Supplementary Information

A Simple and Versatile Microfluidic Cell Density Gradient Generator for Quantum Dot Cytotoxicity Assay

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I. Cell culture

Both HepG2 and MCF-7 cells were cultured in Dulbecco's modified Eagle's minimal essential medium (DMEM, Gibco, Grand Island, NY) containing 10% fetal bovine serum (FBS), 100 U mL^{-1} penicillin G and 100 U mL^{-1} streptomycin. Cells were maintained in a 5% CO_2 humidified incubator at 37 ^{o}C and passaged every two or three days at a subculture ratio of 1:4 using 0.25% (w/v) trypsin solution.

II. The process of generating cell density gradients on the microfluidic chip

The microfluidic cell density gradient generator is conveniently to be handled to generate series of cell densities. The process only needs three steps and could be monitored by microscope with ease. After cell suspension was infused into microchannels, the first micro-images in Figure S1 showed that the microwells were full filled with cells. Then, surplus cells were flushed out by phosphate buffered saline (PBS) twice from inlet and outlet, respectively. The second micro-image illustrated that the redundant cells in microchannels were flushed away. Cells would fell out of microwells when the device was put upside-down and almost no cells left (see in the third micro-images).

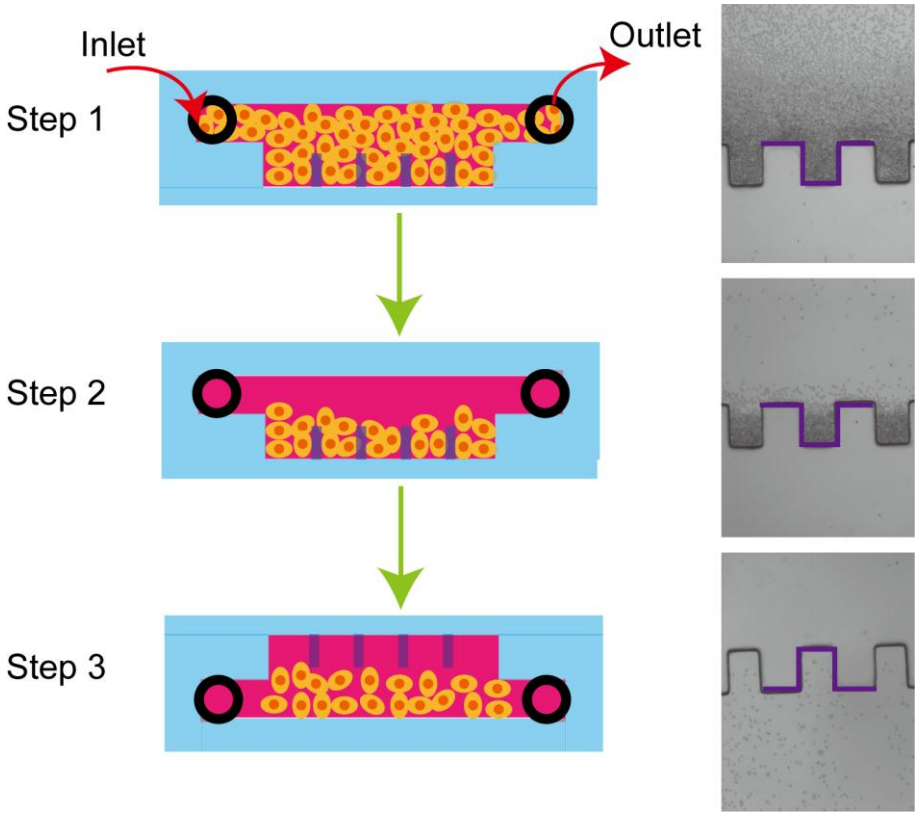

Fig. S1. Schematic representation of the cell density gradient generation process on the microfluidic chip (left) and the corresponding microscope images (right).

