Biosensors and Bioelectronics 26 (2011) 2436–2441

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A portable generic DNA bioassay system based on *in situ* oligonucleotide synthesis and hybridization detection

Chen Wang [a], Dieter Trau [a,b,*]

[a] Division of Bioengineering, National University of Singapore, Singapore
[b] Department of Chemical & Biomolecular Engineering, National University of Singapore, Singapore

ARTICLE INFO

Article history:
Received 19 July 2010
Received in revised form 5 October 2010
Accepted 17 October 2010
Available online 23 October 2010

Keywords:
DNA synthesis
DNA biosensor
Bio-threat detection
Homeland security

ABSTRACT

In this study, we present a portable and generic DNA bioassay system based on *in situ* oligonucleotide synthesis followed by hybridization based detection. The system include two main parts, an oligonucleotide synthesizer and a fluorescence detection system. The oligonucleotide synthesizer is based on microfluidic technology and capable of synthesizing any desired oligonucleotide which can be either used as a primer for PCR based detection (external) or a probe for hybridization based detection (integrated) of a target DNA analyte. The oligonucleotide sequence can be remotely sent to the system. The integrated fluorescence detection system is based on a photodiode to detect Texas Red fluorophore as low as 0.5 fmol. The complete system, integrating the oligonucleotide synthesizer and fluorescence detection system, was successfully used to distinguish DNA from two different bacteria strains. The presented generic portable instrument has the potential to detect any desired DNA target sequence in the field. Potential applications are for homeland security and fast responses to emerging bio-threats.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

State of the art DNA based bioassays for the detection of biological warfare and infectious disease agents such as bacteria and viruses make use of pre-made oligonucleotides that function as either probe or primer (Ivnitski et al., 2003). For in-the-field application of these technologies, portability of such device is an important factor. DNA microarray technology has been proven to be a powerful tool for biological agents detection based on DNA hybridization (Heller, 2002; Stoughton, 2005). However, the expensive and complicated instrument setup made it difficult to be used for in-the-field applications. For other methods such as PCR based technologies, the devices can be fabricated in a very portable format. However, pre-made oligonucleotide primers with known sequence are used to carry out the assays (Kricka and Wilding, 2003; Neuzil et al., 2006). As a consequence, no immediate detection of emerging microbes or viruses or artificially manipulated or naturally mutated agents is possible. Therefore, there is an emerging need for technologies that can timely react to such threats.

Microfluidics is a promising technology due to its versatile use and ease of integration. The invention of microfluidic valves enabled microfluidic devices to manipulate and control fluids flow and therefore dramatically extend their use (Unger et al., 2000; Vieider et al., 1995). Oligonucleotide synthesis is one of such applications where sequential switching of a number of reagents was needed. As far as we know, just a few studies have been conducted on developing microfluidic device for oligonucleotide synthesis (Huang et al., 2007; Lee et al., 2010). However, no work has been done aiming to develop an oligonucleotide synthesizer in a portable format that has potential use for in-the-field applications.

Bioassay detection based on fluorescence such as DNA hybridization with fluorescence labeled targets is a well known powerful method. However, often expensive laser excitation sources and sophisticated detectors were used, which make them not suitable for in-the-field applications (Stoughton, 2005). With the development of LED technology, several studies demonstrated LEDs as cheap and small form factor light source often combined with photodiodes as detector (Li et al., 2005; Novak et al., 2006; Yang et al., 2009). In some studies, an oligonucleotide probe was directly immobilized on the photodiode surface, which limited the reusability of the detector (Li et al., 2005).

In this work, we present a portable and generic DNA bioassay system. The system is able to synthesize any desired oligonucleotide according to a remotely received sequence and consecutively used the oligonucleotide as a hybridization probe in its integrated fluorescence based detector to detect any target DNA in the field. The oligonucleotide synthesizer is based on microfluidic technology and the fluorescence detector is based on a LED light source and a photodiode detector. Alternatively the system

* Corresponding author at: Division of Bioengineering, National University of Singapore, Room EA-03-12, Singapore 117576, Singapore. Tel.: +65 65168052; fax: +65 68723069.
E-mail address: bietrau@nus.edu.sg (D. Trau).
URL: http://www.biosingapore.com (D. Trau).

