Supporting Information

A novel aminoclay-curcumin hybrid for enhanced chemotherapy

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Differential Scanning Calorimetry (DSC) Analysis

The physical state of the Cur in the AC-Cur hybrid was characterized using a Q2000 DSC (TA Instrument Co. Inc.). Each sample was encapsulated separately in hermetical Tzero aluminum pans and the reference pan was an empty hermetical Tzero aluminum pan. The samples were purged in DSC with pure dry nitrogen gas which was set at a flow rate of 50 mL/min, the temperature speed was set at 10 °C/min, and the heat flow was recorded from 50 to 200 °C.

Cellular Uptake Efficiency by Flow Cytometry (FCM)

For quantification of cellular uptake efficiency using FCM (BD FACSCalibur™, BD Biosciences, San Jose, CA, USA), HeLa cells were seeded in 6-well plates and free Cur and the AC-Cur hybrid were used at 2 μg Cur/mL. At various time points post incubation, the media were removed, followed by washing twice with PBS to remove the Cur remained outside of the cells. Afterwards, the cells were detached using trypsin/EDTA, washed twice by PBS. At last, the suspensions of cells were measured on a FCM with excitation at 488 nm, and 10,000 viable cells were evaluated in each experiment. The experiment was carried out in triplicates.

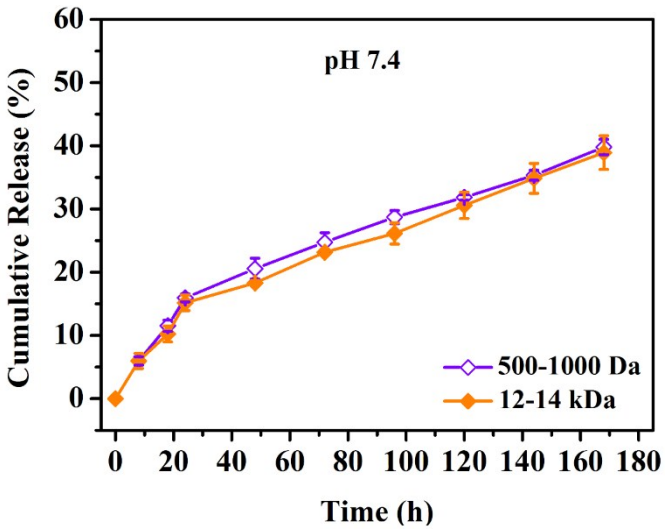


Figure S1. Time-dependent cumulative release of Cur from the AC-Cur hybrid in 0.01 mol/L, pH 7.4 PBS by using dialysis tubing with different MWCO, 500-1000 Da and 12-14 kDa.

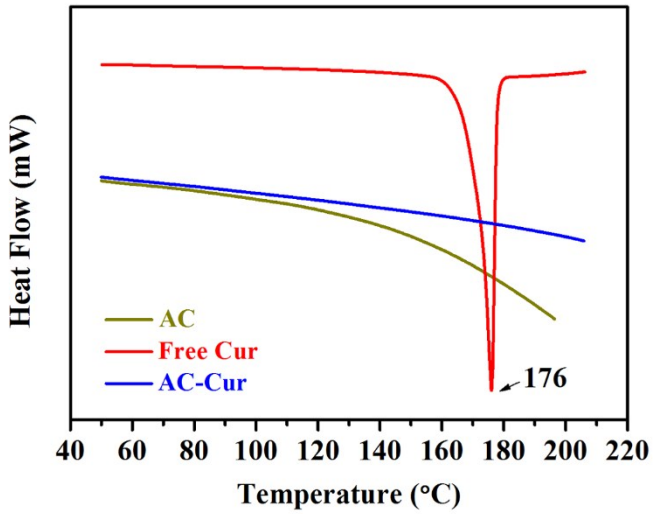


Figure S2. DSC thermogram of free Cur, AC and the AC-Cur hybrid.

