Supporting Information

Mass production and size control of lipid-polymer hybrid nanoparticles through controlled microvortices

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Material preparation for nanoparticle syntheses

The lipid dissolved in 4wt% ethanol aqueous solution in the outer streams of the microfluidic channel was composed of lecithin (soybean, refined, molecular weight ~330 Da; Alfa Aesar, Ward Hill, MA) and DSPE-PEG (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-carboxy(polyethylene glycol); molecular weight ~2850 Da; Avanti Polar Lipids, Alabaster, AL). The mole ratio of lecithin to DPSE-PEG was 7:3. The PLGA (poly(lactide-co-glycoliude); inherent viscosity of 0.55~0.75; Lactel, Pelham, AL) was dissolved in acetonitrile. The final concentrations of the PLGA and lipid solutions were prepared by making their aliquots (PLGA solution (15 mg/mL) and lipid solution (1.5 mg/mL)) and diluting them to create the PLGA-to-lipid ratios used in the experiments.

PLGA / lipid (wt)	PLGA (mg)	Acetonitrile (mL)	PLGA solution (mg/mL)	Lecithin (mg)	DSPE-PEG (mg)	4% ethanol Aq. (mL)	lipid solution (mg/mL)
5	3.750	10	0.375	0.32	1.18	20	0.075
10	7.500	10	0.750	0.32	1.18	20	0.075
25	18.75	10	1.875	0.32	1.18	20	0.075
50	37.50	10	3.750	0.32	1.18	20	0.075
100	75.00	10	7.500	0.32	1.18	20	0.075

Particle sizing

Nanoparticle size was analyzed three times for each sample using dynamic light scattering (DLS) with ZetaPALS (Brookhaven Instruments Corporation, US). The nanoparticle samples of 100 μL were gently suspended and mixed into 200 μL PBS in a disposable low-volume cuvette. All measurements were performed after replacing the organic solvent (i.e. acetonitrile) with purified water to ensure that any size variation observed in the results was not due to the effect of the remaining solvent. Dilution of the solvent was performed by repeated centrifugal purification processes of the solution with purified water.

Particle visualization

Nanoparticles were imaged by transmission electron microscopy (TEM; JEOL JEM-299CX) at an acceleration voltage of 200 kV. Samples were prepared by depositing 10 μL of the nanoparticle suspension onto 200-mesh carbon-coated copper grid. The samples were blotted away for 15 minutes and the grids were negatively stained for 2 minutes with filtered 2% uranyl acetate aqueous solution. The grids were then washed with double-distilled water and air-dried prior to imaging.

Reynolds number and flow rate

Three glass syringes were mounted on syringe pumps (NE-1010-U, Kats Scientific) to regulate flow rates through the device. The flow rates in the outer streams of the lipid and lipid-PEG in water were varied from 10 μL/min to 10 mL/min while the flow rate in the central stream of the PLGA in acetonitrile was varied from 2 μL/min to 2 mL/min. Reynolds number (Re) was calculated using the following equation[31].

$$\mathrm{Re} = \frac{\rho U D_h}{\mu} = \frac{\rho U}{\mu}\frac{2wh}{w+h} = \frac{\rho}{\mu}\frac{2Q}{w+h}$$

where Q is the flow rate; μ represents the fluid's viscosity; w and h represent the channel width (2000 μm) and height (400 μm); ρ represents the fluid's density; U represents the fluid's average velocity. The flow rates and the resulting Reynolds numbers were shown (Figure S1).

