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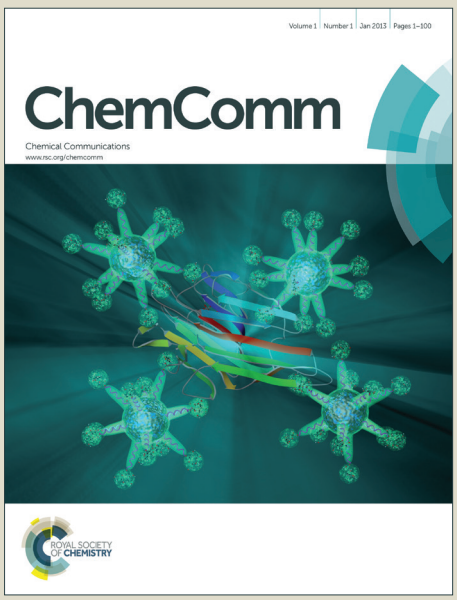

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COMMUNICATION

Multiplex detection of nucleic acids with low cost microfluidic chip and personal glucose meter at the point-of-care

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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A simple assay for multiplex DNA detection has been developed using microfluidic chip and personal glucose meter. By using of this system, multiplex detection of three genotypes of Hepatitis B virus DNA was possible with a detection limit of 10 pM. This point-of-care assay represented a versatile platform for sensitive multiplex target detection.

The development of point-of-care (POC) devices for rapid, on-site and cost-effective detection has long been sought, in home healthcare and medical testing, even for environmental monitoring.[1] In recent years, considerable efforts have been devoted to the development of POC devices.[2-15] Among these devices, some personal portable devices, such as personal glucose meter (PGM) and personal uric acid meter (PUAM), perhaps become the most successful examples of POC device due to their compact size, low cost, reliable quantitative results and simple operation.[4, 11] Generally, PGM or PUAM can only detect blood glucose or uric acid as the unique target. Recently, it has been reported that some targets, including metal ions,[16, 17] proteins,[4, 18] nucleic acids,[11, 19] and small molecules,[4] could be detected by these personal portable devices.

However, these previous works were limited to the detection of single-target. Multiplex target detection has attracted increasing attentions in life research because of its potential for extracting the most information from the smallest amount of sample volume.[20] At present, some efficient methods capable of multiplex target detection have been developed.[21-25] Generally, most of these approaches are based on sophisticated instruments or customized devices that are not currently easily accessible to the public. Hence, there is an unmet need for developing a simple method to detect multiple targets at the POC in both developed and developing countries. Expanding the applicability of personal portable devices in the detection of multiple targets would potentially leverage the POC testing.

Here, to address this unmet need, a novel assay for multiplex detection of nucleic acids was introduced using a portable and low cost microfluidic chip and PGM. Theoretically, this protocol may have the potential to be applicable to the portable detection of other multiple targets, such as proteins and small molecules, at home or in the field, as long as appropriate probes can be found and replaced.

Firstly, the microfluidic chip was fabricated using double-sided adhesive film and the non-lithographic technique. As shown in Fig. 1, the chip was composed of three pieces of inexpensive polystyrene plate, i.e., the cover, middle, and bottom plates. Both sides of the middle plates were attached with double-sided adhesive film. A central channel (48 mm long, 5 mm wide, 0.5 mm deep) and four parallel branch channels (20 mm long, 2 mm wide, 0.5 mm deep) were made by sandwiching the middle plates between the top and bottom plates via the double-sided adhesive film. The total volume of all channels was ca. 200 μL, and each branch channel can accommodate 20 μL of the solution. The microfluidic chip costed less than $ 0.01 in material cost and can be easily assembled at a high throughput (60 s / chip).

The most significant characteristic of this microfluidic chip was that it could achieve multiplex target detection conveniently. Fig 2 showed the operation procedure for multiplex DNA detection by using of this low cost microfluidic chip. The sample was manually pipette into a modified chip from the central channel, and all channels were full of solution within seconds, even the channels of different branches in the microfluidic chip experienced different pressure resists (Fig. 2a). Next, a filter paper was placed at the outlet of the central channel for blotting the solution in central channel through capillary action. The sample solution in all branch channels were retained, because the width of the branch channels was smaller than that of the central channel (Fig. 2b). Since each branch channel constituted a complete assay for target detection, this microfluidic chip could be used for the detection of at least two targets simultaneously.

