Lab on a Chip

Supplementary Information

Detecting miRNA Biomarkers from Extracellular Vesicles for Cardiovascular Disease with a Microfluidic System†

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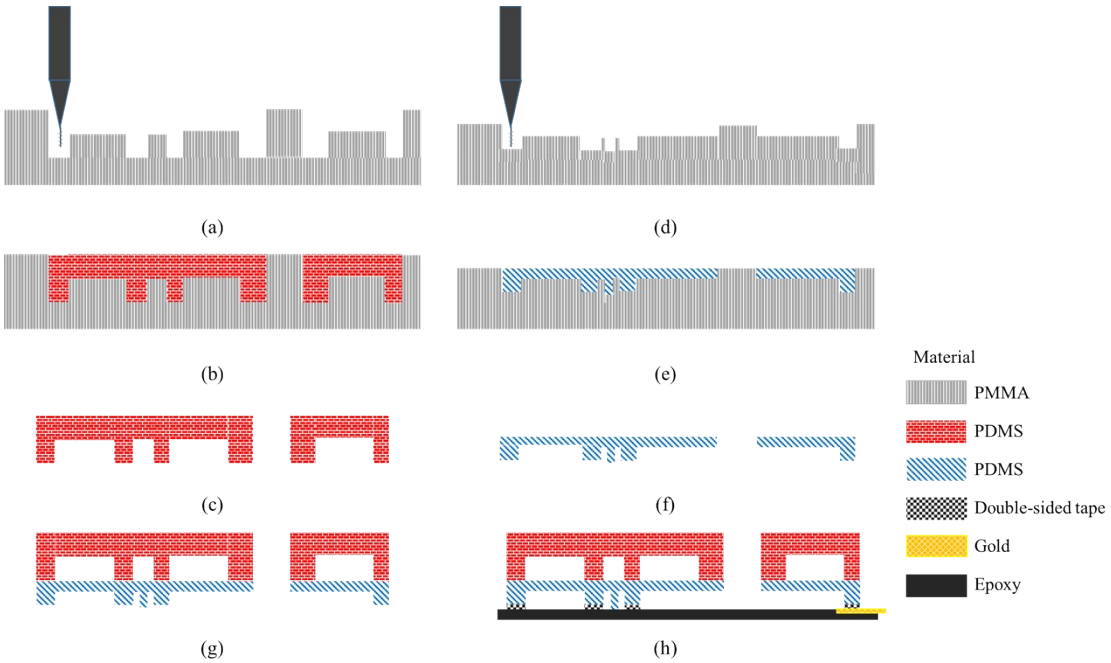
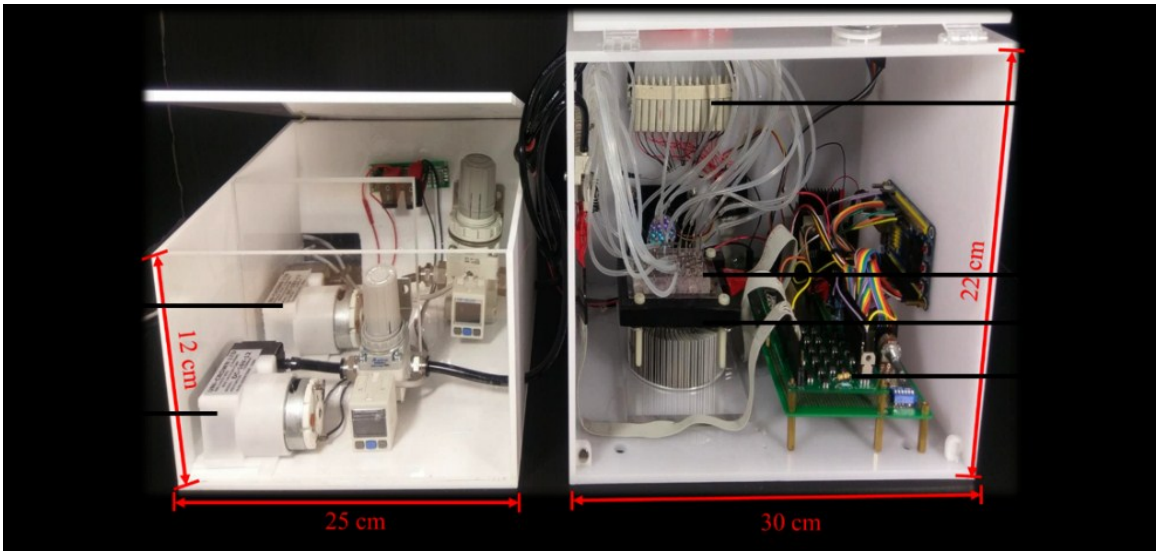


Supplementary Figure S1. A schematic illustration of the fabrication process for the integrated microfluidic chip. (a),(d) Engraving the master mold of the air control layer and the liquid channel layer by a CNC machining process; (b),(e) Casting PDMS in the master mold of the air control layer and the liquid channel layer; (c),(f) Peeling the PDMS layer off the master mold of the air control layer and the liquid channel layer; (g) Bonding two PDMS layers together by the oxygen plasma treatment process; (h) Bonding the PDMS layers with the FET substrate by using a double-sided tape.

