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Supplementary Information

A Label-free Electrical Detection of Exosomal microRNAs using Microelectrode Array

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1. Establishment of stable cell lines

Dulbecco's Phosphate Bufferd Saline (1x DPBS, pH 7.4, 150 mM), Dulbecco's Modified Eagle Medium (DMEM; high glucose), and Advanced DMEM were purchased from Invitrogen. A 1x TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) was purcharsed from Promega. The primary-miR-143 expression vector and primary-miR-146a expression vector was purchased from TaKaRa BIO. Stable HEK293 cell lines which expressed miR-143 or miR-146a were generated by selection with 300 mg/ml Geneticin (Invitrogen). HEK293 cells were transfected with 0.5 ug of the pri-miR-143 expression vector at 90 % confluency in 24-well dishes using a Lipofectamine LTX (Invitrogen) in accordance with the manufacturer's instructions. Twelve hours after the transfection, the cells were re-plated in a 10 cm dish, followed by a 3-week selection with the antibiotic. Ten surviving single colonies were picked up from each transfectant and then cultured for another 2 weeks. The cells expressing the largest amount of miR-143 or miR-146a among transfectants were used as miR-143 or miR-146a stably expressing cells.

2. Characterization of Exosomes

The size and number of exosomes were counted by nanoparticle tracking analysis (NTA) with LM10-HS system (NanoSight, Amesbury UK) on exosomes resuspended in DPBS at 25 °C.

The size of exosomes dispersed in DPBS at 25 °C was measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Marvern Instruments, Malvern, UK). The DLS can determine a relative size

distribution of particle ranging in size between 1 nm and 6 μm.[S1] Figure S1 shows the calculated relative size distribution of exosomes from HEK293 cells for DLS. We observed three components of the size distribution. A strong peak at 70-80 nm was assigned as exosomes released by the cells. The remaining two peaks are attributed to proteins (at 10 nm) and microvesicles (at hundreds nanometers). Note that because larger microvesicles scatter light more significantly than smaller ones, the peak intensity of larger microvesicles is emphasized as compared to the other, revealing apparently increased fraction of the larger ones. By considering this point, the main components of the purified medium were assigned as proteins and exosomes secreted by cells in serum-free medium.

Table S1. The number of exosomes released from the three types of cells.

Cells	Exosomes per cell per 24 h
HEK 293, miR-143-expressed	444
HEK 293, miR-146a-expressed	573
HEK 293, normal	518

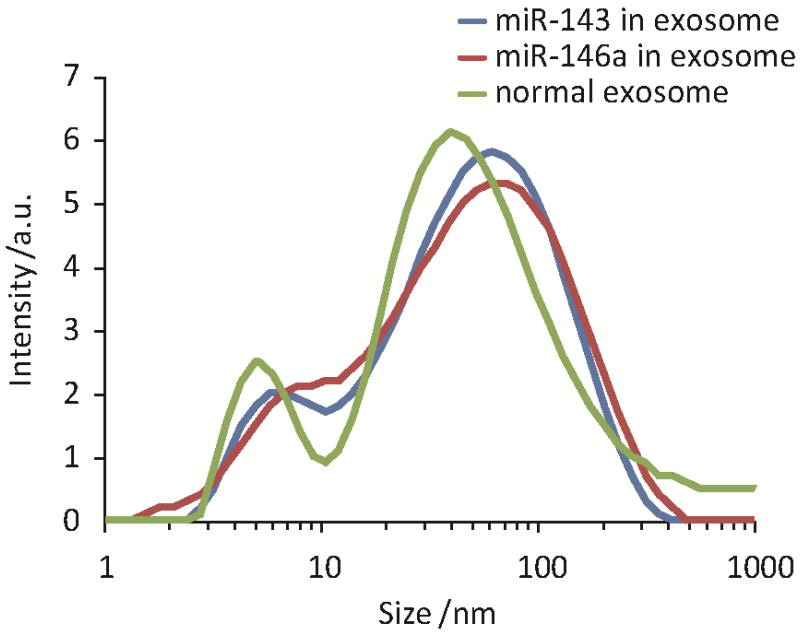

Figure S1. A DLS study for size distributions of microvesicles in exosome fraction collected by ultracentrifuges from serum-free medium after incubation for 24 h.