0956-5663/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2010.10.028

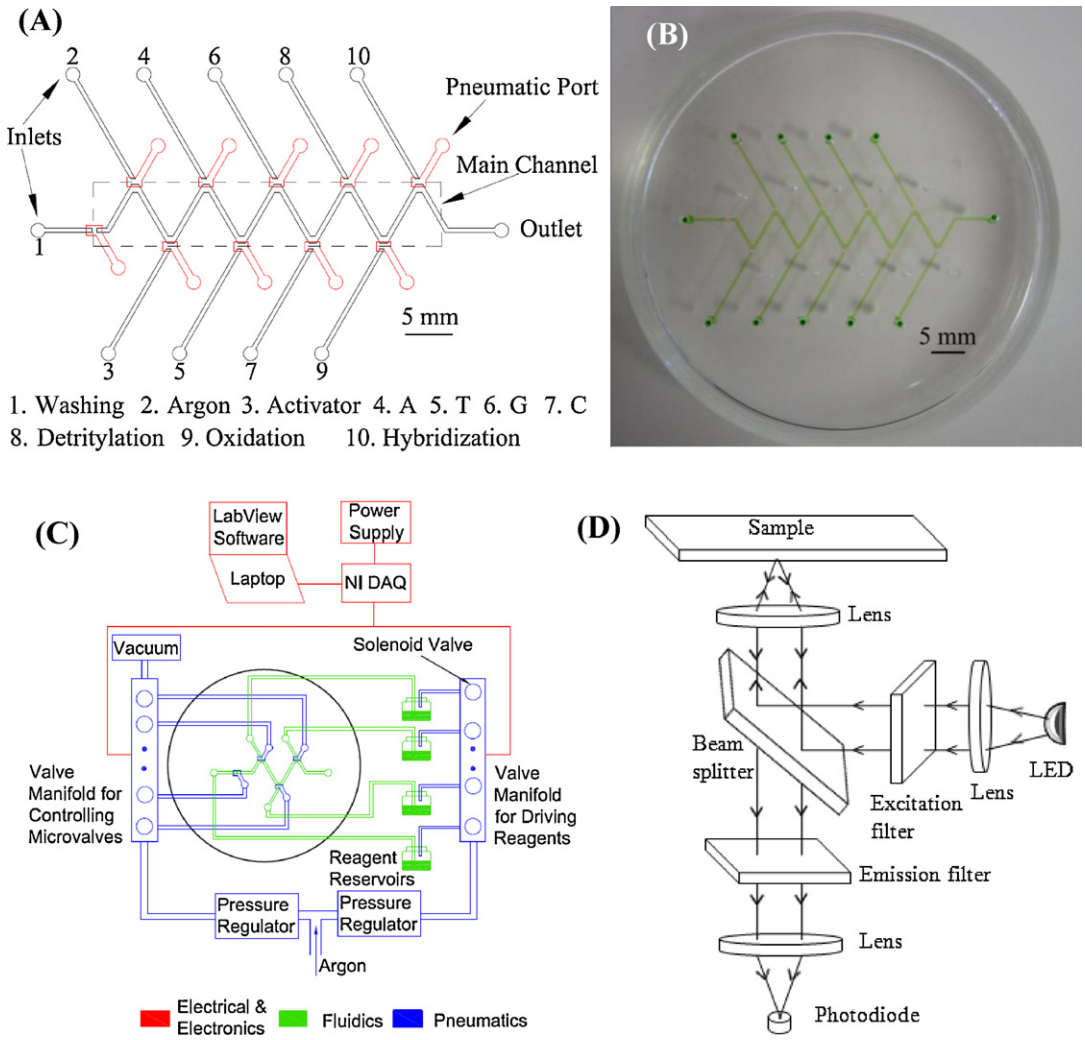

Fig. 1. (A) Design of the microfluidic chip with integrated microvalves. Fluidic channels are in black color and pneumatic channels are in red color. Microvalves are positioned at each turning point of the zigzag shaped main channel. (B) Photograph of the fabricated microfluidic chip. The fluidic channels are filled with green color liquid. (C) Schematic diagram illustrating the design of the oligonucleotide synthesis system. (D) Schematic diagram of the Texas Red fluorescence detection system. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

can be used to synthesize oligonucleotide primers for PCR applications. Both primers and probes were synthesized by the portable system and their functionality was demonstrated by PCR and DNA hybridization experiments. The fluorescence detection system can detect the Texas Red fluorophore as low as 0.5 fmol and was successfully used for differentiating complementary and single-base mismatch DNA in hybridization experiments. The complete system, which integrated the oligonucleotide synthesizer and fluorescence detection system, was successfully used to distinguish DNA from two different bacteria strains. The system is portable and can synthesize DNA "on the run" with potential homeland security or remote analysis applications in-the-field.

2. Experimental

2.1. Oligonucleotide synthesis system

The core part of the oligonucleotide synthesis system is a microfluidic chip with integrated microvalves. The microfluidic chip was completely fabricated by PDMS and has a three-layer structure. The working principle of the microvalve used in this study was similar to previous work (Vieider et al., 1995). Fig. 1A shows a schematic of the microfluidic chip design. The microfluidic chip has 10 inlets and one outlet. Each inlet was connected to one oligonucleotide synthesis reagent. Each reagent was controlled by one microvalve located exactly at the connecting point of the inlet channel and the zigzag shaped main channel. Unlike other designs (Lee et al., 2008; Wang and Lee, 2005), the connecting channel that connect the inlet channel and the main channel was removed. An advantage of our design is that reagents trapped in the connecting channel and leftover in the chip can be easily washed out and therefore cross-contamination among reagents can be minimized. The width of the channels is 500 μm and the height is 50 μm. The total length of the main channel is 70 mm. The total volume of the main channel is around 1.8 μL. The length of the valve seat is 700 μm. A photograph of the fabricated microfluidic chip is shown in Fig. 1B.

