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

Supplementary Information

of

Droplet Microfluidics for Functional Temporal Analysis and Cell Recovery on Demand using Microvalves: Application in Immunotherapies for Cancer

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Comparing effective resistance of each docking site

The effective resistance from the entry of the docking site to the extraction outlet is the most critical parameter for droplet trap and release. The effective resistance for a particular docking site (docking site 1 here) is given as ($R_{effective\ 1} = (R_{11} + R_{12} + R_{13}) + R_{a1}$.

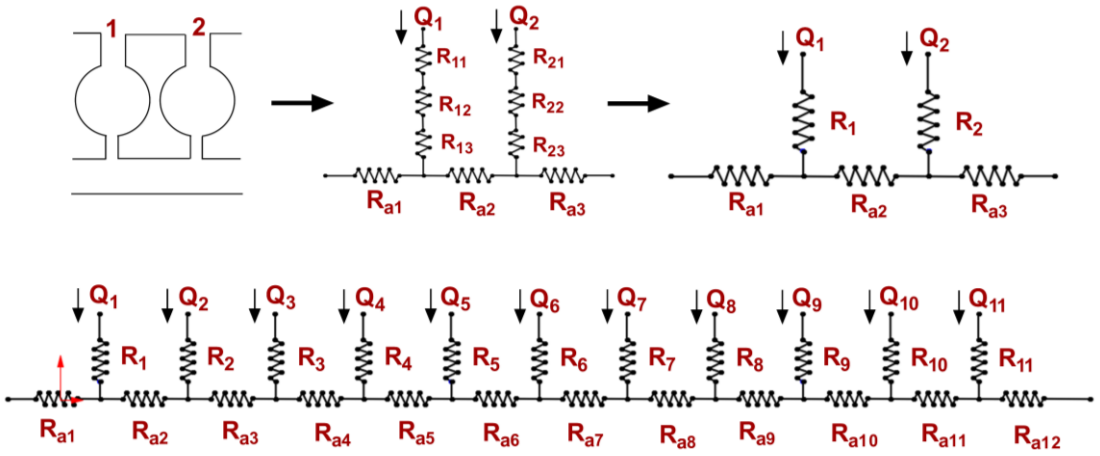

Figure 1: The resistive network of the first two docking sites, with each branch's hydrodynamic resistance as R_{11}, R_{12} and R_{13}. The overall resistance of each docking site is represented as R_1 and R_2.

(c) The resistive network of the first half of the row (11 docking sites) along with all hydrodynamic resistances and corresponding flow rates.

For mathematically calculating the effective resistance for each docking site, we have used

$$R = \frac{12\mu l}{wh^3[1-0.63(h/w)]}$$

Using the equation above, we have calculated the resistance of each individual branch and then each docking site as well,

$R_{effective\,1} = R_1 + R_{a1}$

Similarly, for other docking sites the effective hydrodynamic resistance is being calculated, we have also provided how each docking site resistance is compared to the first docking site resistance which is given as follows:

$R_{effective\,2} = 1.04\, R_{effective\,1}$

$R_{effective\,3} = 1.09\, R_{effective\,1}$

$R_{effective\,2} = 1.13\, R_{effective\,1}$

$R_{effective\,2} = 1.174\, R_{effective\,1}$

$R_{effective\,2} = 1.217\, R_{effective\,1}$

$R_{effective\,2} = 1.261\, R_{effective\,1}$

$R_{effective\,2} = 1.305\, R_{effective\,1}$

$R_{effective\,2} = 1.348\, R_{effective\,1}$

$R_{effective\,2} = 1.392\, R_{effective\,1}$

$R_{effective\,2} = 1.435\, R_{effective\,1}$

We have provided the comparative composition of each docking site in supplementary information.

Encapsulation of cells

The Poisson distribution governs the encapsulation of cells (NK92 cells and K562 cells here) in droplets. It is possible to predict the probability of encapsulation using the Poisson distribution, which is given by,

$$p(k,\lambda) = \frac{\lambda^k e^{-\lambda}}{k!}$$

Where k, λ represents the number of cells in a droplet and the average number of cells per droplet volume. In our case, the initial cell concentration of both types of cells was 3×10^6 cells/mL. The droplet volume is 1.1 nL. λ in this case, is

$$\lambda = \frac{3\times10^6}{mL} \times \frac{1.1\ nL}{droplet} = 3.3\ cells\ per\ droplet$$

Hence, we calculate the probability of having k=0,1,2,3,4…. N cells per droplet, as follows

$$p(0\ cells\ per\ droplet) = \frac{\lambda^k e^{-\lambda}}{k!} = \frac{3.3^0 \times e^{-3.3}}{o!} = 3.6\ \%$$

$$p(1\ cells\ per\ droplet) = \frac{\lambda^k e^{-\lambda}}{k!} = \frac{3.3^1 \times e^{-3.3}}{1!} = 12.17\ \%$$

$$p(2\ cells\ per\ droplet) = \frac{\lambda^k e^{-\lambda}}{k!} = \frac{3.3^2 \times e^{-3.3}}{2!} = 20.1\ \%$$

$$p(3\ cells\ per\ droplet) = \frac{\lambda^k e^{-\lambda}}{k!} = \frac{3.3^3 \times e^{-3.3}}{3!} = 22.1\ \%$$

$$p(4\ cells\ per\ droplet) = \frac{\lambda^k e^{-\lambda}}{k!} = \frac{3.3^4 \times e^{-3.3}}{4!} = 18.23\ \%$$

For the experiments, we focus on 1:1 E:T ratios, however 2:1, 1:2, 2:2, etc. ratios can be observed up to about 5:1, which is controllable based on loading concentration. For the experiment used for this study, we observed 62.9% of droplets were 1:1, 15.7% were 2:1, 11.2% were 1:2 and 4.5% were 2:2. The remaining few percent of droplets were a mix of other various ratios.

Supplementary movies

Movie S1 ("selective droplet release")

Movie showing selective droplet release from a docking site. Droplet gets trapped in a docking site when the valve is off. When the valve is actuated, the droplet starts getting out of the docking site through the connecting exit channel, and finally, the droplet comes out of the docking site. The neighbouring droplet trapped in the docking site is not released, showing how the valve can have selective release

Movie S2 ("NK92 Release"):

Movie displaying the release of NK92 cells encapsulated in a droplet and subsequently held in place in the collection channel.

Movie S3 ("NK92 Viability"):

Movie displaying viability of NK92 cells post-droplet release in collection channel for 1 hour. Cells are labeled with Calcein AM (green) to track viability.

Movie S4 ("K562 Release"):

Movie displaying the release of K562 cells encapsulated in a droplet and subsequently held in place in the collection channel.

Movie S5 ("K562 Viability"):

Movie displaying viability of K562 cells post-droplet release in collection channel for 1 hour. Cells are labeled with Calcein AM (green) to track viability.