SUB-ATTOMOLE DETECTION OF MicroRNA IN TWENTY MINUTES USING POWER-FREE MICROFLUIDIC CHIP: TOWARDS POINT-OF-CARE TESTING

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ABSTRACT

This paper reports the first achievement in sub-attomole microRNA detection on the microfluidic device realized by laminar flow-assisted dendritic amplification (LFDA). This method allows us to detect microRNA at a limit of detection of 0.5 pM from 0.5 µL sample solution, which corresponds to 0.25 attomole, with the detection time of 20 min. Since some miRNAs circulating in blood have been found to have strong relation to specific cancers, this method, together with the advantages of self-reliance of this device, might substantially contribute to the future point-of-care early-stage cancer diagnosis.

KEYWORDS

MicroRNA, Point-of-care (POC), Microfluidics, PDMS, Surface hybridization

INTRODUCTION

MicroRNAs (miRNAs) are short, highly conserved non-protein-coding single-stranded RNAs of typically 18 to 24 bases. MiRNAs repress gene expression in a sequence-dependent manner, and are associated with various human diseases including diabetes, Alzheimer's, and cancer [1,2]. Thus, miRNAs have become increasingly important in determining disease diagnosis and prognosis. Some of miRNAs circulate in human body fluid with extremely low concentration at the early-stage of cancer, and its expression profiling can detect or even classify cancer in human body [3]. Therefore, highly sensitive miRNA detection will open a new field of early-stage cancer diagnosis.

Various techniques, such as quantitative PCR (qPCR), deep sequencing and oligo microarrays, are the major technical platforms for miRNAs profiling with their own strengths and drawbacks [4]. Those techniques allow detection and quantification of miRNAs over the hurdles by intrinsic properties of miRNAs, such as small size, wide range of melting temperature and large number of highly homologous sequence variants. Considering the point-of-care (POC) diagnosis, there are several additional requirements besides high sensitivity, such as short detection time and small sample volume. Those detection processes should be completed on a portable and compact device, which does not require huge equipment or trained personals to operate. Due to those various requirements, none of the current techniques meets all the requirements for POC diagnosis. This paper reports a new method for easy, rapid, and highly sensitive miRNA detection with limit of detection (LOD) of sub-attomole using a power-free PDMS microfluidic-chip by adopting laminar flow-assisted dendritic amplification (LFDA) [5].

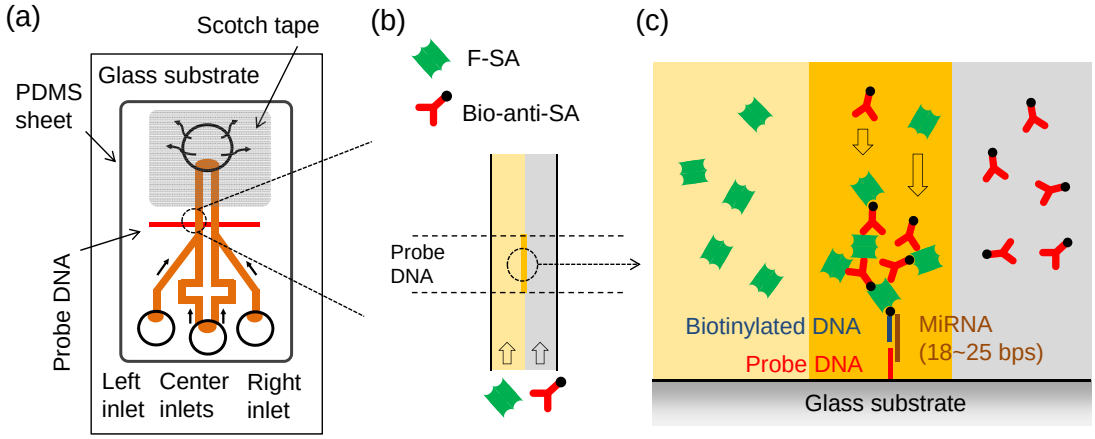

Figure 1. Schematics of the device and LFDA. (a) Schematics of the power-free microfluidic device. PDMS absorbs air in the outlet chamber thus being a self-stand pumping device. (b) Enlarged view of the channel. (c) Enlarged cross-sectional view. F-SA: FITC-labeled streptavidin, B-anti-SA: biotinylated anti-streptavidin.

EXPERIMENTAL

Probe DNAs were patterned onto the glass substrate and a power-free polydimethylsiloxane (PDMS) microfluidic device with a pair of Y shape microchannels was fabricated as reported elsewhere (Figure 1) [6]. Symbols and sequences of oligonucleotides, probe DNAs and miRNAs, used in this study are listed in Table 1. MiR-21 was adopted as a model sequence since it is one of the most well-known miRNAs as cancer markers [7,8]. The sandwich construction was designed to take an advantage of coaxial stacking effect, which enhances the hybridization efficiency [9].

