Electronic Supplementary Information

Synthesis of Surface Functionalized Hollow Microporous Organic Capsules for Doxorubicin Delivery to Cancer Cells

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Experimental Procedures

Preparation of Silica NPs

The hard template in present work is the silica NPs. The surface of silica NPs was fabricated with vinyl groups which act as a seed in emulsion polymerization. In a typical synthesis, TEOS was used as a precursor. In a 4- necked round bottom flask, the precursor (40 ml) and ethanol (500 ml) was strongly agitated by mechanical stirring followed by the addition of $NH_3 \cdot H_2O$ (40 ml). After 24h of stirring, mixture of MPS and ethanol (4ml: 26 ml) was dropped into the silica dispersion. Reaction was stirred for another 24 h to obtain the MPS modified SiO_2 NPs. Later the SiO_2- MPS NPs were separated by centrifugation and washed properly by three cycles of centrifugation and re-dispersion with ethanol, dried in vacuum oven at 60°C for 24h.

Experimental procedure for the Encapsulation of DOX

Doxorubicin. HCl is an anti-cancer drug used in present study. The encapsulation of DOX had been carried out by immersing the Py-HMOCs-FA (100 mg) in 10 ml aqueous solution of Doxorubicin.HCl (500 mg) for 24 h. Later the DOX loaded Py-HMOCs-FA were separated by

centrifugation at 11000 rpm for 20 min followed by washing with excessive amount of water to get rid of unbound DOX. Then the samples were dried in vacuum oven at 60 ºC for 24 h. The filtrate was collected to determine the amount of loaded drug using UV-Vis spectrophotometer. The encapsulation efficiency of Py-HMOCs-FA is calculated by following equation [1]:

$$\boldsymbol{Encapsulation\ efficiency}(\%) = \frac{[DOX]Total - [DOX]supernatant}{[DOX]Total} \times 100 \quad (1)$$

Similarly, the drug loading capacity was determined by applying the following equation [2]:

$$\boldsymbol{Drug\ Loading}\ (\%) = \frac{[DOX]Total - [DOX]supernatant}{Weight\ of\ HMOCs} \times 100 \quad (2)$$

Similarly, for the *in*- vitro testing, variable quantities of DOX (0.1, 0.5, 1.0, 2.5, 5.0 µg/ml) have been loaded on to the HMOCs (10 mg/ ml) to predict the effectiveness of carrier system in the cancer cell lines.

Experimental Procedure for the Release of DOX

The release of DOX was studied at two physiological pH (7.4 and 5.5). In general, the DOX loaded Py-HMOCs-FA (100 mg) was transferred to the semi-permeable bag and soaked in phosphate buffers (50 ml) at body temperature (37 ºC). At pre-determined time interval, 3 ml of the sample was taken out and replenished the same amount immediately. The liquid samples were analyzed by UV-Vis spectroscopy at 480 nm to determine the concentration of DOX released at various intervals. The release kinetics of the DOX. HCl from Py-HMOCs-FA is evaluated by using the equation as under:

$$\boldsymbol{Cumulative\ release}\ (\%) = \frac{Total\ DOX\ Released}{Initial\ Amount\ of\ DOX\ in\ HMOCs} \times 100 \quad (3)$$

Experimental procedure of In-Vitro cytotoxicity and DOX release in Cell lines

To evaluate the cytotoxicity of designed material cell counting kit-8 (CCK-8) was used that determines the cell viabilities via colorimetric assays. Herein the tetrazolium salt, WST-8 was reduced by dehydrogenase activities in cells that imparts a yellow color due to the production of formazan dye. In the cell culture medium, the amount of formazan generated is directly proportional to the number of living cells.

