Supplementary Information

Ultrasound-responsive alkaline nanorobots for the treatment of lactic acidosis-mediated doxorubicin resistance

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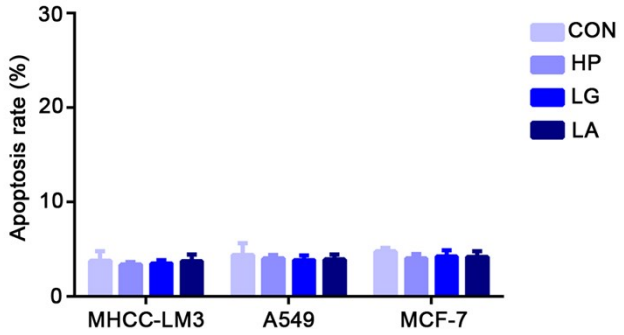

Fig.S1 The apoptosis of MHCC-LM3, A549 and MCF-7 cells induced by pathological conditions, including hypoxia (HP), low glucose (LG) and lactate (LA).

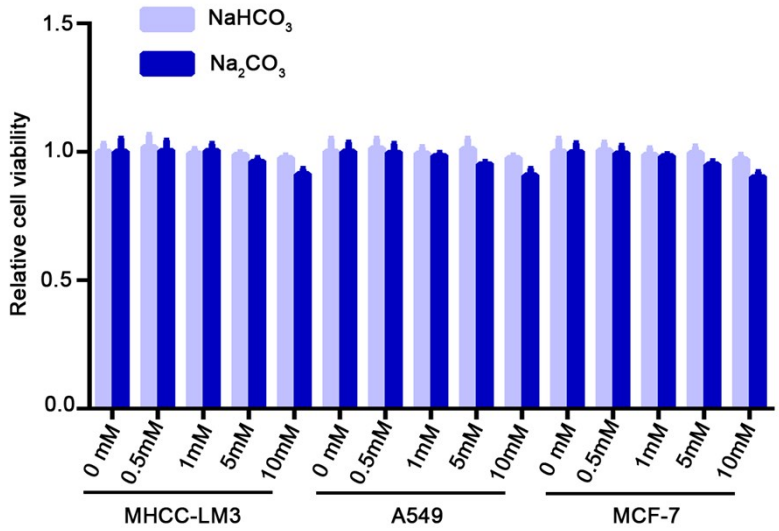

Fig.S2 The cytotoxicity of $NaHCO_3$ and Na_2CO_3 in MHCC-LM3, A549 and MCF-7 cells.

