Autonomous Diagnosis and Therapy: miRNA Detection and Drug Release using Programmable DNA and Biological Nanopore

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ABSTRACT

This paper describes a droplet system which diagnoses and releases DNA drugs autonomously for treatment of a small cell lung cancer (SCLC) using a programmable DNA. Although microRNA (miRNA) has attracted attentions as an early diagnostic marker of cancers, a simple diagnostic system has been aspired because the conventional methods require the time-consuming and multistep procedures. In this study, we constructed the droplet system; one droplet has programmed DNA which detect the miRNA, and then DNA drugs can be transported to the next droplet through the nanopores. We believe this autonomous system can be applied to the drug delivery system.

KEYWORDS: Nanopore, Lipid bilayer, miRNA detection, Diagnosis, DNA drug

INTRODUCTION

It has recently emerged that miRNA regulates gene expression, and its expression levels are elevated in various diseases. Particularly, the detection of miRNA is expected to be an early diagnosis [1]. Although polymerase chain reaction (PCR) and gel-electrophoresis have been used for the detection of miRNA, these methods are time-consuming because large-scale apparatuses or a high operation technique are required. For example, when miRNA is detected by PCR, it is needed a reverse transcription for 65 minutes, PCR for 50 minutes, and then gel electrophoresis for around 1 hour. Molecular beacons, deep sequencing and single-molecule fluorescence have also been applied to miRNA detection; however, these methods still require the labelling and chemical modification of the target.

In recent years, molecular robotics which deals with a programmable artificial molecular system receives an attention, and it is expected that this field can be a key technology to solve various problems in medicine, environment and so on. Here, we constructed the programmable micro medical system for SCLC using DNA and the biological nanopore. The nanopore is a nano-scale pore formed by membrane proteins such as α-hemolysin (αHL). It is able to detect a single molecule with high sensitivity and electrically elucidate single-molecule kinetic pathways [2]. Our system is composed of two droplet; one droplet for SCLC diagnosis has a programmed DNA which detect the miRNA with an energy-driven strand displacement, and then single-stranded DNA (ssDNA) drugs can be transported through the nanopores to the next droplet for SCLC therapy. The drug is an anticancer drug to inhibit mRNA from oncogene. In the case of SCLC, *bcl-2* antisense oligonucleotide called Oblimersen has been developed [3]. In this study, we aimed at simplification of miRNA detection, diagnosis and therapy of SCLC by constructing the autonomous medical system.

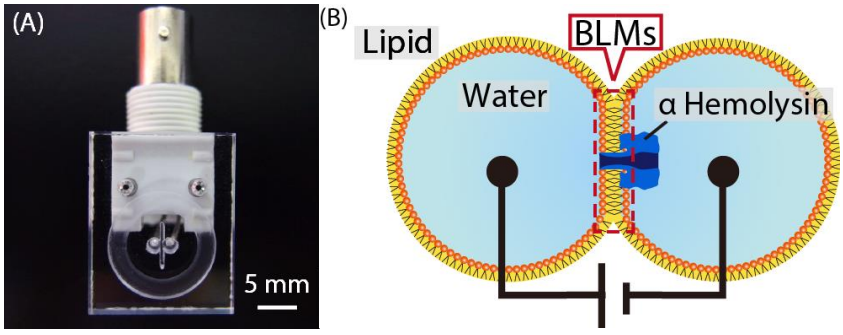

Figure 1: (A) A photograph of the double well chip device fabricated by photolithography for preparing BLMs. (B) We used "droplet contact method" to prepare BLMs. An α-HL nanopore is reconstituted in BLMs.

EXPERIMENTAL

To construct the autonomous medical system, we designed two oligonucleotides using NUPACK; Template DNA and Oblimersen-7. Oblimersen-7 includes the sequence of Oblimersen. Template DNA has the partial complementary sequence to both Oblimersen-7 and miRNA. Template DNA, Oblimersen-7 and miRNA were synthesized by FASMAC Co., Ltd. (Japan). Before testing, the mixture of Template DNA and Oblimersen-7 were heated to 95 °C for 5 min, gradually cooled to room temperature in order to form the double stranded DNA (dsDNA) called Programmed DNA. The mixture of 5 μM Programmed DNA and 5 μM miRNA in buffer solution (10 mM PBS, 500 mM KCl, pH 7.2) was left at room temperature for 90 min to occur strand displacement reactions. The artificial bilayer lipid membranes (BLMs) of 1, 2-diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC) were formed by droplet contact method (DCM) in our device as shown Fig. 1A [4]. In this method, the two monolayers contact together and form BLMs on a parylene C film that partitioned two chambers (*cis* and *trans*) (Fig. 1B). The recording solutions on each side of BLMs contained 500 mM KCl and were buffered with 10 mM PBS (pH 7.2). The αHL was reconstituted in BLMs to form a nanopore from *cis* side. The reaction solution was also added to the *cis* side. The voltage (+120 mV) was given from the *trans* side and the *cis* side was grounded, so that a negatively charged DNA could pass through the nanopore from *cis* to *trans* by a positive voltage. Nanopore currents were recorded with Pico Patch Clamp Amplifier (Tecella, USA), filtered at 8 kHz. The sampling rate was 40 kHz.

