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Thermally-Assisted Ultrasonic Separation of Giant Vesicles

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Abstract

We report on a newly-developed membrane stiffness-based separation of vesicles using a thermally-assisted acoustophoretic approach. By tuning the temperature, we achieved the separation of vesicles of the same size, shape, and charge but with different stiffness values. It was observed that at a specific transition point, the acoustic contrast factor of vesicles changed sign from positive to negative. This change was mainly due to change in the acoustic compressibility of the vesicles, which is inversely proportional to stiffness. The acoustic contrast temperature, corresponding to the temperature at which the acoustic contrast factor switches sign, was determined to be unique to the composition of the vesicles. This unique temperature signature allowed us to develop a separation method of vesicles with distinct membrane stiffness with target outlet purities exceeding 95%. Our studies suggest that this method may be applied for the separation of cells affected by diseases that affect the cellular stiffness.

Graphical Abstract

Using thermo-acoustophoresis, vesicles are separated based on their stiffness at a temperature between the acoustic contrast temperatures of the vesicles.

The lipid bilayer membrane is the common underlying structure that confines the cytoplasm and cellular organelles and gives cells their unique physical properties such as size, density, and stiffness. Various biological changes in cells can affect these properties such as aging,¹ response to an infection,² or drug treatment.³ Changes in stiffness are especially of importance due to recent discoveries linking these changes to invasiveness of cancers,⁴ parasitic infections,⁵ and red blood cell anemia.⁶ The ability to separate cells which have undergone these changes in a viable and unaltered state from their healthy counterparts can be of significant importance to many biomedical fields. Since vesicles are essentially lipid bilayer membranes encapsulating aqueous cores, they have been chosen to be the subjects of

this study in which their properties have been altered to test the feasibility and limitations of thermally-assisted ultrasonic separation.

In standing-wave acoustophoresis the acoustic force can be used to manipulate particles based mainly on the size, density, and compressibility of both the particles and the medium.⁷

For a one-dimensional standing-wave of the planar type, the acoustic radiation force, F_x^{rad} , can be reduced to the one-dimensional form described by:⁸

$$F_x^{\text{rad}} = 4\pi\phi(\rho, \beta)k_x r^3 E_{\text{ac}} \sin(2k_x x) \quad (1)$$

in which the acoustic radiation force along the channel transversal direction, x , is directly related to the particle's radius, r , acoustic energy density, E_{ac} , and the acoustic contrast factor, Φ . The sign of Φ determines the direction of the radiation force and is given by the equation:⁷

$$\phi(\rho, \beta) = \frac{1}{3} \left[\frac{5\rho_p - 2\rho_0}{2\rho_p + \rho_0} - \frac{\beta_p}{\beta_0} \right] \quad (2)$$

where ρ_p and β_p are the density and compressibility of the particles, respectively; and ρ_0 and β_0 are the density and compressibility of the medium, respectively. In a half wavelength resonator microchannel where the width of the channel W equals $\lambda/2$, where λ is the length of the acoustic wave (inset in Fig. 1), the direction of the particle migration depends on the sign of Φ . Negative Φ affords anti-nodal migration, while positive Φ affords nodal migration.⁷ The magnitude and sign of Φ affects the acoustic force (eqn (1)) and consequently the velocity of the migration.

Standing-wave acoustophoresis has been previously reported for various microparticle separations, primarily based on their size and density.^{8–10} Its label-free separation and gentle action on the particles provided most of its development impetus, particularly in the field of cell separations.¹¹ A handful of studies^{12–15} have reported practical separation methods; nonetheless, while highlighting numerous limitations and challenges. Unaltered cell separation using acoustophoresis has been restricted mainly to size contrast.^{16,17} The main observation emerging from these studies was that cells often do not vary much in density to yield a change in Φ enough to use in a separation. Altered cells however, by binding them with elastomeric particles yielded a negative Φ .¹⁸ Similarly by changing the density of the medium by adding large amounts of CsCl salt (220 g L⁻¹), red blood cells were separated from platelets.⁸

Cellular rigidity, as an intrinsic property varies drastically among cells, changing as many as 10 folds in stiffness in some instances.¹⁹ To our knowledge, no previous works were able to take advantage of this vast change for same size and shape cells to yield a separation in acoustophoresis. The reason is that at room temperature, cells predominantly have positive acoustic contrast factors, leading to their swift migration towards the nodal region.²⁰ For that reason, in this study we introduced temperature as a new dimension to acoustophoresis to

yield a change in vesicle compressibility that allowed opposite Φ values for different vesicle systems. We dubbed this approach thermally-assisted ultrasonic separation or thermo-acoustophoresis.