Reynolds number (Re)	Flow rates (mL/min)			
	Lipid (L)	PLGA (C)	Lipid (R)	Total
0.3	0.01	0.002	0.01	0.022
30.6	1.0	0.2	1.0	2.2
76.4	2.5	0.5	2.5	5.5
152.8	5.0	1.0	5.0	11.0
305.6	10.0	2.0	10.0	22.0

Microfluidic device design and fabrication

The microfluidic device had three inlet channels with rectangular cross-sections of dimensions 200 μm wide, 400 μm high, and 10 mm long. These inlet channels converged to form a single outlet channel of rectangular cross-section with dimensions 2000 μm wide, 400 μm high, and 20 mm long. The device was fabricated with polydimethylsiloxane (PDMS) (SYLGARD 184, Dow Corning, Midland, MI) using standard soft-lithography techniques[32].

Flow visualization and microscopy

Flow patterns were visualized using a stereo microscope (Leica M125, Leica Microsystems, Bannockburn IL). The central stream has a 10:1 ratio of deionized water and black ink and the other outer streams have deionized water. Images were taken for the Reynolds number of 30, 75, and 150.

Numerical simulations

Numerical simulations of the flow field were conducted using the commercial CFD solver (SC/Tetra, CRADLE, Beavercreek, OH) in order to solve the non-linear Navier-Stokes equations governing the conservation of mass and momentum within the fluid elements. Advection-diffusion equations were also solved to predict the flow field and the user-defined scalar species. The diffusion coefficients for the scalar species used in the simulations were assumed to be 1e-10 m^2/s corresponding to that of water at approximately room temperature[33]. We assumed a Newtonian fluid having the properties of water at room temperature and no-slip boundary conditions on all the walls. Mesh independence was verified by examining higher density meshes. Flow rates were specified at the three inlets. Convergence limits were set so that velocities converged within 0.1% and mass fractions for the central stream species reached their asymptotic values within 0.01%.

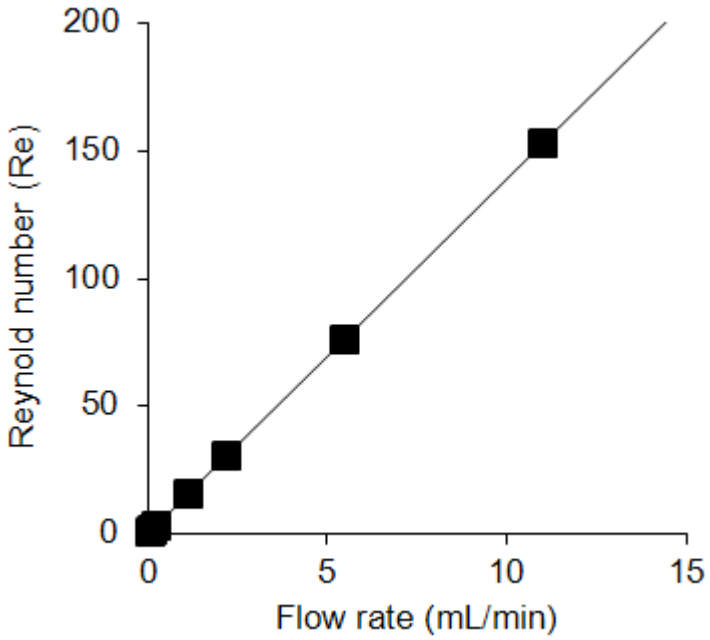

Figure S1. Reynolds number as a function of flow rates used in the experiments.

