Supplementary Information

Surface Modified Titania Nanotubes Containing Anti-bacterial Drugs for Controlled Delivery Nanosystems

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1. Drug Release in *Vitro* Pharmacokinetics of Drug Delivery Nanosystems

Release mechanisms in *vitro* of drug deliver nanosystems were determined by the selected three kinds of release kinetic statistic models described as follows.

Zero-order model was applied to drug delivery nanosystems with a constant release rate which had no associated with drug concentration in release media. The equation for zero order release can be expressed as:

$$M_t / M_\infty = kt \quad (1)$$

Since release rate only depending on the concentration of drug in media in some circumstances, first order model could be applied to explain the release mechanism of the drug delivery nanosystems following Fick's law. The equation for first order release can be described as:

$$\ln(1-M_t / M_\infty) = -kt \quad (2)$$

Release pharmacokinetics of drug delivery naosystems, fabricated by insoluble porous materials as matrix system, were commonly described by Higuchi models, which were based on a square root of a time and process of Fickian diffusion.

$$M_t / M_\infty = kt^{0.5} \quad (3)$$

In above equations, M_t and M_∞ represented the amount of drug release at time t and the final maximum , respectively. Meanwhile, k is the corresponding release rate constant.

2. Cell Culture

Human embryonic kidney (HEK) 293T cells and rat pheochromocytoma PC12 cells were cultured and maintained in Dulbecco's modified Eagle's medium (DMEM) (GIBCO, Invitrogen, USA) with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin at 37 °C in humidity atmosphere containing 5% CO_2. Medium was replaced every 2 days. When grown to 80% ~ 90% confluence, cells were passaged by repeated trypsinization (0.25% trypsin/0.02% EDTA for 2 ~ 3 min) and replanting. Cell numbers were determined with an electronic cell counter device (CASY1, Schärfe Systems, Reutlingen, Germany).

3. Cell Viability Study

The cytotoxicity of the modified TNTs or TNTs against different cell lines, including 293T and PC12 cells was evaluated in *vitro* using the Cell Counting Kit-8 (CCK-8) (Dojindo, Kumamoto,

Japan), according to the manufacturer's manual. In brief, 293T and PC12 cells were seeded in a 96-well plate at a density of 2.5×10^3 cells per well. DMEM (100 μL) containing 10% FBS was added to each well, and incubated for 24 h (37 °C, 5% CO_2). The medium was aspirated off, and then each well was washed three times with 1× PBS. Fresh medium (100 μL) containing modified TNTs or TNTs in PBS at different concentrations (1000 μg/mL, 500 μg/mL, 250 μg/mL, 125 μg/mL, 62.5 μg/mL, 31.2 μg/mL, 15.6 μg/mL) was added in triplicate. The liquid was aspirated off after a 48h incubation and replaced with 100 μL of fresh medium and 10 μL of CCK-8 reagent. The cells were further incubated for 2h, and then the absorbance at 570 nm was measured in a microplate reader (Bio Tek Instruments, Inc.). Negative controls were 293T and PC12 cells incubated without any treatment of nanospheres. The cytotoxicity was expressed as the percentage of cell viability compared to untreated controls.

4. Hemolysis Assay

The blood samples from healthy adult rats were used to evaluate the blood compatibility of modified TNTs and TNTs. Rat red blood cells (RBCs) were collected by removing the serum from the blood after centrifugation and suction. RBCs were purified by washing with PBS five times, and then diluted to 1/10 of their initial volume with PBS solutions. A 0.5 mL suspension of diluted RBCs suspension was then mixed with 1.0 mL 0.9% NaCl as a negative control; 1.0 mL 10% Triton-100 as a positive control; or 1.0 mL of TNTs and modified TNTs suspension (0.9% NaCl) at concentrations ranging from 31.25 to 1000 μg/mL. The mixtures were shaken slightly and then kept still for 2 h at room temperature. The samples were centrifuged, photographed, and the absorbance of the supernatants at 570 nm was measured in a microplate reader (Bio Tek Instruments, Inc.). All experimental protocols and animal handling procedures were approved by the Institutional Animal Care and Use Committee of Sun Yat-Sen University and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

