Supporting Information

Chitosan-based core-shell structured particles for in vivo sustainable gene transfection

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1. Biodegradation of EG-HBC

The synthesized EG-HBC was dissolved in an acetic solution (2%) at a concentration of 1 mg/mL. Then the solution was poured into a petri dish, dried in an air oven at 45 °C to get a thin EG-HBC film. The obtained EG-HBC thin film was immersed into 50 mL of the aqueous solution of lysozyme (0.5 mg/mL). The status of EG-HBC film were recorded in digital photos at different time, as shown in Figure S1. It is clearly seen that a lot of bubbles appear on the film indicating the degradation of EG-HBC begins since the products of the degradation of CS include carbon dioxide. The EG-HBC film cannot be clearly observed after 24 h, and 72 h later, totally disappears.



Figure S1. The biodegradation process of EG-HBC film in the aqueous solution of lysozyme

at room temperature.

2. Zeta potentials of *p*EGFP-loaded CS-based particles.

Zeta potentials of different particles in the aqueous solution of acetic acid were measured by dynamic light scattering (Nano-ZS90, Malvern, UK).



Figure S2. Zeta potential of *p*EGFP-loaded CS-based particles.

3. FACS graphs.

Fluorescence activated cell sorting (FACS) was used to characterize the gene transfection efficiency of pEGFP-loaded on different carriers.



Figure S3. FACS graphs of in vitro gene transfection of *p*EGFP loaded on different carriers (1: CS; 2: TACS; 3: TACS@HBC; 4: TACS@EG-HBC; 5: Lip2000).

3. The luciferase assay.

Hela cell lines (derived from cervix carcinoma cells of human) were cultured in DMEM-high glucose (HyClone) containing heat-inactivated 10% fetal bovine serum (FBS, Gibco-BRL) and 1% penicillin-streptomycin. Then, Hela cells were seeded in 24-well plates at a density of 3×10^4 per well, and grew overnight at 37 °C in CO₂ incubator. The high glucose DMEM in each well was all removed. The wells were washed with 0.5 mL of serum-free DMEM. Then, 100 µL of serum-free DMEM medium containing luciferase reporter gene plasmid (*p*LRG) or *p*LRG-loaded particles (*p*LRG for each well was 4 µg) was injected into each well. For comparison, a group of *p*LRG-loaded Lip2000 was set as the positive control, another group of 100 µL fresh serum-free DMEM medium as the blank control. After 4 hours' incubation, the mediums in all wells were replaced by fresh DMEM medium with 10% FBS. After 48 h, mediums were all replaced with 100 µL of lysis buffer for each well, and after that another 50

 μ L of luciferase detection reagent was added, the result was measured by a microplate reader. As Figure S4 showed,



Figure S4. The luciferase assay of pLRG loaded on different carriers