SUPPLEMENTARY MATERIAL FOR:

Anti-tumor effect of pH-sensitive drug-loaded nanoparticles optimized via an integrated computational/experimental approach

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SUPPLEMENTARY TABLES

Supplementary Table 1. Measurements of DOX release from SPNCD in PBS at different values of pH.

Time (h)	Drug release (%)								
	pH 7.4			pH 7.1			pH 6.8		
1	0.11	0.13	0.11	0	0.11	0.15	3.7	4.9	5.2
2	0.11	0.14	0.15	0	0.12	0.15	8.7	10.2	9.0
5	0.15	0.17	0.17	1.3	1.2	1.7	15.9	17.2	19.5
24	0.18	0.22	0.18	1.8	1.7	1.9	30.3	35.7	36.8
48	0.23	0.25	0.19	2.5	2.7	3.7	38.7	44.1	46.4
Time (h)	pH 6.5			pH 6.0			pH 5.5		
1	12.4	13.0	11.9	6.7	7.9	7.2	12.5	14.3	11.9
2	15.6	15.9	15.5	13.1	13.6	13.2	15.8	17.2	16.3
5	25.5	24.9	25.8	29.3	28.7	29.2	31.2	33.5	33.1
24	51.5	55.3	52.5	58.9	60.3	59.5	65.8	64.9	65.3
48	62.4	67.7	61.8	69.1	69.7	70.2	73.7	77.2	73.3

Supplementary Table 2. Computational model parameters for best-fit equation (1) used to fit SPNCD DOX release at specific pH values (corresponding to those experimentally observed in **Figure 1**). Each SPNCD type represents a different DOX release rate profile (as defined in **Supplementary Figure 6**), where Rate 1 is slowest, Rate 2 is slower, Rate 3 is the experimental baseline (**Figure 1D**), Rate 4 is faster, and Rate 5 displays the fastest DOX release. N is cumulative fraction of drug remaining, t is time and T_A is non-dimensionalized tumor acidity based on the lactate concentration. Parameter n_1 represents the fraction of initial (non-dimensionalized to 1) drug concentration, while n_2 and n_3 represent drug fractions in SPNCD after 24 h under physiological pH and lysosomal pH conditions, respectively.

$$N = \left[n_1 - \left(n_2 - T_A(n_2 - n_3)\right)\right]e^{-(\alpha+\beta T_A)t} + \left(n_2 - T_A(n_2 - n_3)\right) - \gamma t(1 + (1 - T_A))^{\varepsilon}$$

Parameter

| pH	n_1	n_2	n_3	α	β	γ	ε
				Rate 1			
5.5	1.01036	0.00000	0.68975	0.00000	0.13951	0.00132	0.00000
6.0	0.98768	0.20404	1.87712	0.11732	0.00000	0.00053	1.71816
6.5	1.00935	0.79234	0.85908	0.16969	0.42954	0.00100	1.28862
6.8	0.98390	0.77618	3.43632	0.02167	2.90830	0.00150	0.00000
7.1	0.98965	1.28862	1.28862	0.00722	0.11161	0.00120	0.85908
7.4	1.00368	0.00000	0.00000	0.00000	0.00000	0.00002	0.81563
				Rate 2			
5.5	0.98988	0.00000	0.59311	0.00000	0.13228	0.00175	0.00000
6.0	1.00277	0.72508	0.53632	0.15450	0.00000	0.00170	0.85908
6.5	0.98260	0.50369	3.00678	0.05028	1.28862	0.00042	3.00678
6.8	0.98542	0.84669	0.97419	0.12679	1.28862	0.00127	0.85908
7.1	1.00492	0.97578	0.85908	0.01926	1.38803	0.00000	0.00000
7.4	0.99104	0.24719	0.00000	0.00003	0.00000	0.00001	0.54379
				Rate 3 (experimental baseline)			
5.5	0.97549	1.50840	0.29251	0.00000	0.09145	0.00093	0.00000
6.0	0.99074	0.00000	1.13002	0.01962	0.21150	0.00094	0.00000
6.5	0.98885	0.57895	0.90504	0.04469	1.81008	0.00185	1.50840
6.8	0.98975	0.77177	0.30168	0.00241	3.19444	0.00047	2.71512
7.1	0.98975	1.23651	0.30168	0.00241	0.13896	0.00134	0.30168
7.4	1.00772	0.00000	0.00000	0.00000	0.00000	0.00002	0.71827
				Rate 4			
5.5	0.98443	0.00000	0.20527	0.05614	0.23972	0.00143	0.00000
6.0	0.98833	0.00000	0.99640	0.00000	0.91146	0.00085	1.28862
6.5	0.98828	0.44643	0.00000	0.37980	0.99318	0.00329	0.00000
6.8	1.00318	0.64430	0.50223	0.27604	3.86586	0.00042	2.14770
7.1	0.98893	0.93753	3.43632	0.01445	0.61190	0.00000	0.00000
7.4	1.00167	0.00000	0.00000	0.00000	0.00000	0.00002	0.94556
				Rate 5			
5.5	0.98448	0.71509	0.04338	0.04043	0.22665	0.00000	0.00000
6.0	0.98591	0.31285	0.02026	0.03919	0.78125	0.00049	2.57724
6.5	0.98936	0.08409	2.92794	0.38281	0.95346	0.00197	0.98630
6.8	1.00919	0.63263	0.00000	0.34278	3.00678	0.00176	0.42954
7.1	0.98651	0.89169	3.86586	0.00241	0.25692	0.00000	0.00000
7.4	1.00167	0.00000	0.00000	0.00000	0.00000	0.00002	0.94556