In order to perform a systematic study with no interference from other properties, giant vesicles with average diameter of 10 μm have been prepared from various phospholipids to produce vesicles of variable membrane compositions. We hypothesize that due to thermotropic phase transitions, the vesicles had an apparent effect on their mechanical properties, especially compressibility. This effect provided tunability of Φ depending on temperature, thus enabling the existence of a temperature range in which opposite Φ signs existed. Within this temperature “window” the vesicles become mechanically distinct and thus differentiable in the acoustic radiation field, yielding to their separation (Fig. 1).

In a typical experiment, an aqueous suspension of vesicles was continuously injected into a rectangular microfluidic channel (500 μm wide and 90 μm deep, Fig. 2a), etched in a silicon wafer following a standard protocol detailed in the supporting information (ESI[†]). The microfluidic device was fitted with a piezoelectric transducer connected to a frequency generator with adjustable sinusoidal frequency. The chip was initially kept at a temperature as low as 1 $^{\circ}\text{C}$ with the signal generator set to the first harmonic frequency. At that frequency and temperature, the vesicles promptly focused at the nodal region located at the centre of the channel (Fig. 2b). A slow sweep of temperature (0.5–5 $^{\circ}\text{C min}^{-1}$) was then initiated while the vesicles were visually monitored under a fluorescent microscope (ESI[†]). Once the channel temperature reached or exceeded the acoustic contrast temperature, T_{Φ} , the vesicles collectively migrated towards the anti-nodal regions, *i.e.*, the walls in a first harmonic channel (Fig. 2b). Nodal and anti-nodal migrations due to temperature change are shown in Video S1 (ESI[†]).

The first harmonic frequency was determined for the microfluidic device using initial conditions. However, since the speed of sound in the bulk phase is dependent on temperature, substantial temperature increase required adjustments in the frequency to accommodate this change. While this adjustment maintained denser vesicle focusing at both the nodal and anti-nodal zones and helped increase the separation efficiency, it did not change the overall behaviour of the vesicles, and was deemed optional. The effect of the medium’s property change on Φ due to heating/cooling was assessed in Fig. S1 (ESI[†]). By comparing scenarios of (a) only properties of vesicles change versus (b) only properties of medium change due to the T change, it was observed that the reversal of the theoretical Φ was predominantly dependent on the compressibility of the vesicles. The temperature stability of the microchannel was improved using two heat sinks, above and below the Peltier element. At the frequency range around 1.4 MHz, the heat generated due to the ultrasonic actuation lead to 3 to 4 $^{\circ}\text{C}$ increase in the temperature and the Peltier element was adjusted accordingly.

To demonstrate the capability of thermo-acoustophoresis in the separation of vesicles, vesicles with distinct membrane compositions were prepared (ESI[†]). Using a modified solvent-injection method, vesicles were prepared using various molar ratios of the phospholipids DMPC ($T_m=23.9$ $^{\circ}\text{C}$) and DPPC ($T_m=41.4$ $^{\circ}\text{C}$). These phospholipids were

shown to display discernible differences in mechanical properties before and after the main thermotropic phase transition.^{25–27} Hydrated phosphatidylcholines of medium-chain size such as DMPC and DPPC experience a series of thermotropic transitions between different lamellar phases. At a lipid-specific temperature called the pre-transition temperature (T_p), the metastable or stable gel phase ($L_{\beta'}$) undergo a transition to another gel phase known as the rippled phase ($P_{\beta'}$). On further increasing the temperature to a point known as the main transition temperature (T_m), the $P_{\beta'}$ gel phase converts to a fluid (or liquid-disordered) phase (L_{α}).²⁸ The transition temperature observed in this study, T_{Φ} , occurred at a temperature $T_p < T_{\Phi} < T_m$ indicative of a mechanical transition in the membrane property, *i.e.*, membrane compressibility, rather than a distinct phase transition. The observation was corroborated using compressibility data to calculate the T_{Φ} values shown in Fig. 1 which closely matched the T_{Φ} measurements obtained in this study. The T_{Φ} for various molar ratios of DMPC:DPPC vesicles was investigated for both heating and cooling transitions both at $1\text{ }^{\circ}\text{C min}^{-1}$ (Fig. 2c). The heating experiments showed that the transition occurred at approximately the solidus line, right before the transition from ripple to liquid phase occurred. The cooling T_{Φ} was found to be lower, presumably due to the heat capacity in the vesicles. This temperature gap between the transitions was minimized by slowing down the temperature sweep. It is noteworthy that this transition temperature was consistent with the sudden drop in bending rigidity as a result of heating similar to what was observed in previous studies.^{26,27}

