ESI for

Cellular Heterogeneity Identified by Single-Cell Alkaline Phosphatase (ALP) *via* a SERRS-Microfluidic Droplet Platform

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1. Reagents and materials

Gold(III) chloride trihydrate, trisodium citrate (98%), alkaline phosphatase, 5-bromo-4-chloro-3-indolyl phosphate (BCIP), sorafenib, sodium orthovanadate (Na_3VO_4) were purchased from Aladdin Industrial Corporation (Shanghai, China), ALP Live Stain was purchased from Thermo Fisher Scientific (China) Co., Ltd.. Other chemicals of analytical grade were obtained from Sinopharm Chemical Reagents (Beijing, China).

2. Band assignments

Table S1. The SERS vibrational band assignments of BCI.[1]

Peak position (cm^{-1})	Assignment
600	δ (C=C-CO-C)
695	δ (C-C)
777	δ (C-H), δ (C-N-C)
938	γ (C-H), δ (C-C) ring
1167	δ (C-C), ν (C-C) ring
1219	δ (C-H)
1285	δ (C-C)
1331	δ (N-H), δ (C-H)
1571	ν (C=C), ν (C=O)
1615	ν (C=C), δ (C-H)

3. Generation and collection of droplets

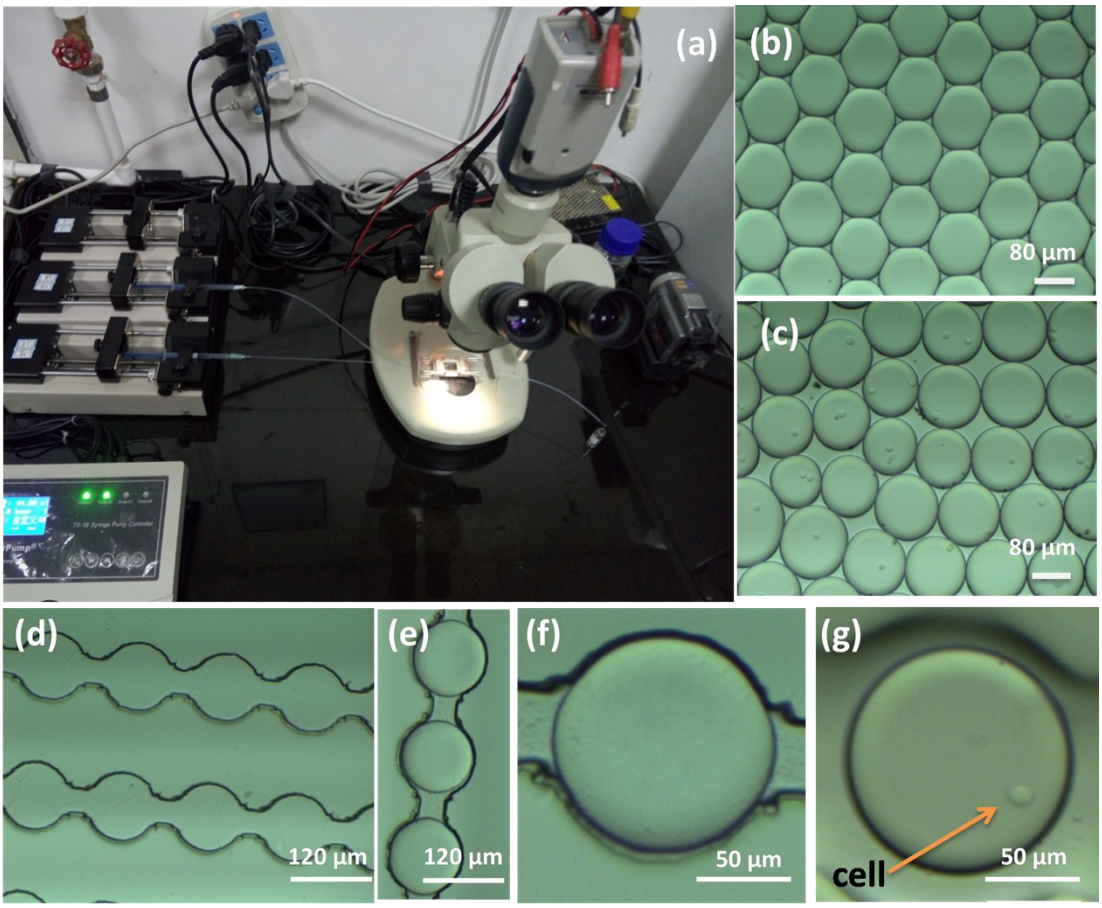

Fig. S1 (a) The setup for monitoring drops with a CCD (SN-435C, IKEGAWA) and illumination bright light. Microscopic images (×10) of collected droplets (b) and droplets containing single cell (c). (d-g) Droplets were re-injected into the single-droplet trains by the infusion of oil in a PDMS chip.

4. Distributions of BCI and Au NPs in cells

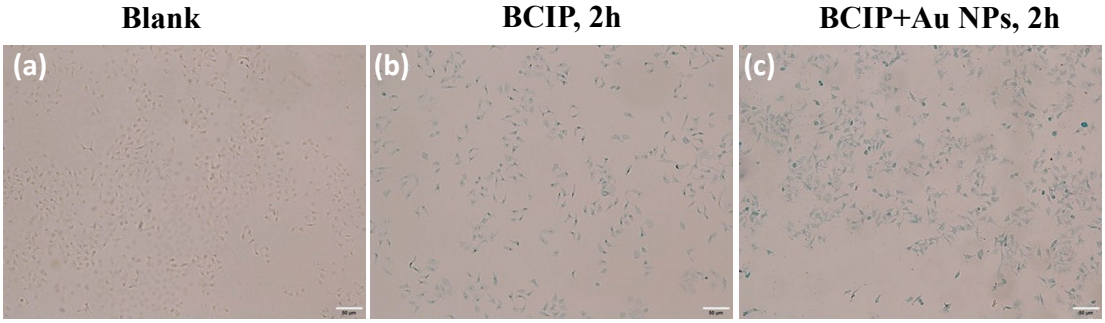

Fig. S2 Bright field images of HepG2 cells after they were incubated without (a) and with 8.0 nM of BCIP (b), BCIP +Au NPs (c) for 2 h. The scale bar is 50 μm.

5. Particle size

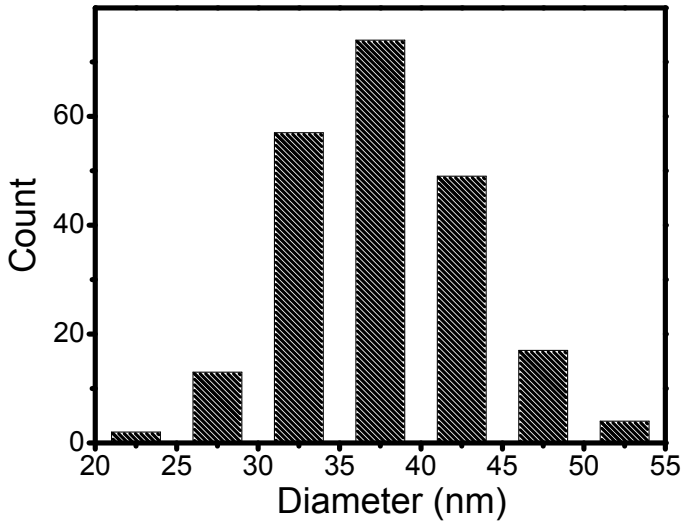

Fig. S3 The statistic particle size of AuNPs.

