SPIRAL CHANNEL FOR FAST AND NOISE-FREE MICRORNA DETECTION

Satoshi Fujii[1], Toshihisa Osaki[1], Nobuo Misawa[1], Koki Kamiya[1], and Shoji Takeuchi[1,2]
[1]Kanagawa Academy of Science and Technology, Kanagawa, JAPAN
[2]Institute of Industrial Science, The University of Tokyo, Tokyo, JAPAN

ABSTRACT

MicroRNA, a short-length RNA known as a biomarker of cancers, was detected by magnet-attached spiral channel and electrical measurement. A designed oligo-DNA bound with magnetic bead was entrapped in spiral channel by magnetic field, and hybridized with microRNA. Thereafter, liposome were exported from the spiral channel by duplex-specific nuclease, and applied to electrical measurement. Finally, ionic current signals were observed specifically when the target microRNA was applied. Whereas, when a mixture of over 10^6 different mock microRNAs were applied, no signals were observed. This indicates a noise-free, high selectivity was achieved in the presented device.

INTRODUCTION

MicroRNA is a short RNA with 22-around nucleotides in length. MicroRNA does not encode a protein, but regulates several metabolism by interacting with other messenger RNAs, therefore, the repression of translation. So far, specific sequence of microRNA had been shown to be up-regulated, or down-regulated dependently on development of cancers in body [1]. Moreover, the target sequence of microRNA differs among multiple cancers, thus though as a potential therapeutic targets for cancer diagnosis. Therefore, many challenges for the accurate detection and quantification of microRNAs have been made, by next-generation sequencer, microarrays, and reverse-transcription quantification PCR methods [2]. For these measurement, a specific technique to purify the short-length RNA is generally required. Additionally, because of lack of the poly(A) tail in microRNA, which is widely used as a universal tag sequence for RNA amplification, binding the tag sequence to the sample microRNA, for the following amplification steps are often used for microRNA detection. Therefore, easier and simpler detection method are required.

Recently, nanopore sensing technologies, which measures the ionic current through the nanopore, has been developed for a de-novo sequencing of DNA/RNAs [3]. Nanopore sensing does not require the binding of tag sequence, thus has been used for the detection of microRNAs [5]. We have also succeeded to detect microRNA in a sequence-specific manner by utilizing the nanopore sensing techniques [6]. However, as this system monitors the transition of all nucleotides through the nanopore, many noise-signals derived from non-specific RNAs were detected, and lowered the signal to noise ratio. Therefore, the system was only applicable to the purified microRNA samples. In this paper, we report the development of the system which does not require the purification of microRNAs as well as binding the tag sequences, thus enabling the easier and simpler diagnosis by using the blood samples in future.

DESIGN

We utilized the magnet field and duplex-specific nuclease for the detection of microRNA. This system was previously developed to detect the microRNA in in a noise-free, and tag-free manner [6]. In this system, oligo-DNA was bound to a magnetic bead, and was entrapped by a magnetic field. A liposome, with nanopore integrated on its membrane, was bound to another termini of oligo-DNA. Detection schematic is as follows: 1. "microRNA hybridize with the oligo-DNA in a sequence-specific manner", 2. "duplex-specific nuclease digest the oligo-DNA and release the liposome from the magnetic entrapment", 3. "liposome fuse to the lipid bilayer formed by droplet contact method [7]", and 4. "generate a nanopore on the lipid bilayer and emits an electrical signal" (Fig. 1a). Although the presented system could detect microRNA, due to the low enzymatic reactivity in bulk, it took 30 minutes at 37°C for the detection. We therefore introduced a spiral-channel, and included the magnetic beads-based reaction within. The spiral channel enhance the interaction of oligo-DNA with the microRNA, and nuclease with duplex strand.

EXPERIMENTAL

We constructed the double-spiral channel on PMMA (Fig. 1b, c). Two double-spiral channels were bonded by thermos compression bonding method to double the reaction volume, and inlet/outlet valves were placed on the edge of the channel. Magnetic sheet were placed on the top of the channel to entrap magnetic beads on the inner wall of the spiral channel. Liposomes composed of cholesterol-oligo-DNA were prepared by gentle-hydration method. DNA was designed to hybridize with the target microRNAs. Then, nanopore protein, alpha-hemolysin was integrated on the liposome membrane by using cell-free translation systems. These liposomes were bounded to magnetic beads, by avidin-biotin system.

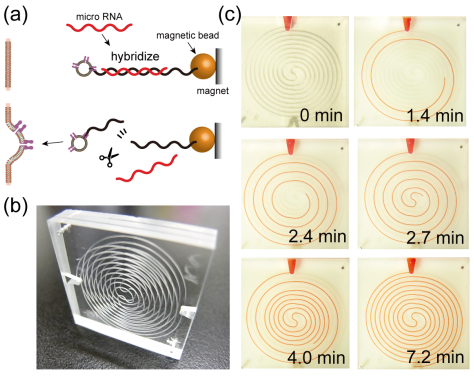

Figure 1: (a) Schematic of microRNA detection. (b) Double-spiral device constructed on PMMA. (c) Time-lapse images of solution flowing the spiral channel.

Then, we constructed the electrical measurement device (Fig. 2a, b). Based on the droplet contact method, we formed a droplet interface bilayer (DIB), and measured the ionic current through the bilayer by contacting the electrodes on droplet, and connecting to the patch-clamp amplifier. We preliminary confirmed that generation of the nanopore on the DIB could be detected and monitored as a step-like signals, in a single-molecule level (Fig. 2c).