5. Cell Apoptosis Study

Microscopic fluorescence images of the 293T and PC12 cells were obtained using an upright fluorescence microscope (Olympus, BX51, Tokyo, Japan). The 293T and PC12 cells were cultured and maintained in modified TNTs or TNTs for 72 h at concentrations ranging from 31.25 to 2000 μg/mL and normal DMEM for 36 h, and then it was stained with Hoechst33342 for nucleus and analyzed using ImagePro Plus 6.0 software (Media Cybernetics, Bethesda, MD, USA).

6. In *Vivo* Drug Release Pharmacokinetics of Drug Delivery Nanosystems

Pharmacokinetics of pure Enro, Enro-TNTs, Enro-NH_2-TNTs and Enro-SH-TNTs released in *vivo* were studied by Winnolin (or PC Nonlin) v6.1 bought from American Pharsight company in Veterinary College, South China Agricultural University. Mathematic models were chosen as non-compartmental M200, one-compartmental M3 and M5 and two-compartmental M11 for

intramuscular injected administration. Parameter items were measured while comprehensively properties of different delivery nanosystems in *vivo* could be analyzed.

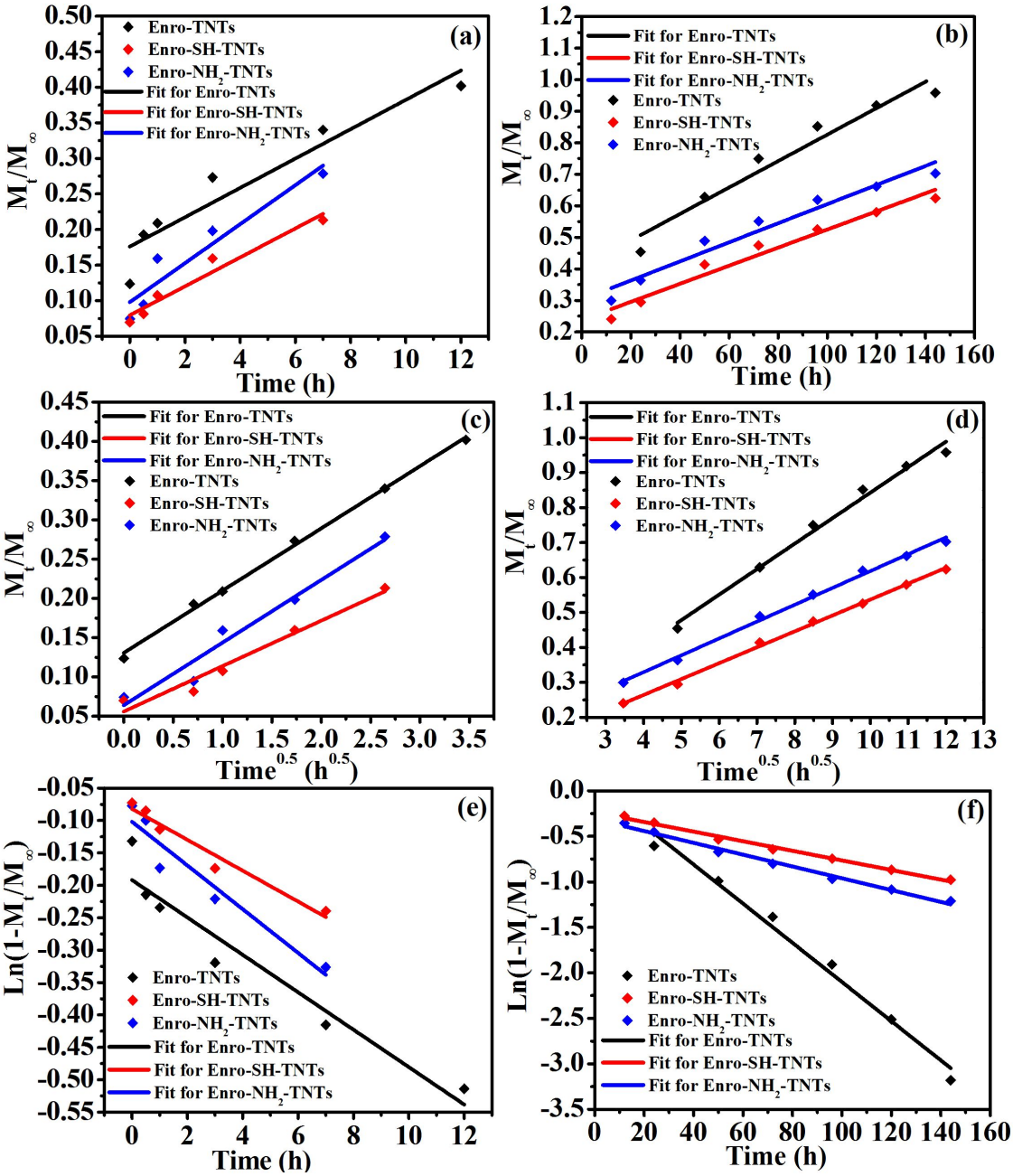

Fig. S1 Pharmacokinetics of in *Vitro* Drug Release experiments: fitted of zero-order equation for (a) Enro-SH-TNTs and Enro-NH_2-TNTs (0~7 h), and Enro-TNTs (0~12 h), (b) Enro-SH-TNTs and Enro-NH_2-TNTs (12~144 h), and Enro-TNTs (24~144 h); fitted of Higuchi equation for (c) Enro-SH-TNTs and Enro-NH_2-TNTs (0~7 h), and Enro-TNTs (0~12 h), (d) Enro-SH-TNTs and Enro-NH_2-TNTs (12~144 h), and Enro-TNTs (24~144 h); fitted of first-order equation for (e) Enro-SH-TNTs and Enro-NH_2-TNTs (0~7 h), and Enro-TNTs (0~12 h), (f) Enro-SH-TNTs and Enro-NH_2-TNTs (12~144 h), and Enro-TNTs (24~144 h).