The chip integrated microvalves were operated through pneumatic ports by three-way normally open solenoid valves (Pneumadyne, US) mounted on a multiple-station aluminum manifold. The microvalves were closed under positive pressure (7.2 psi, relative to atmospheric pressure) and opened under negative pressure (−4.2 psi). All reagents were driven by positive pressure (2 psi, the maximum reagent driven pressure the microvalve can withstand is 9.8 psi) controlled by a two-way normally closed solenoid

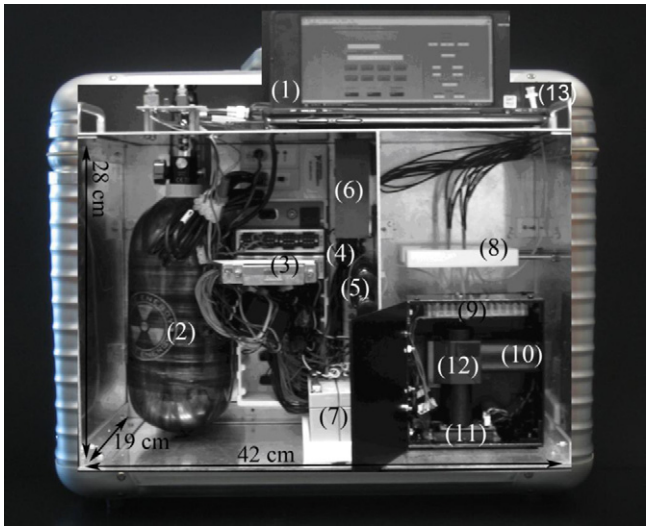

Fig. 2. Photograph of the portable system and artistic impression of its fit into a portable aluminum suitcase: (1) PC, (2) argon tank, (3) NI-DAQ, (4) solenoid valves and manifold, (5) pressure regulators, (6) vacuum pump, (7) batteries, (8) microfluidic chip, (9) synthesis and hybridization reactor, (10) LED, (11) photodiode, (12) lenses with filters and beam splitter, and (13) hybridization sample injection port.

valve mounted on another aluminum manifold. Positive pressure used in the system was provided by pressurized argon gas stored in a tank (6310, Carleton Technologies Inc., US) (pressure tank and regulators are adapted from "paintball equipment"). The pressure was first regulated by a regulator (Custom Products, US) to 20 psi and was then further regulated down to the working pressures (Type 91, Marsh Bellofram, US). The negative pressure was provided by a micro-boxer pump (Cole-Parmer, US). All parts were powered by 12 V DC.

The whole system was controlled by LabView software (National Instrument, US) running on a notebook PC (VGN-P25G, Sony, Japan). The solenoid valves were operated by a 32-Channel digital output module (National Instrument, NI 9476) mounted on a USB CompactDAQ Chassis (National Instrument, NI cDAQ-9172).

Fig. 1C shows the schematic diagram of the microfluidic oligonucleotide synthesizer system. All components were mounted into an aluminum rack with the dimensions of 42 cm × 19 cm × 28 cm. The rack with notebook PC and all parts mounted was inserted into an aluminum suitcase (Rimova GmbH, Germany) for carrying (artistic impression in Fig. 2).

2.2. Texas Red fluorescence detection system

Fig. 1D shows the schematic of the fluorescence detection system. A white color LED (Luxeon Emitter, LXHL-PW01, Lumileds Lighting, US) was used as the excitation light source and was powered by a Stardrive LED driver (1 W, Sumatec Ltd., UK). The white light was first collimated by a lens (f = 25 mm, Edmund Optics, US) and then passed through an excitation filter (HQ575/50x, Chroma, US). The filtered light was reflected by a beam splitter (Z594/750rpc, Chroma, US) and focused onto the sample (spot of ~2 mm diameter). The fluorescence emitted from the sample passed through the emission filter (HQ645/70m-2p, Chroma, US) and was focused by a lens (f = 25 mm) on a high sensitivity, low noise photodiode detector with built-in preamplifier (S8745-01, Hamamatsu Photonics, Japan). The entire excitation spot was focused onto the sensor. The photodiode was powered with ±6 V and the signal from the preamplifier was directly measured with an analog input card (NI 9219 National Instrument, US) without further amplification. All parts were fixed into light tight black aluminum housing.