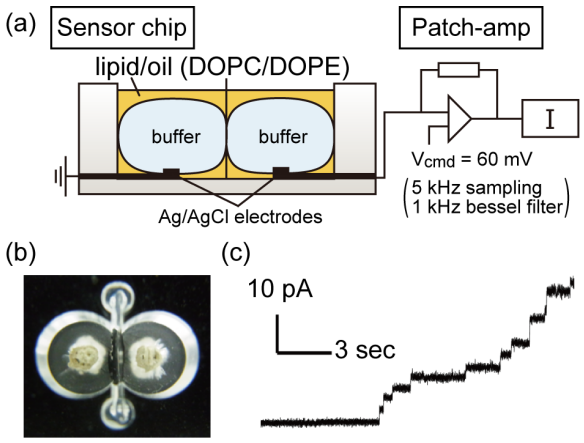

Figure 2: (a) Design of the double well chip to form the lipid bilayer by droplet contact method (b) Microscopic image of the double well chip. (c) Ionic current representing the generation of nanopore on DIB.

RESULTS

Solution applied to the inlet valve of spiral channel spontaneously flowed due to its surface tension, and reached the outlet in 7 minutes (Fig. 1c). The brown-colored magnetic beads were not observed from the outflow, which indicates the successive entrapment of magnetic beads within the channel (Fig. 3). Using this system, we first applied mock microRNA mixture of $>10^6$ different sequences, without using the magnet. In this way, step-like signals, representing the formation of nanopores on DIB, were observed (Fig. 4a). On the other hand, when the magnetic sheet was employed, no signals were observed, indicating the magnetic field in spiral channel was functioning to reduce the nonspecific signals (Fig. 4b). Finally, when the target microRNA was applied, signals were observed (Fig. 4c). This indicates that the system could distinguish the microRNA among a million of different sequences, which shows a noise-free detection.

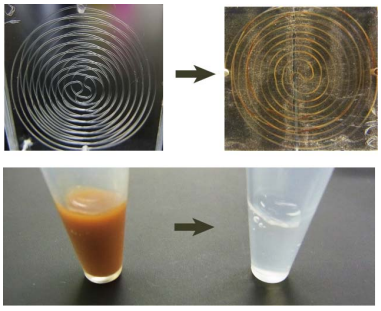

Figure 3: Top image: Spiral channel entrapping the magnetic beads. Bottom image: Solution before and after flowing through spiral channel.

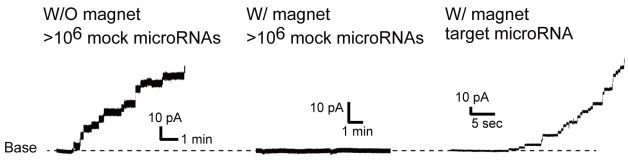

Figure 4: Ionic current monitored by applying microRNA samples of (Left): mock samples without using magnet, (Center): mock samples with using magnet, (Right): target sample with using magnet.

CONCLUSION

We succeeded to detect the microRNA in a sequence specific manner among a million of mock sequences. Compared to a bulk reaction, detection time was reduced from 30 min to 7 min by introducing the spiral channel. Due to this fast, and noise-free system, the presented device will be useful for diagnosis of crude solutions, such as unpurified-blood samples, for onsite diagnosis.

ACKNOWLEDGEMENTS

The authors acknowledge the technical support by Sora Hashino. This work was partly supported by Regional Innovation Strategy Support Program of MEXT, JAPAN, and JSPS KAKENHI Grant Number 15K17454, Japan.

REFERENCES

[1] K. Nakamura, K. Sawada, A. Yoshimura, Y. Kinose, E. Nakatsuka, T. Kimura, "Clinical relevance of circulating cell-free microRNAs in ovarian cancer", *Molecular Cancer*, vol. 15, pp. 48, 2016.

[2] R. M. Graybill, R. C. Bailey, "Emerging Biosensing Approaches for microRNA Analysis", *Anal. Chem.,* vol. 88, pp. 431-450, 2016.

[3] J. J. Kasianowicz, E. Brandin, D. Branton, D. W. Deamer, "Characterization of individual polynucleotide molecules using a membrane channel", *Proc. Natl. Acad. Sci. USA,* vol. 93, pp. 13770-13773, 1996.

[4] X. Zhang, Y. Wang, B. L. Fricke, L. Q. Gu, "Programming nanopore ion flow for encoded multiplex microRNA detection", *ACS Nano.,* vol. 8, pp. 3444-3450, 2014.

[5] T. Osaki, K. Kamiya, S. Fujii, S. Takeuchi, "MicroRNA diagnosis using complementary DNA that brakes transit events through a biological nanopore", *Proc. MEMS,* pp. 315-316, 2016.

[6] S. Fujii, K. Kamiya, T. Osaki, N. Misawa, S. Takeuchi, "Lipid Bilayer-based Noise-free MicroRNA Detection", *Proc. MicroTAS,* pp. 112-113, 2016.

[7] K. Funakoshi, H. Suzuki, S. Takeuchi, "Lipid bilayer formation by contacting monolayers in a microfluidic device for membrane protein analysis", *Anal. Chem.*, vol. 78(24), pp. 8169-8174, 2006.

CONTACT

*S. Fujii; phone: +81-44-819-2037;
fujiis@iis.u-tokyo.ac.jp