Electronic Supplementary Material (ESI) for Analyst.

Supporting information

3D Hanging Spheroid-Filter Plate for High-throughput Drug Testing and CAR T Cell Cytotoxicity Assay

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Supporting Figures

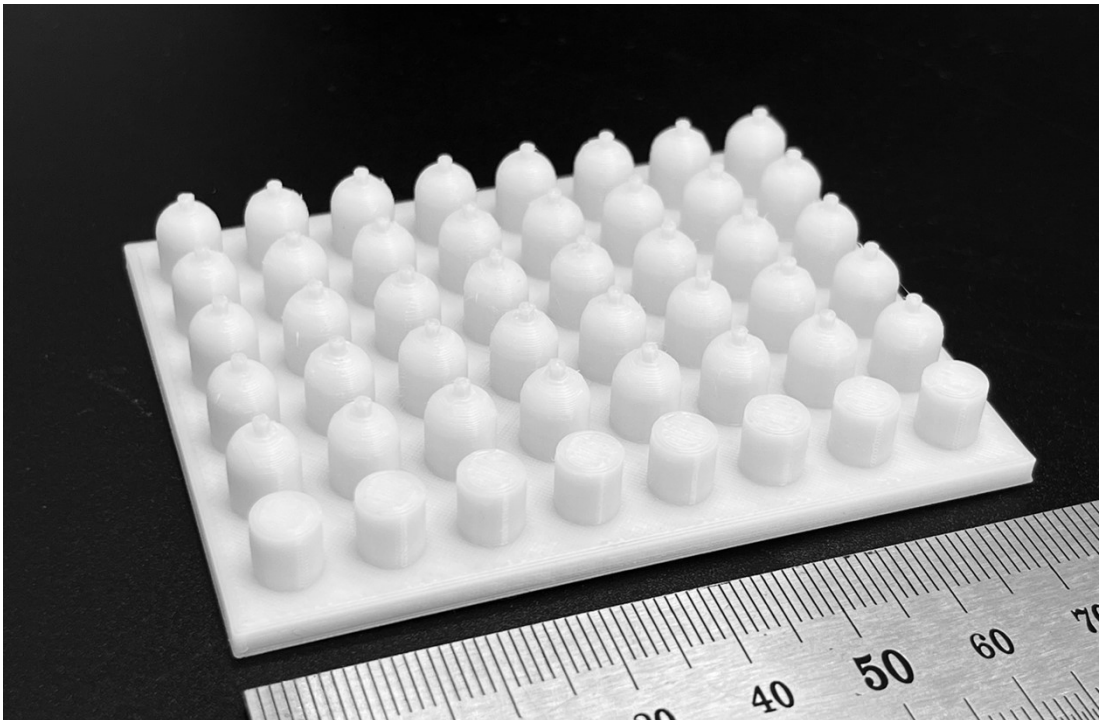

Figure S1. Photograph of cell culture array molds fabricated from ABS filament via 3D printer (Single Plus – 320C, Cubicon).