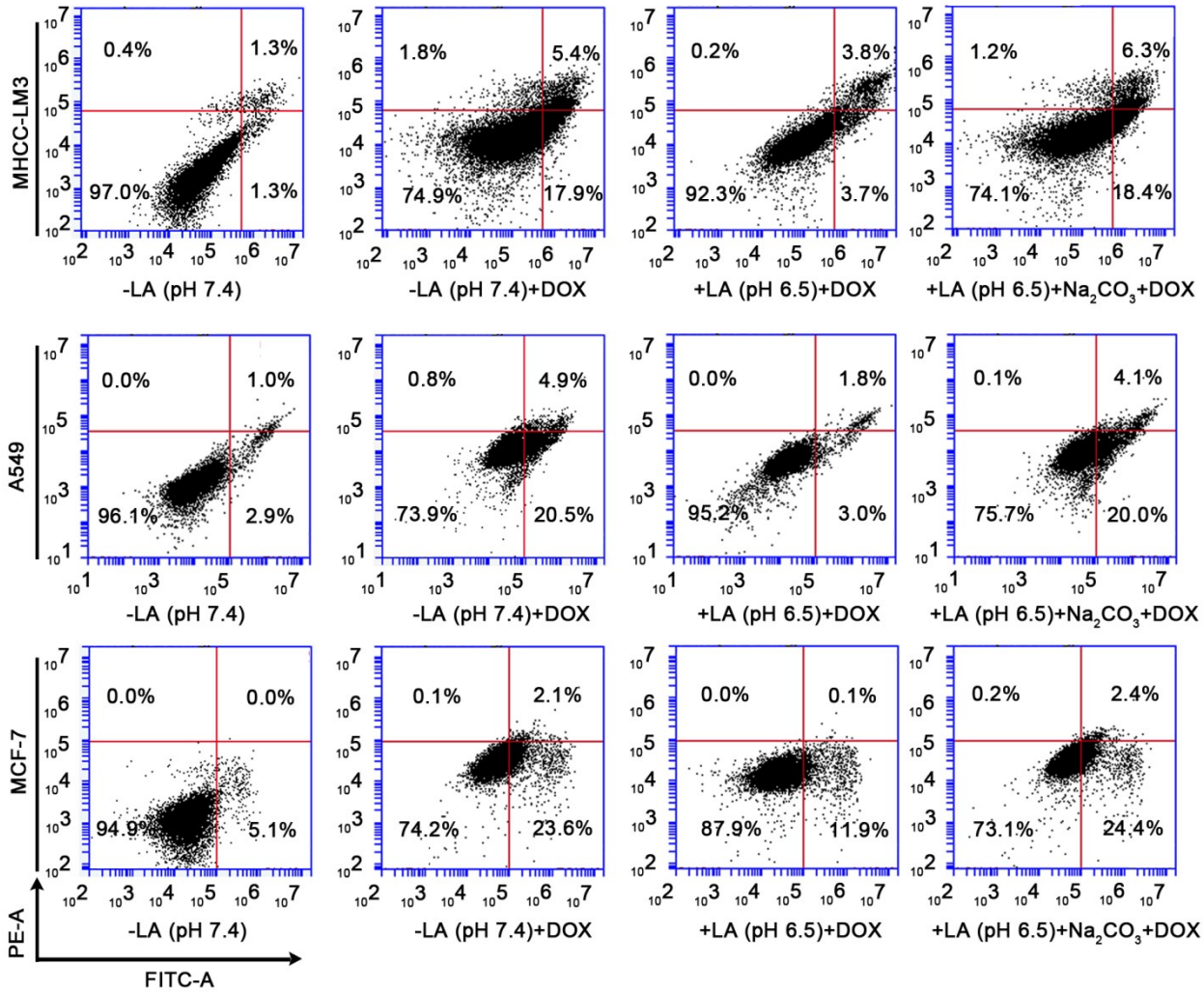

Fig. S3 Cell apoptosis of MHCC-LM3, A549 and MCF-7 cells under different culture conditions for 24 h.

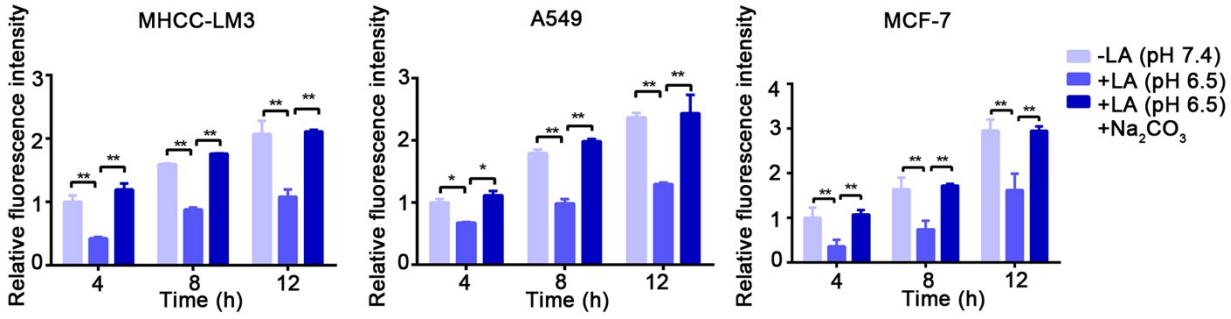

Fig. S4 Flow cytometric measurement of DOX uptake in MHCC-LM3, A549 and MCF-7 cells under different conditions.

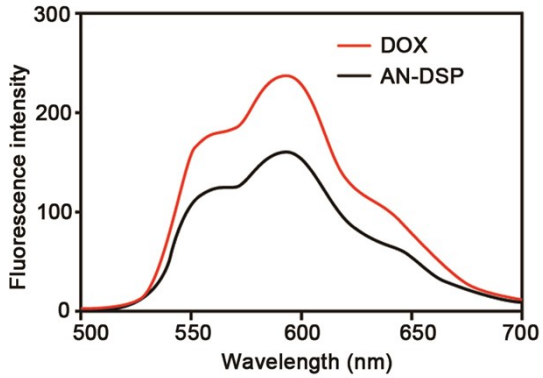

Fig.S5 The fluorescent spectrum of free DOX and AN-DSP.