Table 1. Sequences of oligonucleotides used in this study.

Nucleic acids	Sequence (from 5' to 3')
Aminated probe DNA	NH2- TTT TTT TTT TTT TTT TCA ACA TCA GT
Biotynilated probe DNA	CTG ATA AGC TA-biotin
Target miRNA (miR-21)	UAG CUU AUC AGA CUG AUG UUG A
Random miRNA	UGG UGC GGA GAG GGC CCA CAG U

Experimental protocols were as follows. First, blocking solution (BS) (1 % Roche BR, 0.02 % (w/v) SDS, 5×SSC, 0.05% Tween 20) was injected into all the channels and incubated for three minutes. Next, 10 nM ~ 0.1 pM miRNA sustained in the 0.5 µL BS was injected from the left channel, together with 1 µM of biotinylated probe DNA in 1 µL BS from the center channels, and 0.5 µL BS from the right channel. Finally, after five minutes, fluorescent streptavidin (2.5 µg/mL in BS) was injected from the left and right channels simultaneously with biotinylated anti-streptavidin (25 µg/mL in BS) from the center channels. Fluorescent signals were captured by CCD camera and analyzed by the software (ImageJ). Calibration curves were plotted from the fluorescent signals at the elapsed time when the right channel (Blank signal) started to increase by non-specific binding.

RESULTS AND DISCUSSION

The signal was amplified after trapping the sample miRNA by sandwich hybridization. Figure 2 (a) shows typical image of fluorescence microscopy with the target miRNA of 100 pM at elapsed time of five minutes after the initiation of LFDA. A bright line appeared at the expected position; it appeared only on the area with the aminated probe DNA in the left channel in which the target miRNA had been supplied. This image proved that the LFDA successfully took place in a target-dependent manner. A fluorescence intensity profile plot across the microchannels is shown in Figure 2 (b). In this plot, outer half of the both channels have significant fluorescence from the bulk F-SA solution. Inner half of the both channels have slight fluorescence, which were adopted as background levels. We defined the peak intensity in the left channel from the background level as the signal and the maximum intensity in the right channel from the background level as the blank reference.

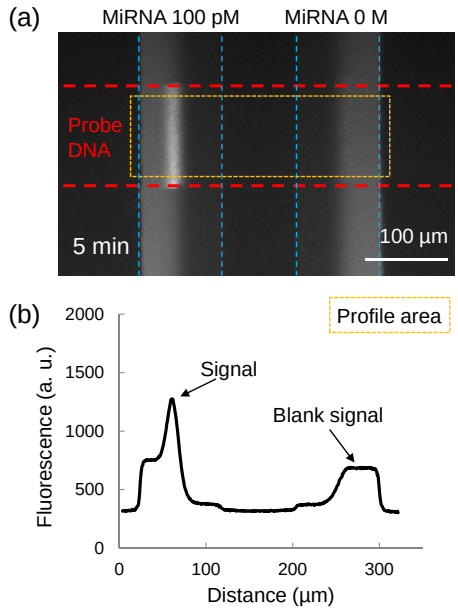

Figure 2. Fluorescent signals and their profiles. (a) Microscopic view of an amplified fluorescent signal after five minutes of LFDA. (b) Profile of fluorescent signals.

Figure 3 shows the time courses of signals generated by the LFDA with various concentrations of the target miRNA. The signals began to rise from the initial value (F-SA bulk fluorescence) at some time, depending on the target concentrations. Generally, a higher concentration caused an earlier rise. The blank sample showed the latest rise, typically after the elapsed time of five minutes, which was probably initiated by nonspecific adsorption of F-SA or B-anti-SA. Once the signals rose, they exponentially grew at similar rates. As a result, higher signals were obtained with higher target concentrations.

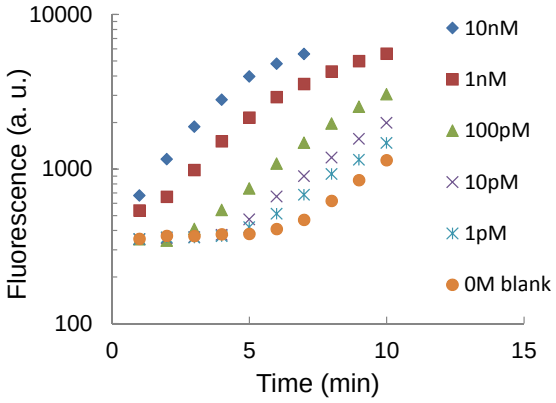

Figure 3. Time courses of amplified fluorescent signals. MiRNA concentrations were varied from 0 M (blank signal) to 10 nM.

