Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary information

A microfluidic platform utilizing anchored water-in-oil-in-water double emulsions to create niche for analyzing single non-adherent cells

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S1 The hydraulic resistance balance of the branch channels and double emulsion anchor channels

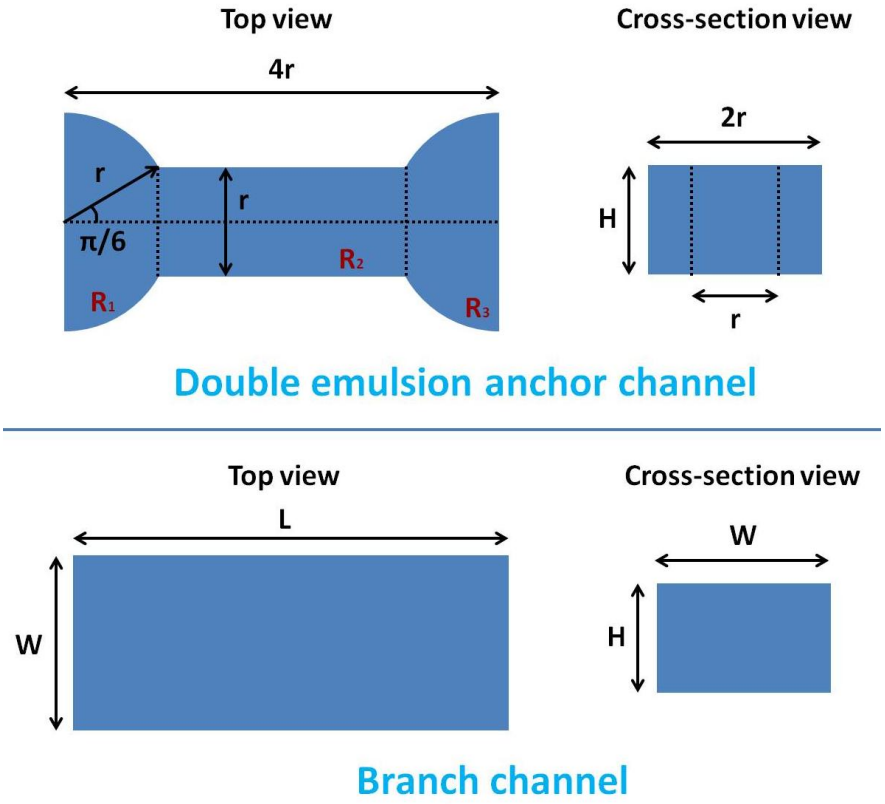

Figure S1

Fig. S1 The illustration showing the top view and cross-section view of a part of the double emulsion anchor channel and the branch channel of our microfluidic chip. According to the chip design, r = 50 μm, H = 120 μm, W = 200 μm, and L represents the length of the branch channels.

According to previous studies (**Ref. S1-1** and **Ref. S1-2**), the hydraulic resistance of a microchannel with rectangular cross-section is defined as:

$$R = \frac{12\mu_0 L}{WH^3}$$

Here, μ_0 refers to the fluidic viscosity. L, W and H refer to the length, width and height of the microchannel.

According to the design of our device, the double emulsion anchor channel is constituted of 21 sub-units illustrated in **Fig. S1**, so the hydraulic resistance R_A of one double emulsion anchor channel can be calculated as:

$$R_A = 21R_{A_u} = 21\left[(R_1 + R_3) + R_2\right]$$

$$= 21 \cdot \frac{12\mu_0}{H^3}\left[\int_{-\frac{\sqrt{3}}{2}r}^{\frac{\sqrt{3}}{2}r} \frac{dx}{2\sqrt{r^2 - x^2}} + \frac{2r + 2\left(r - \frac{\sqrt{3}}{2}r\right)}{r}\right] \approx 835 \times \frac{\mu_0}{H^3}$$