The experimental setup was composed of a personal computer (PC), a home-made control circuit, electromagnetic valves (EMVs), an air compressor, a thermal control module, a field-effect transistor (FET) sensing module and an integrated microfluidic chip. The control circuit was used to regulate EMVs such that the PDMS thin films on the microfluidic devices (including micropumps, microvalves and micromixers) could be pneumatically controlled under the pressure of compressed air, which was supplied by the air compressor. The thermal control module was used to regulate the required temperature during the entire process. The integrated microfluidic chip was used to automate the entire process including EV extraction, EV lysis, target miRNA extraction and miRNA detection by FET.



Supplementary Figure S2. Photographs of the home-made portable control device, which was composed of (a) a positive pressure source (compressor), (b) a negative gauge pressure source (vacuum pump), (c) electromagnetic valves (EMVs), (d) a microfluidic chip, (e) a TE cooler (thermal control module), and (f) a control circuit.

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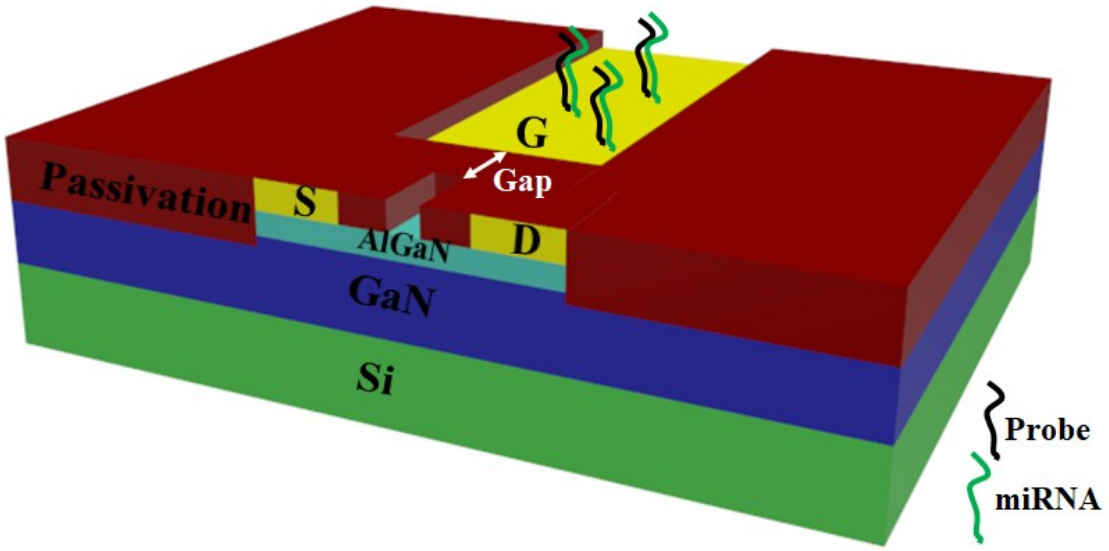
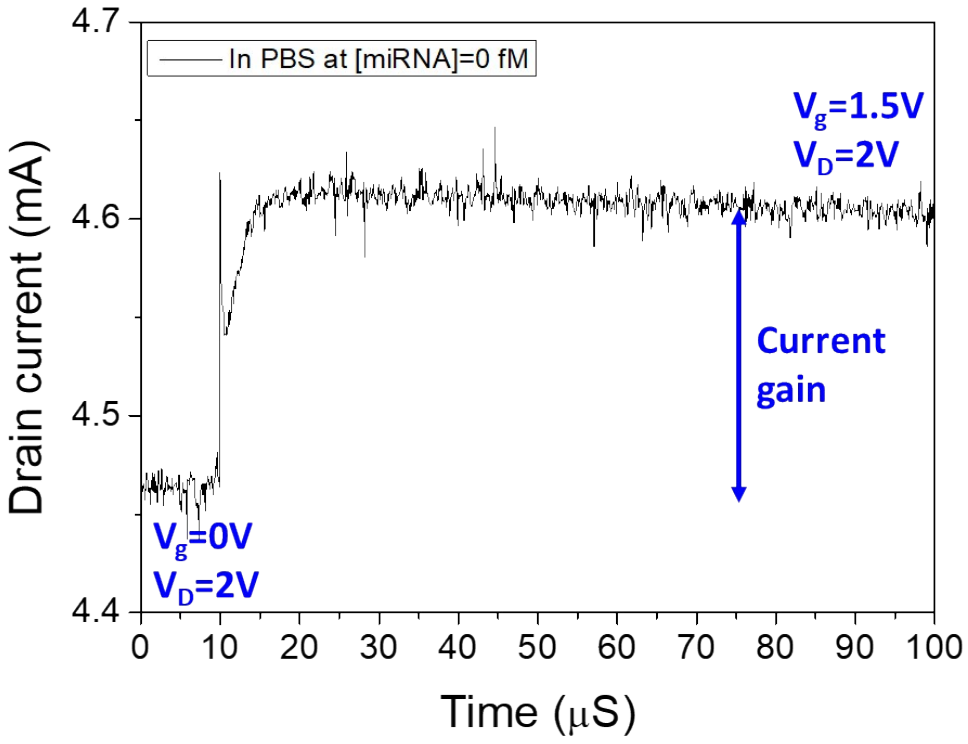