2.3. Integration of fluorescence detection and oligonucleotide synthesis system

The fluorescence detection system was integrated with the oligonucleotide synthesis system to form the portable DNA bioassay instrument. The photograph of the complete integrated system is shown in Fig. 2. The system also comprises an oligonucleotide synthesis and hybridization reactor with heater and temperature sensor (Fig. S1 in supplementary materials). The reactor was fixed inside the fluorescence detection device. The inlet of the reactor directly connected to the outlet of the microfluidic chip by Teflon tubing. After synthesis, the fluorescent labeled DNA target was manually injected using a syringe through a port extended outside of the rack for detection (part 13 in Fig. 2, this port was controlled by valve 10 on the microfluidic chip).

2.4. Materials

PDMS (Sylgard 184) was purchased from Dow Corning (US). Oligonucleotides not synthesized by the system were all purchased from Sigma–Aldrich. The PCR kit (GoTaq PCR core system) was purchased from Promega. Oligonucleotide synthesis reagents including anhydrous acetonitrile, tetrazole solution (0.45 M in acetonitrile), iodine, acetic acid, pyridine, dichloroacetic acid, nitromethane, ethylenediamine and universal controlled pore glass (CPG) were purchased from Sigma–Aldrich. Phosphoramidites were purchased from Glen Research (US). All reagents were directly used as received without further purification.

2.5. Microfluidic chip fabrication

The microfluidic chip was designed having a three-layer structure. A thin flexible PDMS membrane was sandwiched by a fluidic layer and a pneumatic layer. The PDMS membrane was obtained by spin-coating PDMS on silicon a wafer at 2000 rpm followed by peel-off from the wafer resulting in a 35 μm thick membrane. Both fluidic layer and pneumatic layer were fabricated by PDMS micromolding using a masters fabricated by photolithography from SU-8 (Microchem, USA).

The microfluidic chip was fabricated by the following steps. The fluidic layer and the PDMS membrane were first bonded together after treatment in oxygen plasma. The valve seat regions were marked with an oil based permanent marker (Zebra, Japan) before plasma treatment to prevent this region to bind to the PDMS membrane. Then, the other surface of the PDMS membrane was bonded with the pneumatic layer after oxygen plasma treatment. Alignment was done under a stereo microscope. The chip was ready to use after baking at 65 °C overnight.

2.6. Oligonucleotide synthesis

The phosphoramidite method (Gait, 1984) was used to synthesize oligonucleotides in the system. The usual detritylation reagent was substituted by 3% dichloroacetic acid in anhydrous nitromethane (Letsinger, 1988), because PDMS can be badly swelled by dichloromethane (Lee et al., 2003). The usual oxidation reagent was replaced by 0.1 M iodine in 9:1 pyridine/acetic acid (v/v) (Moorcroft et al., 2005). The volume of reagent consumed in each reaction was ~20 μL. The reagents and reaction time for each step is summarized in Table 1. The total time for the extension of one base is 9 min.

Oligonucleotides used as primers for PCR were synthesized on controlled pored glass (CPG) filled into a small column (1 mm diameter; 3 mm lengths). After synthesis, the CPG was harvested and the primer was deprotected and cleaved from the solid support by treatment with 28% ammonium hydroxide at 65 °C. The

Table 1
Reagents and reaction time for oligonucleotide synthesis.

No.	Reaction	Reagent	Time (min)
1	Detritylation	3% dichloroacetic acid in nitromethane	1.5
2	Washing	Acetonitrile	1
3	Coupling	(a) 0.45 M tetrazole in acetonitrile (b) 0.1 M phosphoramidite (for A, T, C or G) in acetonitrile	3
4	Washing	Acetonitrile	1
5	Oxidation	0.1 M iodine in 9:1 pyridine/acetic acid (v/v)	1.5
6	Washing	Acetonitrile	1

primer was then vacuum dried and dissolved in water. Primers were directly used for PAGE analysis and PCR without further purification.

Oligonucleotides used as a probe for DNA hybridization experiments in the integrated fluorescent detector were synthesized on microscope glass slides. The surface of the glass slide was modified with 2% N-(3-triethoxysilylpropyl)-4-hydroxybutyramide in 95% ethanol for 4 h at room temperature followed by curing under vacuum at 120 °C for 1 h. After synthesis, the oligonucleotide was deprotected by ethylenediamine:ethanol (1:1, v/v) for 2 h at room temperature.

2.7. PAGE, PCR and DNA hybridization

Polyacrylamide gel (15%) electrophoresis (PAGE) was used to confirm the length of the synthesized oligonucleotides (the ratio of acrylamide:bis-acrylamide was 29:1). Samples were heated to 95 °C for 2 min and then immediately chilled by transferring onto ice. After sample loading, the gel was run for 1.5 h at 200 V, stained with SYBR® Gold and imaged with a G:BOX gel documentation system (Synoptics, UK). The synthesized oligonucleotide was then used as a reverse primer to amplify *Escherichia coli* 16S ribosomal RNA. The 596 bp PCR product was analyzed in agarose gel electrophoresis.