Similarly, the hydraulic resistance of one branch channel R_B can be calculated as:

$$R_B = \frac{12\mu_0 L}{WH^3}$$

According to the device design, the length L and the width W of the branch channel (**Fig. S1**) are (8700 + 6000) μm and 200 μm respectively, so we can estimate the value of R_B as:

$$R_B = 12 \cdot \frac{8700 + 6000}{200} \cdot \frac{\mu_0}{H^3} = 822 \times \frac{\mu_0}{H^3}$$

Therefore, hydraulic resistance of the double emulsion anchor channel and the branch channel is almost the same, although there are some irregular channels connecting to the outlets for both branch channel and double emulsion anchor channel. This hydraulic balance can benefit the loading process of DEs into the microchambers as well as DE anchorage in the microchambers.

Reference:

S1-1 F. Guo, K. Liu, X.-H. Ji, H.-J. Ding, M. Zhang, Q. Zeng, W. Liu, S.-S. Guo and X.-Z. Zhao, *Appl. Phys. Lett.*, 2010, 97, 233701-233703.

S1-2 K. W. Oh, K. Lee, B. Ahn and E. P. Furlani, *Lab Chip*, 2012, 12, 515-545.

S2 Generated cell-laden W/O/W DEs that was being loaded into the microchambers

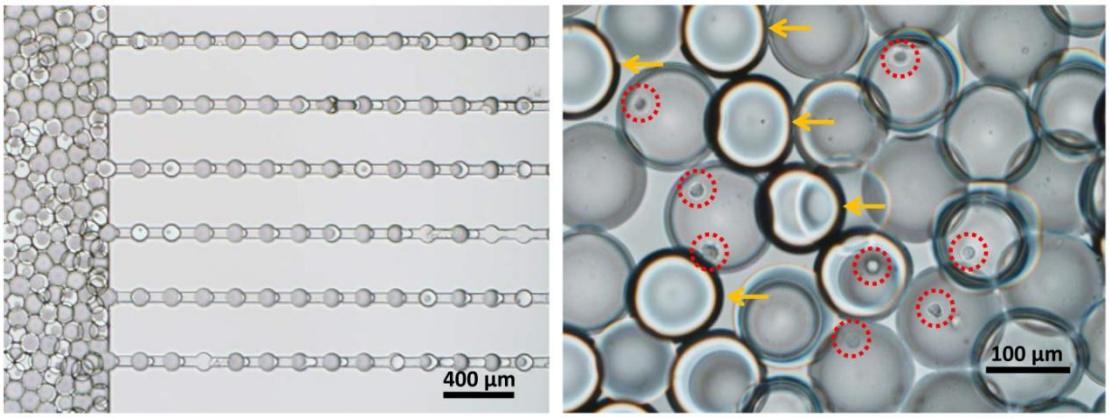

Figure S2

Fig. S2 Generated cell-laden W/O/W DEs to load into the microchambers. The right image is a magnification. Red circles indicate the encapsulated cells, and yellow arrows figure out single emulsions (SEs) generated together with DEs due to the unstable droplet generation process of flow-focusing jetting regime.

