Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supporting Information

Multicompartmentalized vesosomes containing DOX loaded liposomes and 5FU loaded liposomes for synergistic tumor treatment

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S1. DLS measurements of vesosomes

The measurements show that the individual DPPC liposomes (DPPC:Chol = 80:20 in molar ratio) averaged about 160 nm in diameter (Figure S1a), and individual DOPC liposomes (DOPC:Chol = 80:20 in molar ratio) averaged about 650 nm in diameter (Figure S1b). It showed two clear peaks when we mixed up two kinds of liposomes (Figure S1c). In vesosomes, there were also two distinct peaks (Figure S1d), which indicates the DPPC liposomes were encapsulated in DOPC liposomes.

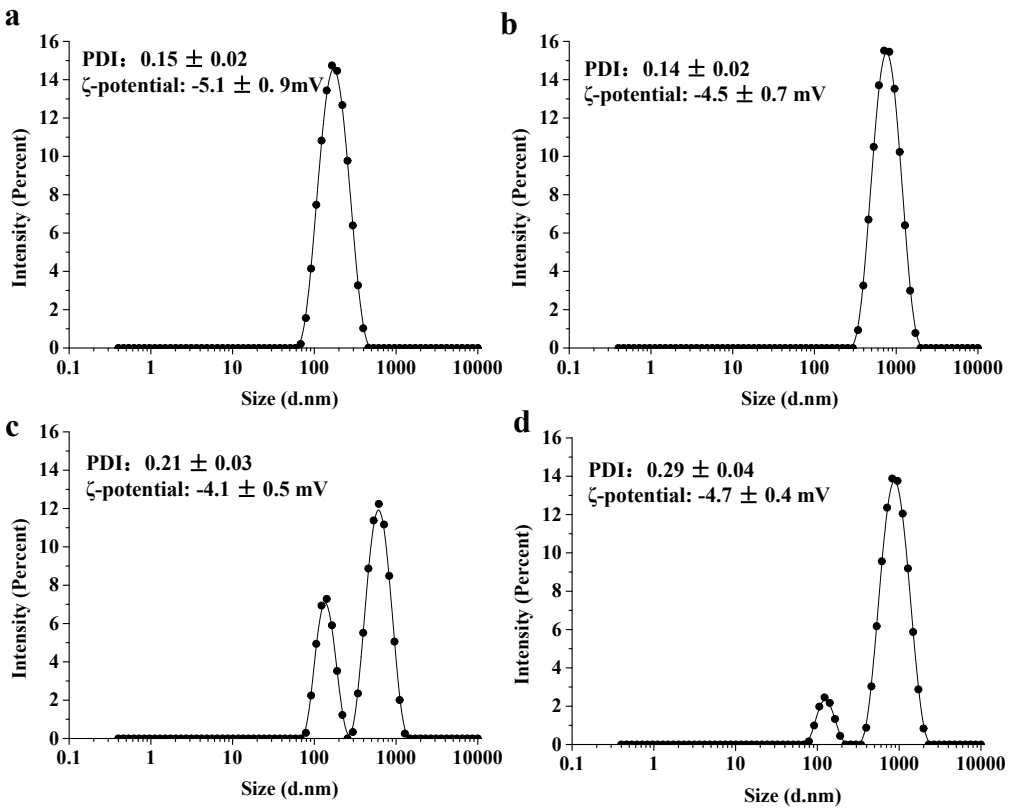


Figure S1. ζ-potential and DLS measurements of (a) individual DPPC liposomes, (b) individual DOPC liposomes, (c) the mixture of DPPC liposomes and DOPC liposomes, and (d) vesosomes.