Here, three genotypes of Hepatitis B virus (HBV) DNA were used as model target DNAs. As illustrated in Fig. 1, three probes (i.e., Capture-B, Capture-C and Capture-D) corresponding to HBV-B, HBV-C, HBV-D sequences, and a random probe (Capture-R) serving as a negative control, were respectively immobilized on the surface of four branch channels using biotin-avidin linkage.[26, 27] The sequences of oligonucleotides used here were listed in Table S1 of Electronic Supplementary Information (ESI). As the target DNA was added, it hybridized with capture probes immobilized on the channel surface. Since the three capture probes have only three or four bases differences, the target DNA may hybridize with these three capture probes. When high-stringency washing buffer was introduced, mismatched hybrids between target DNA and noncomplementary capture probes were removed. Then, report probe (i.e., invertase-DNA conjugate) was added and subsequently captured by the target DNA, forming a "sandwich" hybridization structure. The report probe was synthesized and characterized as shown in Fig. S1 of ESI. Upon addition of sucrose, the bound report probe can catalyze the hydrolysis of sucrose into glucose. The glucose product in different branch channels spontaneously entered into the test strips of a PGM through capillary action (Fig. 2c-f). Finally, by analysing the different PGM signal values obtained from different channels, the concentration of targets can be determined respectively and simultaneously (see also section 4 in ESI).

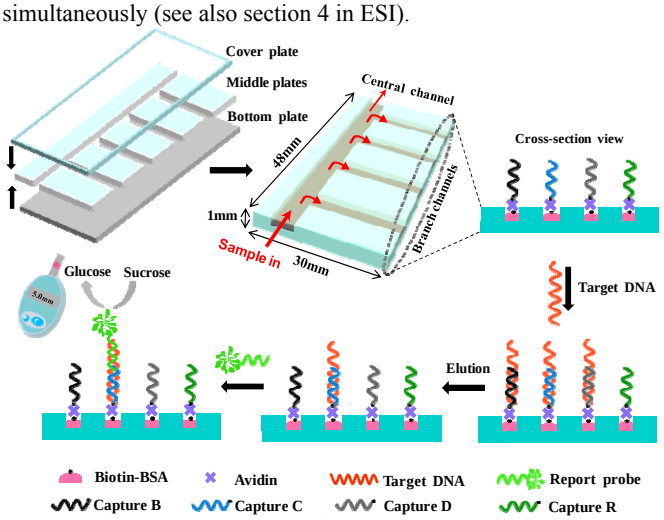

Fig. 1 Fabrication process of the microfluidic chip and the schematic illustration of the microfluidic chip for HBV-C detection by using of a personal glucose meter.

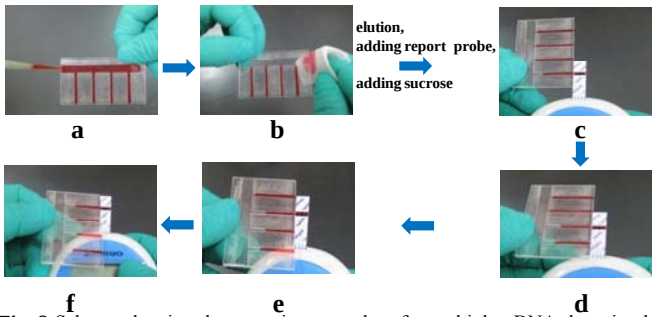

Fig. 2 Scheme showing the operation procedure for multiplex DNA detection by using of this microfluidic chip. (a) A small volume (200 μL) of sample was introduced into the microfluidic chip, and all channels were full of solution within seconds. (b) A filter paper was used to quickly blot the solution in central channel, and the sample was then separated into four branch channels. (c-f) After glucose produced in different branch channels, PGM was used to test the glucose concentration. The red dye filled in microfluidic chip was used for visualization.

In this assay, the concentration of high-stringency washing buffer played an important role in the performance of the detection system. Based on the melting temperature (T_m) differentiation between the matched hybrids and the mismatched hybrids, the deionized formamide was chosen as elution buffer and the concentration of deionized formamide was optimized. As shown in Fig S2a of ESI, with increasing deionized formamide concentration, the dehybridization of mismatched hybrid process was gradually obvious and tended to stable values. Thus, 50% deionized formamide was used in our experiment. Other factors, such as the concentration of sucrose, the catalytic reaction time and the reaction temperature, were also optimized. As shown in Fig. S2b-d of ESI, 500 mM sucrose concentration, reaction time of 50 min and 25 °C were used in the following experiment.