The velocity of the migration of the vesicles was proportional to the net force acting on the vesicles. In the transversal direction, these forces include the acoustic radiation force and the Stokes' drag force. Therefore, by increasing the temperature, the net force increases due to larger Φ and lower drag force due to lower viscosity of the medium. The average velocity of the migration can also be experimentally obtained using time-lapse micrographs. For example, in Fig. 3 the average transversal velocity was approximately $15\text{ }\mu\text{m s}^{-1}$. This velocity was mainly dependent on the magnitude and sign of Φ , the distance from the wall, and vesicles' diameter.

The existence of a membrane-specific temperature below which vesicles exhibit a positive Φ , and above which they assume a negative Φ offers us the possibility of separating these vesicles (Fig. 1). Two vesicle samples 100:0 and 70:30 DMPC:DPPC were selected to demonstrate the separation. The T_{Φ} for these two vesicles was $2.7\text{ }^{\circ}\text{C}$ (pure DMPC with $T_{\Phi}=23.2\text{ }^{\circ}\text{C}$ and 70:30 DMPC:DPPC $T_{\Phi}=25.9\text{ }^{\circ}\text{C}$). The separation temperature was selected as the approximate mid-point of $24.5\text{ }^{\circ}\text{C}$. At this separation temperature, the mixture of vesicles was shown to be randomly dispersed in a channel that was not acoustically excited (Fig. 4a). However, once the transducer was switched on ($f=1.40\text{ MHz}$ and 50 Vpp), the separation of vesicles promptly started and completed in less than 30 seconds. The distribution of vesicles can be seen in Fig. 4b with red-stained 100:0 DMPC:DPPC at the walls and the blue-stained 70:30 DMPC:DPPC at the centre of the channel. This distribution was quantified using averaged fluorescent intensity in the direction normal to the flow and the relative intensities are shown in Fig. 4c. In addition, the purity of each of the vesicle systems recovered from the outlets were 93–97%. Further improvement of the separation can be achieved by optimizing variables such as volumetric flow rate, separation temperature, and the amplitude of the acoustic radiation. The effect of the separation

temperature of the vesicles mixture was also investigated for separation of 100:0 DMPC:DPPC and 0:100 DMPC:DPPC. Separation experiments were conducted over different separation temperatures ranging from 17–43 °C while the separation window was determined to be 23.2–37.6 °C (Fig. 4d). At a temperature (23.6 °C) slightly higher than the T_{Φ} for DMPC (23.2 °C), the purities were 92% and 93% for DMPC and DPPC, respectively. This was due to the fact that right after the transition temperature Φ would not have reached a large enough value to produce high acoustic radiation force. In this case, some smaller vesicles, not hydrodynamically big enough, may migrate slower. When the separation temperature was chosen closer to the midpoint of the two T_{Φ} values (29 °C), the purities were improved to 98% for both DMPC and DPPC. The deviation from the mean value also decreases at higher temperature, indicating a more reliable separation at the midpoint. At a temperature right below the T_{Φ} of DPPC (37.2 °C), purities of 96% and 95% were obtained for DMPC and DPPC, respectively. This drop from the higher purity for the midpoint could also be explained by the lower Φ values for DPPC. However, the purities for both outlets were improved compared to $T=23.6$ °C due to the reduced drag force of the medium at higher temperatures.

In real cells, T_{Φ} is dependent on membrane and media compositions that may include in addition to phospholipids, proteins, cholesterol/triglycerides, and polysaccharides. From thermotropic transition data previously reported we expect T_{Φ} to range from 5 to more than 50 °C. For wild-type *E. coli* W945 the cytoplasmic membrane transition was reported as 5 °C,²⁹ while for rat small intestinal enterocytes as 23–39 °C,³⁰ and for human red blood cells thermotropic transitions were observed at various temperatures, some as high as 50 °C.³¹ We recognize that temperatures exceeding 43 °C for extended periods of time may pose harm to cells,³² however, many cells/vesicles can still be separated using thermo-acoustophoresis, making this technique biomedically relevant.