The cell toxicity of Py-HMOCs and Py-HMOCs-FA was analyzed for normal cell lines and cancer cell lines respectively. In general, 100 µl of cells suspension (5000 cells/ Well) were dispensed in the 96- well plate. Initially the plate was incubated for 24 h in a humidified incubator at 37ºC and 5 % CO_2. Then different concentrations of materials (1- 250µg/ mL) had been added to the plate and incubated again for 48 h. Afterwards, 10 µL of CCK-8 solution was added to each well and avoid the bubble formation that might affect the optical density

(OD). reading. Incubate the plate again for 1-4 h. Later, the absorbance at 450 nm was measured using the microplate reader. Similarly, the absorbance was noted again after the addition of 10 µL of 1% w/v SDS or 0.1 M HCl. The absorbance was measured again after 48 h of incubation which remains constant, suggesting the biocompatibility of HMOCs.

Experimental procedure for the Cell internalization assay and DOX release

To observe the release of DOX in cells, the DOX loaded Py-HMOCs and Py-HMOCs-FA were dispensed into the cell medium and incubated for 1 and 6 h respectively. Afterwards, the supernatant was removed and cells are washed with PBS buffer saline for 2 times. The intracellular internalization of DOX was recorded by measuring the fluorescent signals using the confocal laser scanning microscope.

Experimental procedure for the flow cytometry

To validate the cell internalization flow cytometry analysis were conducted. Pure DOX and Py-HMOCs-FA were added to the culture medium and incubated for 4 h at 37ºC. Afterwards the supernatant was removed and samples were washed repeatedly with PBS and flow cytometry was proceeded.

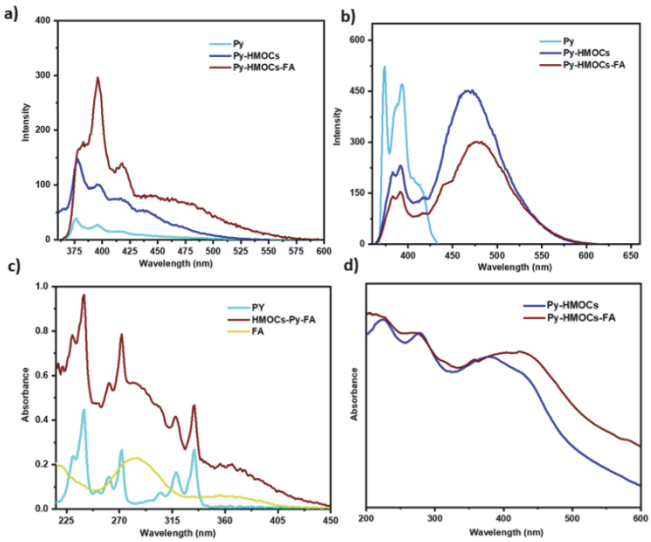

Figure S1: a) Fluorescence spectra of Py-HMOCs and Py-HMOCs-FA in pure water. b) Fluorescence spectra of Py-HMOCs and Py-HMOCs-FA in DMSO-water mixture. c) UV absorbance spectra of Py-HMOCs and Py-HMOCs-FA in solid state. d) UV absorption spectra of Py-HMOCs and Py-HMOCs-FA.