Parameter for the calibration curve was defined as the ratio of signal over blank reference at the elapsed time when the blank reference in the right channel started to rise by non-specific binding (Figure 4). The data points of the calibration curve were fitted with the four-parameter logistic function and the LOD was evaluated by the 3σ criterion. As a result, we obtained an LOD of 0.5 pM, which corresponds to 0.25 amol. This LOD, in terms of the number of molecules, is below the LOD in most of the previous reports concerning solid-phase hybridization assays of miRNA without enzymatic amplification [10]. Compared to conventional methods, such as qPCR, sequencing and microarray, which usually require a few hours to an overnight incubation, detection time was drastically shortened. This is also the first achievement of this LOD in 20 min among any existing techniques that can detect or quantify miRNA. Therefore, we have proven that the combination of the power-free microfluidic device and the LFDA is effective for the simple, rapid, and sensitive detection of miRNA.

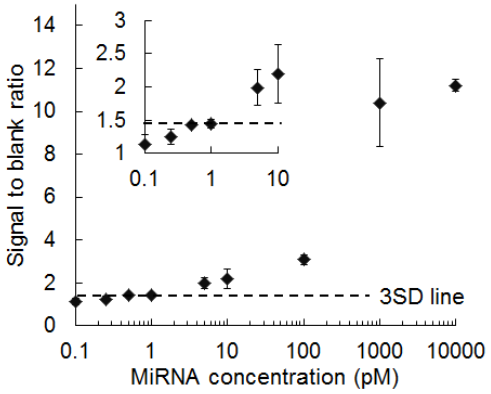

Figure 4. Signal to blank ratio vs. miRNA concentration. Inset is the expanded view at low concentrations.

CONCLUSIONS AND OUTLOOK

This paper reported a new method for easy, rapid, and highly sensitive miRNA detection with a limit of detection (LOD) of sub-attomole adopting LFDA using the power-free PDMS microfluidic device. In our previous study, miRNA detection by sandwich hybridization was proven to be capable of multiple sequence detection from the same sample solution [9]. This capability strongly support the fact that miRNA detection by this device is a promising technology in cancer diagnosis, because each cancer is considered to have only a few number of corresponding marker miRNAs, which may exclude the need for screening hundreds of sequences by conventional array chip.

For practical application, the optical and electrical equipment for signal output should be miniaturized or combined with existing electronic devices [11]. Those challenges might effectively demonstrate the potential of this method to the commercial production. Rapidness, simple operation, small required sample volume and portability of the device are ideal advantages for point-of-care cancer diagnosis. Therefore, further study might contribute to improve healthcare environment even in resource poor environments, such as in developing countries, and may give both industrial and societal impact for the global health.

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REFERENCES

[1] Lu J, et al. (2005) MicroRNA expression profiles classify human cancers. *Nature* 435: 834–838.
[2] Esquela-Kerscher A, Slack FJ (2006) Oncomirs - microRNAs with a role in cancer. *Nature Reviews Cancer* 6: 259–269.
[3] Mitchell PS, et al. (2008) Circulating microRNAs as stable blood-based markers for cancer detection. *Proc. Natl. Acad. Sci. USA* 105: 10513-10518.
[4] Baker M (2010) MicroRNA profiling: separating signal from noise. *Nature Methods* 7: 687-692.
[5] Hosokawa K, Omata M, Maeda M (2007) Immunoassay on a power-free microchip with laminar flow-assisted dendritic amplification. *Analytical Chemistry* 79: 6000-6004.
[6] Hosokawa K, Sato T, Sato Y, Maeda M (2010) DNA detection on a power-free microchip with laminar flow-assisted dendritic amplification. *Analytical Science* 26: 1053-1057.
[7] Jazbutyte V, Thum T (2010) MicroRNA-21: from cancer to cardiovascular disease. *Current Drug Targets* 11: 926-935.
[8] Thum T, et al. (2008) MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. *Nature* 456: 980-984.
[9] Arata H, Komatsu H, Han A, Hosokawa K, Maeda M (2012) Rapid microRNA detection using power-free microfluidic chip: coaxial stacking effect enhances the sandwich hybridization. Analyst 137: 3234-3237.
[10] Duan D, et al. (2011) Label-free high-throughput microRNA expression profiling from total RNA. *Nucleic Acids Research* 39: doi:10.1093/nar/gkr774.
[11] Zhu H, et al., (2011) Optofluidic fluorescent imaging cytometry on a cell phone. *Analytical Chemistry* 83: 6641-6647.

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