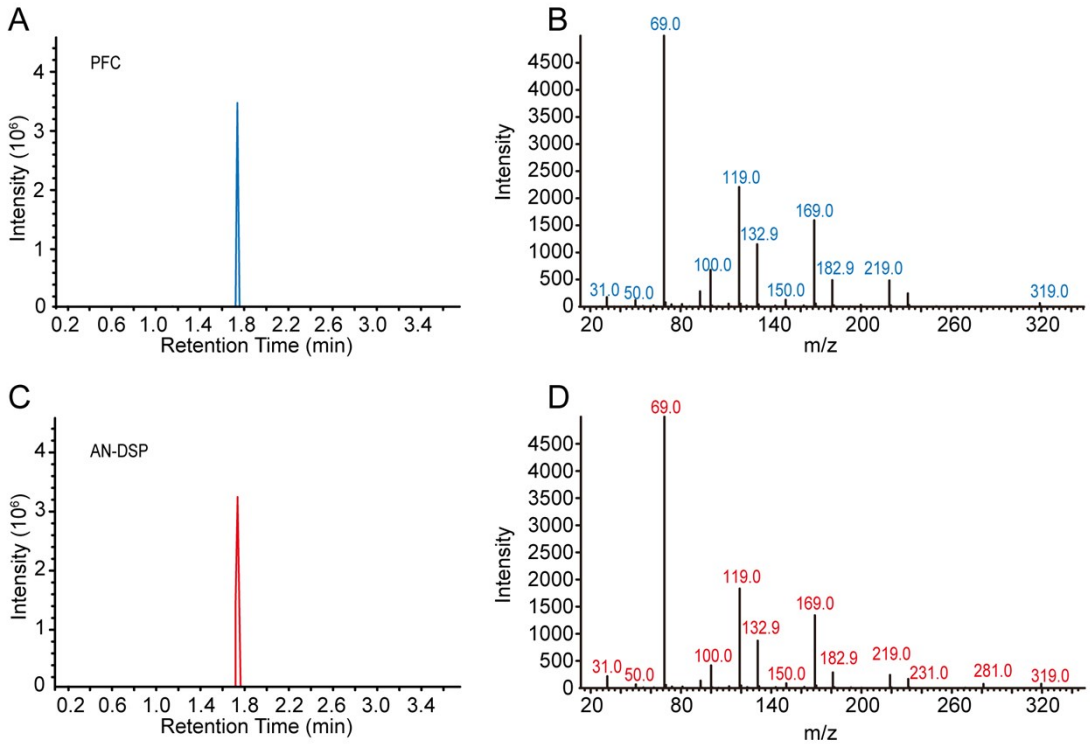

Fig.S6 GC-MS of PFC and AN-DSP.

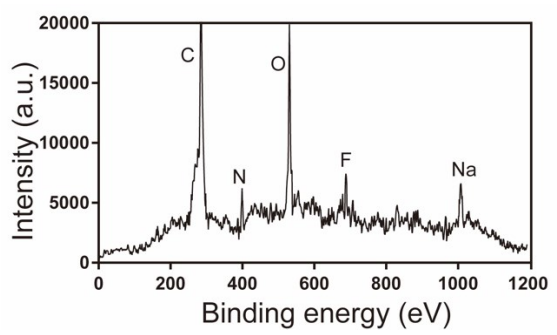

Fig.S7 Energy dispersive spectroscopy of AN-DSP.

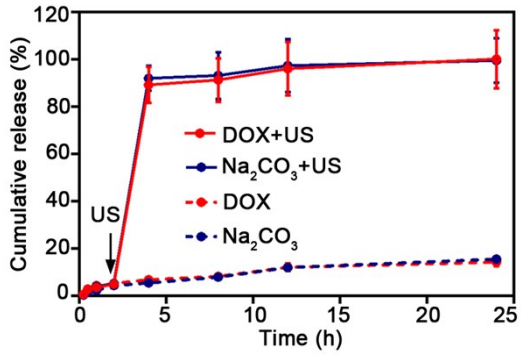

Fig.S8 *In vitro* release profiles of DOX and Na_2CO_3 from AN-DSP in pH 6.5 with or without ultrasound.