Under the optimized conditions, various samples containing HBV-C target concentrations in the range of 10 pM ~ 100 nM were detected. As shown in Fig. 3, the PGM signals obtained from the Capture-C modified channel were elevated obviously along with the increase of the HBV-C target concentration. Meanwhile, the Capture-B and Capture-D modified branch channels displayed relatively low PGM signals. When the concentration of HBV-C target reached 50 nM, PGM signal reached a saturated plateau. According to the 3σ rule,[28] the signal value could be observed even at a DNA concentration as low as 10 pM. Moreover, The detection method provided good repeatability with a RSD of 1.5% (n=5).

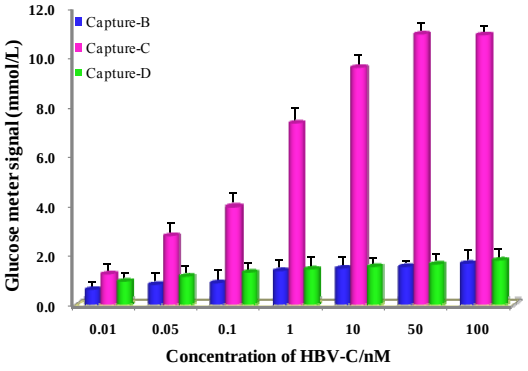

Fig. 3 The glucose meter signals for HBV-C detection at various concentrations. The background value obtained from control channel (i.e., Capture-R modified branch channel) was deducted from each measured values using PGM. Error bars show the standard deviations of measurement taken from five experiments.

To achieve rapid multiplex DNA detection in a single assay using this microfluidic chip, the sample solutions including single or multiple target DNAs were introduced into seven identical chips respectively. As shown in Fig. 4, when HBV-B was added into the chip, high PGM signal could be obtained only at the Capture-B modified branch channel; while low PGM signals were obtained at the Capture-C or Capture-D modified channels, respectively. Similar results occurred as HBV-C or HBV-D were added respectively. As two target DNAs, such as HBV-B and HBV-C, were introduced into the chip, high PGM signals were observed at the Capture-B and Capture-C modified channels; while low PGM signal was obtained

at the Capture-D modified channels. For other mixture samples including two target DNAs, similar results were obtained. More interestingly, high PGM signals could be detected on three branch channels when three target DNAs were injected. These results indicated that this approach was capable of the multiplex DNA detection simultaneously.

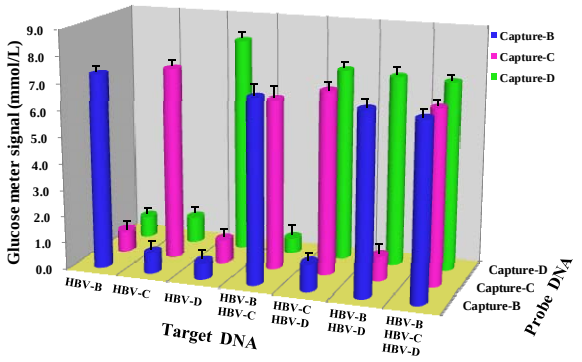

Fig. 4 Multiplex detection of target DNA using the microfluidic chip. The sample solutions containing single or multiple target DNAs. The concentration of each target DNA strand was 1 nM. The background value obtained from control channel (i.e., Capture-R modified branch channel) was deducted from each measured values using PGM. Error bars show the standard deviations of measurement taken from five experiments.

Conclusions

In conclusion, we have developed a simple approach for portable detection of three genotypes of HBV DNA by using low cost chip and easily accessible PGM. This assay possessed several excellent features. First, the chip was easy to fabricate and convenient to operate; Second, this assay did not require any specialized skills or sophisticated instruments; Moreover, we have extended the use of PGM for quantitative detection of multiple targets in one sample. This approach may be altered for the detection of other targets by simply changing the probes. These features establish a simple and universal platform which could be potential for the development of POC research. For prospective development, it is worthwhile to optimize the design of the microfluidic chip for further reducing the assay time and decreasing the reagent consumption.

This work was supported by the National Natural Science Foundation of China (21190040, 21375034, 21175035), National Basic Research Program (2011CB911002), International Science & Technology Cooperation Program of China (2010DFB30300), and Program of Young Teacher Grown of Hunan University.

Notes and references

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Electronic Supplementary Information (ESI) available: Experimental details, surface modification and characterization, synthesis and characterization of invertase-DNA conjugate, optimized procedure. See DOI: 10.1039/c000000x/

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