Supporting Information

Size-dependent endocytosis and a dynamic-release mode of nanoparticles

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1. Materials

Lecithin from soybean (Sinopharm Chemical Reagent Co., Ltd.), chitosan (Haidebei Co., Jinan, China; deacetylation degree ≥ 95%), Rhodamine B (AR, Aladdin Co., Ltd.), phosphate buffered saline (PBS, pH 7.2~7.5, 0.01 M, GE Healthcare Life Sciences, Utah, USA), fluorescein diacetate (FDA, Sigma) were used as purchased. 1640 RPMI culture medium, fetal bovine serum (FBS) and penicillin-streptomycin solution were purchased from Gibco (USA) and used without further purification. Deionized water was used in all experiments.

2. Characterization

The size and zeta potential data of nanocapsules were measured by dynamic light scattering (DLS) on a zeta potential analyzer (Brookhaven, USA). The detailed morphology of nanocapsules was recorded by transmission electron microscopy (TEM, Hitachi H-7650B, Japan) with accelerating voltage of 80 kV. UV analyses were carried out by a UV/Vis/NIR spectrophotometry (Lambda 750, PerkinElmer,

USA). The cell morphology was observed using an optical microscope (Leica DMI3000 B, Germany). The fluorescent intensity in cells (485/535 nm) were collected by a microplate reader (VICTORTM X3 PerkinElmer 2030 Multilabel Plate Reader). The orbital shaker (ZD-9566, HLD laboratory equipment Co., China) and a peristaltic pump (BT/101S, Baoding Lead Fluid Technology Co., Ltd., China) were linked to the dynamic release study.

3. Methods

3.1 Preparation

Table S1. Diameters (nm) of NP1, NP2 and NP3 with different amount of Rhodamine B.

Rh.B (mg/mL) / Sample	0.5	1	3	5
NP1	207±28	185±21	166±17	150±13
NP2	82±10	74±10	62±9	50±4
NP3	56±6	50±5	42±2	27±1

3.2 Stability

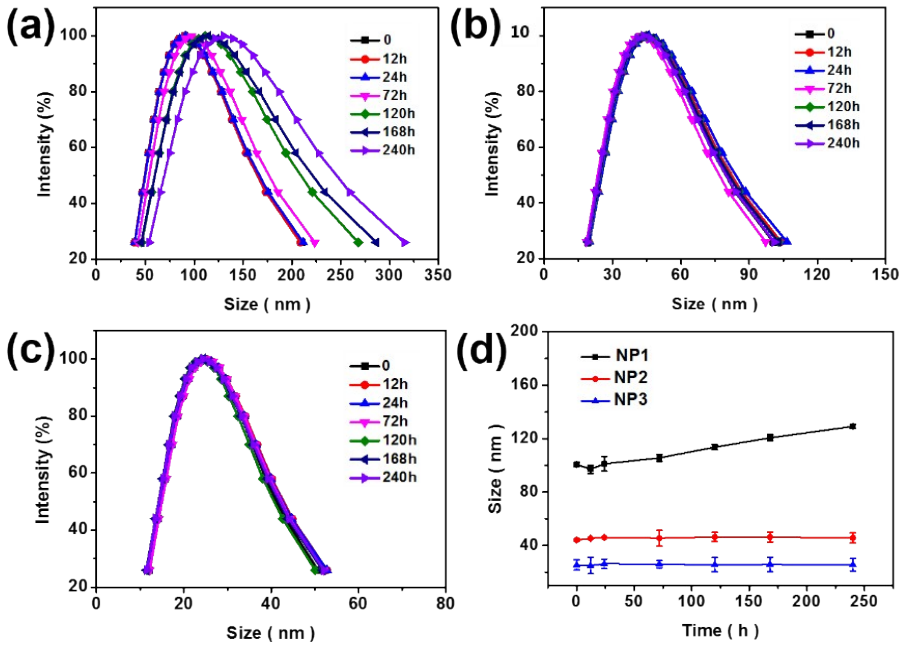

Figure S1. Size distribution of a) NP1, b) NP2 and c) NP3 after certain time; d) size variation of NP1, NP2 and NP3 after certain time.

3.3 Encapsulation efficiency

To identify the encapsulation efficiency (defined by the formula F1) of nanocapsules, we measure the standard linear of the calibration curve of Rhodamine B firstly (Fig. S2). Then, nanocapsules dispersion (5 mL) was centrifuged (8000 rpm, 3 mins), and the upper liquid was measured (UV, 554 nm) to calculate the free Rhodamine B value. The loading ratio of Rhodamine B in nanocapsules was calculated as the equation F1 and listed in Tab. S2.

$$\text{Encapsulation efficiency (EE)} = \frac{\text{actual amount of drug loaded in nanoparticles}}{\text{theory amount of drug loaded in nanoparticles}} \times 100\% \quad \textbf{F1}$$