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3. Characterization of SAM on the Gold Electrode

For determining probe density of the ssDNA in the mixed SAM, chronocoulometry (CC) was performed at a pulse period of 1000 ms and the potential stepped from 125 mV to −300 mV (v.s. Ag/AgCl) in 15 mM DPBS with/without 50 μM hexaammineruthenium (III) chloride (RuHex) using an Autolab PGSTAT 302 (Eco Chemie, Utrecht, The Netherlands). Packing density of the SAM on the gold electrode was determined by cyclic voltammetry using the Autolab PGSTAT 302. A conventional three-electrode cell, consisting of a platinum wire (0.25 mm in diameter, 99.98%) as a counter electrode and the Ag/AgCl reference electrode, was used with a salt bridge. Desorption of an alkanethiol SAM was performed by scanning the potential from −0.2 to −1.3 V at a scan rate of 20 mV/s in a solution of 0.5 M KOH and 3.3 M KCl. All voltammograms were taken after the sample solutions were purged with nitrogen gas for 30 min.

Figure S2a indicates the relationship between the molar ratio in the immobilization solution and the surface density of the ssDNA probe calculated by chronocoulometric measurement.[S2] It was found as molar ratios of the DNA probe to SB or MCH decreases from 1:1 to 1:10, the surface density decreases from 0.04 to 0.02 chains nm^{-2} independent of the type of SAM. The distance between the adjacent DNA probes was calculated to be 5.0-7.1 nm, suggesting that the utilized probe provided moderate interstitial space for DNA hybridization to form a double helix (2.3 nm in diameter). The total surface density of the mixed SAM calculated from cyclic voltammetry[S3] was about 2-5 chains nm^{-2} (Figure S2b), which is roughly consistent with the saturation coverage of the SAM on Au(111).[S4] The density of the SB and MCH SAM increased monotonically with increased concentration of thiols in the immobilization solution from 20 (at 1:1) to 110 (at 1:10) μM.

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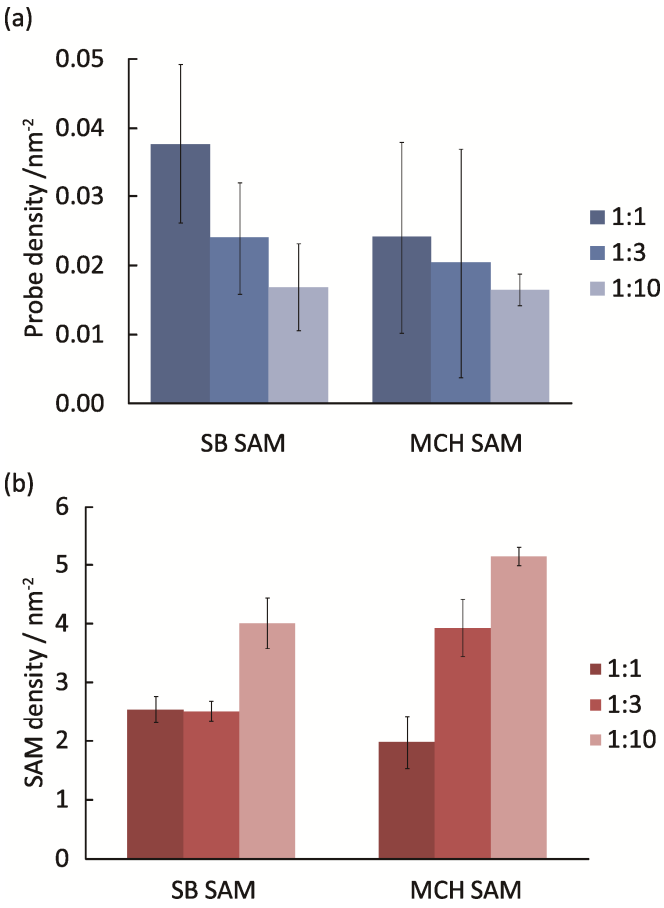

Figure S2. Characterization of the mixed SAM on the gold microarray by electrochemical techniques. (a) Surface density of DNA probe in the mixed SAM at the different molar ratio between the thiol-derivatized probe DNA and backfilling alkanethiols (SB or MCH). (b) Total packing density for each SAM.

