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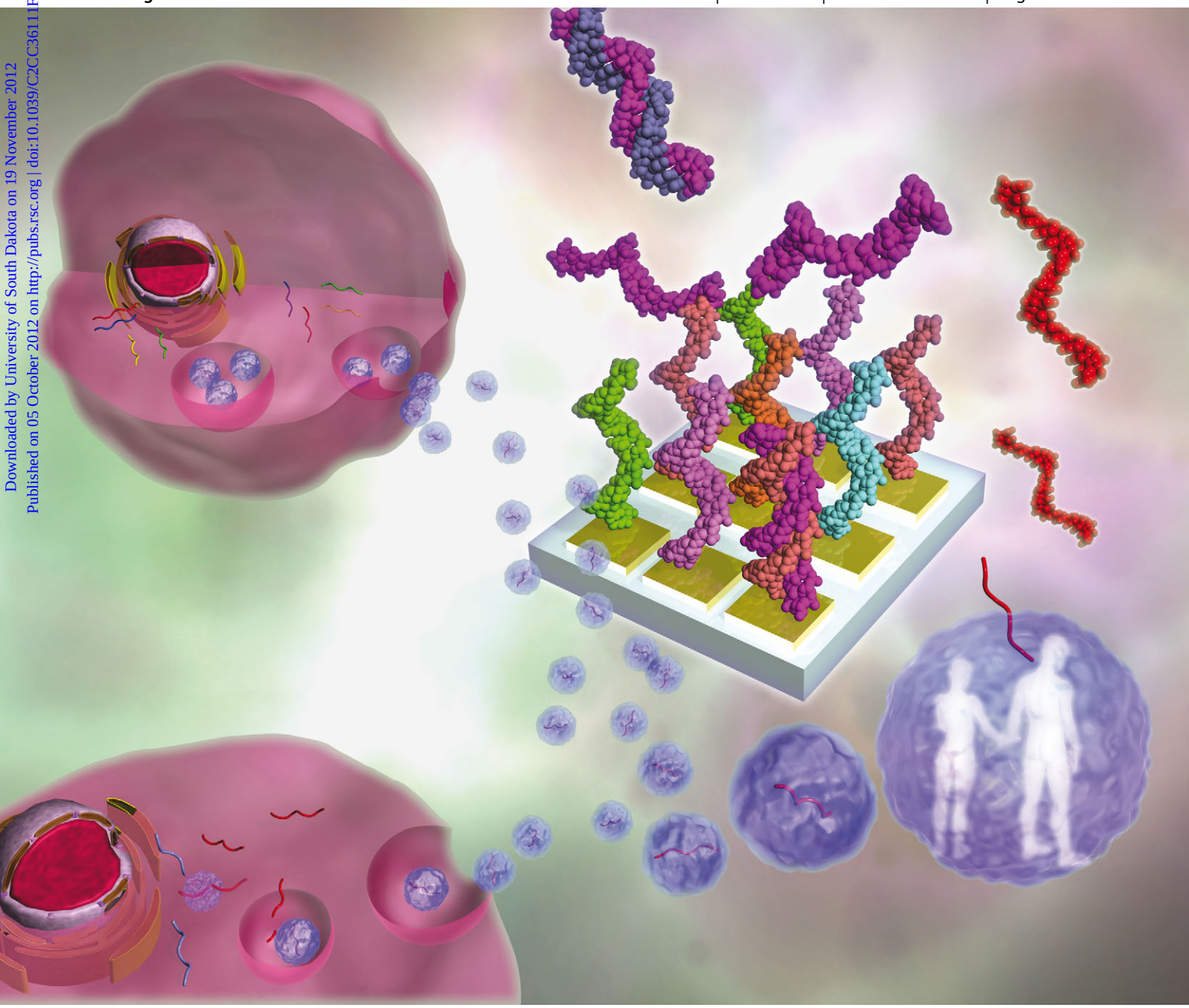

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COMMUNICATION

A label-free electrical detection of exosomal microRNAs using microelectrode array†

Tatsuro Goda,[a] Kozue Masuno,[a] Junko Nishida,[a] Nobuyoshi Kosaka,[b] Takahiro Ochiya,[b] Akira Matsumoto[a] and Yuji Miyahara*[a]

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We report a method for detecting microRNAs encapsulated in exosomes using a microelectrode array in semiconductor-based potentiometry after RT-PCR. The inherent miniaturization of the electrical biosensor meets requirements for massively parallel analysis of circulating microRNA as a noninvasive biomarker.

