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

Supplemental information

Isolation and quantification of extracellular vesicle-encapsulated microRNA on an integrated microfluidic platform †

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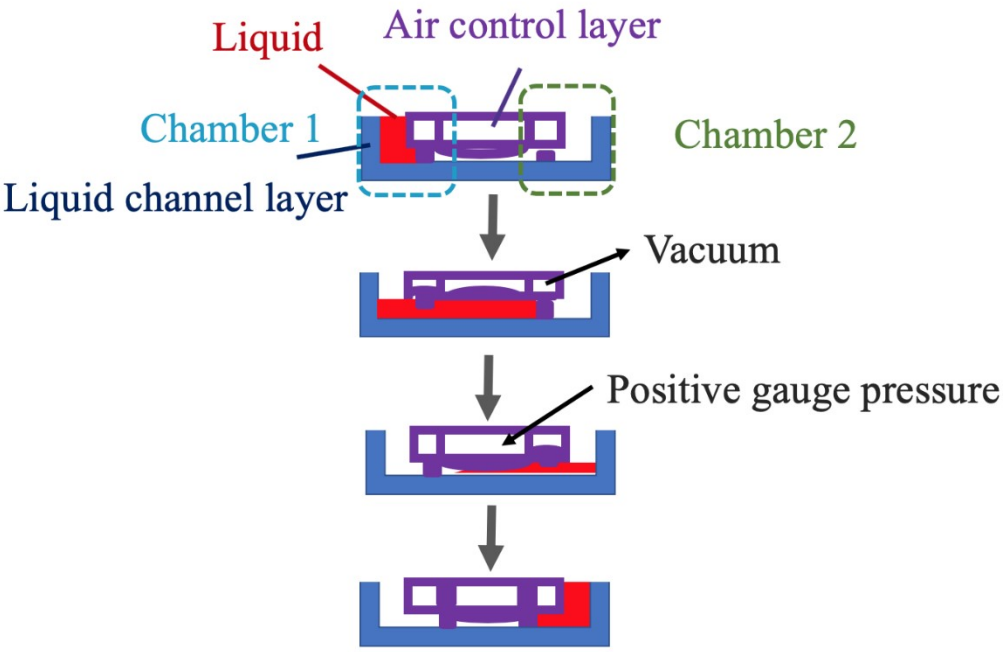

Fig S1. The structure and working mechanism of the micropump. Firstly, the PDMS membrane in the air control layer was deformed downwards under a positive gauge pressure, and the liquid chambers in the circular micropumps were depleted. After the liquid was loaded into the sample inlet with a pipette manually, the liquid in the reagent chamber was isolated by a normally-closed microvalve. Then, the closed microvalve was elevated by vacuum, and the membranes were lifted accordingly, thus causing liquid to be transported into the micropump because of the applied negative gauge pressure (i.e. vacuum). A positive gauge pressure was then injected to close the microvalves near the inlets; thus, a specific volume of liquid would be drawn into the micropump. Afterwards, by lifting the microvalve near the reaction chamber and compressing the membrane of the micropump under a positive gauge pressure, the liquid would be squeezed into the outlets. Thus, a fixed volume of liquid could be precisely transported from different reagent chambers by the well-controlled micropump.

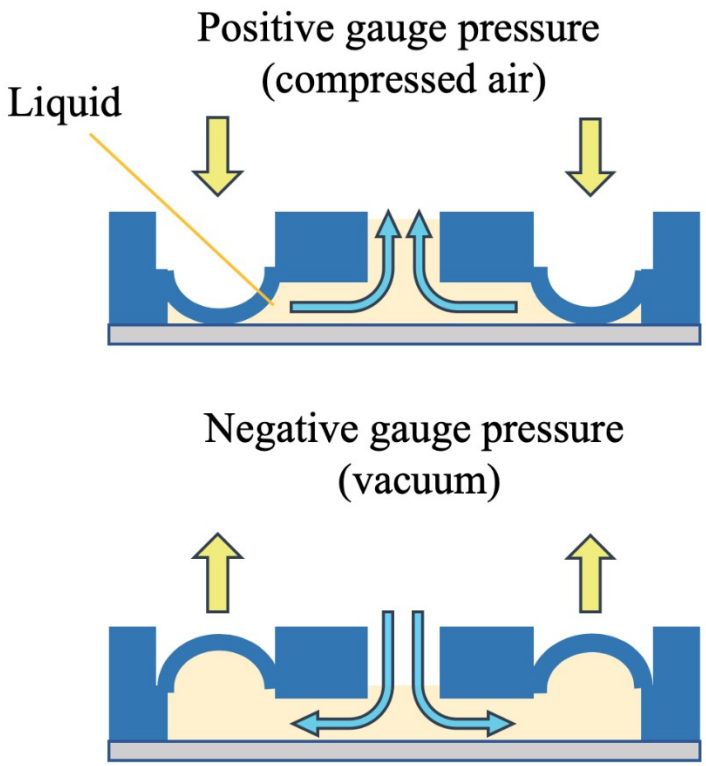

Fig S2. The structure and working mechanism of the micromixer. The vortex-type micromixer comprised an air control layer and a liquid channel layer. While alternating positive (compressed air) and negative (vacuum) gauge pressures were applied with an optimized mixing frequency (2 Hz) on the air control layer, the PDMS membrane would be activated to induce a vortex-like flow to gently mixing the plasma sample with different reagents.