III. Design of microwells on the microfluidic cell density gradient generator

The width and length of microwell on the microfluidic cell density gradient generator decide the number of cells contained in it so optimization of the two parameters is vital for this work. The optimized results summarized in Figure 2b and the micro-images were provided in Figure S2. The length was changed from 100 to 500 μm and the width was ranged from 50 to 300 μm. Cells full filled microwells easily when the length was less than 300 μm while they were flushed away at the length below 200 μm so the optimized length

was 300 μm. Cells in microwells which were more than 200 μm wide were always flushed away and they couldn't enter into the microwells which widths were below 150 μm. As a result, the best width was 200 μm.

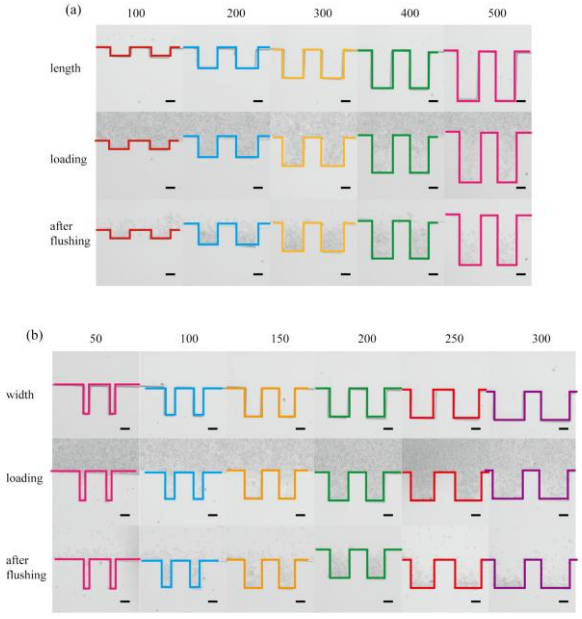

Fig. S2. Optimize length (a) and width (b) of chambers on the microfluidic cell density gradient generator (scale bar: 100 μm).

IV. Intracellular reactive oxygen species (ROS) variation under series of quantum dot (QD) treatment

In order to give more details about intracellular ROS variation after QD treatment under different cell densities, series of QD solutions (0, 10, 15, 20, 25, 30 μg mL^{-1}) were detected on the microfluidic cell density gradient generator we designed. The highest fluorescence of ROS was observed in the first channel adjacent with only one microwell when the QD concentration was below 10 μg mL^{-1} while it was found in the eighth channel with eight

microwells under the QD concentration more than 10 μg mL^{-1} (Figure 5c and S3). Fluorescence intensity of ROS decreased from 255 to 50 in the first channel with QD concentration increasing from 10 to 20 μg mL^{-1}. In the eighth channel, the most intense fluorescence of ROS was observed when the QD concentration was 20 μg mL^{-1} and the fluorescence also decrease instead of increasing accompanying the QD concentration growing more than 20 μg mL^{-1}. As illustrated in the text, the reason was that cell detachment happened in these channels when QD cytotoxicity was severe and fluorescence of ROS quenched easily in the detached cells.