6. Poisson distribution

Single cell studies required that each of droplet has encapsulated one cell. However, the majority of droplets contained no cell at all because of the encapsulation process following Poisson statistics. In our work, the produced droplets encapsulating individual cells was mainfested in Fig. S4, where yellow arrows highlight the cell-bearing droplets. The Poisson distribution of cells encapsulated into droplets is given as following:

$$f(\lambda ;n) = (\lambda^{n}\ e^{-\lambda}) / n!$$

where n is the number of cells in the droplets and λ is the average value of cells encapsulated into per droplet. We have evaluated the distributions under the different densities of cells into every droplets. We have calculated the values of λ was 0.15, 0.3, and 0.6 respectively, which are typical values of interest for single cell experiments. It can ensure that very few droplets containing multiple cells. In our work, the value of λ used is about 0.3 which is in good agreement with those results calculated from Poisson statistics (Fig. S4) under the density of cells about 3.0×10^{6} cells/mL. Therefore, we have obtained the probability of single cells encapsulated into one droplet was about～20% while ensuring that fewer than 6% have two or more cells. Although the number of single-cell-bearing droplets is rather low, it could not influence the detection of single cells in this work, because the high production and screening rate can be achieved with microfluidic devices to obtain the single cells encapsulated into one droplet.

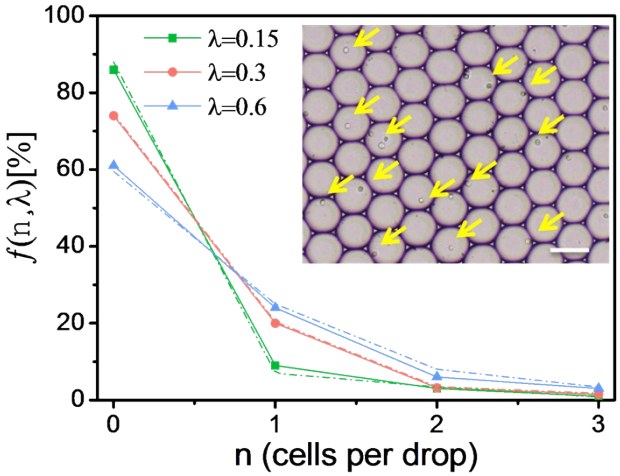

Fig. S4 The probability of a droplet encapsulating a single cell. n is the number of cells in the drops and λ is the average number of cells per drop. Dashed and solid lines show the predicted values from Poisson statistics and experimental results. Insert is the micrograph showing cells in drops.

7. Distributions of BCIP and AuNPs on cells in droplet

To display the locations of BCIP and AuNPs on cell in the droplet, we traced the diffusion of them in droplet during the ALP catalytic reaction by the bright field imaging. As shown in Fig. S5(a), a small amount of AuNPs are close to the cell when the reaction time is 0.5 h. As the reaction time prolonged, more and more AuNPs were absorbed on the cell (a-d).

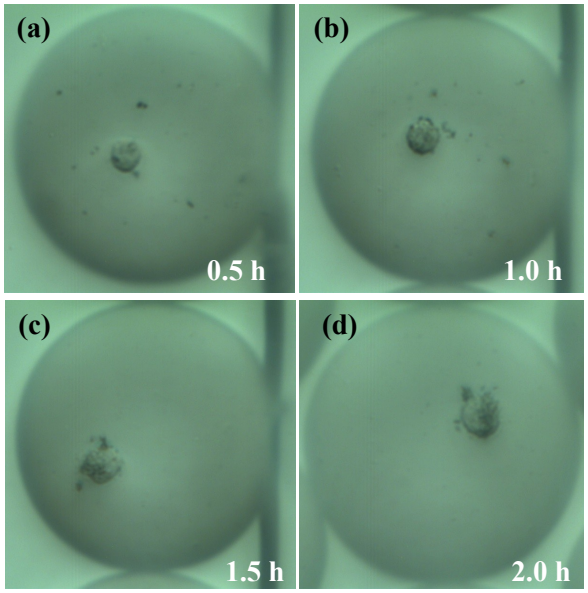

Fig. S5 Bright images of HepG2 cells with AuNPs and BCIP in the droplet at the catalytic reaction time of 0.5 (a), 1.0 (b), 1.5 (c), and 2.0 h (d), respectively.

