Supporting Information

Pneumatically Controlled Microfluidic Synthesis of Polymeric Nanoparticles for mRNA Delivery

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Supplementary Figures

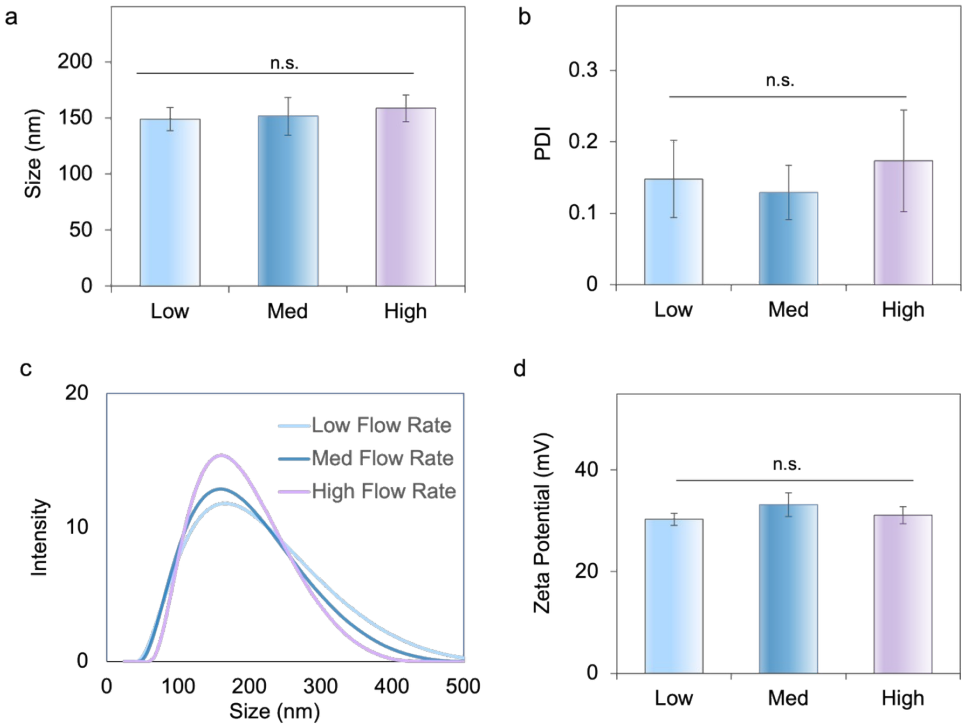

Fig. S1 Physicochemical characterization of PPH nanoparticle synthesized under different liquid flow rate (high: 1.2 µL/s, med: 0.6 µL/s, low: 0.2 µL/s) with fixed air flow rate. (a) Hydrodynamic size. (b) Polydispersity index. (c) Size distribution. (d) Zeta potential. (Data are presented as mean ± SD (n = 3). * p< 0.05, ** p<0.01, n.s. = statistically insignificant)

