### **Electronic Supplementary Information**

# Convenient tumor 3D spheroid arrays manufacturing via acoustic excited bubbles for in-situ drug screening

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#### This PDF file includes:

Fig. S1 to S4

#### Other Supplementary Materials for this manuscript include the following:

Movie S1: Acoustic wave turns on the process of cell aggregation from the dispersed state into spheres (MP4).

Movie S2: Bright and fluorescent fields after cell sphere agglomeration stabilization (MP4).

## Figures



**Fig. S1.** (A) Bright-field image of bubble array. Scale bar is 500  $\mu$ m. (B) Bright-field image of a single acoustic bubble forming a cell spheroid. Scale bar is 50  $\mu$ m. (C) Formation of bright-field images of the cell spheroid. Scale bar is 250  $\mu$ m. (D) Fluorescence image of cell spheroid after curing. Scale bar is 300  $\mu$ m. (E) Changes in the volume of the hydrogel encapsulating the cells with time. Scale bar is 150  $\mu$ m.



**Fig. S2.** (A) Bidirectional gradient distribution of drug/dye reflected in the hydrogel area array. Scale bar is 600  $\mu$ m. (B) Concentration distribution in each channel. (C) Cellular activity after acoustic and light curing, stained with FDA/PI. Scale bar is 300  $\mu$ m. (D) Number of live and dead cells before and after acoustic and light effects.



**Fig. S3.** (A) Value-added of cells over time after the action of collected acoustic bubbles. Scale bar is 200  $\mu$ m. (B) OD450 values of acoustic-treated and non-acoustic-treated cells were measured with CCK-8. A linear fit was made to the OD450 curve over time, with means and variances from four independent groups.



**Fig. S4.** (A) Number of live-dead cells in the cell spheroids array at each concentration. (B) the live-dead cells of single cells at 7.2 mg mL<sup>-1</sup> and 18 mg mL<sup>-1</sup> concentrations, respectively. Scale bar is 900  $\mu$ m.