S3 W/O/W DE trapping mechanism

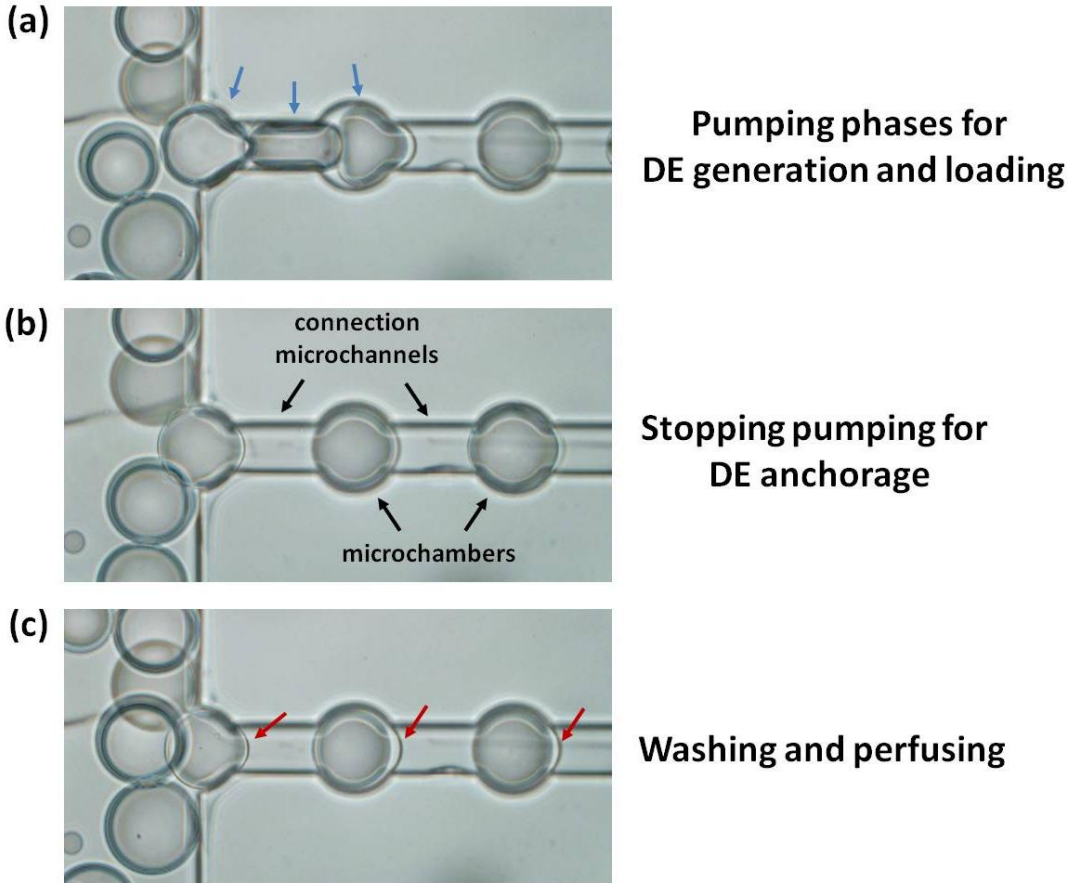

Figure S3

Fig. S3 Trapping mechanism of W/O/W DEs into the microchambers. **(a)** The loading process of W/O/W DEs during the droplet generation. Blue arrows indicate DEs that are deformed by the pressure from other DEs to go across the microchannel or to leave the microchamber. **(b)** The anchorage of W/O/W DEs in the microchambers after their generation. **(c)** W/O/W DEs that kept trapped during washing and solution perfusion processes. Red arrows indicate the tiny deformation of anchored W/O/W DEs caused by the washing or perfusion flow.

Due to the hydraulic force that generated by **OA** phase whose flow rate was large enough, W/O/W DEs with proper size (regulated by flow rates of **IA**, **OP** and **OA** phases, as shown in **Fig. 3(b)** and **3(c)**) collided with each other. Due to the function of surfactants (PVA in aqueous phases and PDMS precursor in oil phase), these DEs did not merge but pressed each other into the anchorage microchannels, as indicated by blue arrows in **Fig. S3(a)** and shown in **Movie S3**. Subsequently, when all the microchambers were filled with W/O/W DEs, DE generation procedure was stopped. The flows inside the chip were also stopped. At this time, those DEs which were rightly in the microchambers, restored their sphere shape due to surface tension. Since

that the size of those DEs (around 100 μm) was bigger than the connection microchannel (50 μm) and their surface tension was large enough, they did not transform to go across the narrow connection microchannel. Thus then these DEs were trapped in the microchambers (**Fig. S3(b)**). During the wash and perfusion processes, because the flow rates used for wash or perfusion (≤ 400 μL/h) were much smaller than **OA** flow rate used for DE generation (≥ 2000 μL/h), the hydraulic force on the anchored W/O/W DEs was tiny to cause limited deformation (as indicated by red arrows in Fig. **S3(c)** and shown in **Movie S4**). Thus then the size of these DEs were still larger than the anchorage microchannels, and DEs could not get across the connection microchannels to flow downstream. Therefore, during the wash or perfusion procedure, the anchored W/O/W DEs kept trapped in the microchambers.