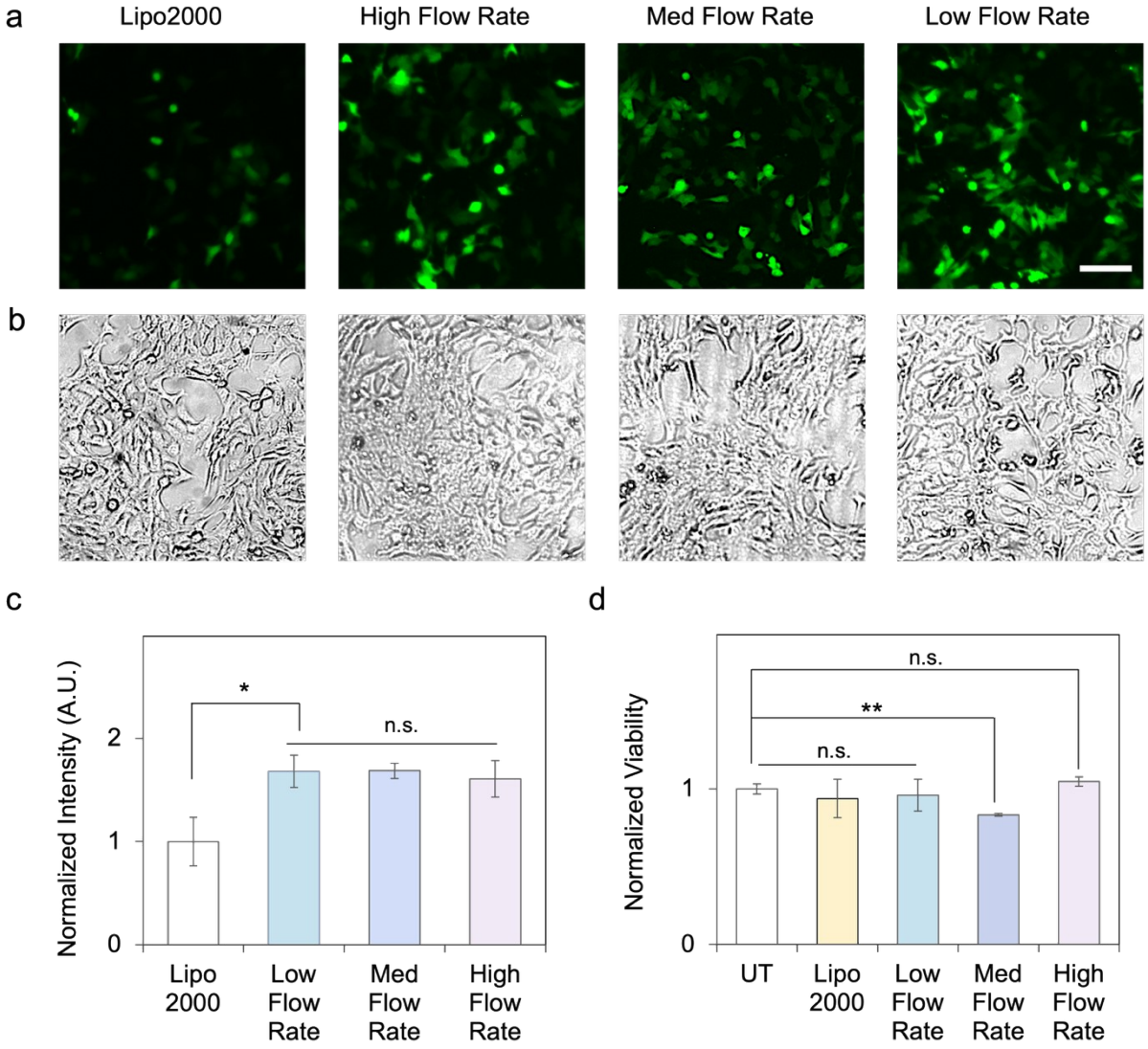

Fig. S2 Transfection efficiency and cytotoxicity of PPH nanoparticles in 4T1 cells. (a) Fluorescence images showing GFP expression in 4T1 cells transfected with GFP-mRNA-loaded PPH nanoparticles synthesized under different air flow rates. mRNA loaded Lipofectamine 2000 (Lipo2000) serves as a positive control. (b) Bright-field images of 4T1 cells treated with Lipo2000 and PPH nanoparticles at an mRNA dose of 2 μg/mL. (b) Bright field images of 4T1 cells treated with mRNA loaded Lipo2000 and PPH at a mRNA concentration of 2 μg/mL. (c) Histograms of GFP fluorescence intensity in cells treated with Lipo2000-mRNA and PPH nanoparticles generated under low, medium, and high flow rates. Fluorescence intensities are normalized to the Lipo2000-mRNA group (set to 1). (d) Cell viability following 24-hour treatments with Lipo2000 or PPH nanoparticles. Viability is normalized to untreated controls. (Scale bar = 50 μm. Data are shown as mean ± SD (n = 3). n.s., not significant.)

a

Chip Components	Specification
Hamilton microliter syringes	50 uL and 500 uL syringes (with needle of gauge 22)
NE-300 'Just Infusion'TM Syringe Pump	/
PTFE Tube	28 gauge, 12 cm
PDMS	SYLGARDTM168 Silicone Elastomer Kit
Glass Slide	/
Two part epoxy	Gorilla Glue

b

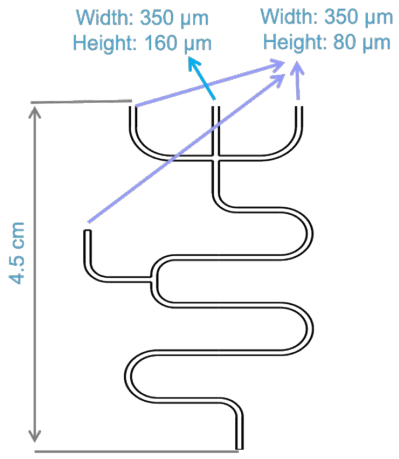

Fig. S3 Specification of the microfluidic device components. (a) Components of the microfluidic chip platform. (b) Geometry and dimensions of the microfluidic channels used in the device.