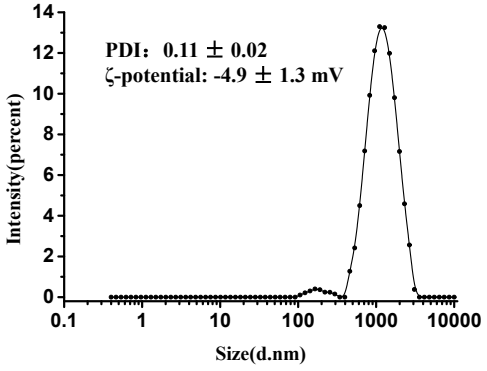


Figure S2. ζ-potential and DLS measurements of vesosomes after filtration

S2. Release profiles of liposomes and vesosomes

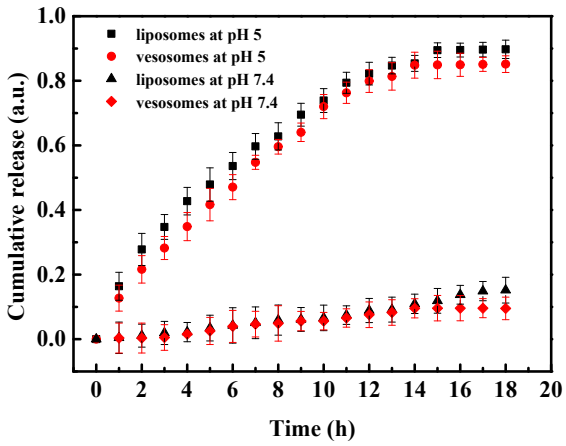
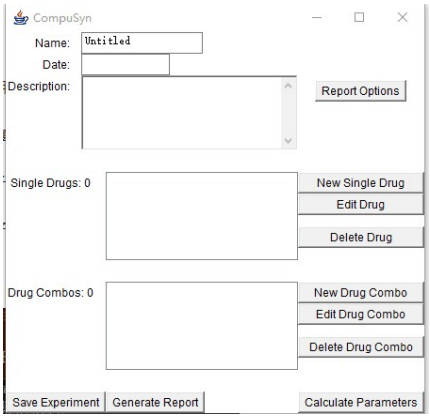


Figure S3. DOX release kinetics of DPPC liposomes and vesosomes at different pH. (n=3, error bar = standard error)

S3. Calculation of CI

The user interface of CompuSyn software is shown as below. Fill the corresponding parameters, and click the "calculate parameters" button, the software will finish the work within a blink.



S4. Determination of drug concentration in vesosomes

Calibration curves of 5FU and DOX are shown in Figure S3 and S4 respectively.

The calibration curve fitting of 5FU is:

$$y = 0.014\ (0.003) + 0.062\ (0.002)\ x \qquad (1)$$

The calibration curve fitting of DOX is:

$$y = 0.0044\ (0) + 0.071\ (0)\ x \qquad (2)$$

The values of intercepts and slopes are expressed as mean value (standard error) in both equations. R^2 values are both 0.99.

According to the calibration curves, the concentrations of 5FU and DOX in free drugs were 0.100 ± 0.004 μg mL^{-1} and 1.000 ± 0.060 μg mL^{-1}, respectively. The same amount of drugs were used to prepare the drugs loaded vesosomes. After purified by Saphadex, vesosomes were broken down by Triton X-100 and vortex, suspensions were filtered by dialysis bag. The supernatant were tested by UV-vis spectrum. 5FU was tested at 265 nm, and DOX was tested at 495 nm. The concentrations of 5FU and DOX in vesosomes were 0.047 ± 0.024 μg mL^{-1} and 0.45 0± 0.072 μg mL^{-1}.

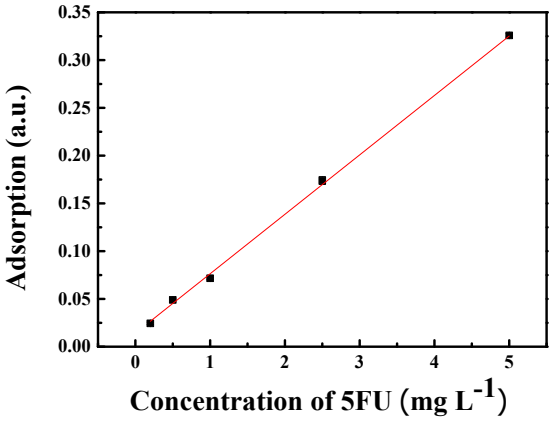


Figure S4. Calibration curve of 5FU

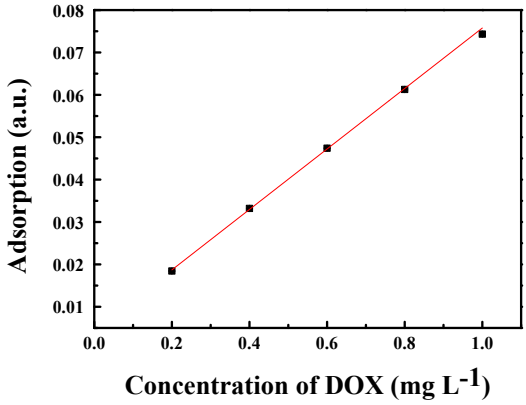


Figure S5. Calibration curve of DOX

S5. Quantitative diagnostic plot

Isobolograms in Figure S5 demonstrate the equivalent dose of individual DOX or 5FU where the combination displays comparable effect. As shown in Figure S5c, the equivalent lines represents additive effect, while the area above the line represents antagonism, and the area below the line represents synergism, respectivley. As to vesosomes, the isobologram indicates that more data points drop in the synergistic area, and both liposomes and vesosomes group has dose reduction of DOX compared to free drug group.