Table. S1 Pharmacokinetics of different drug deliver nanosystems release in *vitro*

Models	Drug deliver system	Range of time (h)	Equation	R^2
Zero-order ($M_t/M_\infty = kt + K$)	Enro-TNTs	0~12	$M_t/M_\infty = 0.0206t + 0.1760$	0.8795
		24~144	$M_t/M_\infty = 0.0042t + 0.4069$	0.9372
	Enro-NH_2-TNTs	0~7	$M_t/M_\infty = 0.0274t + 0.0979$	0.8800
		12~144	$M_t/M_\infty = 0.0030t + 0.3027$	0.9492
	Enro-SH-TNTs	0~7	$M_t/M_\infty = 0.0203t + 0.0795$	0.9383
		12~144	$M_t/M_\infty = 0.0028t + 0.2381$	0.9605
Higuchi ($M_t/M_\infty = kt^{0.5} + b$)	Enro-TNTs	0~12	$M_t/M_\infty = 0.0793t^{0.5} + 0.1306$	0.9969
		24~144	$M_t/M_\infty = 0.0729t^{0.5} + 0.1135$	0.9905
	Enro-NH_2-TNTs	0~7	$M_t/M_\infty = 0.0798t^{0.5} + 0.0638$	0.9496
		12~144	$M_t/M_\infty = 0.0482t^{0.5} + 0.1365$	0.9954
	Enro-SH-TNTs	0~7	$M_t/M_\infty = 0.0579t^{0.5} + 0.0558$	0.9527
		12~144	$M_t/M_\infty = 0.0454t^{0.5} + 0.0821$	0.9973
First-order ($Ln(1-M_t/M_\infty) = -kt + b$)	Enro-TNTs	0~12	$Ln(1-M_t/M_\infty) = -0.0289t-0.1918$	0.9174
		24~144	$Ln(1-M_t/M_\infty) = -0.0215t+0.0517$	0.9802
	Enro-NH_2-TNTs	0~7	$Ln(1-M_t/M_\infty) = -0.0337t-0.1018$	0.9055
		12~144	$Ln(1-M_t/M_\infty) = -0.0064t-0.3115$	0.9895
	Enro-SH-TNTs	0~7	$Ln(1-M_t/M_\infty) = -0.0238t-0.0821$	0.9492
		12~144	$Ln(1-M_t/M_\infty) = -0.005t-0.2370$	0.9898

