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

Supplementary Information

Thermally-Assisted Ultrasonic Separation of Giant Vesicles

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Chip Fabrication

The microfluidic channel used as the separation device was fabricated *via* standard photolithography and anisotropic wet etching. Briefly, the front side of a 4-inch <100> silicon wafer (WRS Materials, San Jose, CA) pre-coated with a low-stress silicon nitride layer was first spin coated by photoresist AZ 4620 (MicroChemicals GmbH, Ulm, Germany), followed by a soft-bake at 110 °C for 2 min. The photoresist was subsequently exposed using a contact-mode mask aligner (Model 800 MBA, OAI, San Jose, CA) with an exposure energy of 400 mJ/cm^2 and then developed using developer AZ® 400K (AZ Electronic Materials, NJ) diluted by DI water at a volume ratio of 1:3. Upon approving the feature quality by optical microscopy (Unitron Versamet Optical Microscope, Commack, NY), the residual resist inside the channel was removed by an oxygen plasma treatment (CS-1701, MARCH, Concord, CA). The descum procedure was performed using 100 mTorr pressure of O_2 and 400 watts of power with 60 sccm flow rate for 45 s. The developed pattern was then etched with CF_4 plasma to remove the nitride layer. In the next step, the remaining photoresist was removed using a Remover PG solution (MicroChem, Newton, MA) kept at 65 °C for 30 min. The treatment was followed by a rinse in isopropyl alcohol and water. The underlying silicon material was subsequently removed *via* wet-etch processing. A preferential silicon etchant (PSE-200) (Transene Company, Inc., Danvers, MA) was utilized to carry out the Si etching. In order to remove the remaining nitride layer, the wafer was washed with an aluminum etchant (Transene Company, Inc., Danvers, MA) for 240 min at 180 °C. The wet etching was monitored and stopped when silicon was etched to the desired depth measured using an optical profilometer (Nanovea, Irvine, CA). Following that, the wafer was cleaned using a piranha solution, ethanol and DI water to remove any debris left from the prior processing. After rinsing and drying, the wafer was again examined using the optical microscope. Finally, a 4-inch Pyrex® wafer (Praezisions Glas & Optik GmbH, Iserlohn, Germany) containing holes created manually by an ordinary drill (220-01 WorkStation™, Dremel, Racine, WI) was anodically bonded to the substrate via heating up the wafer at 500 °C for 15 min with 500 V of potential difference provided by a high-voltage power supply (Model 247, Keithley Instruments Inc., Cleveland, OH).

Experimental Setup

The width and depth of the main channel were 500 and 90 μm, respectively. The length of the main channel was 20 mm. A disk-shaped piezoelectric transducer (12.75 mm in diameter) with wrap-around electrode pattern (APC International, Ltd, Mackeyville, PA) was bonded to the back of the chip underneath the main separation channel. An AC signal of sinusoidal form was generated by a function generator (DG4062, RIGOL Technologies Inc, Beaverton, OR) and then amplified by a power amplifier (Model 2348, TEGAM Inc, Geneva, OH). The applied voltage amplitudes were monitored using an oscilloscope (TDS 2014B, Tektronix Inc, Beaverton, OR). Chip temperature was controlled throughout all experiments using a Peltier element (Farnell, London, UK), which was connected to a DC power supply (Model 72-2010, TENMA, Washington, OH). For separation runs, the inlet and outlet flows were controlled by syringes (BD Luer-Lok™, Franklin Lakes, NJ) connected to two syringe pumps (New Era Pump Systems Inc, Farmingdale, NY). One pump was connected to the chip inlet, infusing the vesicle specimen from a 3 mL syringe. The other pump was set in withdrawal mode and connected to two plastic syringes to control the flow in the separation chip. Both pumps were connected via PVC tubing with luer ends (TUBING LUER M-F 24", Cole-Parmer, Vernon Hills, IL) to the chip outlets. The flow rate used in all experiments was 15 μL min^{-1}. To conduct the separation experiments, the device was loaded onto the stage of a fluorescence microscope (Axio Scope.A1, Carl Zeiss Microscopy GmbH, Oberkochen, Germany). After the separation runs, the purity of the vesicles collected at the outlets was calculated using the concentrations measured by a calibrated fluorescent-intensity-based quantification approach.