S4 Calibration of colorimetric intensity vs. Rhodamine B (Rho-B) concentration

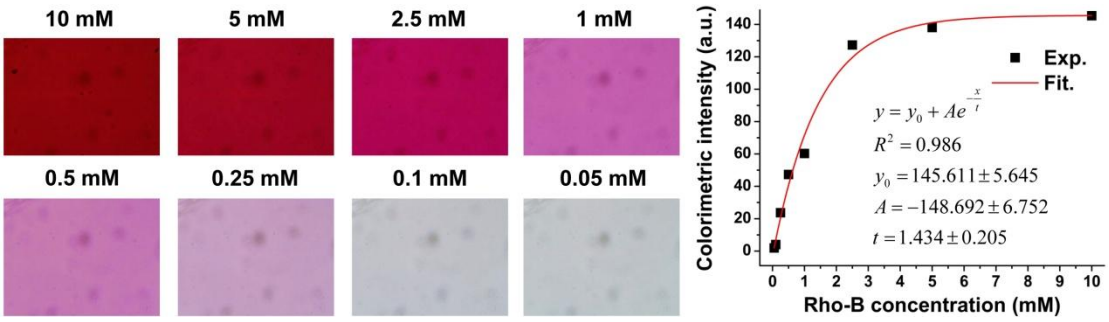

Figure S4

Fig. S4 Calibration of colorimetric intensity vs. Rhodamine B (Rho-B) concentration. Color images of Rhodamine B aqueous solutions with different concentrations (0.05 mM, 0.1 mM, 0.25 mM, 0.5 mM, 1 mM, 2.5 mM, 5 mM and 10 mM, respectively) are demonstrated under the same snapping condition. After the corresponding colorimetric intensity is obtained through image analysis by Image Pro Plus software, standard calibration curve is set up between the colorimetric intensity and the concentration of Rhodamine B.

S5 Concentration variation of Rhodamine B in the interior of a W/O/W DE during 100 μL/h continuous perfusion

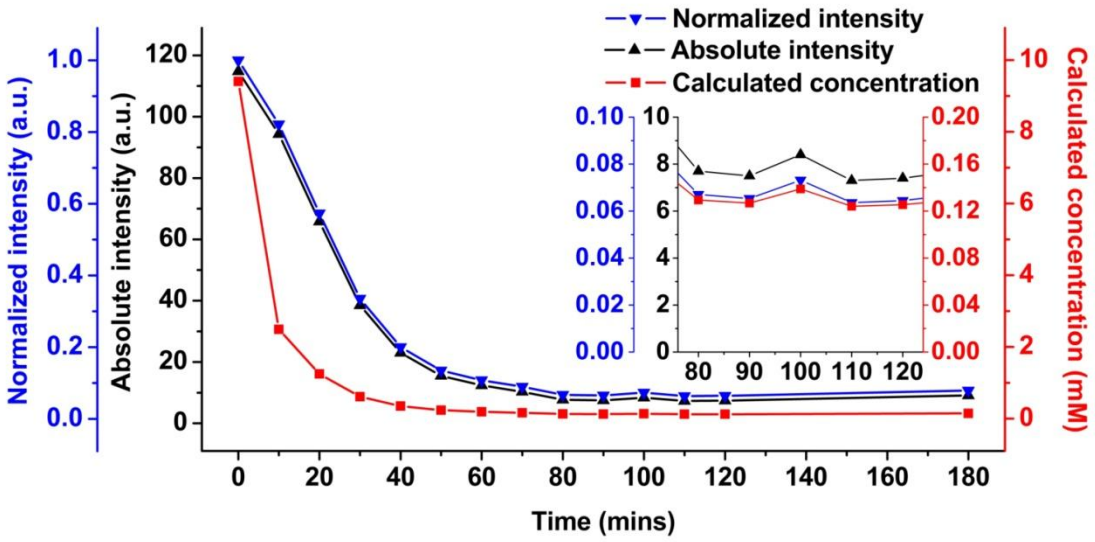

Figure S5

Fig. S5 Concentration variation of Rhodamine B in the interior of a W/O/W DE during 100 μL/h continuous perfusion. Black and blue curve respectively show the absolute and relative colorimetric intensity of the DE interior. Red curve shows the calculated concentration of Rhodamine B according to the standard curve in **Fig. S2**.