Synthesized oligonucleotide probes were used to differentiate different bacteria strains. The bacteria genomic DNA (*Bacillus cereus* ATCC 14579 and *Salmonella enterica* subsp. *enterica* ATTC 13311) was first amplified by asymmetric PCR. The ratio of Texas Red labeled primer and non-labeled primer was 10:1. Therefore, more fluorescence labeled single-strand target DNA can be produced. The hybridization was taken place at 45 °C for 1 h in 5× SSC buffer containing 0.1% SDS followed by washing with 0.1× SSC at 37 °C for 5 min. The total time for conducting the whole detection including synthesis of a typical 20 mer oligonucleotide probe, hybridization and fluorescence measurement was 6.5 h.

The following oligonucleotide sequences were used in this work: P1: GGTTGCGCTCGTTGCGl; P2: CCAGCAGCCGCGGTAA; T1: CGCAACGAGCGCAACC[6-FAM]; P3: GCTACAACTGCTTATG[Amine]; P4: GCTACCACTGCTTATG[Amine]; T2: CATAAGCAGTTG-TAGC[TxRd]; 16S-F: CCTACGGGAGGCAGCAGT; 16S-R: [TxRd]CGTTTACGGCGTGGACTAC; *Bacillus* probe: CGCGCAGGTG-GTTTCTTAA; *Salmonella* probe: GGTCTGTCAAGTCGGATGTG.

3. Result and discussion

3.1. Oligonucleotide synthesis by the system

3.1.1. Primer synthesis

To evaluate the function of the oligonucleotide synthesis system, a 16mer oligonucleotide primer "P1" was synthesize on CPG. The oligonucleotide was then cleaved from the CPG and analyzed by PAGE. The PAGE image in Fig. 3A shows that the main product of the synthesized oligonucleotide (lane 3) has the same mobility as the commercial HPLC purified oligonucleotide of the same sequence (lane 2). This confirmed that the 16mer full length oligonucleotide

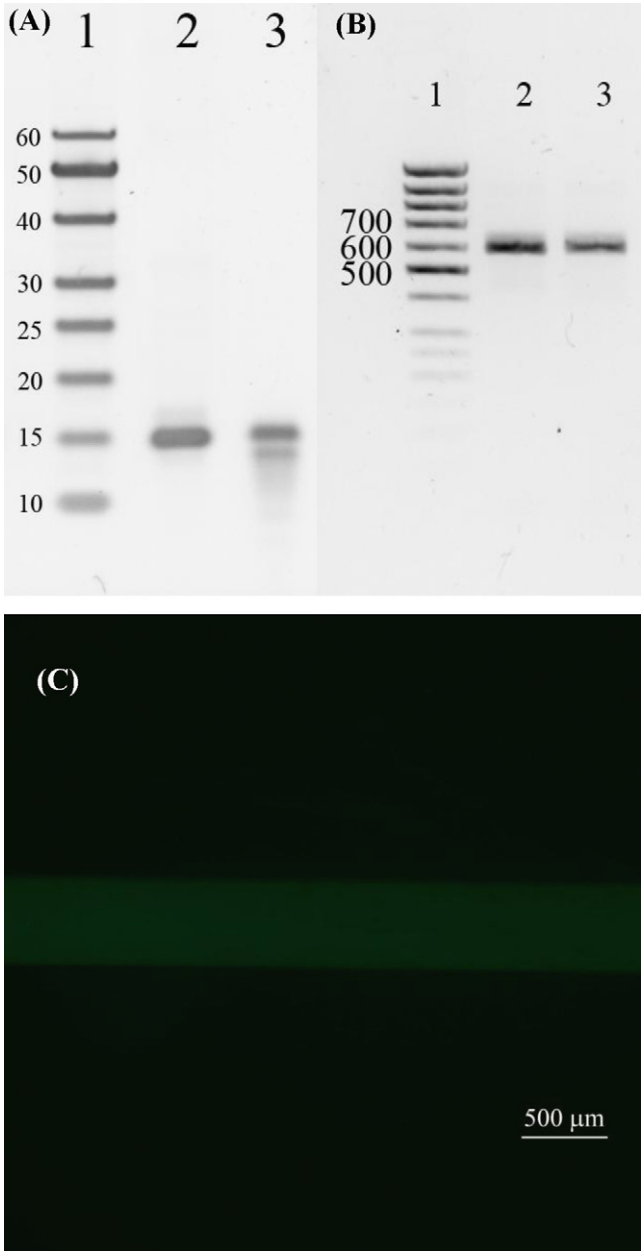

Fig. 3. (A) PAGE image for length confirmation of synthesized oligonucleotide primer. Lane 1: oligonucleotide ladder. Lane 2: HPLC purified oligonucleotide with the same sequence. Lane 3: oligonucleotide synthesized by the portable system. (B) Gel electrophoresis image of the PCR product. Lane 1: 50 bp DNA ladder. Lane 2: positive control with both forward and reverse primer purchased. Lane 3: PCR product amplified by using the synthesized oligonucleotide in our system as reverse primer. (C) Fluorescence image of synthesized oligonucleotide probe hybridizing with a 6-FAM labeled complementary oligonucleotide.