RESULTS AND DISCUSSION

When miRNA did not exist, we could observe a long current blockade as shown in Fig. 2A. Programmed DNA maintained its duplex structure because the strand displacement was not occurred without miRNA. The diameter of dsDNA (2.0 nm) is larger than that of αHL nanopore (1.4 nm). Therefore, Programmed DNA was captured in αHL, and current was blocked for a long time. On the other hand, if there was miRNA, spike-like current blockades were observed associating with translocations of Oblimersen-7 released by strand displacement reactions, that can be considered as the diagnosis and treatment for SCLC (Fig. 2B). The diameter of ssDNA (1.0 nm) is smaller than that of αHL nanopore, so that ssDNA drug can pass thorough the nanopore. However, there was some possibility that those spike-like blockades resulted from miRNA which failed to combine with Template DNA. In order to conform those signals came from Oblimersen-7, we gathered signals of miRNA and compared Oblimersen-7-like signals and miRNA signals about their blocking rates and duration time by *t*-test respectively. As a result, a significance different is appeared between their blocking rates, while not appeared between duration times at translocations ($P<0.01$). The above analysis indicates that Oblimersen-7 was truly released and moved into the next droplet for treatment. Namely, it was shown that the autonomous system using programmable DNA and αHL worked well.

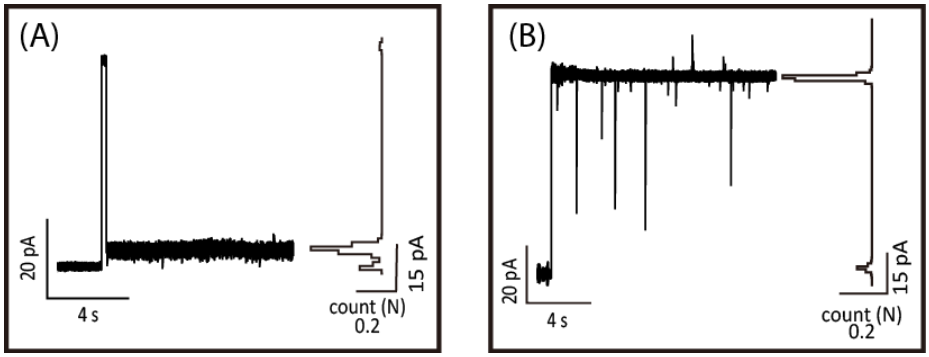

Figure 2: (A) The typical current and time trace when miRNA does not exist at a voltage of +120 mV. The histogram of that trace is also shown. (B) The typical current and time trace when miRNA exists at a voltage of +120 mV. The histogram of that trace is also shown.

CONCLUSION

We constructed the programmable system using DNA and αHL nanopore, which can detect miRNA, diagnoses SCLC and release ssDNA drugs autonomously. As a result, this system enables to simplify the method and shorten the time for miRNA detection, comparing to conventional methods. Furthermore, this device can release the drugs simultaneously. We believe that our medical system can be applied to a drug delivery system, which detects miRNA, diagnoses and treats diseases autonomously in the body.

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REFERENCE

[1] R. Garzon, A. Calin, C. M. Croce "MicroRNAs in cancer" *Ann. Rev. Med.* **60**, 167-179(2009)

[2] Y. Wang, D. Sheng, Q. Tan, M. X. Wang, L. Gu "Nanopore-based detection of circulating microRNAs in lung cancer patients" *Nat. Nanotech.* **6**, 668-673(2011)

[3] C. M. Rudin, G. A. Otterson, A. M. Mauer, M. A. Villalona-Calero, R. Tomek, B. Prange, C. M. George, L. Szeto, E. E. Vokes "A pilot trial of G3139, a bcl-2 antisense oligonucleotide, and paclitaxel in patients with chemorefractory small-cell lung cancer" *Annals of Oncology*. **13**, 539-545 (2002)

[4] R. Kawano, T. Osaki, H. Sasaki, M. Takinoue, S. Yoshizawa, S. Takeuchi "Rapid detection of a cocaine-binding aptamer using biological nanopores on a chip" *J. Am. Chem. Soc.* **133**, 8474-8477 (2011)

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