SUPPLEMENTARY FIGURES
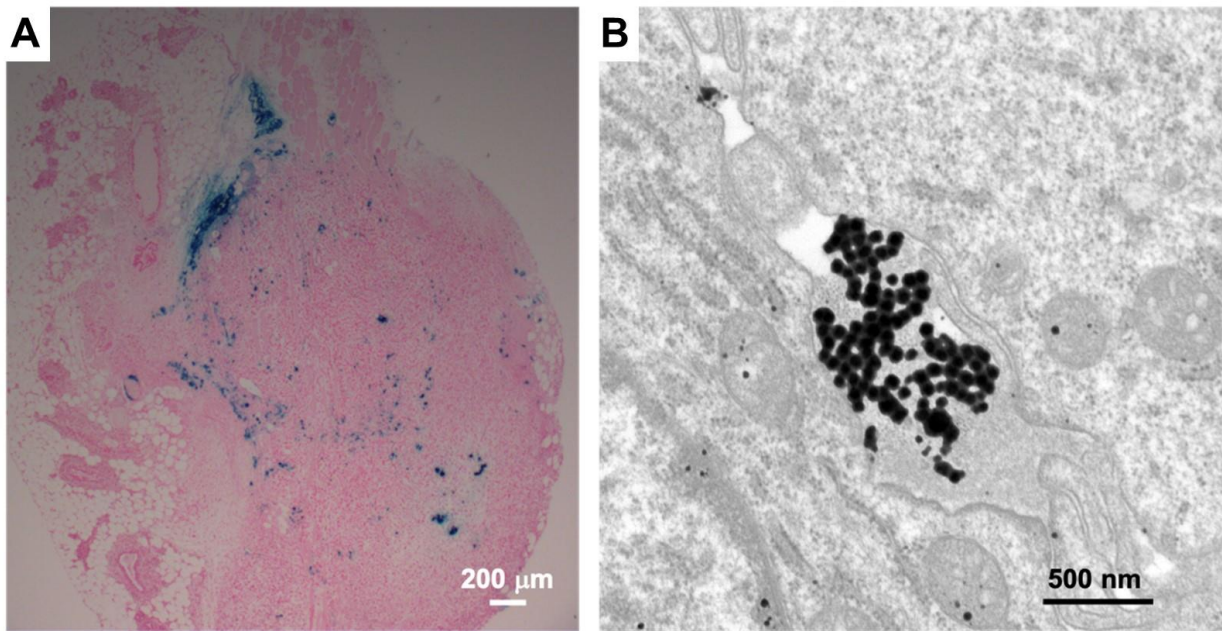
Supplementary Figure 1. Evaluation of SPNCD distribution and degradation in tumor tissue. (A) Representative tumor tissue slice (pink) stained with Prussian blue shows SPNCD distribution after intra-tumoral injection in mice. (B) Transmission electron microscopy (TEM) image of tumor slice shows degradation of SPNCD (black).