MicroRNAs (miRNAs) are a recently discovered class of naturally occurring small noncoding single-strand RNAs that conduct post-transcriptional gene regulation.[1] Many miRNAs have been found in extracellular space, protected from degradation by forming a miRNA–protein complex or by being encapsulated into microvesicles and exosomes.[2,3] Exosomes are vesicles (40–100 nm) of endocytic origin released into the extracellular space upon fusion of multivesicular bodies with the plasma membrane.[4,5] Extracellular transfer of stable miRNAs enveloped in exosomes proposes a mechanism of intercellular genetic exchange, raising the possibility of circulating miRNAs in body fluids as a novel non-invasive biomarker.[6–8] Expression profiles of miRNAs are generally determined with fluorophotometry or Northern blot after reverse transcription (RT)-PCR.[9] Alternatively, label-contained biosensors have been proposed as PCR-free detectors in recent years.[10–13] Multiplexed detection and particles-based high-throughput screening are relevant to identify expression patterns in tumor tissues and cancer cells.[14–16] Semiconductor-based label-free sensing is proposed for detecting innate charges of target nucleotide following hybridization on a sensor surface.[17,18] This type of biosensor is amenable to miniaturization through integrated circuit technology, which potentially allows systematic analysis of miRNA expressions in an array format without the requirement for optical assistance.

Here we present a model study on PCR-contained electrical readout of exosomal miRNAs from genetically modified HEK293 cells to abundantly secrete and export exosomal miR-143 or miR-146a to the medium (Fig. 1).[9] Down-regulation of miR-143 is related to colorectal cancer[19] and osteosarcoma metastasis,[20] whereas miR-146 level is involved in immune response[21] and breast cancer metastasis.[22] The hybridization events were directly transformed into potentiometric signals and were monitored using an electrometer in real-time. Exosomes were collected from the serum-free supernatant of the cultured cells by centrifugation (10 000 × *g*), filtration and ultracentrifugation (100 000 × *g*). Exosomes were characterized by nanoparticle tracking analysis (NTA; LM10-HS system, NanoSight) and dynamic light scattering (DLS) (Table S1 and Fig. S1, ESI†). The miR-143-expressed, miR-146a-expressed, and intact cells were found to release nearly the same amount of exosomes (444–573 particles per cell per 24 h) independently on their genetic modifications, being roughly consistent with the amount released by normal fibroblasts in culture.[23] We confirmed the presence of CD63 as a representative exosomal marker by Western blotting. The Western analysis was consistent with previous observation that CD63 is heavily glycosylated and displays a broad range between 30 and 60 kDa in non-reducing conditions.[24] A simultaneous process of exosomal digestion and RT can minimize the latent risk for enzymatic degradation under extracellular environments. The PCR amplicons that retain the original miRNA sequences were captured by hybridization with probe DNAs on the sensor in the microarray format. Formation of a self-assembled monolayer (SAM) provides a simple route to functionalize the electrode in the form of a nanometer-scaled robust and reproducible film. To capture the amplified cDNA, a 5′-SH-$(CH_2)_6$-DNA was immobilized together with sulfobetaine-3-undecanethiol (SB). The backfilling SB reduces nonspecific adsorption by forming a thick hydration shell onto the electrode.[25,26] The mixed SAM gave fine control of the probe density by simply changing the molar ratios of the two components (Fig. S2, ESI†). In potentiometry, the hybridization event directly changes the surface potential by inducing Coulomb charges. We used 1.5 mM Dulbecco's PBS (DPBS, pH 7.4) to gain effective electrical readout of DNA charges by minimizing the screening effect in the salt solution. The calculated solution Debye length was 8.1 nm,[27] corresponding to the length of a 24 *mer* DNA duplex.

