HYDRODYNAMICALLY-INDUCED DROPLET MICROVORTICES FOR CELL PAIRING APPLICATIONS

Xuhao Luo[1,], Braulio Cardenas-Benitez[1,**], and Abraham Lee[1,*]**
[1]*Department of Biomedical Engineering, University of California Irvine, Irvine, CA, USA*

ABSTRACT

We present a water-in-oil droplet microfluidic trap array capable of modulating the distance between co-encapsulated cell pairs through microvortex formation. We demonstrate that vortex shape and periodicity can be directly controlled by the continuous phase flow rate. Explicit equations for the recirculation time inside droplet microvortices were derived by approximating the velocity fields through analytic solutions for the flow inside and outside of a spherical droplet. Comparison of these expressions against Particle Tracking Velocimetry (PTV) measurements of K562 (leukemia) cells circulating inside 50 μm droplets showed excellent theoretical agreement.

KEYWORDS: Droplet Microfluidics, Microvortices, Cell-pairing

INTRODUCTION

Droplet compartmentalization of cells has led to the development of single-cell analysis technologies, spanning from sequencing methods to cell-pairing microfluidic technologies, which have impacted the design and study of checkpoint blockade and adoptive T-cell transfer therapies [1]. Our preliminary results indicate that co-encapsulation of effector immune cells with their target enables real-time immunometabolic function assessment through glycolytic state determination via NADH autofluorescence [2]. Notable microfluidic devices to study immune cell interactions include hydrodynamic traps that bring cells into close contact [3], and devices that actively control cell position through actuation (e.g. with microelectrodes [4]). While droplet-based assays comparatively possess a throughput advantage because they are not restricted to a set number of traps per device, they cannot directly control cell pairing position and interaction frequency. We addressed this shortcoming with a microdevice capable of trapping droplets and modulating their internal viscous stress to create microvortices of well-defined shape and periodicity, which were used to control the cell-to-cell distances of K562 (leukemia) cells. Lastly, we theoretically and experimentally demonstrated that outer phase flow rate inversely alters recirculation time within a droplet, in agreement with the developed vortex periodicity expressions (R^2=0.996).

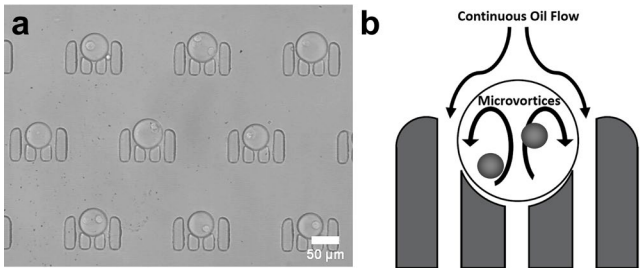

Figure 1: (a) Bright-field image of trapping array with droplets encapsulating K562 cell pairs. (b) Schematic of dispersed and continuous phase flow streamlines of a trapped droplet.

EXPERIMENTAL

Trap arrays with 40μm height were fabricated by soft-lithography. PDMS pillars were separated by 10μm to allow bilateral oil flow around trapped droplets. A separate microdevice was used to generate the 50μm water-in-oil droplets. The dispersed phase consisted of 1x PBS, 16% Optiprep and 0.01% Triton X-100, and HFE7500 oil with fluorosurfactant was selected as the continuous phase. Microvortices inside droplets were induced by controlling the continuous phase flow rate between 2-20 μLmin^{-1}, and PTV was used to measure the particle motion.

RESULTS AND DISCUSSION

We approximated the droplet of viscosity η_i with a circle of radius R_0, immersed in an unbounded fluid of viscosity η_o (Fig. 2a). Setting -$U_0\hat{\mathbf{z}}$ far from the liquid-liquid interface, the solution of the steady-state Stokes equations leads to the Hadamard-Rybczynski velocity fields for the inner and outer phases. Closed particle pathlines are periodic on both $r(t)$ and $\theta(t)$ coordinates, with period T, which is given by:

$$T = \frac{2\tau}{\sqrt{(r_0/R_0)^2-1}}\left[K\left(\frac{r_0^2}{R_0^2-r_0^2}\right) - F\left(\phi, \frac{r_0^2}{R_0^2-r_0^2}\right)\right] = \tau\, f(r_0, R_0)\,,\ \tau = \frac{2R_0}{U_0}\left(1+\frac{\eta_i}{\eta_o}\right), \quad (1)$$

where r_0 is the vortex starting point, $K(m)$ and $F(\phi, m)$ are the complete and incomplete elliptic integrals of the