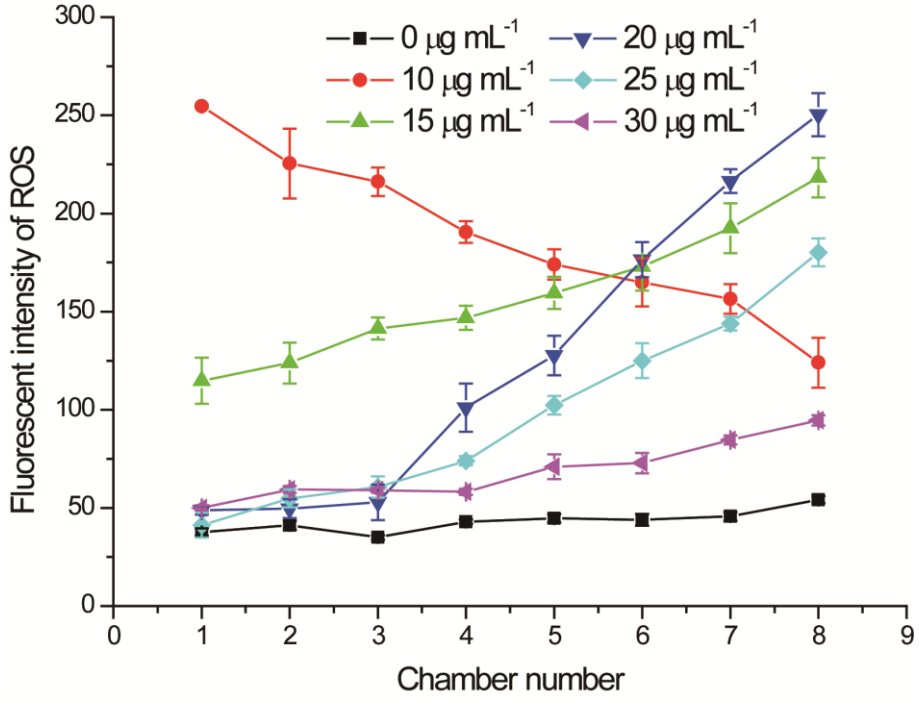

Fig. S3. Intracellular ROS variation of HepG2 cells under series of QD treatment at different cell densities.

V. Cytotoxicity of anticancer drug detected on the microfluidic cell density gradient generator

Cytotoxicity of anticancer drug Act D was detected on the microfluidic cell density gradient generator under different cell densities just like QD cytotoxicity assay. Stocking solution of Act D (8 mM) was diluted into the desired concentrations (0, 0.1, 0.2, 0.3, 0.4, 0.5 μM) by cell culture medium. Cell apoptosis and intracellular ROS variation as the two cytotoxic indexes were detected by Hoechst 33342 and DHE, respectively. As shown in Figure S4, the tendency of cell apoptosis and intracellular ROS variation was similar as cells treated by QDs. Drug cytotoxicity also was observed to be cell density-dependent. At the fixed concentration, cells with low density suffered more intense drug cytotoxicity and cell apoptosis was higher. Fluorescence of ROS decreased with the cell density increasing when the drug concentration was below 0.3 μM. The most intense fluorescence of ROS was not observed in the channel adjusting with one microwell when drug concentration was more than 0.3 μM because most of apoptotic cells detached and their intracellular ROS vanished. All of these results demonstrated that the microfluidic cell density gradient generator we designed also could be used to assay drug cytotoxicity under different cell densities.

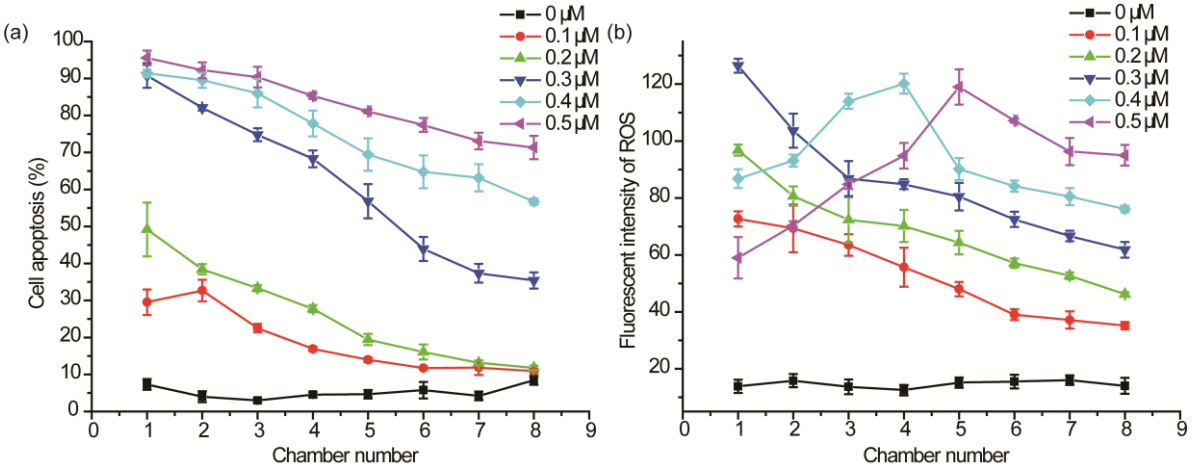

Fig. S4. Cytotoxicity of anticancer drug Act D detected on the microfluidic cell density

gradient generator. Cell apoptosis (a) and intracellular ROS variation (b) of HepG2 cells treated by Act D for 24 h under different cell densities.