Fig. 2a–d show the changes in the interface potential after treatment with the TaqMan solution containing dNTP and polymerase (*i.e.*, negative control) and the target solution

[a] *Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan. E-mail: miyahara@tmd.ac.jp; Fax: +81 3 5280 8095; Tel: +81 3 5280 8097*
[b] *Division of Molecular and Cellular Medicine, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan*

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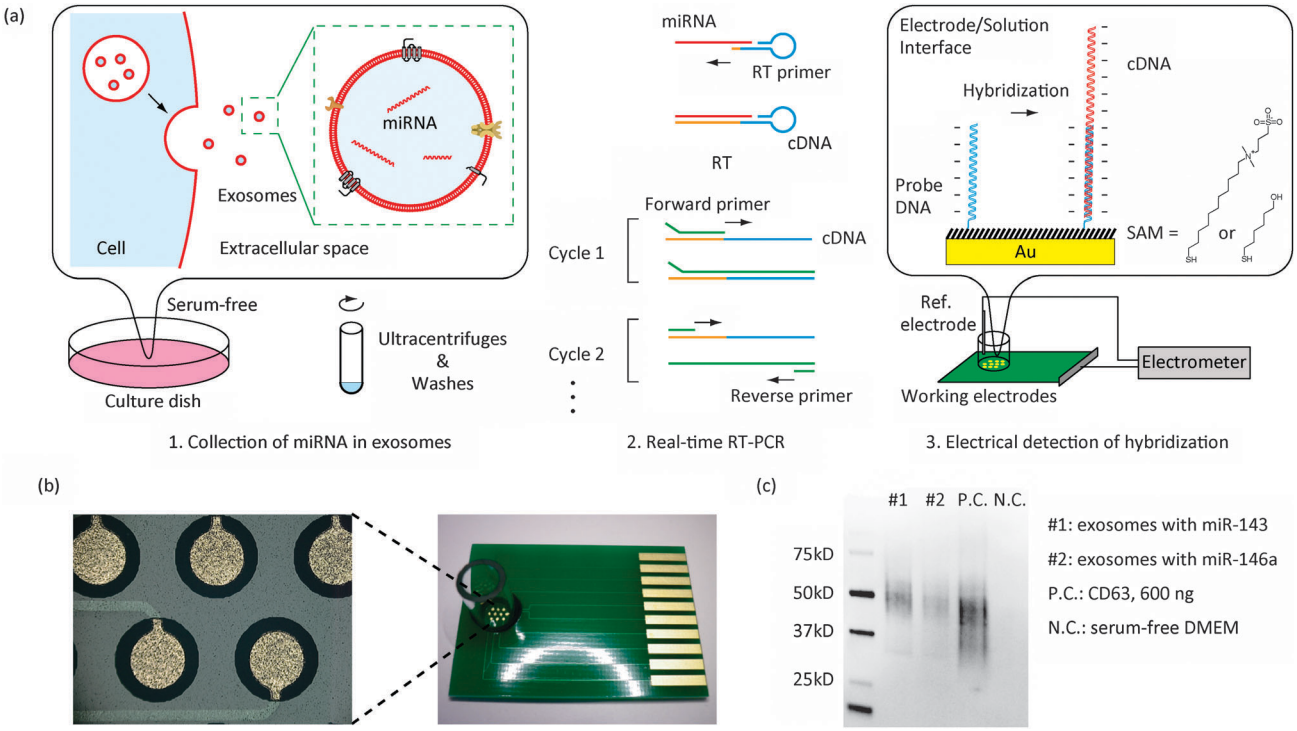

Fig. 1 (a) Schematic illustration of potentiometric biosensors using microelectrodes array for detecting exosomal miRNA from model HEK293 cells after RT-PCR. (1) Exosomes were collected from serum-free medium by ultracentrifugation, (2) miRNA was transformed into cDNA by the stem-loop RT primer, followed by PCR, and (3) an electrometer was used for direct electrical detection of the charges in target cDNAs following hybridization. (b) Photographs of the 10-microelectrode array (scale bar: 500 μm) with a glass chamber on a print-circuit board used in the study. (c) Western blots on CD63 for identifying exosomes collected from the serum-free supernatant.

containing 2 nM heat-denatured PCR amplicon from miR-143 or miR-146a in exosomes. Although the potential on each channel decreased after incubation in the control solution because of the nonspecific adsorption of dNTP and polymerase, the potential was further changed following incubation with the PCR amplicon containing the target sequence. Statistically significant differences were observed solely for target sequences, while the mismatch pairs failed to generate the signal. The signals for the full-match pairs were attributed to hybridization between the negatively charged target cDNA and the probe DNA immobilized on the microelectrode. Fig. 2e indicates that potentiometry was able to distinguish target at a concentration of >20 pM with dynamic ranges of two orders of magnitude. The sensitivity for target cDNA was -6.5 mV per decade at the range of 2–200 pM ($R^2 = 0.99$). The signal intensity was closely related to the probe density (Fig. S3, ESI†). Since the hybridization efficiency is almost 100% under the probe density investigated (*i.e.*, 0.02–0.04 chains nm^{-2}), the observed potential shift was quantitative to the amount of hybridized cDNA.[28] Hybridization kinetics reached a plateau after an initial 20 min (Fig. S4, ESI†). The quick equilibration of the electric signal meets the requirements for rapid monitoring of miRNA. Further, the effect of SAM type on the signal and noise was investigated. Fig. 2f displays the hybridization signals for the SB and 6-mercapto-1-hexanol (MCH) SAMs. The MCH SAM was unable to distinguish the nonspecific signals from the specific ones. We attribute the failure on the MCH SAM to increased susceptibility to nonspecific adsorption compared with SB SAM.[25,26]