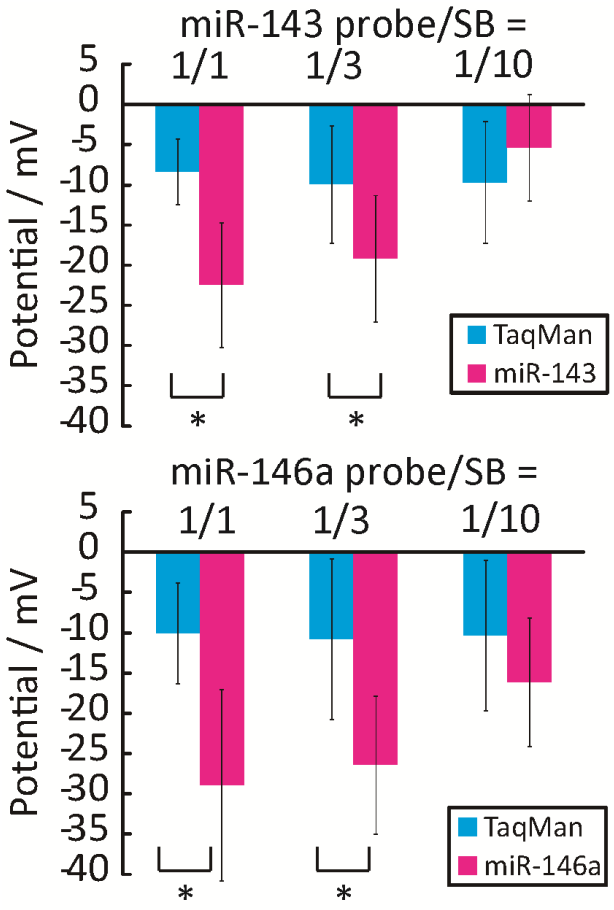

Figure S3. The effect of Probe density on the signal intensity in potentiometry.

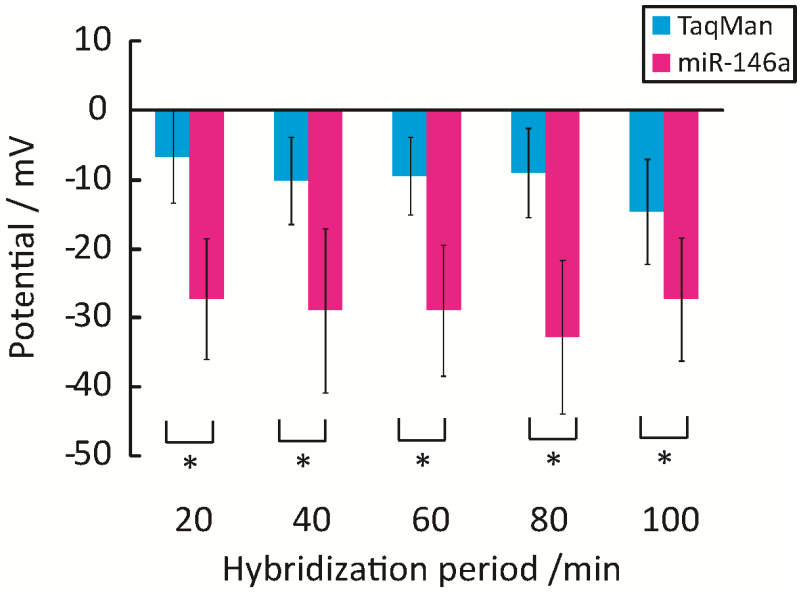

Figure S4. The effect of potentiometric signals on the incubation period. * $p<0.001$.

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Additional References

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