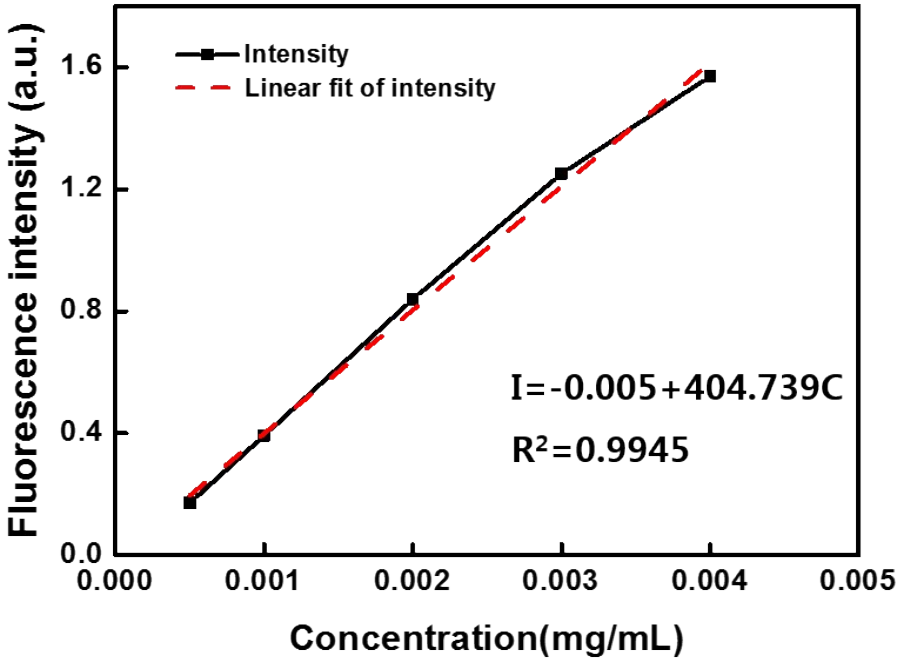

Figure S2. Linear regression of Rhodamine B (solution).

Table S2. The Rhodamine B in nanocapsules and the loading ratio of nanocapsules[a].

Number	Rhodamine B value (A) (before/after lyophilization-dissolving circles)	Loading ratio[b] (%)
NP1	2.89/2.74	64/66
NP2	2.72/2.66	66/67
NP3	2.60/2.53	68/69

[a] Total amount of Rhodamine B was calculated as 8.10 according to the standard linear.

[b] Loading ration (%) = [1- (Rhodamine B value/8.10)] * 100%

3.4 Biocompatibility of NPs

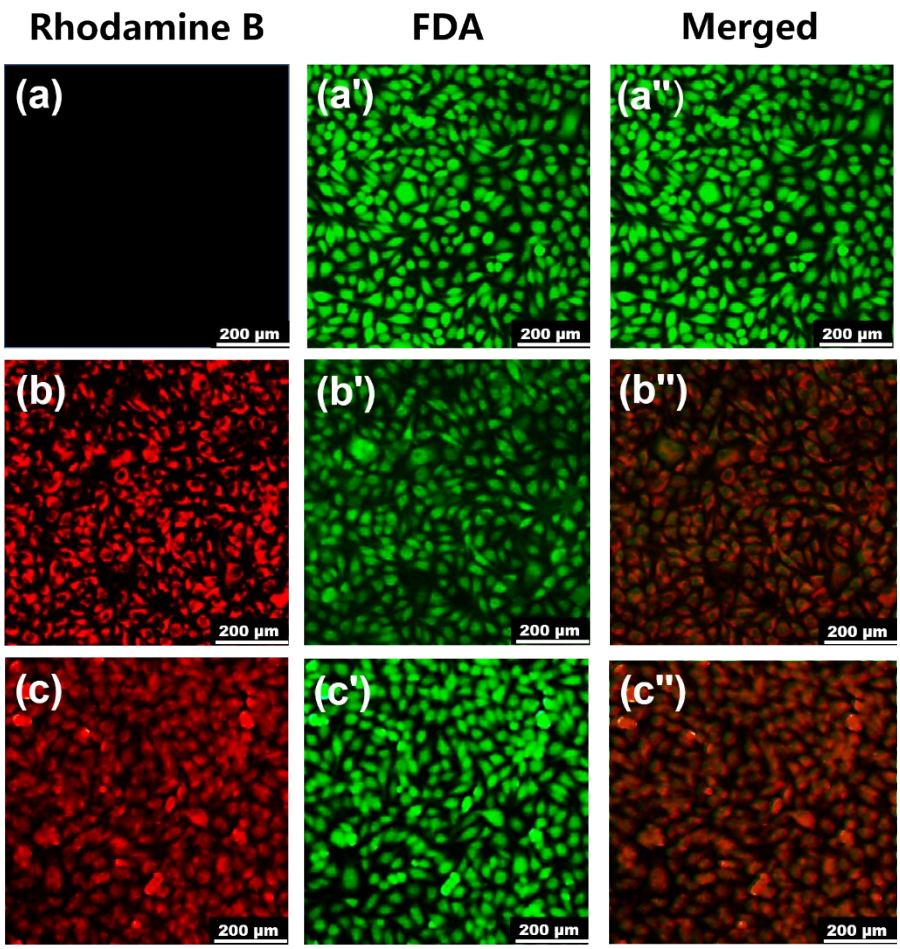

Figure S3. Fluorescent images of L929 cells incubated in a) culture medium only, b) culture medium with NP1, c) culture medium with NP2. Incubation time: 10 min. a'), b'), c') images of live cells (green) stained by FDA. a'') the merged image of a) and a'), b'') and c'') were similarly merged.