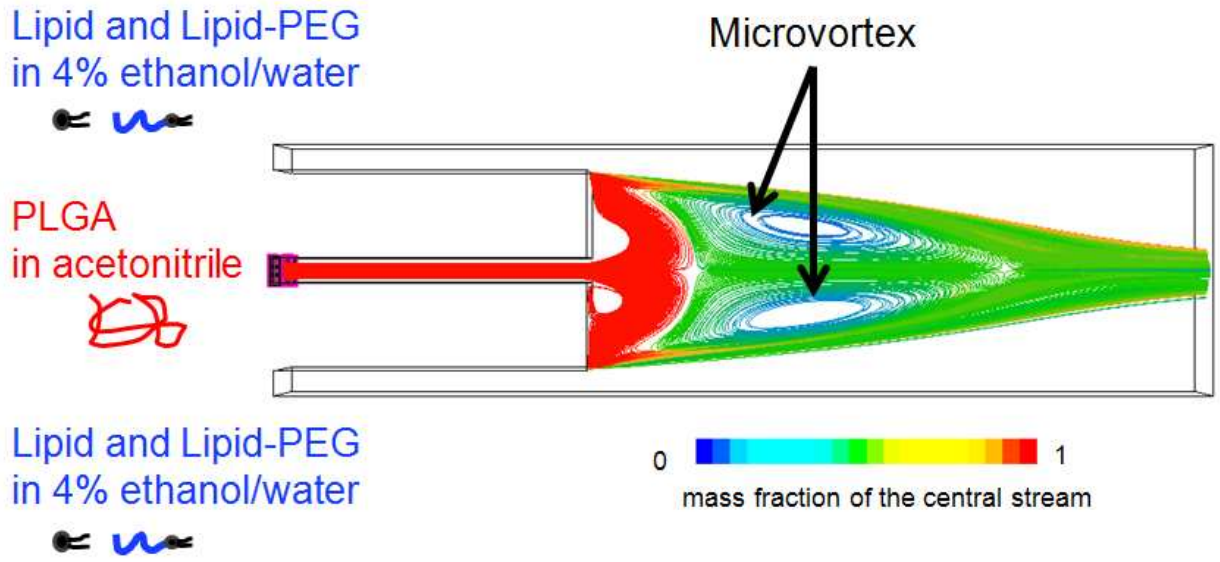

Figure S2. Streaklines and mass fraction of the center stream of PLGA in the microfluidic platform showing formation of symmetric microvortices and mixing downstream

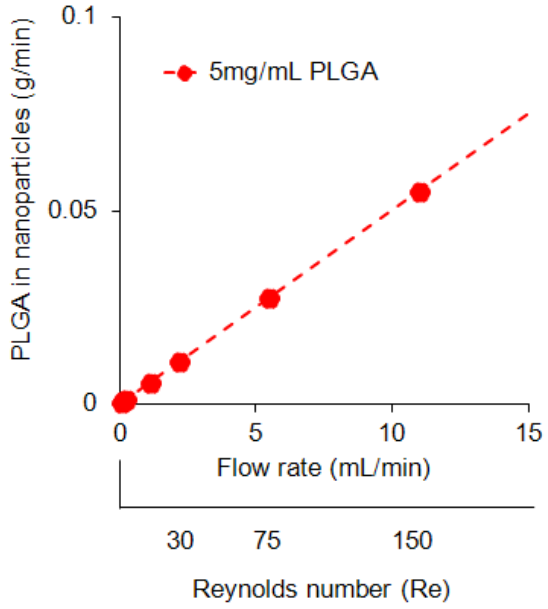

Figure S3. The PLGA amount included in the nanoparticles produced in the microvortex platform. After removing the free lipids with the purification process, the PLGA is assumed to be essentially 100% effective as previously reported.

