Supporting Information For

Polydopamine decorated orlistat loaded nanoparticles with enhanced cytotoxicity against cancer cell lines

Xiaqing Zhou, Tzu-Lan Chang, Shuang Chen, Tianchi Liu, Haoyu Wang, Jun F. Liang*

Department of Chemistry and Chemical Biology, Charles V. Schaefer School of Engineering and Sciences, Stevens Institute of Technology, Hoboken, New Jersey 07030, USA

*Corresponding author. E-mail address: jliang2@stevens.edu；Phone number: (+1)2012165640

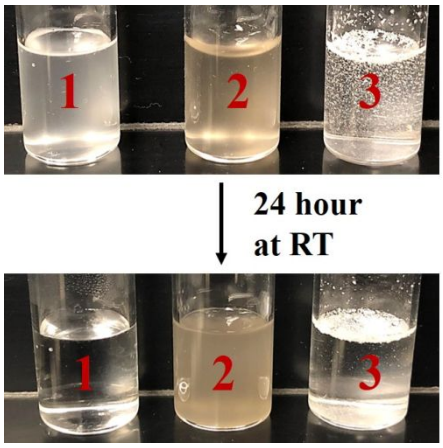

Figure S1. Dispersion test: images of suspensions from orlistat-loaded oil droplet (without PDA coating) (1), orlistat-loaded PHC (2), free orlistat in D.I. water (3) immediately after preparation or left at room temperature for 24 h. The final concentration of orlistat was 1 mg/mL.

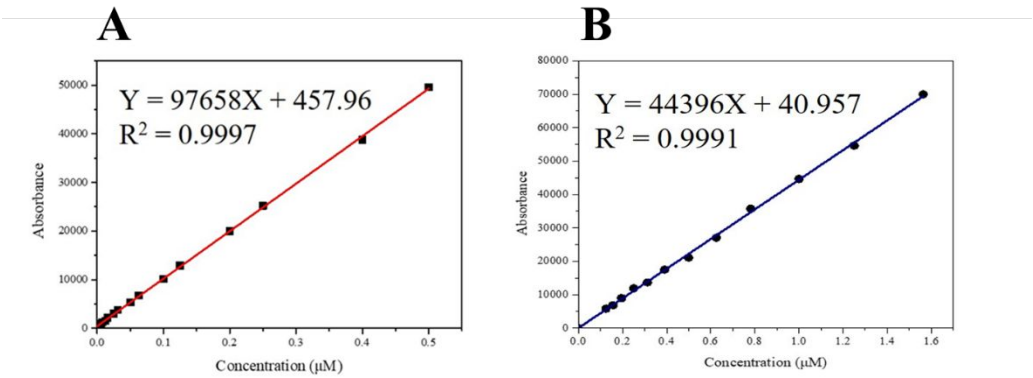

Figure S2. The standard curve of nile red in octane (A) and DMSO (B) for quantification.

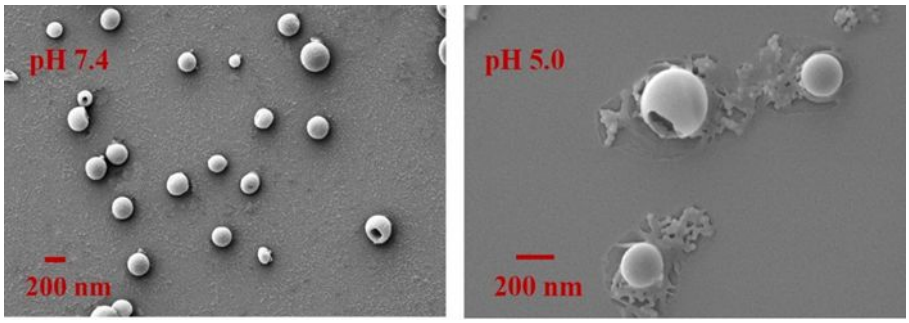

Figure S3. SEM images were taken after orlistat-loaded PHC incubation for 4 h at pH 7.4 and pH 5.0 PBS, respectively. As shown in these two images, the PDA layer was relatively intact in physiological conditions (left image), demonstrating that the PDA layer was stable at neutral pH and was capable of maintaining the structures of the NPs. However, the PDA layer was peeled off and aggregated around the NPs (right image),

indicating that the PDA layer might be detached or partially hydrolyzed from the surface of NPs to unlock the channel under acidic conditions.