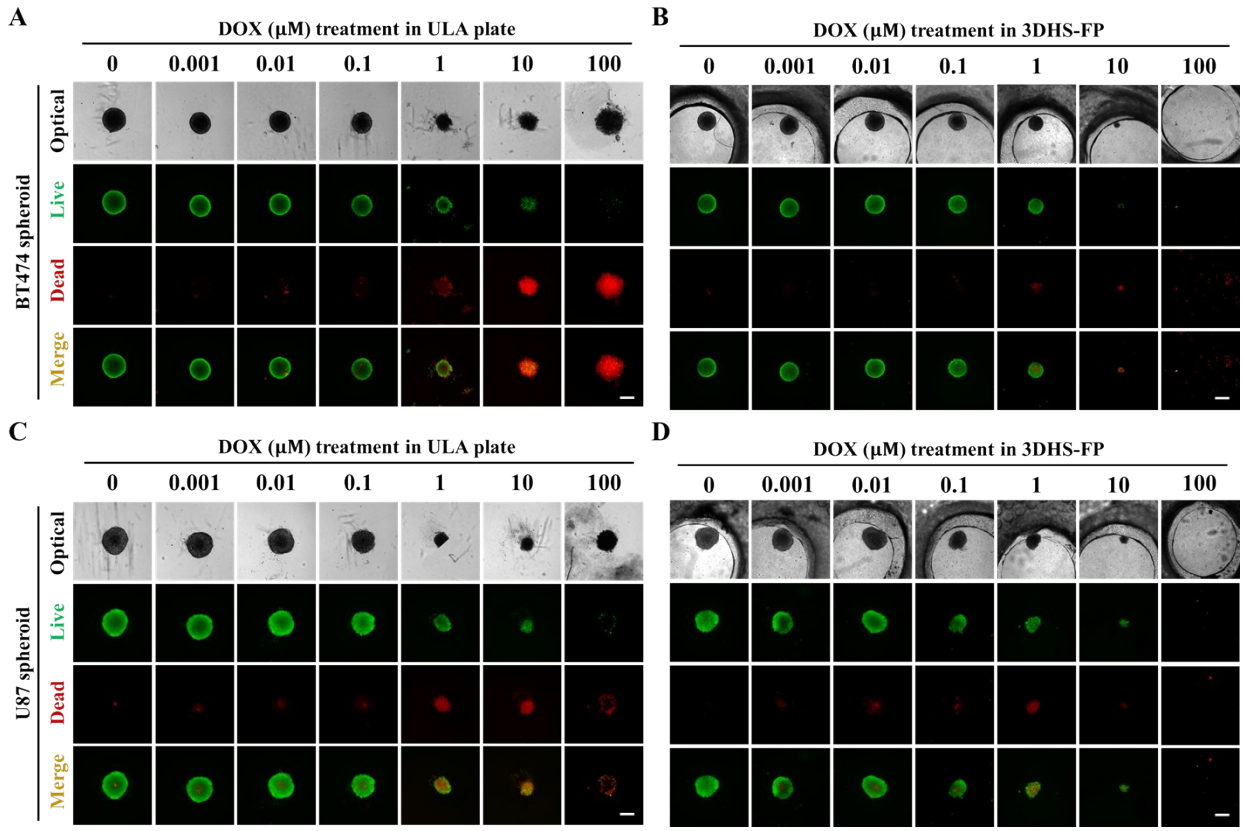

Figure S2. BT474 and U87 spheroids were treated with varying concentrations of DOX (0-100 µM) for a period of 3 days, using either ULA plates or the 3DHS-FP. (A) BT474 spheroids treated with doxorubicin at different concentrations in ULA plates or (B) within the 3DHS-FP. Scale bar: 200 µm. (C) U87 spheroids treated with doxorubicin at different concentrations in ULA plates or (D) within the 3DHS-FP. Scale bar: 200 µm. Optical and fluorescent images of the treated spheroids depict live cells stained with calcein-AM (green) and dead cells stained with ethidium homodimer-1 (red).