8. Confocal fluorescent microscopic images of different cells lines

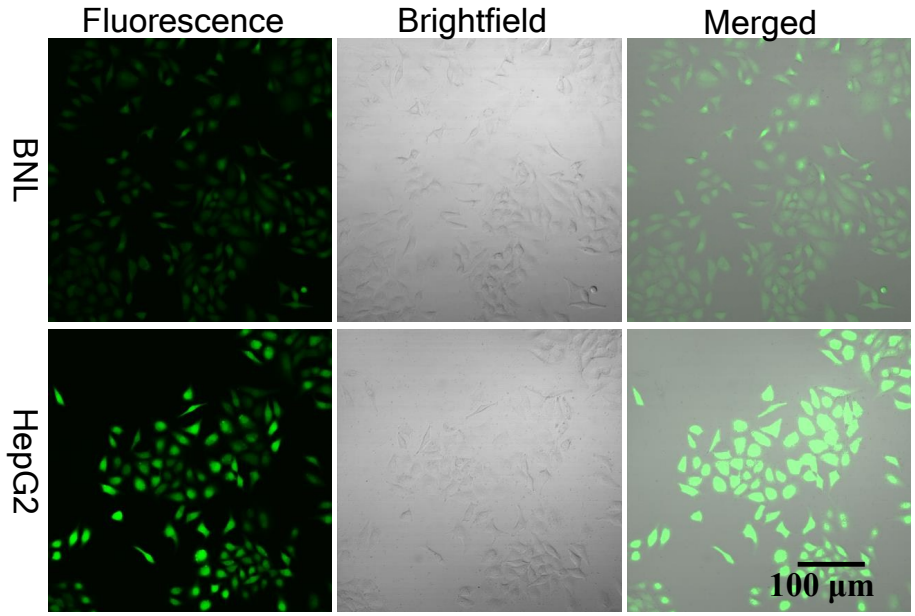

Fig. S6 Confocal fluorescent microscopic images of BNL.CL2 cells and HepG2 cells after they were treated with 2.0 μL of AP Live Stain (500×), respectively.

9. Dynamic expression of ALP in response to sorafenib

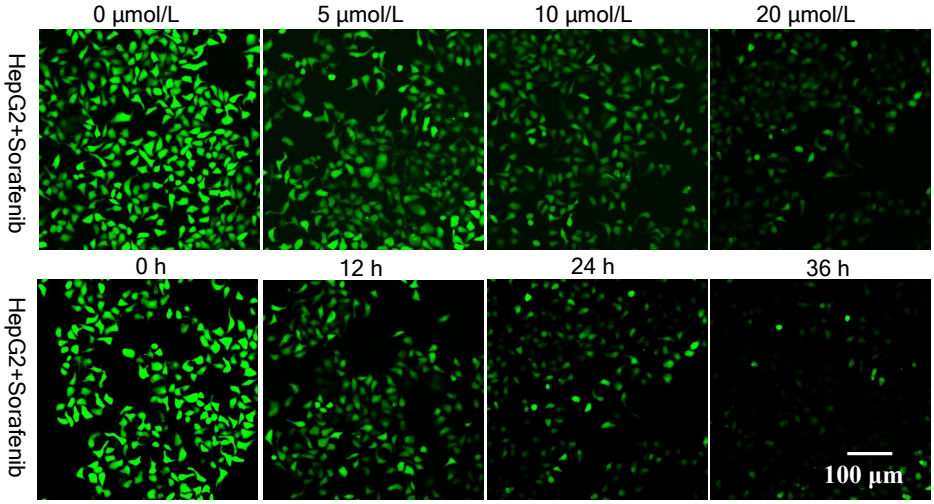

Fig. S7 Confocal fluorescent images of HepG2 cells treated with different concentrations of sorafenib (top panel) or same concentration of sorafenib but different time (bottom panel). The ALP amount are highlighted with 2.0 μL of AP live stain.

References

1 C. M. Ruan, W. Wang and B. H. Gu, *Anal. Chem.,* 2006, **78**, 3379.