S6 The impact of PVA on the long-term culture of non-adherent cells

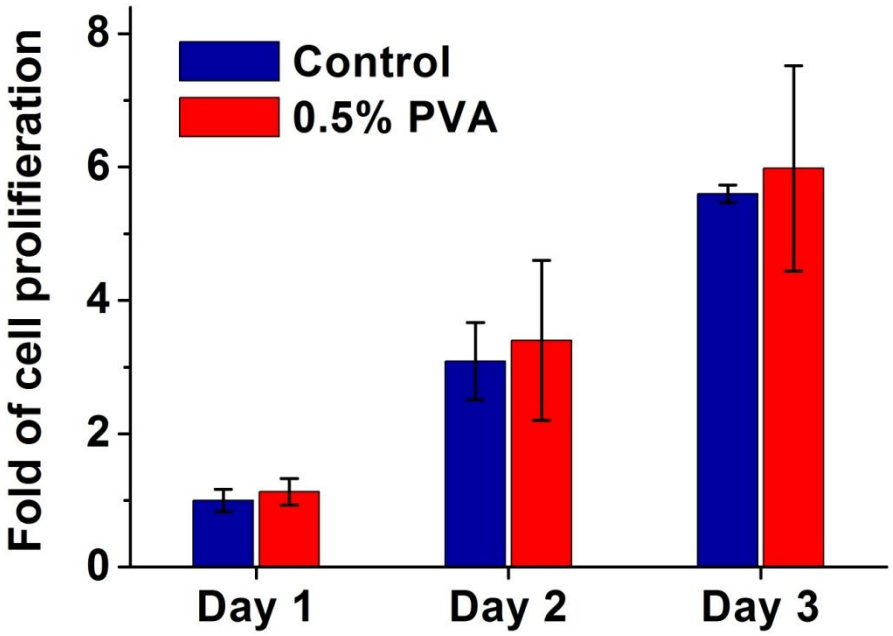

Figure S6

Fig. S6 The impact of 0.5 % (w/v) PVA on the cell growth of TF-1 human erythroleukemia cells, studied by MTT assays for 3 days.

MTT assays were implemented to investigate the impact of PVA on the viability and proliferation of non-adherent cells (TF-1 human erythroleukemia cells).

Briefly, 0.5 % (w/v) PVA was dissolved in RPMI-1640 and then the solution was sterilized by 0.22 μm filtration. 5×10^4 /mL cells suspended in RPMI-1640 (with 10 % serum) (i.e. control groups) or RPMI-1640 (with 10 % serum and 0.5 % PVA) (i.e. PVA groups) was seeded into a 24-well plate and then incubated (37 ℃, 5 % CO_2, humid) for 1, 2 or 3 days, respectively. Subsequently, cells in each well were resuspended in RPMI-1640 without serum, and MTT solution (10 % v/v) was added for further 4 h incubation. Then centrifugation was implemented and all the sediments were collected and dissolved in DMSO. The absorbance at 570 nm of the solution was measured using a microplate reader (Infinite F50, Tecan, Switzerland). All the experiments were carried out three times to obtain corresponding data.

It could be seen from **Fig. S6** that, comparing to the control groups (TF-1 cells cultured normally), cells cultured using RPMI-1640 doped with 0.5 % PVA had no distinct difference in cellular proliferation.