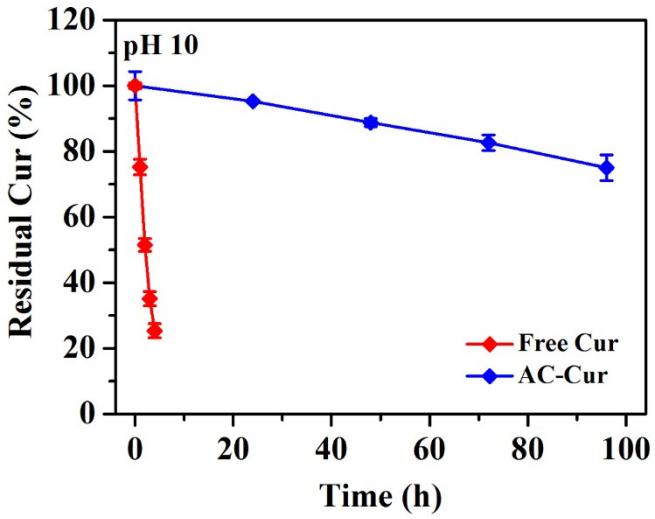


Figure S3. The changes of the UV-Vis absorption intensities as a function of incubation time of free Cur and the AC-Cur hybrid with the same pH value (pH 10) at different incubation times at room temperature in darkness.

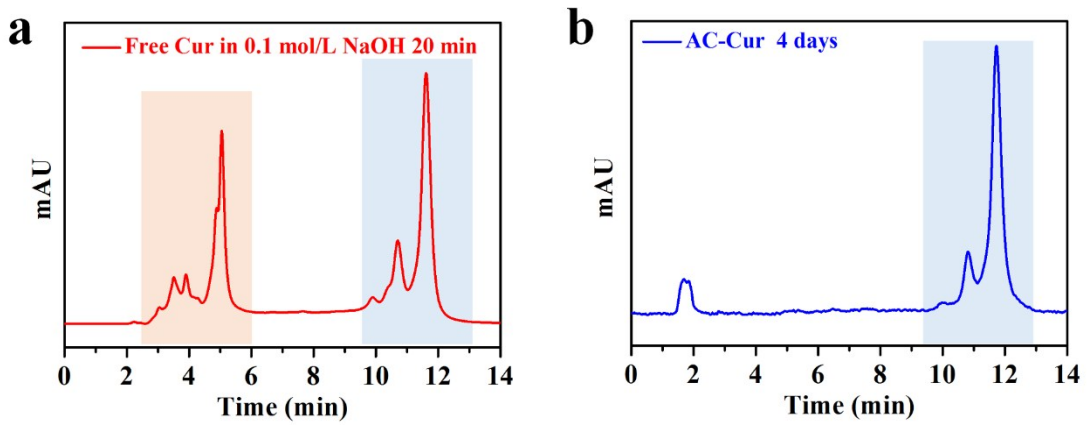


Figure S4. High Performance Liquid Chromatography (HPLC) analysis of the degradation products of (a) free Cur and (b) the AC-Cur hybrid with the same pH values (pH 10) for different incubation times at the room temperature in darkness. HPLC (VP10A, SHIMADZU, Japan) with column Hypersil BDS C18 (5 mm × 250 mm, 5 μm) and the mobile phase consisted of acetonitrile and 5 % (w/v) of acetic acid at the volume ratio of 48:52. The flow rate was set at 1.0 mL/min and the analysis wavelength was at 310 nm for Cur by UV detector. All the analysis was performed at 25 °C. The pink area was the major degradation products of Cur (i.e. vanillin, ferulic acid and feruloylmethane); the blue areas were the residual Cur.