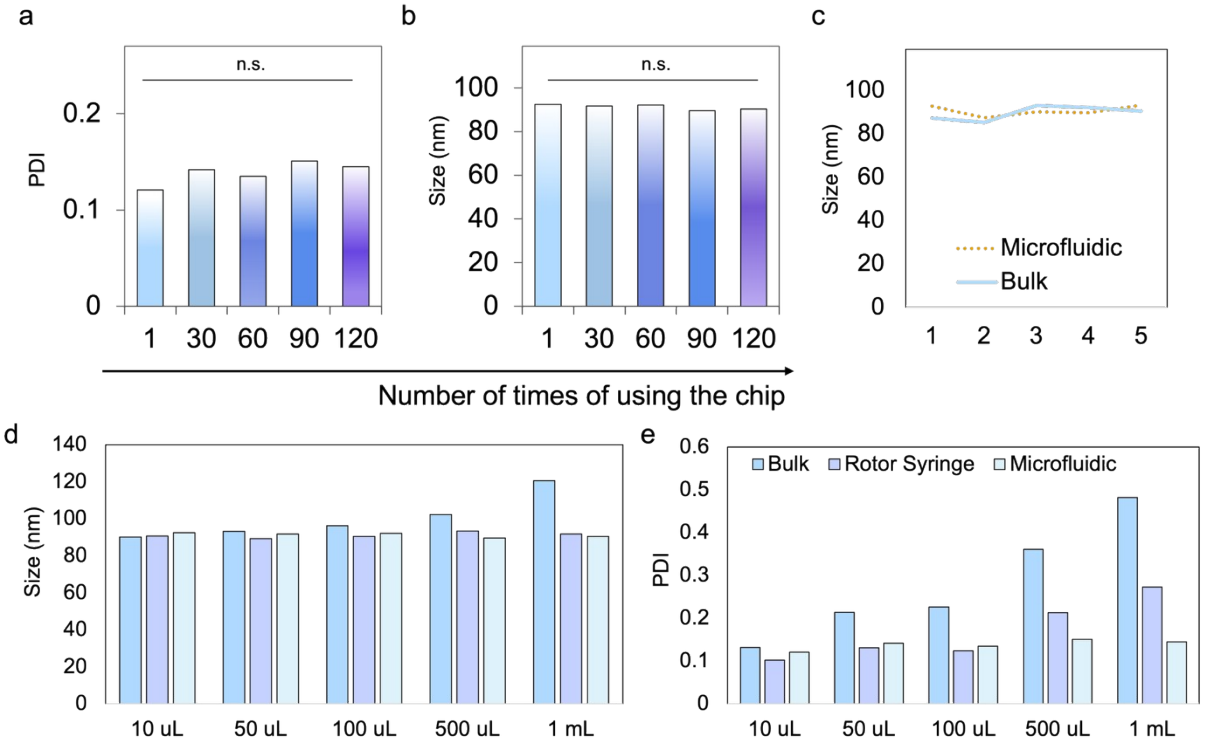

Fig. S4 Long-term operational stability and scalable production of PPH nanoparticles. (a) PDI of PPH NPs synthesized using the microfluidic platform over 120 production cycles. (b) Corresponding hydrodynamic size of the PPH NPs collected across the 120 cycles. (c) Hydrodynamic size of PPH NPs (10 µL production volume) synthesized via different mixing methods across five independent replicates (n = 5) by using bulk pipette mixing and microfluidic synthesis. (d) Effect of production scale on the hydrodynamic size of PPH NPs. Synthesis was scaled from 10 µL up to 1 mL volumes comparing bulk pipette mixing, rotor-syringe method and the microfluidic chip platform.