S7 Long-term culture of non-adherent cells encapsulated in anchored W/O/W DEs on-chip

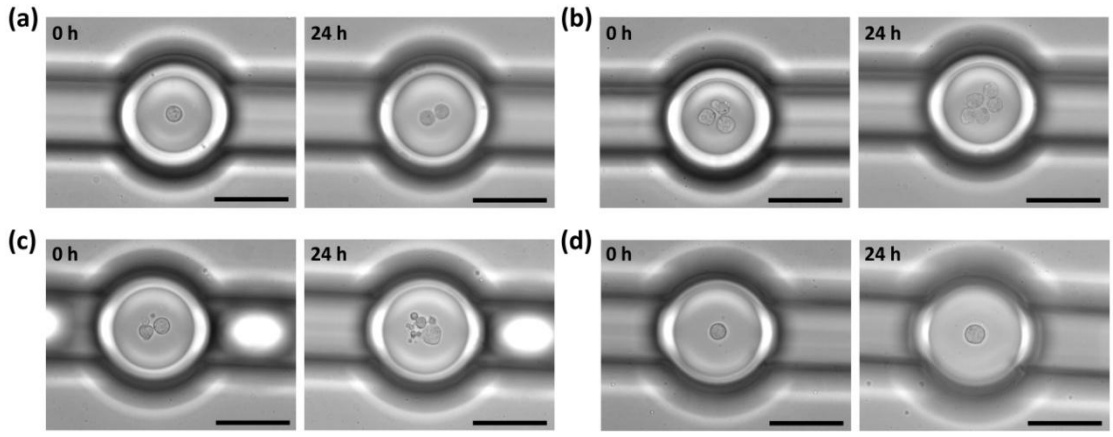

Figure S7

Fig. S7 Long-term culture of TF-1 cells encapsulated in anchored W/O/W DEs on-chip. **(a-b)** Typical microscopy images showing cells that proliferated after 24 h culture. **(c)** Typical microscopy images showing that some cells underwent apoptosis and ruptured after 24 h culture. **(d)** Typical microscopy images showing the cell that did not proliferate but still kept good morphology in 24 h. Scale bars, 50 μm.

On-chip culture of TF-1 human erythroleukemia cells in anchored W/O/W DEs was implemented to investigate the ability of our devices for long-term culture of non-adherent cells.

At first, TF-1 cells were encapsulated in anchored W/O/W DEs as described in the "***FDA perfusion to detect cellular metabolism***" part of ***Experimental*** section in the manuscript. After cell-laden W/O/W DEs were anchored on-chip and excessive DEs were removed, RPMI-1640 doped with 0.5% (w/v) PVA kept continuously perfusing those cell-laden W/O/W DEs during the whole experiment. All the set-up was placed in the humid ambient at 37 ℃ and 5 % CO_2 atmosphere. RPMI-1640 culture medium used here was mixed with fetal bovine serum (10 % v/v) and Penicillin-Streptomycin (1 % v/v).

Total 54 W/O/W DEs containing TF-1 cells (ranging from 1 to 7 cells each DE) maintained about 30 h. After 24 h culture, cells in 30 W/O/W DEs (about 55 % of 54) proliferated (as representatives in **Fig. S7(a)** and **S7(b)**), while some cells in 6 DEs (about 11 %) underwent apoptosis like the one in **Fig. S7(c)** and cells in the rest 18 DEs (about 34 %) contained did not proliferate nor go apoptosis (similar to **Fig. S7(d)**). However, when the DEs were incubated around 30 h, almost all those cells died and ruptured.

Movie S1 The generation procedure of cell-laden W/O/W DEs in the two adjacent flow-focusing structures.

Movie S2 The generation procedure of W/O/W DEs before loading into the microchambers.

Movie S3 The loading procedure of the generated W/O/W DEs into the microchambers.

Movie S4 The medium perfusion procedure to enable mass transfer between the interior and exterior of a cell-laden W/O/W DE.

Movie S5 The perfusion procedure to monitor cellular uptake and conversion metabolism. Live cells take in FDA and emit green fluorescence.

Movie S6 The wash procedure to remove excessive FDA dye after FDA perfusion.