levels in serum. (mean ± SEM, n = 5). Statistical analysis was performed using one-way ANOVA. *P < 0.05, **P < 0.01.

### Stability and in vitro release profile of R848-PLGA-NP@PE-OVA

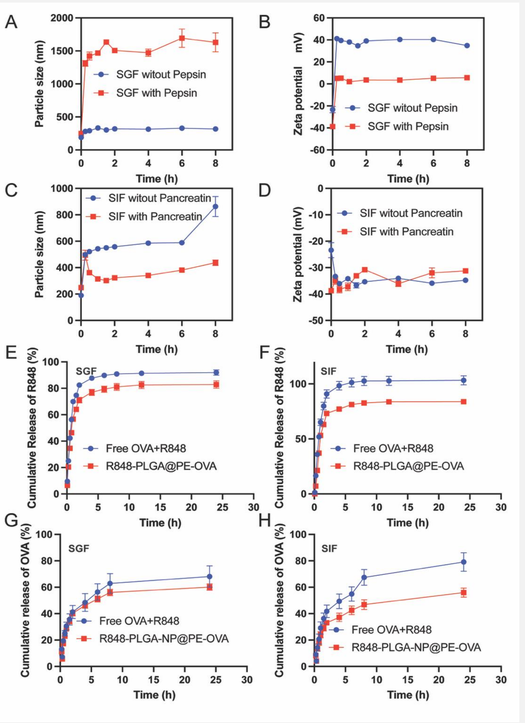



Figure 3 Stability and in vitro release profile of R848-PLGA-NP@PE-OVA (A-D) Stability assessment of R848-PLGA-NP@PE-OVA in SGF with or without pepsin and SIF with or without pancreatin by measuring particle size and zeta potential (E-F) In vitro release profiles of R848 in SGF and SIF. (G-H) In vitro release OVA in SGF and SIF. n=3, All data are represented as mean ± SD.

## Conclusions and Future Work

Pickering emulsion-based oral vaccine delivery system stabilized by R848-PLGA-NP, can elicit both humoral and cellular immune responses. The R848-PLGA-NP@PE-OVA system not only improves DC maturation and antigen presentation but also significantly enhances systemic and mucosal immunity by upregulating OVA-specific IgG and IgA levels. These findings highlight the potential of PLGA-based Pickering emulsions as an innovative platform for oral vaccine delivery, particularly for mucosal immunization strategies. Furthermore, this approach can be extended to other GI-related vaccines and cancer immunotherapies.

**ACKNOWLEDGEMENTS**

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