Time (h)	fluorescence	ID (%)
0	9613800	100.00
0.17	7152849.997	74.40
0.5	3953749.997	41.13
1	1858920.003	19.34
2	929580.0002	9.67
5	459019.9997	4.77
9	193850.0002	2.02
14	89020.00016	0.93
24	39809.99977	0.41
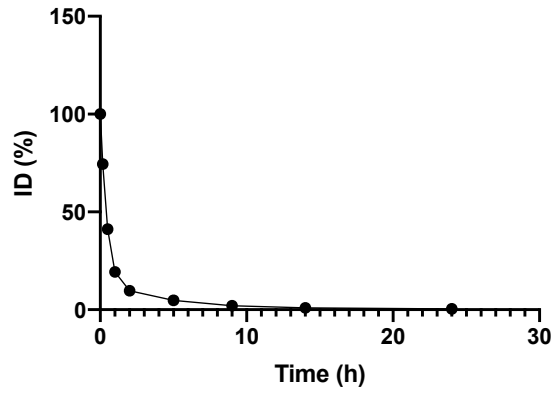
Supplementary Figure 2. Experimental data of *in vivo* SPNCD washout as measured in blood circulation of mice. Percent injected dose (ID %) = (Concentration of SPNCD at specific timepoint) / (initial SPNCD concentration) x 100%.
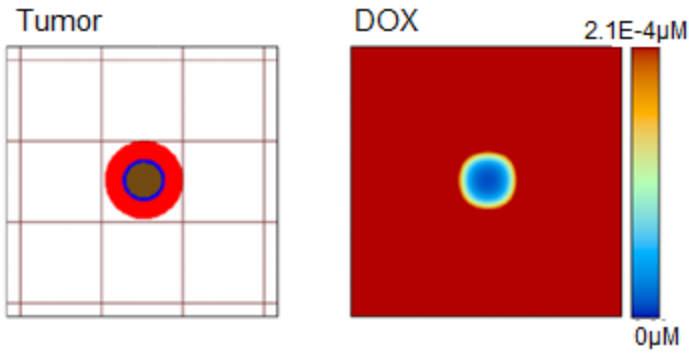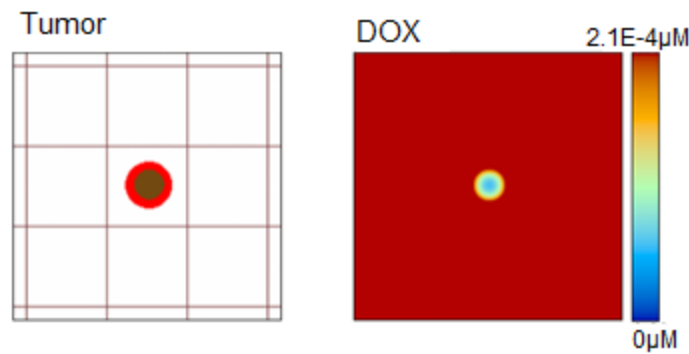
Supplementary Figure 3. Calibration of model-simulated drug effect. Representative images of calibration of drug effect parameter via simulation of an avascular spheroid. In the tumor panels, red denotes proliferating tissue, blue indicates hypoxic