Preparation of Vesicles

For the preparation of vesicles, two phosphatidylcholines containing linear saturated fatty acyl chains namely, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) were purchased from Avanti Polar Lipids (Alabaster, AL). Both lipids were used without further purification. In brief, a solution of the desired lipid in the form of a lyophilized powder was first prepared in the water-miscible solvent tetrahydrofuran (≥99.9%, inhibitor-free) (Sigma-Aldrich, St. Louis, MO). The weight percent of the dissolved lipid in the solvent was between 1-3%. In the next step, a fluorescent dye was introduced to the solution to label the lipid membrane of the vesicles. Dyes used in this study were Laurdan (6-dodecanoyl-2-dimethylaminonaphthalene) (AnaSpec, Inc., Fremont, CA) and Nile red (9-diethylamino-5-benzo[α]phenoxazinone) (Acros Organics, Geel, Belgium). After adding the dye, distilled water was added dropwise with a rate of 10 ml/h to the solution while the mixture was being magnetically stirred at room temperature. A syringe pump (New Era Pump Systems, Inc, Farmingdale, NY) was used for the water dripping. To allow the solvent to evaporate, the sample was left open to sit in the ambient atmosphere for 24 hours, followed by a vacuum treatment when needed. The prepared vesicles were visualized using an optical microscope (AxioCam ICc 1, Carl Zeiss Microscopy GmbH, Oberkochen, Germany) operating in transmission and reflected modes.

Calculation of Acoustic Contrast Factor

In a standing acoustic field, the direction towards which a compressible spherical particle migrates is determined by the acoustic radiation force[1], described as:

$$F^{rad} = -\nabla U^{rad} \tag{1}$$

where U^{rad} is the acoustic potential and the diameter of the particle is assumed to be much smaller than the wavelength of the applied acoustic wave. For a one-dimensional planar standing wave, if the induced acoustic pressure amplitude is assumed to be of the form:

$$p(x,y,z) = p_a cos(k_x x) \tag{2}$$

in which $k_x = 2\pi/\lambda$ and $\lambda = 2w$, w being the channel width, the acoustic radiation force can be then reduced to the one-dimensional form[2]:

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

in which the acoustic radiation force along the direction x (the direction perpendicular to the flow) is directly related to the particle radius, r, acoustic energy density, E_{ac}, and the acoustic contrast factor, $\phi(\rho,\beta)$, which is a function of the compressibility and density of the particles/vesicles and medium:[3]

$$\phi(\rho,\beta) = \frac{1}{3}\left[\frac{5\rho_v - 2\rho_0}{2\rho_v + \rho_0} - \frac{\beta_v}{\beta_0}\right] \tag{4}$$

where ρ_v is the density of vesicles, ρ_0 the density of the medium, and β_v and β_0 are the compressibility values for the vesicles and medium, respectively.

Since the internal volume of giant vesicles is extremely large compared to its enclosing membrane, it is safe to assume that the density ratio is very close to unity. Therefore, ϕ is here is mainly dependent on the compressibility ratio of the vesicles and medium.

The elastic properties of aqueous media and vesicle suspensions can be evaluated by using the relationship:[4]

$$\beta_S = \frac{1}{\rho u^2} \tag{5}$$

where β_S, u, and ρ are the adiabatic compressibility, the speed of sound, and the density of the vesicles suspension, respectively. The parameter usually determined in ultrasonic velocimetry experiments is sound velocity number ($[u]$), which is defined by the equation:[4]

$$[u] = \frac{u - u_0}{u_0[L]}$$

(6)

where, u and u_0 denote the speed of sound in the vesicle suspension and in the solvent, respectively, and $[L]$ is the lipid concentration, usually given in mg/cm^3.