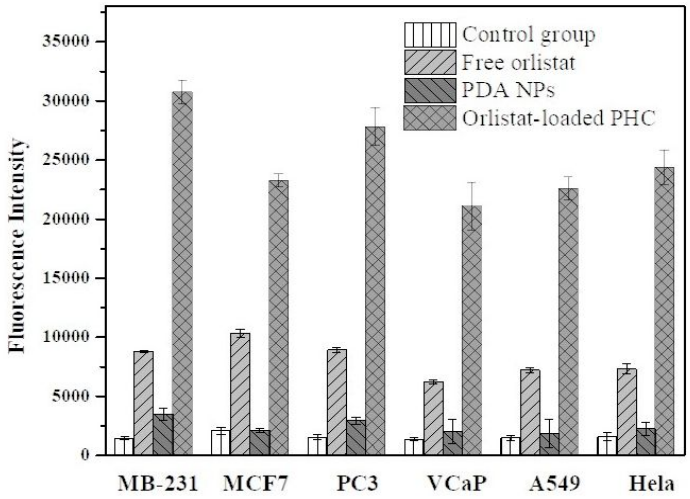

Figure S4. Reactive oxygen species (ROS) production was measured on six indicated cancer cell lines using the fluorescent dye DCFDA [1]. In details, cells were cultured in 96-well plates at 10000 cells/well and treated with medium (control group), 12.5 μg/mL free orlistat, 6.8 μg/mL PDA NPs, and 12.5 μg/mL orlistat-loaded PHC. After 6 h of treatment, DCFDA dye was added to each well at a final concentration of 50 μM. The cells were further incubated at 37 ℃ for 30 mins, followed by washing with PBS. The fluorescence in each well was then quantified at 495 nm excitation using a fluorescence microplate reader. In all cancer cell lines studied, the fluorescence intensities from free orlistat and PDA NPs groups were higher than that of the control group, indicating that both free orlistat and PDA could more or less induce intracellular ROS generation, which was consistent with the previous reports [2-4]. In addition, compared with free orlistat and PDA groups, the expression of ROS from the orlistat-loaded PHC group was found to be significantly elevated in all cancer cell lines demonstrating that after orlistat formulation was endocytosed into cancer cells, PDA and orlistat synergistically enhanced the anticancer properties by increasing the production of ROS. Among these cell lines, MDA-MB-231 cell exhibited the highest fluorescence intensity, which was consistent with its being the most sensitive to this orlistat formulation (Table S2 and S3).

Table S1. Characterization of orlistat-loaded PHC at pH 8.0 (A), 7.4 (B), 6.0 (C), and 5.0 (D). The particle size, Zeta Potential, and PDI were listed.

Type	Zeta-potential (mV)	Particle size (d.nm)	PDI
A	-22.7±4.1	239.4±9.2	0.161
B	-15.5±2.9	246.2±6.7	0.126
C	-10.7±0.9	224.1±4.0	0.184
D	+6.5±1.8	219.5±13.3	0.132

Table S2. Cytotoxicity (IC_{50} value, μg/mL) of free orlistat, PHC/free orlistat, and orlistat-loaded PHC after 4-hour incubation against different cell lines.

	Free orlistat	**PHC/ Free orlistat**	**Orlistat-loaded PHC**
MDA-MB-231	198.2±2.9	151.3±6.7	9.12±2.8
Activity increase*		*1.3*	*21.7*
MCF7	90.7±4.8	86.6±12.8	12.3±1.1
Activity increase		*1.0*	*7.4*
PC-3	85.1±5.7	60.3±0.9	13.6±1.4
Activity increase		*1.4*	*6.3*
VCaP	245.1±11.3	201.5±6.9	21.5±2.1
Activity increase		*1.2*	*11.4*
A549	150.8±7.3	109.1±6.2	30.9±2.4
Activity increase		*1.4*	*4.9*
Hela	146.2±3.4	120.3±4.9	19.7±3.8
Activity increase		*1.2*	*7.4*
MCF 10A	286.2±17.7	257.1±12.7	72.9±3.2
Activity increase		*1.1*	*3.9*

*In comparison with free orlistat.

Table S3. Cytotoxicity (IC_{50} value, μg/mL) of free orlistat, PHC/free orlistat, and orlistat-loaded PHC after 24-hour incubation against different cell lines.

	Free orlistat	**PHC/Free orlistat**	**Orlistat-loaded PHC**
MDA-MB-231	86.3±7.3	65.2±3.6	3.19±0.39
Activity increase*		*1.3*	*27*
MCF7	55.2±5.1	40.8±7.4	4.84±0.12
Activity increase		*1.3*	*11.4*
PC-3	52.8±1.7	37.2±3.9	6.59±0.66
Activity increase		*1.4*	*8.0*
VCaP	104.5±7.7	90.6±6.2	7.12±1.1
Activity increase		*1.2*	*14.7*
A549	87.1±5.7	53.9±8.7	17.6±2.0
Activity increase		*1.6*	*4.9*
Hela	67.7±8.2	56.1±5.3	8.3±0.91
Activity increase		*1.2*	*8.2*
MCF 10A	228.3±7.6	216.0±9.1	47.8±4.4
Activity increase		*1.0*	*4.8*

*In comparison with free orlistat.

References

1. Mohamed, S.; Veeranarayanan, S.; Minegishi, H.; Sakamoto, Y.; Shimane, Y.; Nagaoka, Y.; Aki, A.; Cheruvathor Poulose, A.; Echigo, A.; Yoshida, Y.; Maekawa, T.; Kumar, S. Cytological and subcellular response of cells exposed to the type-1 RIP curcin and its hemocompatibility analysis. *Sci. Rep.* 2014; Vol. 4.

2. Fujiwara, J.; Sowa, Y.; Horinaka, M.; Koyama, M.; Wakada, M.; Miki, T.; Sakai, T. The anti-obesity drug orlistat promotes sensitivity to TRAIL by two different pathways in hormone-refractory prostate cancer cells. *Int. J. Oncol.* 2012; Vol. 40, p 1483-91.

3. Kant, S.; Kumar, A.; Singh, S. M. Tumor growth retardation and chemosensitizing action of fatty acid synthase inhibitor orlistat on T cell lymphoma: implication of reconstituted tumor microenvironment and multidrug resistance phenotype. *Biochim. Biophys. Acta.* **2014,** *1840* (1), 294-302.

4. Nieto, C.; Vega, M. A.; Marcelo, G.; Martín del Valle, Eva M. Polydopamine nanoparticles kill cancer cells. *RSC Adv.* **2018,** *8* (63), 36201-36208.