In summary, we have demonstrated the possibility of separating vesicles based on their membrane stiffness using thermally-assisted acoustophoresis in a microfluidic device. This technique allowed us to exploit the unique acoustic contrast temperatures of vesicles to yield a separation at $T_{\Phi 1} < T_{sep} < T_{\Phi 2}$. Under this separation temperature condition, vesicles migrated in opposite directions, towards the central region or the wall regions of the channel. High separation efficiencies were achieved and we anticipate that it can be further improved in the future. We envision that this separation technique can prove to be of value as a stand-alone or in junction with other separation methods that can yield medical separations and diagnostics, especially in cancers, infections, and red blood cell anemia research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Notes and references

1. Phillip JM, Aifuwa I, Walston J, Wirtz D. Annu. Rev. Biomed. Eng. 2015; 17:113–41. [PubMed: 26643020]
2. Lee GYH, Lim CT. Trends Biotechnol. 2007; 25:111–118. [PubMed: 17257698]
3. Lam, Wa; Rosenbluth, MJ.; Fletcher, DA. Blood. 2007; 109:3505–3508. [PubMed: 17179225]
4. Di Carlo D. J. Lab. Autom. 2012; 17:32–42. [PubMed: 22357606]
5. Suwanarusk R, Cooke BM, Dondorp AM, Silamut K, Sattabongkot J, White NJ, Udomsangpetch R. J. Infect. Dis. 2004; 189:190–194. [PubMed: 14722882]
6. Barabino, Ga; Platt, MO.; Kaul, DK. Annu. Rev. Biomed. Eng. 2010; 12:345–367. [PubMed: 20455701]
7. Bruus H. Lab Chip. 2012; 12:1014–1021. [PubMed: 22349937]
8. Petersson F, Åberg L, Swärd-Nilsson AM, Laurell T. Anal. Chem. 2007; 79:5117–5123. [PubMed: 17569501]
9. Wood CD, Evans SD, Cunningham JE, O'Rourke R, Wälti C, Davies AG. Appl. Phys. Lett. 2008; 92:10–13.
10. Fernandez Rivas D, Cintas P, Gardeniers HJGE. Chem. Commun. 2012; 48:10935.
11. Burguillos, Ma; Magnusson, C.; Nordin, M.; Lenshof, A.; Augustsson, P.; Hansson, MJ.; Elmér, E.; Lilja, H.; Brundin, P.; Laurell, T.; Deierborg, T. PLoS One. 2013; 8:1–11.
12. Franke T, Braunmüller S, Schmid L, Wixforth a, Weitz Da. Lab Chip. 2010; 10:789–794. [PubMed: 20221569]
13. Nam J, Lim H, Kim D, Shin S. Lab Chip. 2011; 11:3361–3364. [PubMed: 21842070]
14. Ding X, Lin S-CS, Lapsley MI, Li S, Guo X, Chan CY, Chiang I-K, Wang L, McCoy JP, Huang TJ. Lab Chip. 2012; 12:4228. [PubMed: 22992833]
15. Antfolk M, Antfolk C, Lilja H, Laurell T, Augustsson P. Lab Chip. 2015; 15:2102–2109. [PubMed: 25824937]
16. Thévoz P, Adams JD, Shea H, Bruus H, Soh HT. Anal. Chem. 2010; 82:3094–3098. [PubMed: 20199060]
17. Li P, Mao Z, Peng Z, Zhou L, Chen Y, Huang P-H, Truica CI, Drabick JJ, El-Deiry WS, Dao M, Suresh S, Huang TJ. Proc. Natl. Acad. Sci. U. S. A. 2015; 112:4970–4975. [PubMed: 25848039]
18. Shields CW, Johnson LM, Gao L, López GP. Langmuir. 2014; 30:3923–3927. [PubMed: 24673242]
19. Faria EC, Ma N, Gazi E, Gardner P, Brown M, Clarke NW, Snook RD. Analyst. 2008; 133:1498–1500. [PubMed: 18936825]
20. Ward M, Turner P, DeJohn M, Kaduchak G. Curr. Protoc. Cytom. 2009:1–12.
21. Heimburg T. Biochim. Biophys. Acta - Biomembr. 1998; 1415:147–162.
22. Krivanek R, Rybar P, Prenner EJ, McElhaney RN, Hianik T. Biochim. Biophys. Acta - Biomembr. 2001; 1510:452–463.
23. Mabrey S, Sturtevant JM. Proc. Natl. Acad. Sci. U. S. A. 1976; 73:3862–3866. [PubMed: 1069270]
24. Cheng WH. Biochim. Biophys. Acta. 1980; 600:358–366. [PubMed: 7407119]
25. Dimova R, Pouligny B, Dietrich C. Biophys. J. 2000; 79:340–356. [PubMed: 10866960]
26. Yi Z, Nagao M, Bossev DP. J. Phys. Condens. Matter. 2009; 21:155104. [PubMed: 21825357]
27. Woodka AC, Butler PD, Porcar L, Farago B, Nagao M. Phys. Rev. Lett. 2012; 109:058102. [PubMed: 23006210]
28. Marsh, D. Handbook of Lipid Bilayers. 2nd. CRC Press, Taylor & Francis Group, LLC; 2013.
29. Melchior DL, Steim JM. Annu. Rev. Biophys. Bioeng. 1976; 5:205–238. [PubMed: 182064]
30. Brasitus T, Tall R, Schachter D. Biochemistry. 1980; 19:1256–1261. [PubMed: 7370232]
31. Forte T, Leto TL, Minetti M, Marchesi VT. Biochemistry. 1985; 24:7876–7880. [PubMed: 3004567]
32. Wiklund M. Lab Chip. 2012; 12:2018–2028. [PubMed: 22562376]