The basic value of $[u]$ as a function of temperature is determined by sound velocity experiments. The density of the vesicles in the solution (ρ), determined by densitometry experiments, is also used to determine the apparent specific partial volume of the solution (φ_V), which is defined by:[5]

$$\varphi_V = \frac{\left[1 - (\rho - \rho_0)/[L]\right]}{\rho_0} = \frac{1}{\rho_0} - [\rho]$$

(7)

whereby ρ_0 denotes the density of the solvent and $[\rho] = (\rho - \rho_0)/(\rho_0[L])$ denotes the concentration increment of density. Based on the values of the specific volume and the sound velocity concentration increment, one could estimate the changes of the specific adiabatic compressibility (φ_K/β_0) of the vesicles during the phase transition by the following equation:[5]

$$\frac{\varphi_K}{\beta_0} = -2[u] - \frac{1}{\rho_0} + 2\varphi_V$$

(8)

whereby β_0 is the coefficient of adiabatic compressibility and φ_K/β_0 is the changes of the volume compressibility of the vesicles suspension relative to the solvent. The apparent specific compressibility can be also expressed as:[5]

$$\frac{\varphi_K}{\beta_0} = \varphi_V + [\beta_S] \tag{9}$$

where $[\beta_S] = (\beta_S - \beta_{S0})/(\beta_{S0}[L])$ is the concentration increment of the adiabatic compressibility, in which β_S and β_{S0} denote the adiabatic compressibility of the vesicles suspension and solvent, respectively. The calculated value of β_S would represent the compressibility of the suspension, which can be then used to find the value of the compressibility of the vesicles by:

$$\beta_{S,suspension} = f_0 \cdot \beta_{S0} + f_v \cdot \beta_{S,v} \tag{10}$$

in which f_0 and f_v are the volume fractions of the water and vesicles, respectively, and β_{S0} and $\beta_{S,v}$ denote the compressibilities of water and vesicles, respectively.

The variation of the absolute value of ϕ against temperature is plotted in Fig. S1. Since these values are a function of the lipid concentration used in the velocimetry and densitometry experiments, in the main text only the relative values are shown.

In Figure S1a the properties of both DPPC vesicles and medium change as a function of temperature have been taken into consideration and predicted a T_{ϕ} of 36.5°C. In figure S1b, the properties of the medium were considered independent of temperature (fixed values for room temperature conditions) and predicted a T_{ϕ} of 36.9°C. In figure S1c the properties of vesicles were considered independent of temperature (fixed values for room temperature conditions) and ϕ remained positive over the range of temperature, thus a T_{ϕ} does not exist. Similar observations were also obtained for DMPC vesicles suspensions. For both systems, the variations of the bending rigidity with temperature begin with a sharp decrease in the gel phase at temperatures around T_m of the lipid. The drop is then followed by an increase in the fluid phase after the T_m and then a small decrease between T_m+3 °C and T_m+6 °C ending with a stabilization at temperatures above T_m+6 °C.[6–10]

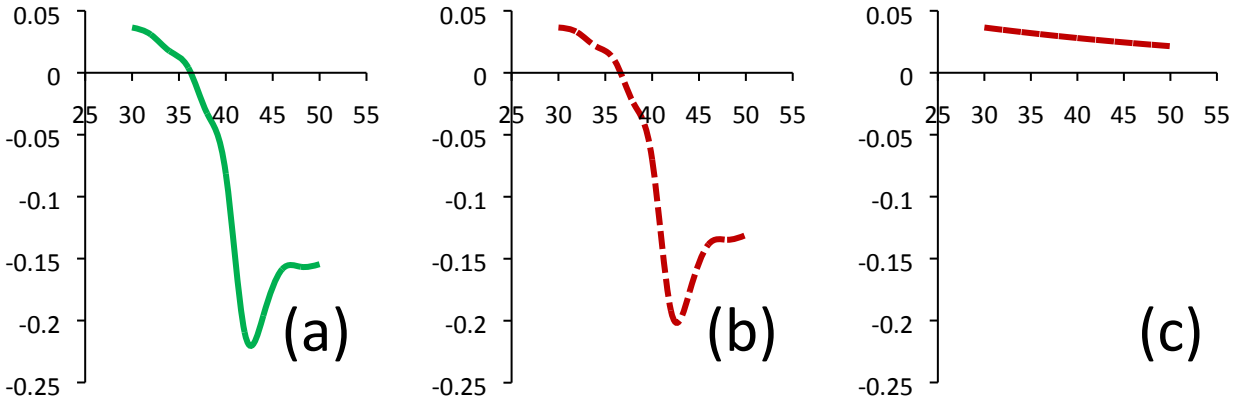

Figure S1 Change of ϕ with temperature for a (a) DPPC vesicles, (b) considering a scenario where the adiabatic compressibility of the bulk medium retains room temperature value at various temperatures, and (c) considering a scenario where the adiabatic compressibility of the vesicles retains room temperature values at various temperatures.