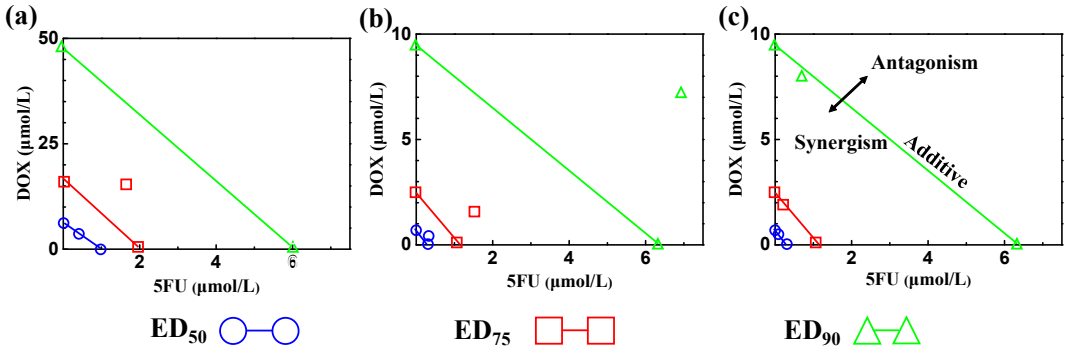


Figure S6. Isobolograms of (a) free drug combination, (b) drug combination in liposomes and (c) drug combination in vesosomes. Blue circle, red square and green triangle represent the experimental data. Blue, red and green lines represent isobolograms of effect dose (ED) of 50%, 75% and 90% inhibitory efficacy, respectively.

The favourable DRI (>1) allows dose reduction that leads to toxicity reduction in the therapeutic application. DRI-Fa diagrams indicate that free drug combination could not take full advantages of 5FU (data dots in the unfavourable dose reduction area as shown in Figure S6c). Although liposome group data points all drop in favourable dose reduction area, the affection of DRI is declining with the increasing of Fa. As to vesosome group, the DRI value of 5FU is increasing, which indicates a better effect in the high Fa area.

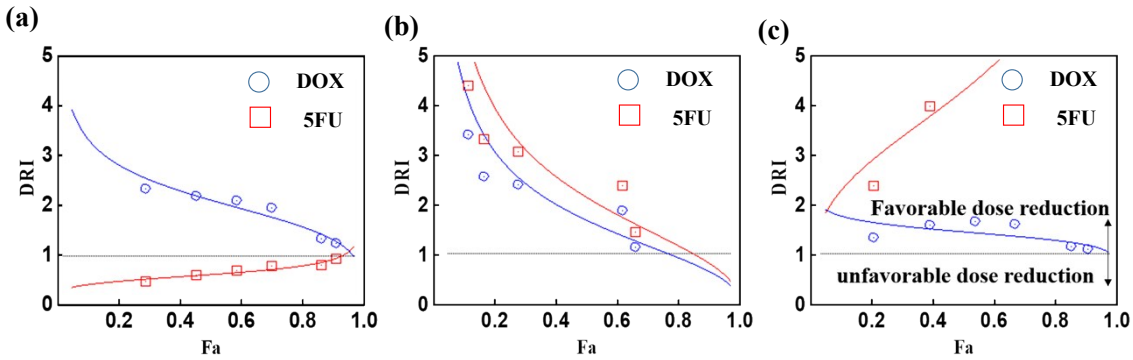


Figure S7. Dose reduction index (DRI) with respect to fraction of affection (Fa) diagrams of (a) free drug combination, (b) drug combination in liposomes and (c) drug combination in vesosomes.

S6. Tolerance of various formulations on mice

Various formulations were injected intravenously to mice, tolerance of each formulation was depicted in Figure S7.

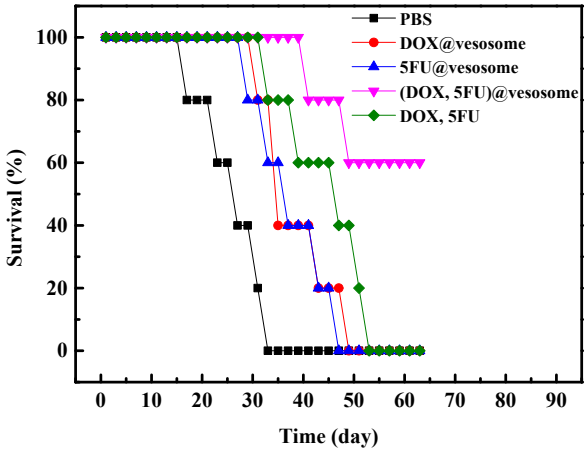


Figure S8. Survival curve of tumor-bearing mice treated by (DOX, 5FU)@vesosome and other controls. (Initial n = 5, and varies according to survival number of mice).