tissue, and brown indicates necrotic tissue. Rectangular lines denote capillaries acting as source of drug but without interaction with the tumor tissue. A drug effect parameter value of 5.3 was found to be equivalent to the 48-h IC_{50} experimentally measured with MDA-BM-231 breast cancer cell spheroids *in vitro* (2).
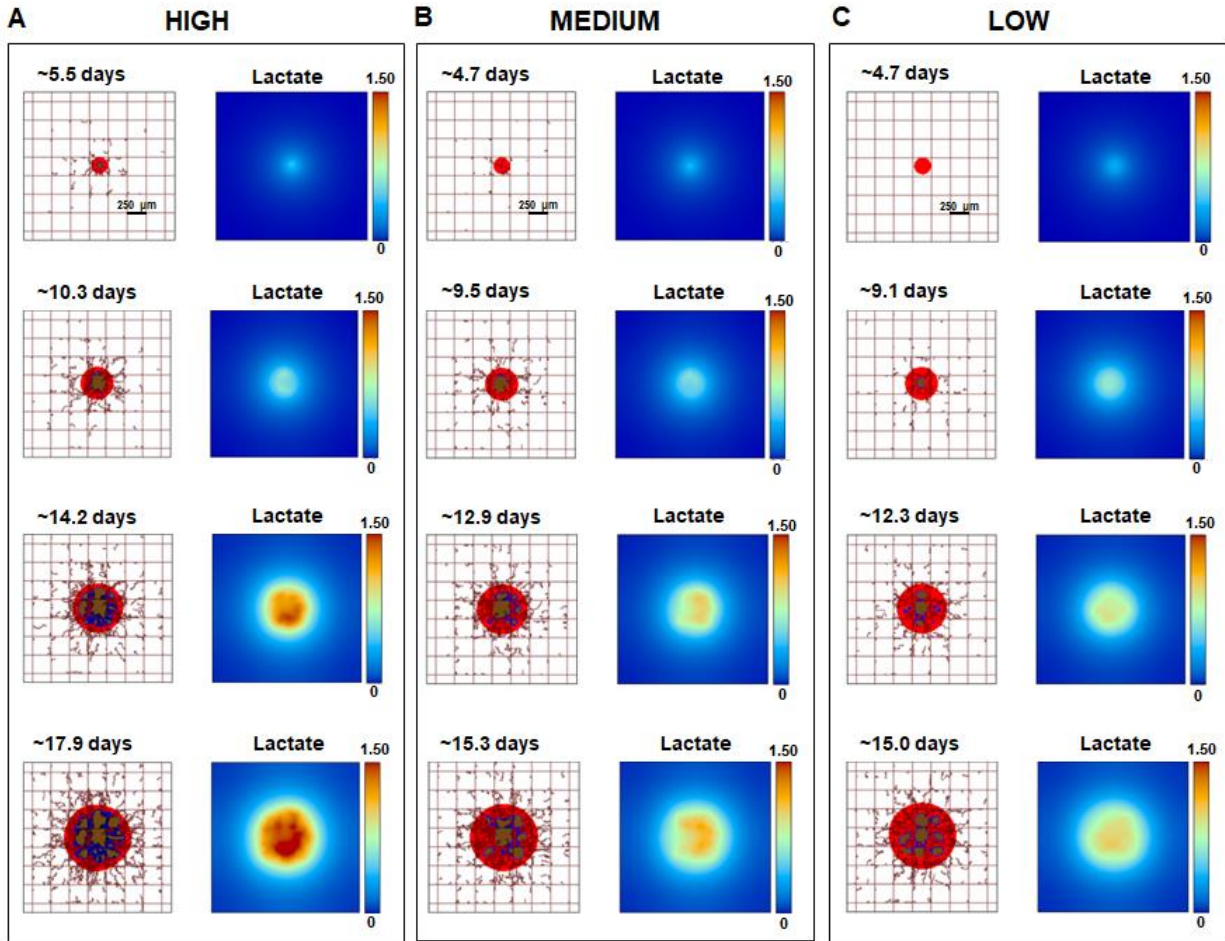
Supplementary Figure 4. Simulation of tumor growth as a function of tissue vascular heterogeneity. (A) HIGH, (B) MEDIUM, and (C) LOW vascular heterogeneity are shown. In the tumor panels, red denotes proliferating tissue, blue indicates hypoxic tissue, and brown indicates necrotic tissue. Pre-existing capillary grid is shown as rectangular lines along with sprouts growing due to angiogenesis. Lactate is shown in non-dimensional units. Bar: 250 μm.