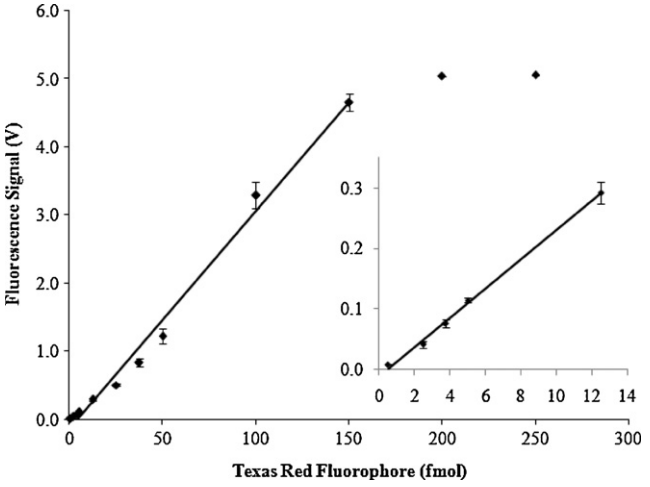

Fig. 4. Calibration curve for Texas Red fluorescence detection system. The lower range of the calibration curve (corresponding to amount of Texas Red fluorophore from 0.5 fmol to 12.5 fmol) is enlarged.

was the major product of the synthesis. The main impurity in the product is n-1 mer.

The synthesized 16mer oligonucleotide was then used as reverse primer in a PCR to amplify *E. coli* 16S ribosomal RNA. The PCR product had a length of 596 bp. The sequence of the forward primer "P2" is given in the experimental section. The gel electrophoresis image (Fig. 3B) shows that the target was successfully amplified (lane 3) when using the oligonucleotide synthesized by the portable synthesizer as a reverse primer. Therefore, the sequence and biological functionality of the synthesized oligonucleotide was successfully demonstrated.

3.1.2. Probe synthesis

To further test the function of the system, an oligonucleotide probe "P1" was synthesized on a surface modified glass slide. After synthesis, the probe was hybridized with a complementary target DNA "T1" labeled with 6-carboxyfluorescein (6-FAM). The fluorescence image in Fig. 3C shows that the complementary DNA was successfully hybridized at the channel region of the reactor to the synthesized oligonucleotide probe, which also confirmed the sequence and biofunctionality of the synthesized oligonucleotide. The function of the portable oligonucleotide synthesis system was confirmed by synthesizing oligonucleotides both on CPG as primer and on glass slide surface as probe. Their sequence and biofunctionality were successfully tested by PCR and DNA hybridization.

3.2. Fluorescence detection system

3.2.1. System characterization

To characterize the Texas Red fluorescence detection system, a serial dilution of Texas Red labeled oligonucleotide in water ranging from 1 nM to 500 nM was prepared. Texas Red was chosen because it is a commonly used fluorophore for labeling DNA. Also at the peak emission wavelength of Texas Red (620 nm), the photo sensitivity of the photodiode is higher (comparing with its sensitivity at peak emission wavelength of 6-FAM 520 nm). Then 0.5 μL of each dilution was spotted onto a glass slide (corresponding to 0.5 fmol to 250 fmol). As the size of the spot was smaller than the size of the excitation light spot, all fluorophores can be excited. Fig. 4 shows that the detection system can detect Texas Red fluorophores from 0.5 fmol to 150 fmol with good linearity (R^2 = 0.989).

The signal was saturated when the amount of fluorophore was above 200 fmol. It is worth noting that at the lower range (0.5–12.5 fmol), the linearity of the detection system was even better (R^2 = 0.996) and this is coincident with the range of fluorophores from DNA hybridization in this study (shown in the following). It was reported that the density of immobilized DNA probe on commercial ammine-reactive glass slides was above 10^{12} probes/cm^2 (Gong et al., 2006) and on thiol reactive glass $\sim 9 \times 10^{13}$ probes/cm^2 (Cheung et al., 2003). A rough estimate yields a maximum oligo probe density of about 170 fmol/mm^2 (the total exited surface area in the flow channel is 1 mm^2); however the real number might be much smaller.

