# **Supporting Information**

# Thiolated Nanoparticles Overcome the Mucus Barrier and Epithelial Barrier for Oral Delivery of Insulin

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#### 1. Methods

## 1.1 Cell viability

The cell viability after incubation with CS NPs and PC6/CS NPs was also evaluated by CCK-8 kits on Caco-2 cells. Briefly, we incubated Caco-2 cells with CS NPs (CS concentration was 3 mg/ml) and PC6/CS NPs (CS concentration was 3mg/ml and PC6 concentration was 0.1mg/ml, 0.2 mg/ml and 0.5 mg/ml respectively) for 2 h and tested the cell viability using CCK-8 kits.

#### 1.2 Bioactivity of insulin loaded in CS NPs and PC6/CS NPs

The bioactivity of insulin loaded in CS NPs and PC6/CS NPs with a final insulin concentration of 50  $\mu$ g/ml was tested using CD spectrum (Circular Dichroism Spectrophotometer, Jasco, Japan). Standard insulin solution was prepared by dissolving free insulin with the same concentration.

### 1.3 Particle stability of CS NPs and PC6/CS NPs

In order to evaluate the stability of CS NPs and PC6/CS NPs at different pH, the morphology of CS NPs and PC6/CS NPs incubated in PBS at pH 5.0, 6.8 and 7.4 respectively was observed by TEM.

#### 1.4 Release of insulin from CS NPs and PC6/CS NPs

200 μl NPs solution loading FITC-insulin with a final FITC-insulin concentration of 50 μg/ml was added into dialysis bag (Mw: 8000-14000 Da) and incubated in 20 ml PBS with different pH (5.0, 6.8, 7.4) at 37°C, 110 rpm. 200 μl dialysis medium was collected and replaced by the blank PBS with the same volume at different time intervals. Collected samples were used to analyze the release of FITC-insulin from CS NPs and PC6/CS NPs and determined by fluorescence spectrophotometer.

# 2. Figures

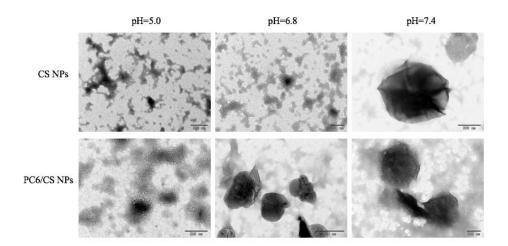


Figure S1. The morphology of CS NPs and PC6/CS NPs (unstained with phosphotungstic acid) observed by TEM after dispersed in PBS with different pH for 30 min.

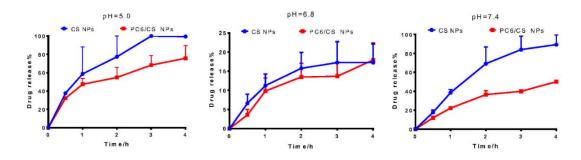


Figure S2. The release profile of insulin from PC6/CS NPs compared with that from CS NPs at different pH.