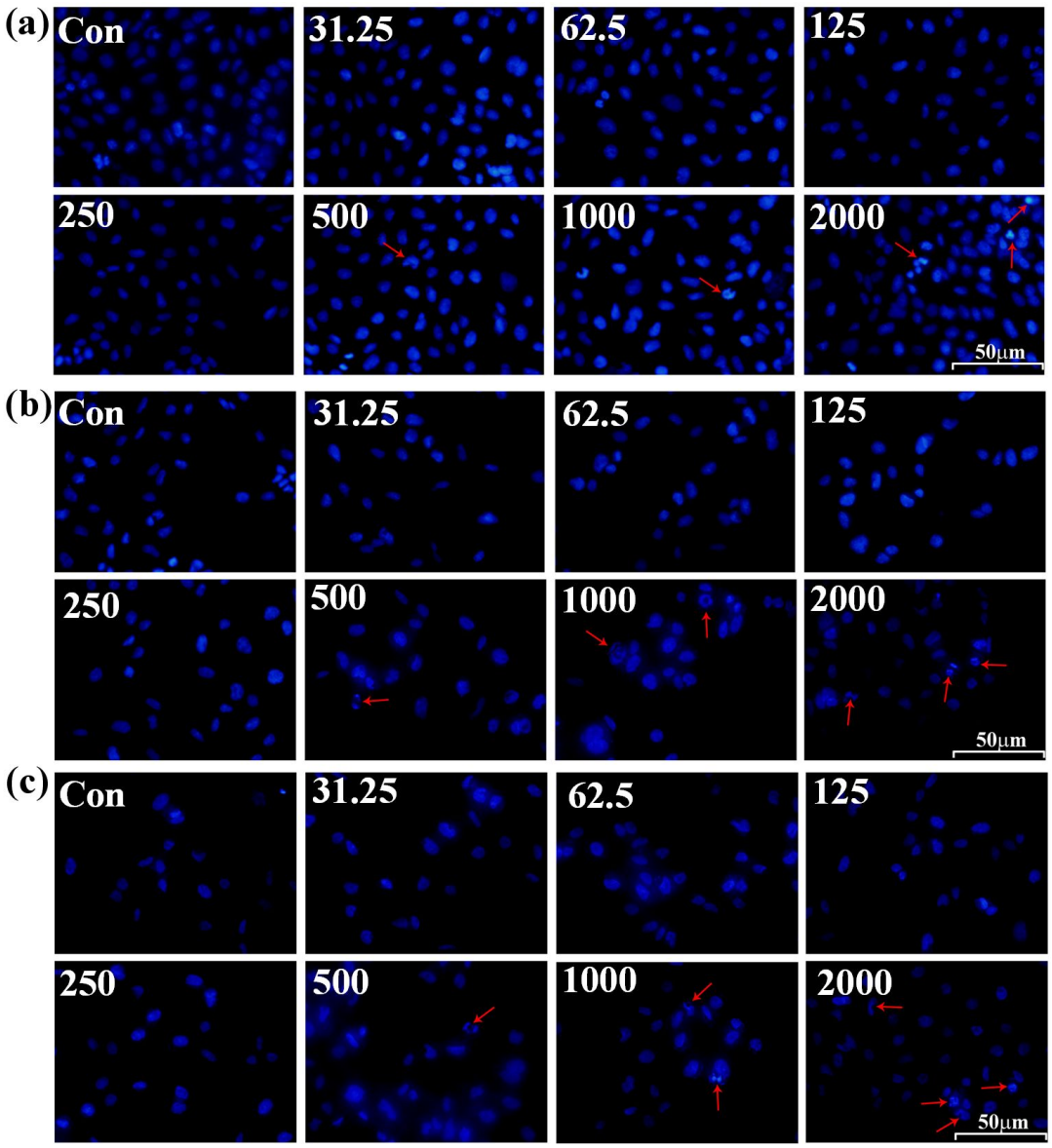

Fig. S2 Immunofluorescence microscopy analysis of apoptosis in 293T cells induced by different samples (a) TNTs, (b) NH_2-TNTs and (c) SH-TNTs at different concentrations (ranging from 31.25 to 2000 μg/mL) for 72 h. Red arrows indicated apoptosis cells. These drug delivery materials did not have any significant effect on the nucleuses (blue) stained with hoechst33342. Scale bar: 50 μm.

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Table. S2 Pharmacokinetic items for different drug deliver system *in vivo* of yellow-further chickens (n=6) administration with formulation containing drug as dosage of 10 mg/kg (Enro/BW)

Formulation	Enro solution	Enro-TNTs	Enro-NH_2-TNTs	Enro-SH-TNTs
MRT(h)	9.52±0.08	13.37±0.24$^{\alpha}$	22.08±1.10$^{\alpha,\beta}$	24.01±1.51$^{\alpha,\beta}$
$AUC_{0\text{-}24h}$ (μg·h/mL)	13.27±0.35	15.95±0.65$^{\alpha}$	26.01±1.23$^{\alpha,\beta}$	34.50±1.65$^{\alpha,\beta}$