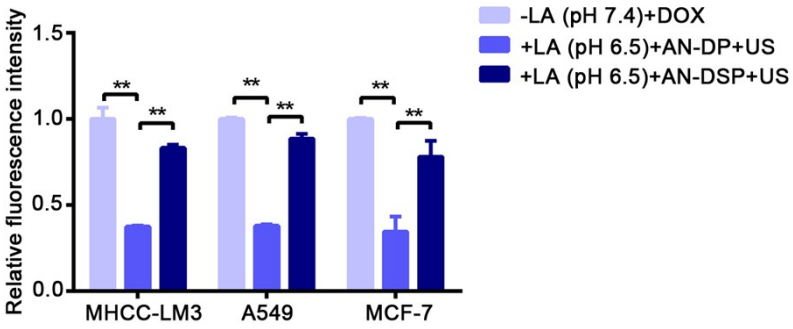

Fig. S9 Flow cytometric measurement of DOX uptake in MHCC-LM3, A549 and MCF-7 cells under different conditions.

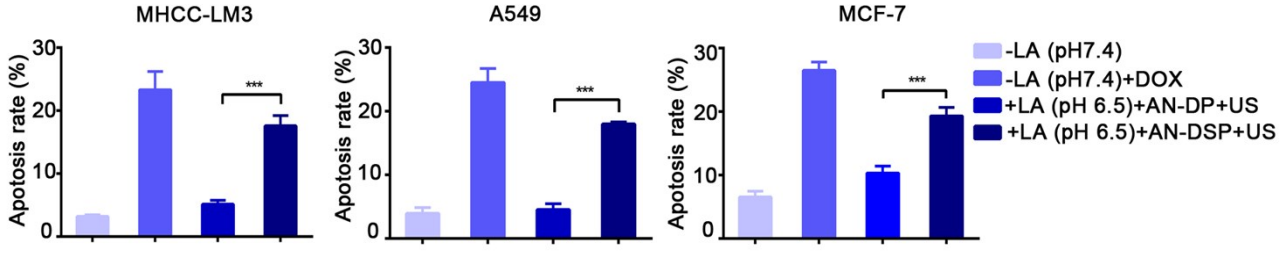

Fig. S10 Cell apoptosis of MHCC-LM3, A549 and MCF-7 under different culture conditions.

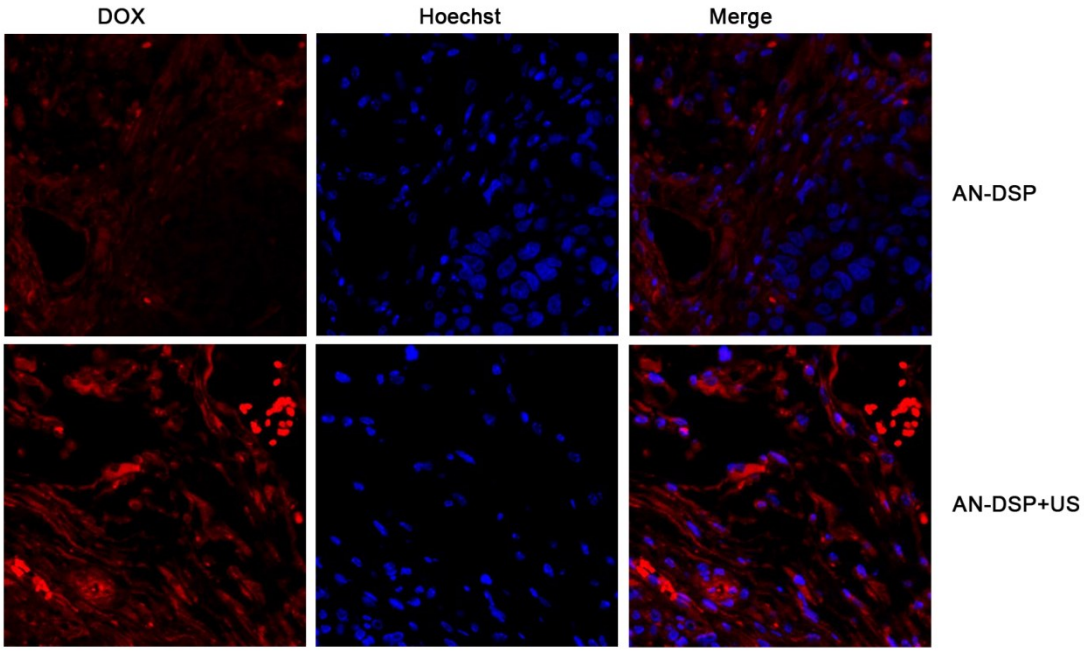

Fig. S11 Cellular uptake of DOX in tumor tissues with or without ultrasound.