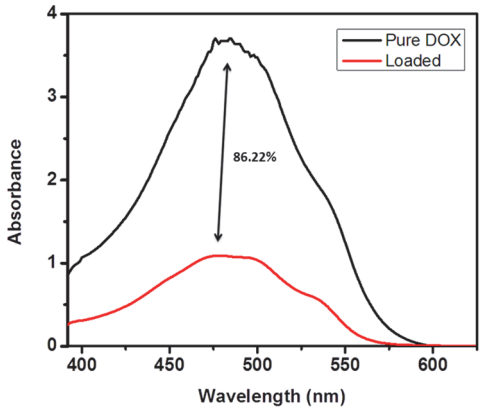

Figure S2: UV- Vis absorbance of free DOX and encapsulation DOX in Py-HMOCs-FA

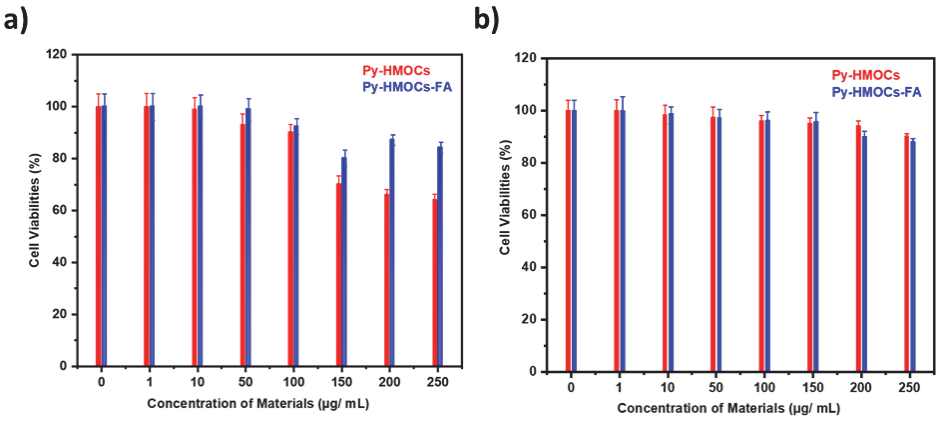

Figure S3: The cell viabilities of a) 2932 and b) MCF-7 cell lines when treated with Py-HOMCs and Py-HMOCs-FA with different concentrations.

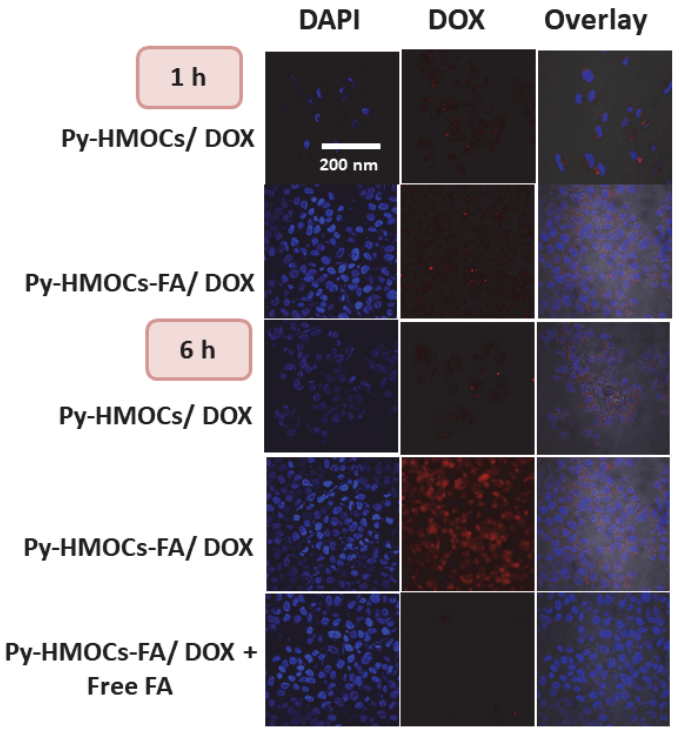

Figure S4: Confocal Microscopic images of DOX delivery to MDA-MB-231 cell lines at different time by Py-HMOCs, Py-HMOCs-FA-DOX and Py-HMOCs-FA-DOX + Free FA. (Scale bar = 200 nm)