A

Proliferating tissue fraction vs HIGH, MEDIUM, LOW

B

Tumor Vessel SA (μm^2) vs HIGH, MEDIUM, LOW
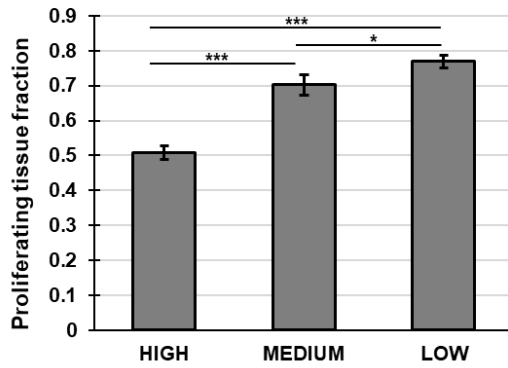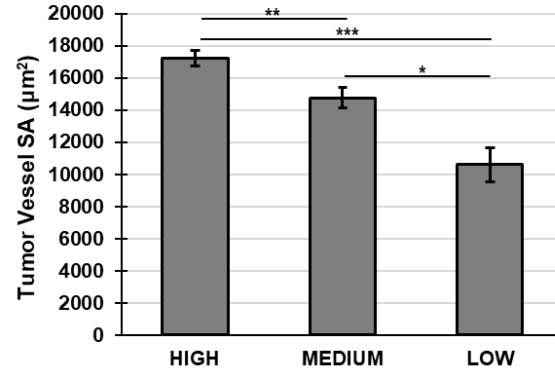
Supplementary Figure 5. Quantification of tumor tissue characteristics based on vascular heterogeneity. (A) Proliferating tissue fraction and (B) tumor vessel surface area (SA) were quantified at the start of therapy simulations. Error bars represent $\pm$SD (n=5). *P < 0.05; **P < 0.001; ***P < 0.0001.
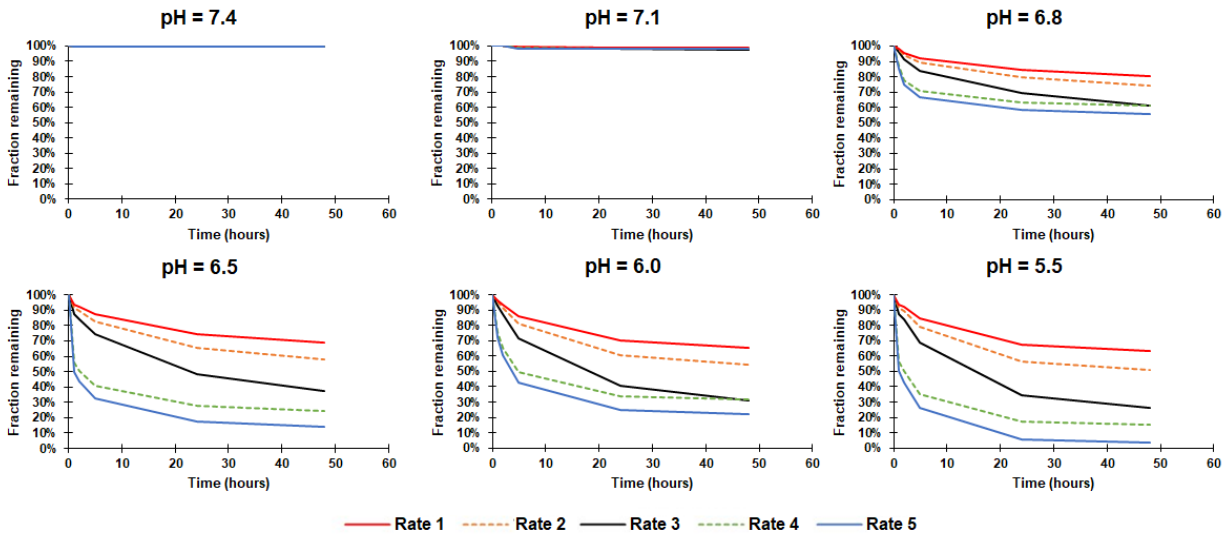
Supplementary Figure 6. Best fit equations of DOX release profiles from SPNCD at each pH value. Drug fraction remaining is shown over time as calculated by the values from the best-fit equation (**Supplementary Table 2**). Rate 1 is slowest release, Rate 2 is slower, Rate 3 is the experimental baseline **(Figure 1D)**, Rate 4 is faster, and Rate 5 has the fastest DOX release.

REFERENCES

1.   Curtis LT, Rychahou P, Bae Y, Frieboes HB. A Computational/Experimental Assessment of Antitumor Activity of Polymer Nanoassemblies for pH-Controlled Drug Delivery to Primary and Metastatic Tumors. Pharm Res. 2016;33(10):2552-64.
2.   Gebeyehu A, Surapaneni SK, Huang J, Mondal A, Wang VZ, Haruna NF, et al. Polysaccharide hydrogel based 3D printed tumor models for chemotherapeutic drug screening. Sci Rep. 2021;11(1):372.