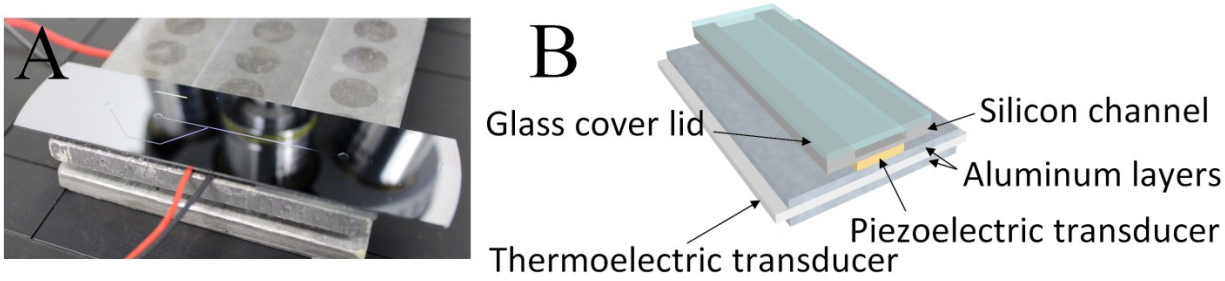

Figure S2 (a) A close-up photograph of the experimental setup showing the chip and electrical connections of the piezoelectric and thermoelectric transducers. (b) Schematic illustration of the different parts of the separation device.

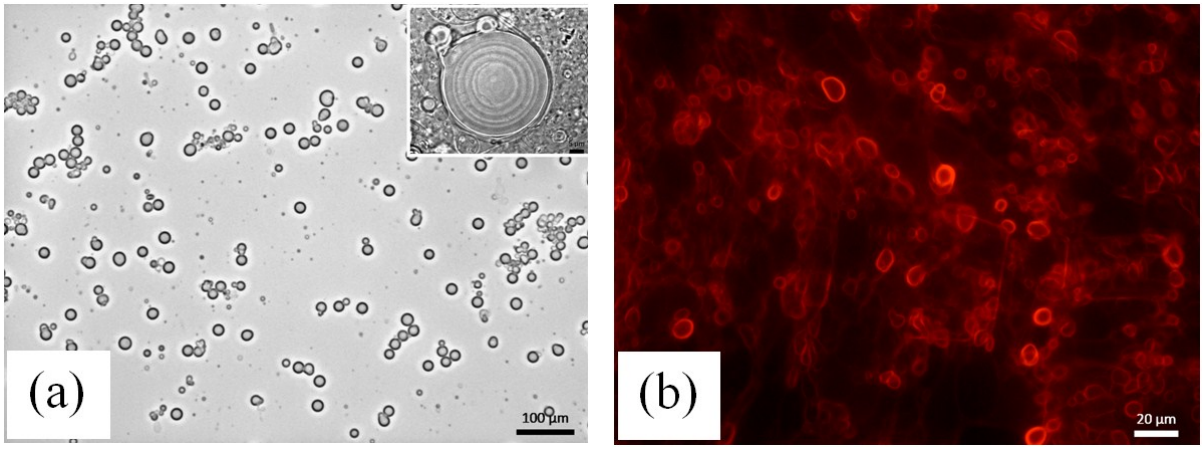

Figure S3 Optical micrographs of the DMPC vesicles: (a) Transmission-mode image shows a uniform population of vesicles (The inset shows a giant multilamellar vesicle in higher magnification) and (b) fluorescent image of the same sample.

Videos Information

Video S1 DPPC vesicles responding to the acoustic actuation by migrating to the center region of the channel at T = 32.0 °C followed by migration to the wall at T=37.2 °C.

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