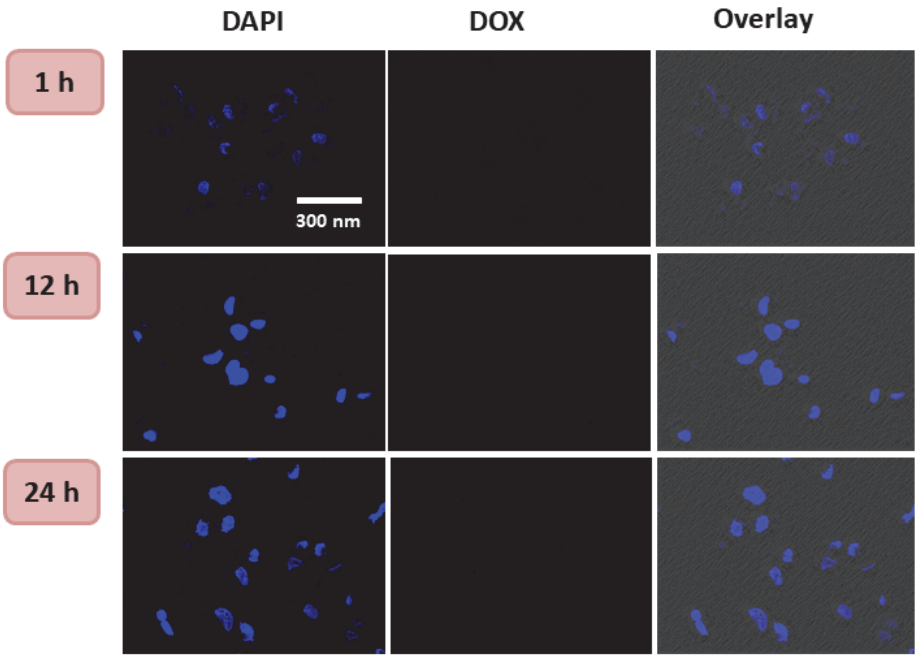

Figure S5: Confocal Microscopic images of DOX delivery to 2932 cell lines were studied at different time by incubating with Py-HMOCs-FA-DOX. (Scale bar = 300 nm)

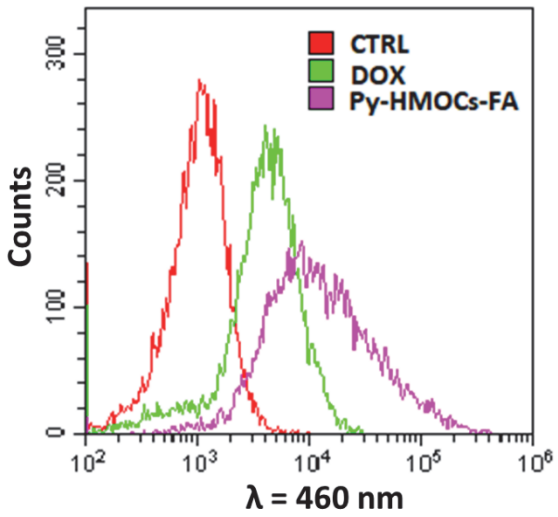

Figure S6: Flow Cytometry analysis of MCF-7 cell lines incubated with free DOX and Py-HMOCs-FA for 4 h. (CTRL is cell medium)

Table S1: Textural properties of Py-HMOCs and Py-HMOCs-FA

Samples	SBET[a] (m^2/g)	SL [b] (m^2/g)	M.A[c] (m^2/g)	PV[d] cm^3/g)	M.P.V[e] (cm^3/g)
Py-HMOCs	758	1010	464	2.37	0.19
Py-HMOCs-FA	695	946	314	1.66	0.11

[a] Surface area calculated through nitrogen adsorption desorption isotherm at 77.3K using BET equation. [b] Surface area calculated through nitrogen adsorption desorption isotherm at 77.3K using Langmuir equation. [c] t-polt micopore area. [d] Plot area calculated by nitrogen sorption isotherm at P/P_0= 0.0995, 77.3K. [e] Plot micropore volume.

Table S2: Elemental analysis results of Py-HMOCs and Py-HMOCs-FA.

Samples	Carbon (%)	Nitrogen (%)	Hydrogen (%)
Py-HMOCs	81.05	0.00	6.07
Py-HMOCs-FA	81.98	2.36	6.74

References

1. P. Yousefpour, F. Atyabi, E. V. Farahani, R. Sakhtianchi and R. Dinarvand, *Int J Nanomedicine*, 2011, **6**, 1487-1496.
2. M. Rajan and V. Raj, *Int J Pharm Pharm Sci*, 2012, **4**, 255-259.