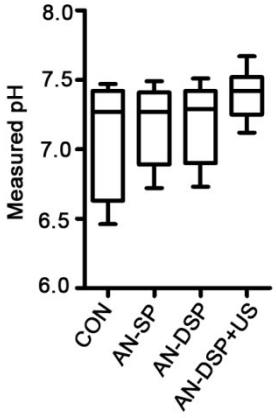

Fig. S12 The measurement of tumor pH. Data are presented as the means ± s. d, n=15.

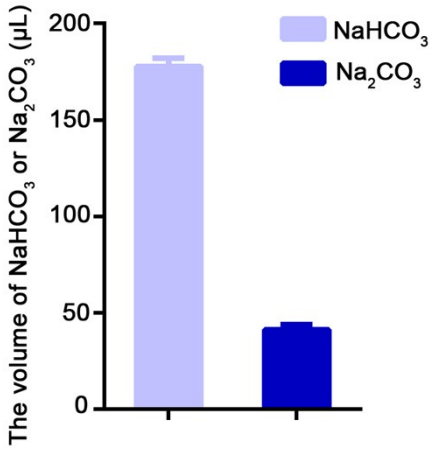

Fig.S13 The ability of $NaHCO_3$ (0.2 M) and Na_2CO_3 (0.2 M) to neutralize 1 mL acidic culture medium (pH 6.5) to pH 7.4.

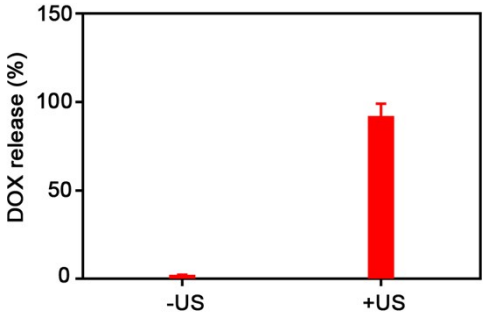

Fig. S14 The release of DOX from AN-DSP under ultrasound using an phantom model composed of agar, glycerol and distilled degassed water (3:4:93, w/w/w).

Table S1. The characterization of different formulations.

Formulations	Particle size	Zeta potential	EE_{DOX}%	EE_{Na2CO3} %	EE_{PFC}%	DL_{DOX}%	DL_{Na2CO3}%	DL_{PFC}%
AN	148.2±2.1	-23.1±0.03	-	-	-	-	-	-
AN-DSP	150.2±1.4	-25.1±0.2	87.4±2.7	73.2±1.4	12.1±0.2	4.5±0.2	7.3±0.1	3.2±0.04
AN-DP	149.2±2.5	-24.1±0.6	89.1±1.9	-	15.3±0.1	4.0±0.3	-	3.5±0.02
AN-SP	151.5±0.9	-24.9±0.4	-	74.1±2.4	11.0±0.3	-	7.9±0.2	3.9±0.05

Abbreviations: AN (ultrasound-responsive alkaline nanorobots), AN-DSP (ultrasound-responsive alkaline nanorobots containing DOX, Na_2CO_3 and PFC), AN-DP (ultrasound-responsive alkaline nanorobots containing DOX and PFC), AN-SP (ultrasound-responsive alkaline nanorobots

containing Na_2CO_3 and PFC), EE_{DOX}% (encapsulation efficiency of DOX), EE_{Na2CO3}% (encapsulation efficiency of Na_2CO_3), EE_{PFC}% (encapsulation efficiency of PFC), DL_{DOX}% (loading content of DOX), DL_{Na2CO3}% (loading content of Na_2CO_3), EE_{PFC}% (loading content of PFC).

Table S2. The hematological parameters of the mice.

Parameter	Control	AN-DSP
WBC (10^9/L)	4.8±1.6	4.3±0.9
RBC (10^{12}/L)	6.6±1.03	6.9±1.4
ALT (IU/L)	48.0±3.6	46.4±2.9
AST (IU/L)	171.5±22.5	183.9±45.1
TP (g/L)	42.9±3.4	47.3±4.9
ALB (g/L)	27.4±3.9	24.9±5.2
HGB (g/L)	121±21.7	112.6±19.4
UREA (mmol/L)	6.4±1.2	7.3±1.1

Abbreviations: WBC: white blood cell, RBC: red blood cell, ALT (alanine aminotransferase), AST (aspartate aminotransferase), TP (total protein), ALB (albumin), HGB: hemoglobin, UREA (urea nitrogen).