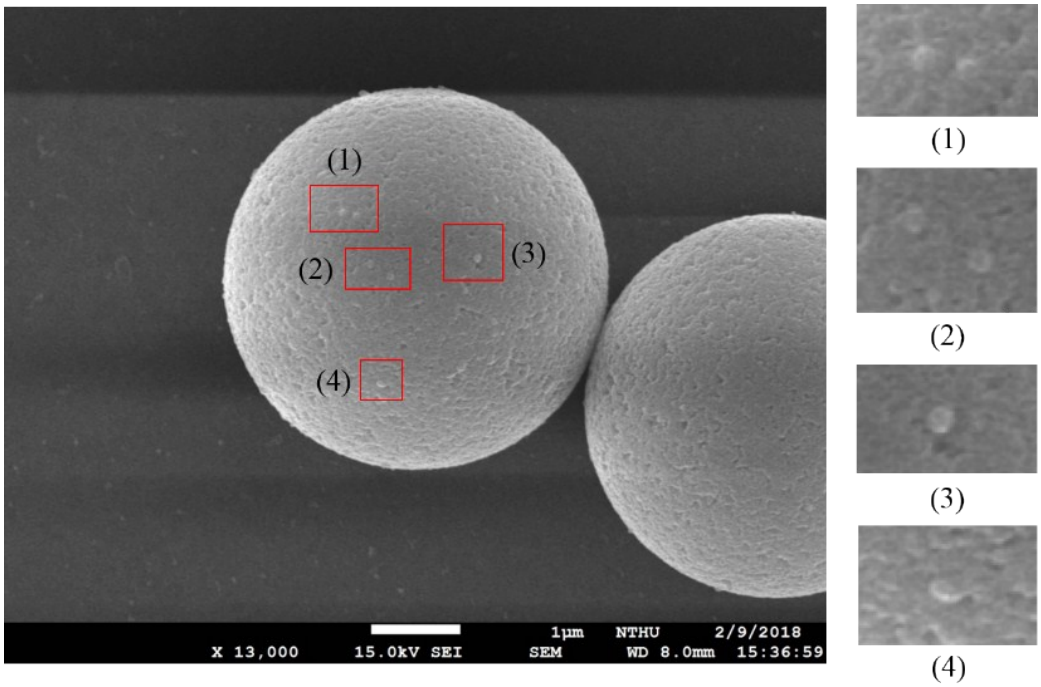
Supplementary Figure S3. Schematic illustration of AlGaN/GaN HEMT sensor capturing target miRNA using specific probes immobilized on the gate electrode.



Supplementary figure S4. Typical drain current response of AlGaN/GaN HEMT sensor.