3.2.2. Detection of complementary and single-base mismatch DNA hybridization

The fluorescence detection system was then tested by DNA hybridization to a surface immobilized probe. Two amine labeled oligonucleotides ("P3" and "P4") were immobilized on amine modified glass slide through disuccinimidyl suberate (DSS) linker. These two oligonucleotides had one base difference in the middle of the sequence. A Texas Red labeled oligonucleotide "T2" complementary to "P3" but with a single mismatch to "P4" was then hybridized to both immobilized probes. After washing, the hybridization was measured by the integrated fluorescence detection system. From the result of our system, the signal for complementary hybridization was 0.190 V and the signal for single-base mismatch was just 0.018 V. Both signals fell in the range where the system has higher linearity. The ratio of these two signals was 10.6, indicating that the fluorescence detection system successfully discriminate the fluorescence signal from complementary and single-base mismatch hybridization. For comparison, the fluorescence images (not shown) of the complementary and single-base mismatch hybridization were also taken (Qimaging CCD camera Retiga 4000R, and Olympus BX41 microscope) and the fluorescence intensity was measured. The ratio of the fluorescence intensity was 8.4, which was lower than the ratio of the signals measured by our system. This indicates that at this range, the performance of our fluorescence detection system was comparable to and even slightly higher than that of our microscope system.

3.3. Bacterial differentiation

The complete portable DNA bioassay instrument was used to differentiate two bacteria strains. Two bacteria strains that may cause foodborne illness from *Bacillus* spp. and *Salmonella* spp. were selected in this study (Chiang et al., 2006). 16S ribosomal RNA of these two strains was amplified by asymmetric PCR which produced more ssDNA for hybridization. The same primer pair was used to amplify 16S rRNA of the two strains ("16S-F" and "16S-R"). The reverse primer was labeled with Texas Red at 5′ end to generate the fluorescence signal for detection. Two probes, each was specific to one strain, were synthesized in the system. Each probe was then hybridized with both Texas Red labeled PCR product and the hybridization results were examined by the fluorescence detection system.

As shown in Fig. 5A, Texas Red labeled PCR products from two different bacteria strains hybridized only to the synthesized probe that was specific to its strain. The fluorescence signals from specific hybridization were much stronger than that from cross-hybridization. Therefore, these two bacteria strains were successfully differentiated by the portable instrument. It can also been seen that the fluorescence signals from specific hybridization were not as high as that from previous oligonucleotide-probe hybridization. This could be due to the fact that the PCR product had a length of 475mer and the length of the target DNA could affected hybridization efficiency (Liu et al., 2007) thus led to smaller signals. To determine the detection limit for Texas Red labeled PCR product, different amount of *Bacillus* PCR product was hybridized to the syn-

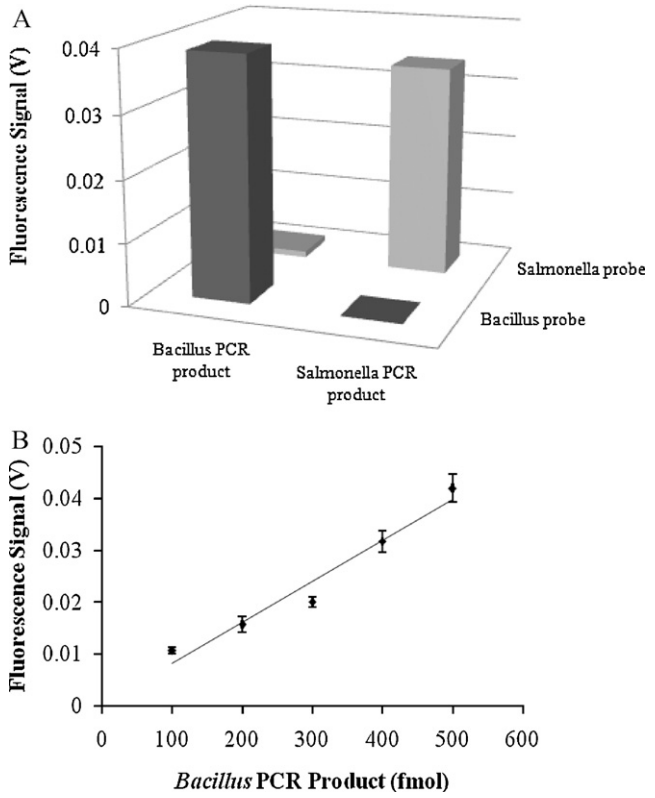

Fig. 5. (A) 3D bar graph showing the fluorescence signal from specific and cross-hybridization between PCR products of two different bacteria strains and synthesized strain specific probes. (B) Hybridization of different amount of *Bacillus* PCR product to the synthesized strain specific probe.

thesized probe and the fluorescence signal was measured (Fig. 5B). The extrapolated detection limit for *Bacillus* PCR product was found to be 97 fmol. Data from Figs. 4 and 5B and previous estimated probe surface densities suggest that only a very small percentage (<1%) of the PCR product (labeled via a Texas Red labeled primer) was actually hybridized to the synthesized oligo probes; this is understandable considering the large excess of the complementary PCR strand competing for the Texas Red labeled strand and the short hybridization times.