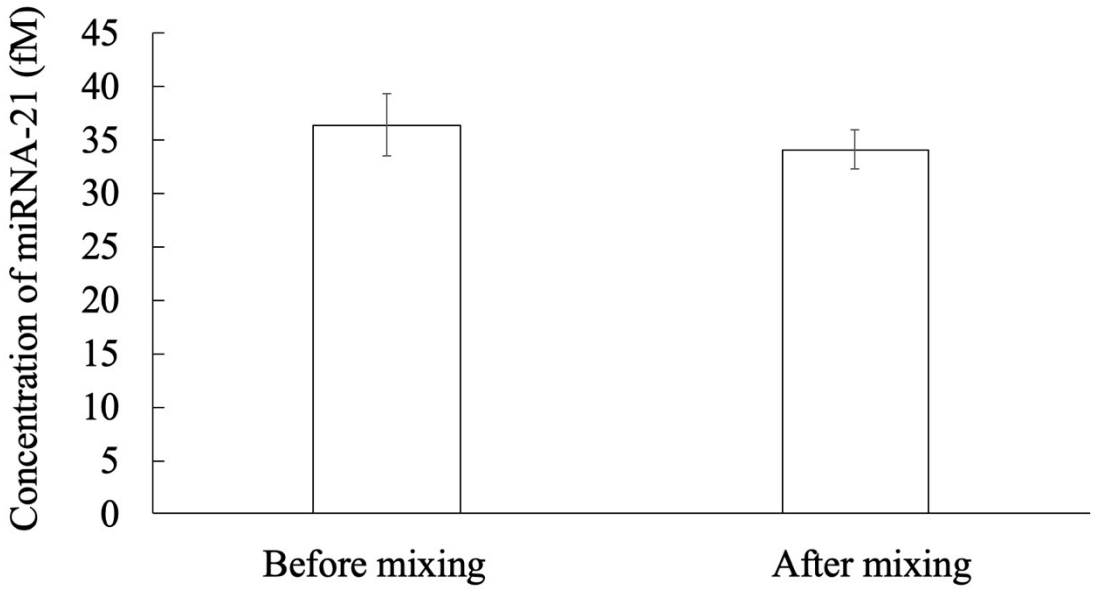

Fig. S3 Concentration of extracted miRNA-21 before and after mixing process at 4^{o}C for 4 hr.

Table S1. Capture rates of extracellular vesicles (EVs) with anti-CD63 coated magnetic beads. Error terms represent standard deviations (n=5).

	EVs in plasma	EVs in waste	Captured EVs
EVs concentration (10^{10} particles/mL)	123.2 ± 1.9	49.8 ± 2.7	73.2 ± 3.5
Capture rate (%)	59.5 ± 2.3		

Table S2. On-chip capture rate of microRNA-21 from pretreated samples incubated with cDNA-coated magnetic beads. Error terms represent standard deviations (n=5).

	Tested-microRNA sample	Supernatant	Captured
Ct value	24.96 ± 0.02	25.99 ± 0.01	25.99 ± 0.01
microRNA-21 concentration (fM)	157.55 ± 0.12	78.86 ± 0.06	78.86 ± 0.06
Capture rate (%)	50.06 ± 0.04		

Table S3. On-chip and on-bench capture rates of miRNA-21 from pretreated sample

		Tested-microRNA sample	Supernatant	Captured
On-chip (20 min)	Ct value	24.96 ± 0.02	25.99 ± 0.01	25.99 ± 0.01
	miRNA-21 concentration (fM)	157.55 ± 0.12	78.86 ± 0.06	78.86 ± 0.06
	Capture rate (%)	50.06 ± 0.04		
On-chip (12 hr)	Ct value	24.96 ± 0.02	26.37 ± 0.11	26.04± 0.05
	Log miRNA-21 concentration (fM)	157.55 ± 0.12	61.10 ± 0.69	76.26 ± 0.31
	Capture rate (%)	48.40 ± 2.0		
On- bench (20 min)	Ct value	24.96 ± 0.02	25.23 ± 0.33	27.99 ± 0.04
	Log miRNA-21 concentration (fM)	157.55 ± 0.12	131.41 ± 2.08	20.57 ± 0.25
	Capture rate (%)	13.06 ± 0.16		
On- bench (12 hr)	Ct value	24.96 ± 0.02	26.34 ± 0.12	26.21± 0.07
	Log miRNA-21 concentration (fM)	157.55 ± 0.12	62.34 ± 0.69	68.03 ± 0.44
	Capture rate (%)	43.18 ± 0.28		

with different mixing time (n=4).

Table S4. The expected and measured concentrations of cDNA in the blind tests, from which inaccuracy rates were computed. Error terms represent standard deviation (n=3).

	Expected concentration (aM)	Measured concentration (aM)	Inaccuracy (%)
Sample 1	12.50	11.93 ± 0.21	4.56 ± 1.68
Sample 2	6.25	5.65 ± 0.10	9.60 ± 1.60
Sample 3	25.00	27.84 ± 0.29	11.36 ±1.16