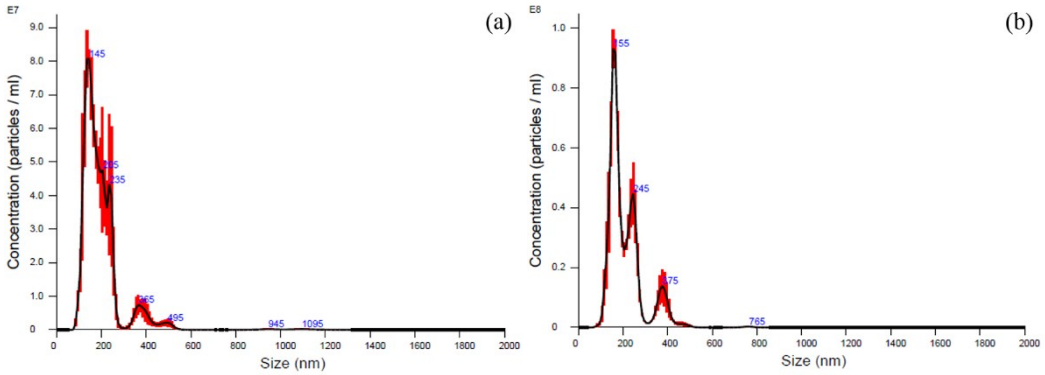
Supplementary Table 1. Experimental procedures for detecting miRNA from EVs using the integrated microfluidic chip

Step	Preparation	Procedure	Temp. (°C)	Volume (μL)	Time (min)
EVs extraction	EVs CD63-magnetic beads 1×PBS Isolation buffer (0.1% BSA in 1×PBS)	Collect beads by magnet	Room temperature	100	3
		Mix	4	100	40-240
		Collect beads by magnet and remove supernatant	Room temperature	-	3
		Wash	Room temperature	100	1/6
EVs lysis	Lysis buffer	Mix and lysis	4	100	10
miRNA isolation	miRNA probes-coated magnetic beads Wash buffer 1 Wash buffer 2 Elution buffer	Collect beads by magnet	Room temperature	100	3
		Mix	30	100	10-80
		Collect beads by magnet and remove supernatant	Room temperature	-	3
		Wash	Room temperature	100	1/6
		Wash	Room temperature	100 3 times	1/2
		Elute and transfer	70	100	3
FET detection	Probe-modified FET biosensor	miRNAs bind to probe	43	100	5
		Detect	Room temperature	-	-
Total assay time		About 290 min *(Using the optimized conditions described in this study)*			