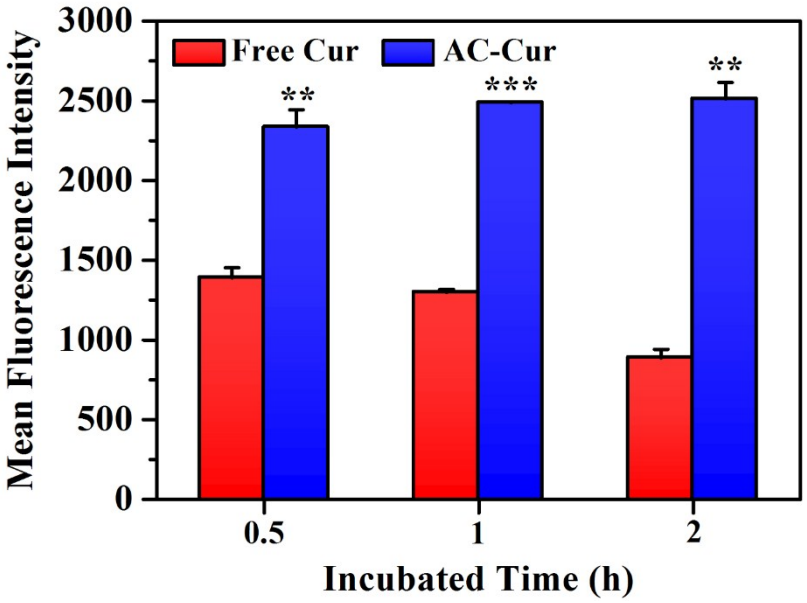
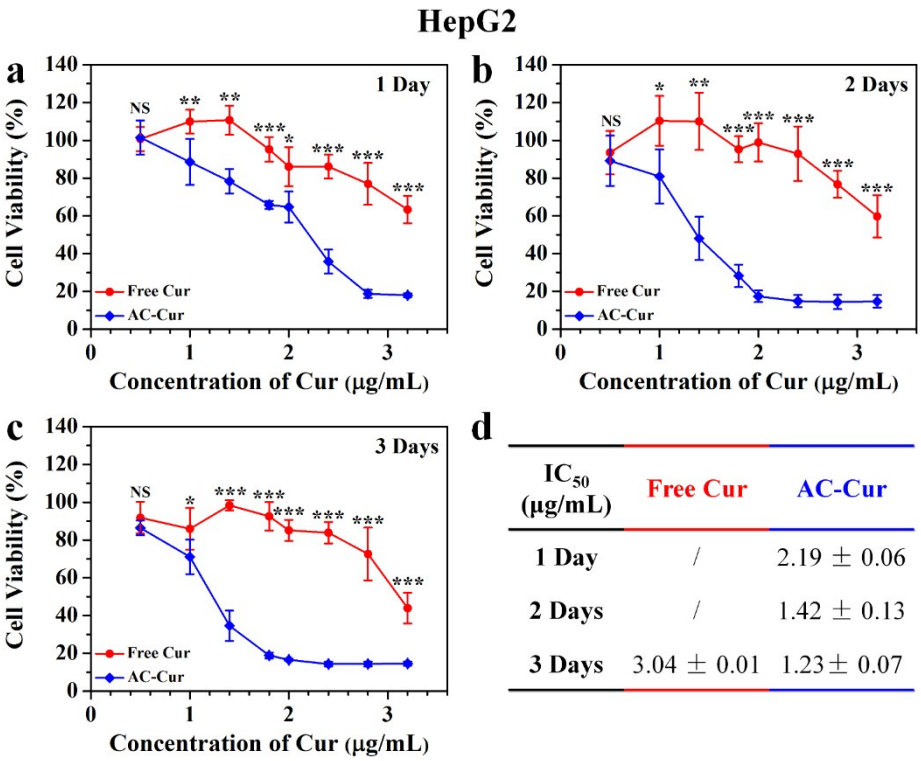