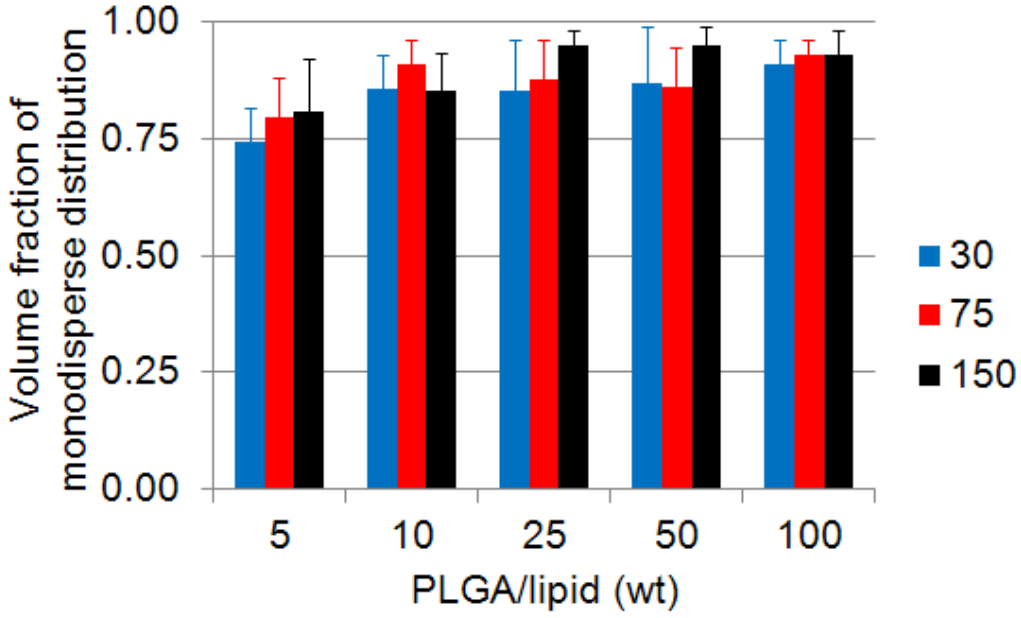

Figure S4. Volume fraction of monodisperse nanoparticle distribution.

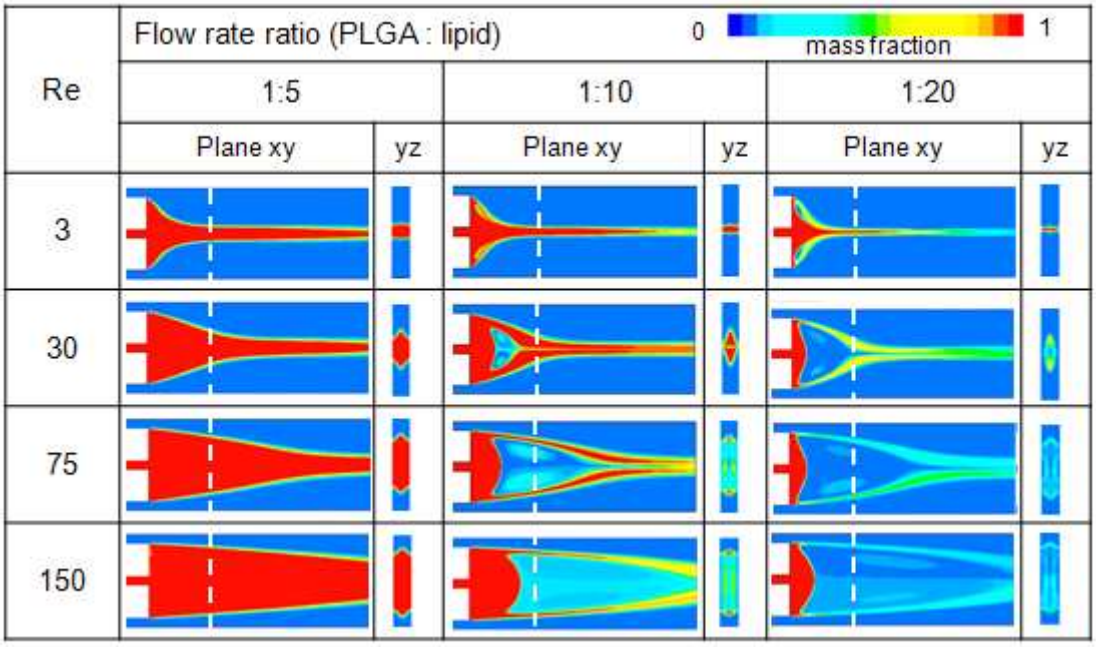

Figure S5. Computational fluid dynamics simulations showing microvortex patterns with variations of flow rate ratios of polymer to lipid streams and Reynolds numbers

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