Fig. 1. Determination of the temperature window of separation for DMPC and DPPC vesicles. The $\Phi > 0$ indicates nodal focusing while $\Phi < 0$ indicates anti-nodal focusing. With $T_{\Phi, \text{DMPC}} < T_{\text{separation}} < T_{\Phi, \text{DPPC}}$, opposite focusing is achieved. The relative values of Φ were calculated (ESI†) using vesicle compressibility data from the literature.^{21,22} Inset: Schematic of the direction of the acoustic radiation force. Black arrows denote the direction of the momentum transfer and the grey arrows denote the direction of the net force and thus the resulting motion.

Fig. 2.

(a) Schematic illustration of the separation device showing an overlaid acoustic half-wave.

(b) Experimental 2.5D view micrographs of the relative fluorescence intensity of the DMPC vesicles focused in nodal region at $T < T_\phi$ and its transition to the anti-nodal region at temperature $T > T_\phi$. The T_ϕ of DMPC was 23.2 °C.

(c) Thermotropic isomorphous phase diagram of the DMPC:DPPC lipid binary mixture. Data were obtained from the mean value of the theoretical data and the experimental data obtained by electron spin resonance (ESR) and differential scanning calorimetry (DSC) studies (solidus (dotted) and liquidus (dashed) curves).^{23,24} The empty circle markers denote the measured T_ϕ during the heating experiment while full diamond markers denote the measured T_ϕ during the cooling curve. The heating T_ϕ nearly overlapped with the solidus line over the molar ratio range. The error bars represents the maximum and minimum observed T_ϕ .

Fig. 3. Time-lapse fluorescent micrographs of the transition from nodal focusing to anti-nodal focusing of DPPC vesicles as the temperature exceeded $T_{\Phi}=37.6^{\circ}\text{C}$ at time $t=0$ s. The focusing was complete at time $t=30$ s.

Fig. 4.

Monitoring the performance of the separation experiment of 100:0 DMPC:DPPC and 70:30 DMPC:DPPC. (a) Optical fluorescence micrographs of 100:0 (red) and 70:30 (blue) DMPC:DPPC vesicles dispersed in the microchannel at 24.5 °C with the acoustic transducer switched off. (b) Same vesicles in A after 3 seconds of the transducer switched on showing 100:0 DMPC:DPPC completed migration towards the pressure antinodes (red) and 70:30 DMPC:DPPC completed migration towards the pressure node (blue) (scale bars in both A and B images are 150 μm). (c) Relative fluorescence intensity scanned in the direction normal to the flow for both red and blue emissions. (d) Target outlet purities at 6 different temperatures over the allowable separation window for pure DMPC and DPPC vesicle systems. The error bars represents the standard deviation for $n = 3$.