4. Conclusion

In this work, we have developed a portable and generic DNA bioassay system to detect any desired DNA target with known sequence. To the best of our knowledge, this was the first portable system developed for *in situ* synthesis of oligonucleotide and integrated detection of DNA targets by hybridization to the synthesized probe oligonucleotide. The core part of the oligonucleotide synthesizer is a specially designed microvalves integrated microfluidic chip aiming to minimize the cross-contamination among synthesis reagents. The microfluidic synthesizer is easy to operate and capable of synthesizing any sequence on demand. The Texas Red fluorescence detection device based on a LED excitation light source and a photodiode detector was integrated into the system. The fluorescence detector can detect the Texas Red fluorophore at amounts down to 0.5 fmol and the detection limit for a typical PCR product was 97 fmol. The system could discriminate complementary and single-base mismatch hybridization. Furthermore, different bacteria strains were successfully distinguished by synthesizing strain specific probes and detecting the hybridization signals within the portable instrument. The total time from remote oligonucleotide sequence submission to the system, oligo synthesis and detection of a target DNA was 6.5 h. The system presented in this work has the potential to detect any DNA sequence in-the-field. We envisage that our system could help to enable fast responses to emerging bio-threats for homeland security and in pandemics.

Acknowledgements

This work was fully supported by the Defence Research and Technology Office of the Singapore Ministry of Defence (POD0412401). The authors thank Dr. Shakil Rehman for his help on designing the fluorescence detection device.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.10.028.

References

Cheung, M.K.L., Trau, D., Yeung, K.L., Carles, M., Sucher, N., 2003. Langmuir 19, 5846–5850.
Chiang, Y.C., Yang, C.Y., Li, C., Ho, Y.C., Lin, C.K., Tsen, H.-Y., 2006. Int. J. Food Microbiol. 107 (2), 131–137.
Gait, M.J. (Ed.), 1984. Oligonucleotide Synthesis: A Practical Approach. IRL Press, Oxford.
Gong, P., Harbers, G.M., Grainger, D.W., 2006. Anal. Chem. 78 (7), 2342–2351.
Heller, M.J., 2002. Annu. Rev. Biomed. Eng. 4, 129–153.
Huang, Y., Castrataroab, P., Leeac, C.-C., Quake, S.R., 2007. Lab Chip 7, 24–26.
Ivnitski, D., O'Neil, D.J., Gattuso, A., Schlicht, R., Calidonna, M., Fisher, R., 2003. Biotechniques 35 (4), 862–869.
Kricka, L., Wilding, P., 2003. Anal. Bioanal. Chem. 377 (5), 820–825.
Lee, C.-C., Snyder, T.M., Quake, S.R., 2010. Nucleic Acids Res. 38 (8), 2514–2521.
Lee, J.-H., Kaplan, J., Lee, W., 2008. Biomed. Microdevices 10 (4), 489–498.
Lee, J.N., Park, C., Whitesides, G.M., 2003. Anal. Chem. 75 (23), 6544–6554.
Letsinger, R.L., 1988. Nucleoside Trichlorodimethylethyl Posphite Esters. Northwestern University, Evanston, IL, United States.
Li, J., Xu, C., Zhang, Z., Wang, Y., Peng, H., Lu, Z., Chan, M., 2005. Sens. Actuators B 106 (1), 378–382.
Liu, W.-T., Guo, H., Wu, J.-H., 2007. Appl. Environ. Microbiol. 73 (1), 73–82.
Moorcroft, M.J., Meuleman, W.R.A., Latham, S.G., Nicholls, T.J., Egeland, R.D., Southern, E.M., 2005. Nucleic Acids Res. 33 (8), e75.
Neuzil, P., Zhang, C., Pipper, J., Oh, S., Zhuo, L., 2006. Nucleic Acids Res. 34 (11), e77.
Novak, L., Neuzil, P., Pipper, J., Zhang, Y., Lee, S., 2006. Lab Chip 7, 27–29.
Stoughton, R.B., 2005. Annu. Rev. Biochem. 74, 53–82.
Unger, M.A., Chou, H.-P., Thorsen, T., Scherer, A., Quake, S.R., 2000. Science 288 (5463), 113–116.
Vieider, C., Ohman, O., Elderstig, H., 1995. The 8th International Conference on Solid-State Sensors and Actuators, 1995 and Eurosensors IX. Transducers '95, pp. 284–286.
Wang, C.-H., Lee, G.-B., 2005. Biosens. Bioelectron. 21, 419–425.
Yang, F.-B., Pan, J.-Z., Zhang, T., Fang, Q., 2009. Talanta 78 (3), 1155–1158.