This is the first report describing such a non-optical label-free electrical sensing of miRNA. Collection of exosomal miRNA from cell-culturing medium was carried out for demonstrating our ability to directly assess miRNAs from real samples. We believe this technique will offer reliable and reproducible miRNA biosensing. There are increasing needs for developing personal care systems to analyze deregulation of circulating miRNAs. A majority of currently available miRNA detection strategies fails to meet the requirements for rapid monitoring and point-of-care uses. The presented system, yet RT-PCR contained, is simple in view of both analytical setup and sample handling, compared to conventional ones. Compared with other methods capitalizing on molecular or fluorescent labels, label-free schemes suffer from low signal-to-noise ratio due to the relatively high noise level caused by detection of molecular species that are nonspecifically adsorbed onto the sensing surfaces. As demonstrated here, an inherent drawback to the label-free sensing can be overcome through a designer interface that exhibits excellent antifouling properties against biomolecules.[25,26] An anti-fouling surface such as an SB SAM is a key issue to obtain high S/N ratio for real world applications.

Direct electrical readouts of reliable PCR amplicons copied by exosomal miRNA are achieved using hybridization-based potentiometry with microelectrode array. Hybridization on the anti-fouling SAM produces specific signals. The technique herein described, which is rapid, specific, free from optical assistance, reliable without labels or reporter molecules, and compatible with IC technology, may open a path to electrical

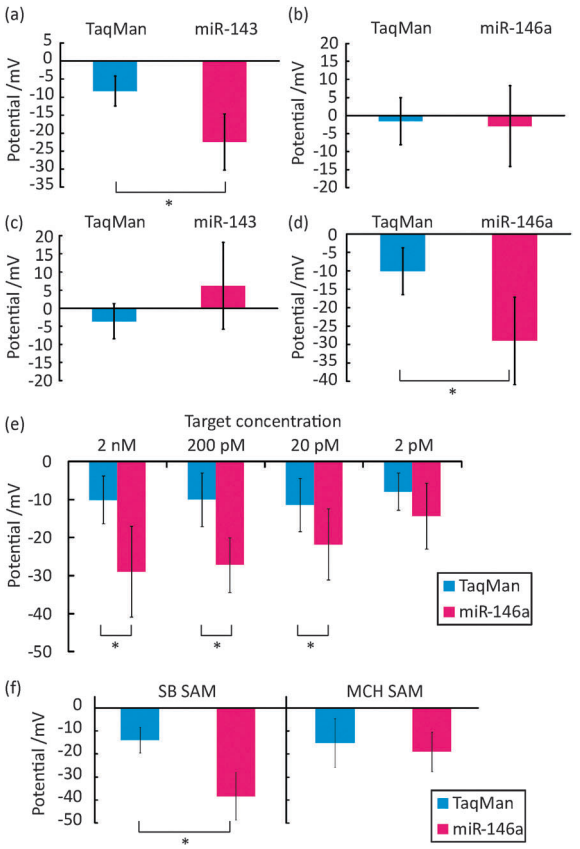

Fig. 2 (a–d) Potentiometric variations after incubation with TaqMan solution (*i.e.*, background signal) and the PCR-treated 2 nM cDNA solution (*i.e.*, specific signal) on the microarray electrode; the combination of probe/target is miR-143/miR-143 (a), miR-143/miR-146a (b), miR-146a/miR-143 (c), and miR-146a/miR-146a (d). (e) The effect of target concentrations on the potentiometric signals. (f) The effect of potentiometric signals on the type of SAM at 2 nM miR-146a; * $p < 0.001$.

diagnostics of miRNA for early cancer detection in a non-invasive manner.

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