$AUC_{0\text{-}\infty}$ (μg·h/mL)	13.30±0.18	16.12±0.48$^{\alpha}$	29.55±1.81$^{\alpha,\beta}$	40.47±2.58$^{\alpha,\beta}$
C_{max} (μg/mL)	2.76±0.01	2.13±0.09	1.98±0.01	2.63±0.14
T_{max} (h)	1.0±0.0	2.0±0.0$^{\alpha}$	3.9±0.1$^{\alpha,\beta}$	4.0±0.0$^{\alpha,\beta}$
$T_{1/2k\alpha}$ (h)	0.18±0.01	0.40±0.01$^{\alpha}$	0.51±0.07$^{\alpha}$	0.71±0.01$^{\alpha,\beta}$
$T_{1/2\beta}$ (h)	4.40±0.06	6.49±0.44$^{\alpha}$	12.92±0.98$^{\alpha,\beta}$	16.02±1.89$^{\alpha,\beta}$
Cl-F (mL/h)	0.75±0.01	0.77±0.04$^{\alpha}$	0.34±0.02$^{\alpha,\beta}$	0.25±0.02$^{\alpha,\beta}$

MRT: mean resident time; $AUC_{0\text{-}24}$: the area under the concentration–time curve from zero to 24 h; $AUC_{0\text{-}\infty}$: the area under the concentration–time curve from zero to infinity; C_{max}: peak concentration; T_{max}: time of peak; $T_{1/2k\alpha}$: half-time of absorption; $T_{1/2\beta}$: half-time of elimination; Cl-F: clearance.

$^{\alpha}$ Significant difference with Enro solution, $P<0.05$

$^{\beta}$ Significant difference with Enro-TNTs, $P<0.05$