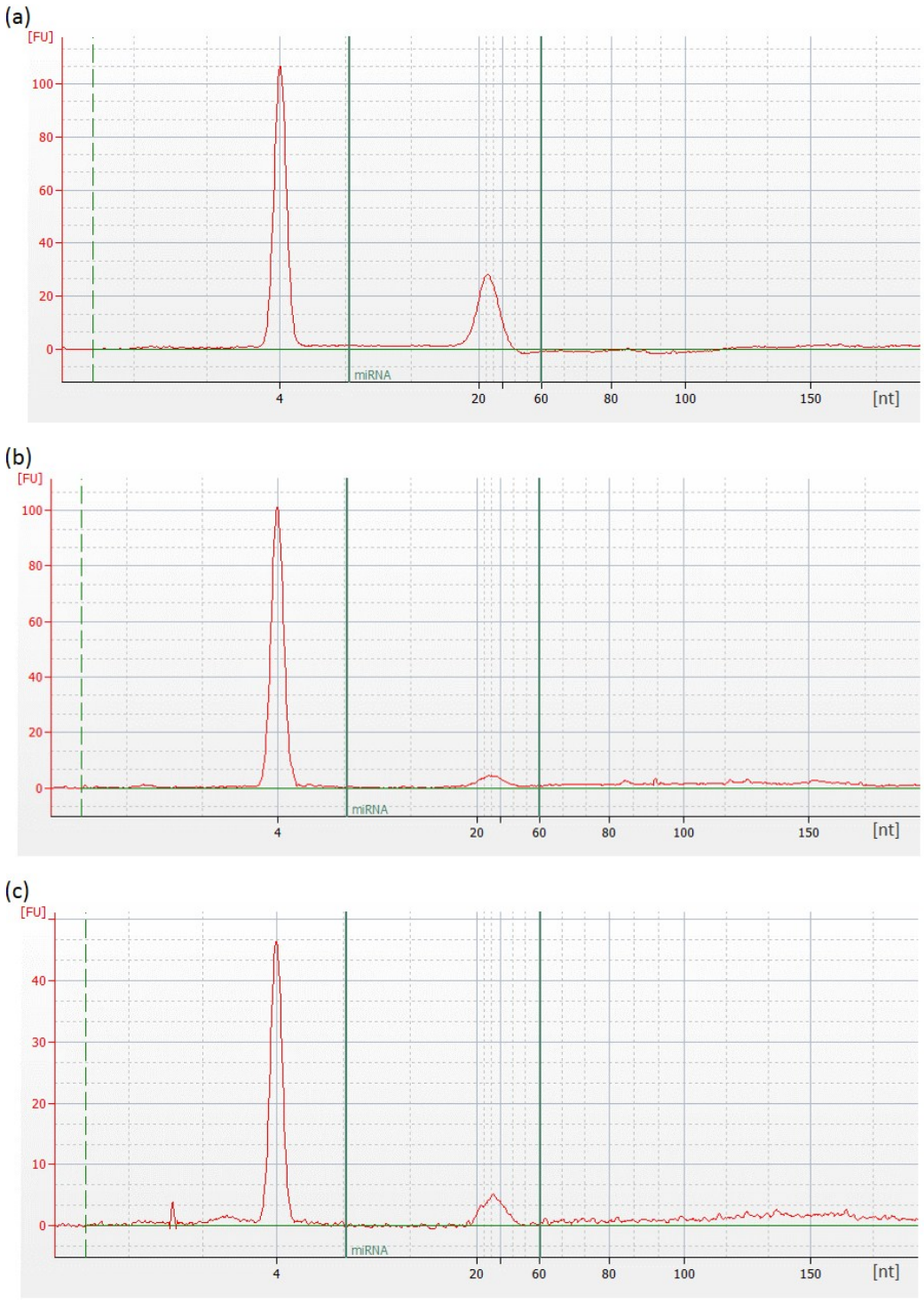


Supplementary Figure S5. The zoomed-in SEM images of captured EVs on the surface of magnetic beads showed that the morphology of captured EVs were still intact and sphere-like.

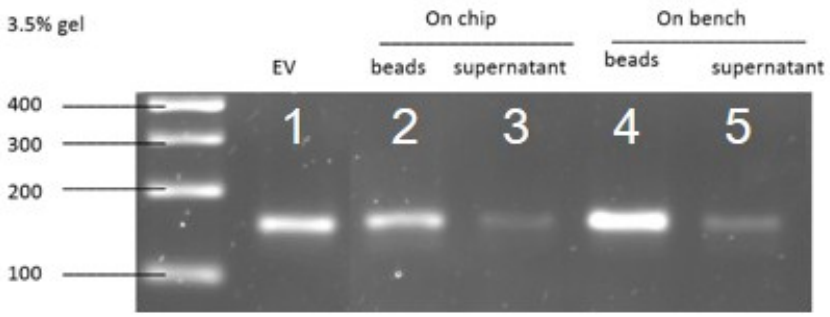
Total volume of media was dependent on how much EVs we needed to be used for experiments, which was about 15 mL of cell culture medium. After purifying and concentrating EVs from cell culture medium by ultracentrifugation and resuspended in PBS, the purified EVs solution was 1000 times diluted in PBS which was filtered through 0.22-μm filters twice and then examined by nanoparticle tracking analysis (NTA, NanoSight LM10-HS, Malvern Instruments, England). Measured particles concentration was $8.89 \times 10^8 \pm 4.80 \times 10^7$ particles/ mL which was counted from three repeated experiments, as shown in Supplementary Figure 6(a). The real particles concentration was 8.89×10^{11} particles/ mL. After EVs extraction by using magnetic beads for 4 hours, the supernatant was 500 times diluted in PBS and measured particles concentration was $8.12 \times 10^8 \pm 4.04 \times 10^7$ particles/ mL which was counted from three repeated measurements, as shown in Supplementary Figure 6(b). The real particles concentration was 4.06×10^{11} particles/ mL.



Supplementary Figure S6. (a) Original particles concentration of purified EVs sample was measured to be 8.89×10^{11} particles/ mL by NTA, and (b) the supernatant after EVs extraction was 4.06×10^{11} particles/ mL.



Supplementary Figure S7. Quality control of extracted RNAs. Bioanalyzer analyses of RNAs isolated from (a) the ultracentrifuged pellets from conditioned MDA-MB-231 cell culture medium, (b) EVs captured on magnetic beads, and (c) the supernatant. Most RNAs were within the miRNA range.



Supplementary Figure S8. RT-PCR performed on RNA extracted from EVs isolated on chip and on bench. Agarose gel shows RT-PCR products performed on RNAs extracted from lane 1: the ultracentrifugation pellet from conditioned MDA-MB-231 cell culture medium, lane 2: EVs captured on-chip, lane 3: the on-chip supernatant, lane 4: EVs captured on-bench after mixing with magnetic beads for 18 hours in the microcentrifuge tube, and lane 5: the on-bench supernatant.