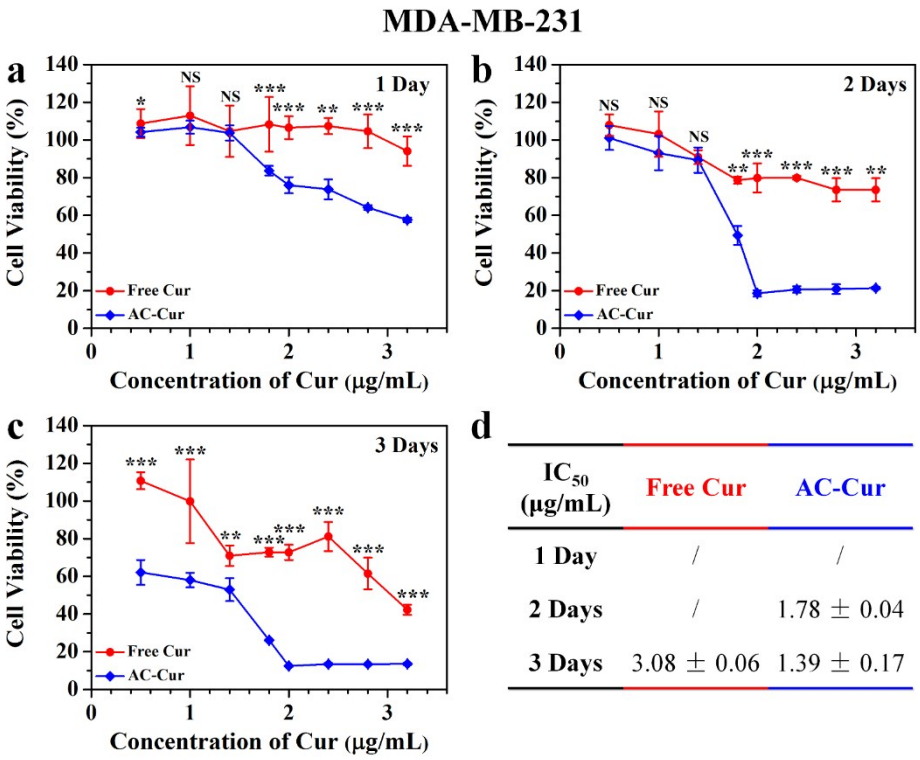
Figure S5. Mean fluorescence intensity of free Cur and the AC-Cur hybrid by FCM. Free Cur and the AC-Cur hybrid (Cur: 2 μg/mL) were added to HeLa cells at 0 h, and evaluated at 0.5, 1 and 2 h post incubation. Data are expressed as mean ± SD (n=3).



d

IC_{50} (μg/mL)	Free Cur	AC-Cur
1 Day	/	2.19 ± 0.06
2 Days	/	1.42 ± 0.13
3 Days	3.04 ± 0.01	1.23 ± 0.07

Figure S6. HepG2 cell viability after the treatment of free Cur and the AC-Cur hybrid for (a) 1 day; (b) 2 days; and (c) 3 days (n=4). Cell viability of control (untreated HepG2 cells): 100%. (d) Comparison of the IC_{50} (μg/mL) values. Data are expressed as mean ± SD (n=4).



d

IC_{50} (μg/mL)	Free Cur	AC-Cur
1 Day	/	/
2 Days	/	1.78 ± 0.04
3 Days	3.08 ± 0.06	1.39 ± 0.17

Figure S7. MDA-MB-231 cell viability after the treatment of free Cur and the AC-Cur hybrid for (a) 1 day; (b) 2 days; and (c) 3 days (n=4). Cell viability of control (untreated MDA-MB-231 cells): 100%. (d) Comparison of the IC_{50} (μg/mL) values. Data are expressed as mean ± SD (n=4).

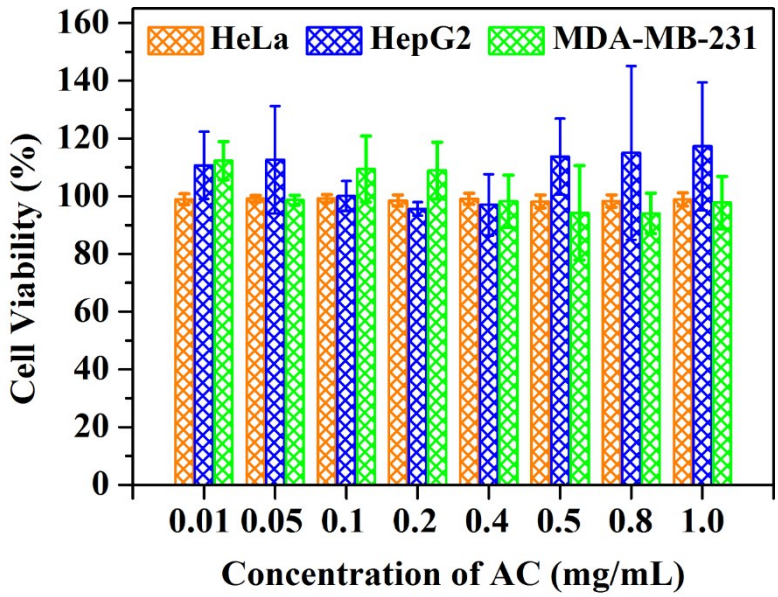


Figure S8. Effects of the AC on HeLa, HepG2 and MDA-MB-231 cell viability. Concentration-dependent cytotoxic effects of the AC were evaluated after 3 days' incubation by CCK-8 assay.