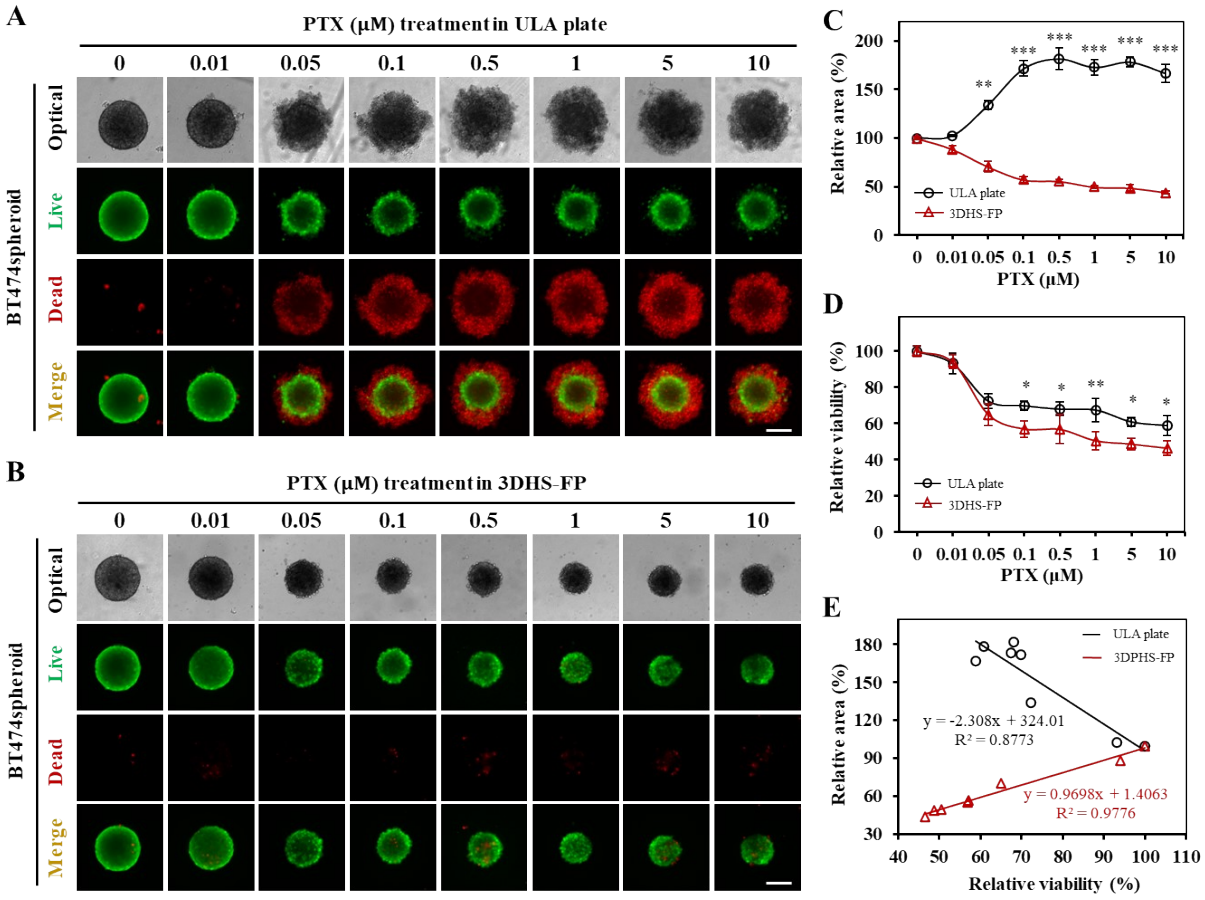

Figure S3. BT474 spheroids were subjected to different concentrations of PXT treatment for a duration of 2 days, both on ULA plates and within the 3DHS-FP. (A) Representative optical images and live/dead staining images of BT474 spheroids, both untreated and treated with varying paclitaxel concentrations. Live cells are labeled with calcein-AM (green), while dead cells are marked with ethidium homodimer-1 (red). Scale bar: 200 μm. (B) Relative spheroid area and (C) viability of untreated and treated spheroids on each plate. The data is presented as the mean ± standard error of the mean (SEM) (n=5). Statistical analysis was performed using Student's *t*-test: **p*<0.05, ***p*<0.01, ****p*<0.001. (D) Linear regression analysis depicting the relationship between spheroid viability and spheroid area. (E) Regression linear analysis between viability and area of spheroid.

Supporting Movie

Movie S1. The process within the 3DHS-FP platform involves separating dead cells and HER2-CAR T cells from the surviving tumor spheroid. Within the 3DHS-FP, upon a spheroid reaching approximately 300 μm in diameter, 10 μL of RPMI-1640 with a specific quantity of HER2-CAR T cells (3,000 cells) was introduced. The video demonstrates how some dead cells drift away from the spheroid located in the separation array (Figure 2G and 2H). This footage lasts for 30 seconds, capturing each frame at one-second intervals.