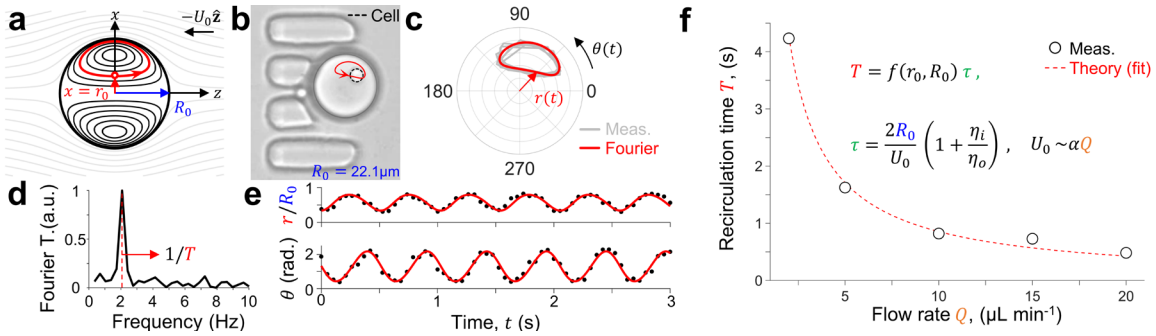

Figure 2: (a) Streamlines inside and outside droplets. (b) A K562 cell circulating in a microvortex. (c) PTV of cell in (b). (d) Fourier transform of $r(t)$. (e) Time-course plots of the cell coordinates r(t),$\theta(t)$. (f) Measured T vs. external phase flow rate.

first kind, respectively, with $\phi = \sin^{-1}[(R_0{}^2 - r_0{}^2)/r_0{}^2]$, and τ is a characteristic time constant.

A bright-field image in Fig. 2b shows a circulating K562 cell upon externally applying 20 μLmin^{-1}. Particle Tracking Velocimetry (PTV) reveals the hemisphere-like path traced by the cell in Fig. 2c, which has been represented by the first two Fourier harmonics (line). A Fourier transform of $r(t)$ in Fig. 2d illustrates the periodicity of the coordinate time-course data (Fig. 2e), peaking at T for the first harmonic. By comparing the measured T v.s flow rate Q, an inverse relationship arises, as predicted from the characteristic time τ in eq. (1). Because the external flow speed U_0 appears in eq. (1), rather than Q, a functional fit was performed, justified by the fact that for Stokes flow, $U_0 \sim \alpha\, Q$. Values of α=1.5x10^6 m^{-2} with R^2 =0.996 support the hypothesis that Hadamard-Rybczynski-like vortices arise in droplets; further, α-values are plausible, as 20 μL min^{-1} translates into U_0 ~500μm s^{-1}.

In Fig. 3a-c, we demonstrate that the microvortex analysis in Fig. 2 is applicable to two-particle systems. Monitoring of the compound vector $\mathbf{r}_c = \mathbf{r}_1 - \mathbf{r}_2$ components reveals the time-course of cell-to-cell distance (Fig. 3b), which retains periodicity albeit with asymmetric vortex patterns (Fig. 3c). Therefore, our main results – vortex recirculation time control, and periodic modulation of cell-to-cell distance through passive hydrodynamics inside compartmentalized microfluidic units– are applicable to droplet-based immuno-analysis, where the transient scanning interaction and proximity between effector and target cell need to be deterministically regulated while retaining pairwise-correlated information to facilitate the study of immunological synapses.

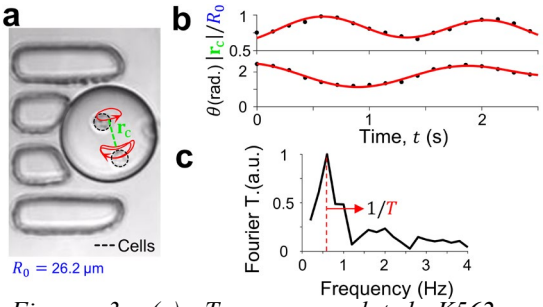

Figure 3. (a) Two encapsulated K562 cells circulating in microvortices. (b) Time-course of compound position vector, and its Fourier transform

CONCLUSION

Through experimental and theoretical understanding of flow patterns in droplets, we demonstrated the feasibility of modulating the position of encapsulated cells, thus providing a critical tool for studying dynamic cell-cell communication. This general particle manipulation approach is thereby applicable to a broad range of applications including drug screening, immunology, immunotherapy, and tissue engineering.

ACKNOWLEDGEMENTS

Authors acknowledge support from the National Science Foundation and industrial members of the Center for Advanced Design and Manufacturing of Integrated Microfluidics (NSF I/UCRC award no. IIP 1841509) Y8-002.

REFERENCES

[1] Segaliny, A. I. *et al.*, *Lab Chip* 18, 3733–3749, 2018.
[2] Palomba, F. *et al.*, *Biophys. J.* 120, 359a, 2021.
[3] Dura, B. *et al.*, *Nat. Commun.* 6, 5940, 2015.
[4] Yoshimura, Y. *et al.*, *Anal. Chem.* 86, 6818–6822, 2014.

CONTACT

* A.P. Lee; phone: (949) 824-9691; aplee@uci.edu ; ** These authors contributed equally to this work.