A FIELD-DEPLOYABLE SYSTEM FOR AUTOMATED MOLECULAR TESTING USING MODULAR MICROFLUDICS

M. L. Hupert,[1] H. Wang,[1] H.-W. Chen,[1] W. Stryjewski,[2] D. Patterson,[1,2] M. A. Witek,[1] P. Datta,[1] J. Goettert,[1] M. C. Murphy,[1] and S. A. Soper[1,2]

[1]Louisiana State University, USA; [2]BioFluidica Microtechnologies, USA

ABSTRACT

We describe the development of a fully integrated, modular microfluidic system for molecular analyses. The processing pipeline was carried out in a 3-D microfluidic system and included cell lysis, solid-phase extraction (SPE) of DNA, continuous-flow polymerase chain reaction (CFPCR), continuous-flow ligase detection reaction (CFLDR), and zip-code array detection. Chip operation was provided by electronic and hydraulic controls located off-chip. Optical detection of array events was achieved with a fluorescence reader consisting of a VCSEL and CCD. As an example, the detection of *E. coli* O157:H7 and *Salmonella* is presented.

KEYWORDS: integrated system, modular microfluidics, molecular testing

INTRODUCTION

Portable and autonomous systems with full integration of the sample processing pipeline are of particular interest for point-of-use applications, such as pathogen detection, forensics, and personalized medicine. Microfluidic-enabled systems are a promising approach as they allow rapid molecular processing, provide automated sample handling and reagent delivery, and minimize the possibility of contamination by using enclosed architectures [1]. In order to avoid sample-to-sample cross-contamination and abide to the requirements of sensitive applications, it is essential that microfluidic chips are produced as low-cost disposable units.

Integrated microfluidic systems are typically made of glass, silicon or glass-polymer hybrids. They incorporate most of the sample handling components (valves, pumps, HV electrodes, heaters, optical detectors) on the chip itself [1]. This strategy leads to rather complicated fabrication processes and as such makes the chips too expensive for realizing one-time use applications. In the system presented herein, we used a modular architecture realized through polymers and operated with a compact system incorporating all of the peripherals such as pumps, valves, heaters, and the optical reader. The module materials were optimized to match task specifications.

EXPERIMENTAL

Double-sided hot-embossing using high-precision micromilled brass mold masters was used for the one step fabrication of polymer modules (polycarbonate (PC) and poly(methyl methacrylate) (PMMA)), possessing both microfluidic networks and structural elements such as thermal separation groves, interconnection ports, and an optical waveguide. After embossing, selected areas of the chip were exposed to UV-light for activation of the SPE bed and oxygen plasma for microarray

978-0-9798064-1-4/μTAS2008/$20©2008CBMS

spotting [2-4]. A non-contact spotting system was used for fabricating the micro-arrays [4]. PC and PMMA modules were assembled using thermal fusion bonding.

All peripherals were assembled using customized components or parts made in-house. Control and data acquisition electronics were designed and assembled in-house (see Fig. 1). Instrument control software was written in-house.

RESULTS AND DISCUSSION

The microfluidic manifold was manufactured as a stack of 2 flow-through modules. A PC module was used for sample and reagent loading, cell lysis, SPE, CFPCR, and CFLDR. A PMMA module was used for detection of the ligation products using microarray readout (Fig. 2 A, B). Sample transport between modules was provided by fluidic interconnects made of Tefzel™ tubing inserted into conical ports in the backsides of the modules. This interconnect provided low dead volume, worked as a self aligning feature and could withstand pressures in excess of 600 psi.

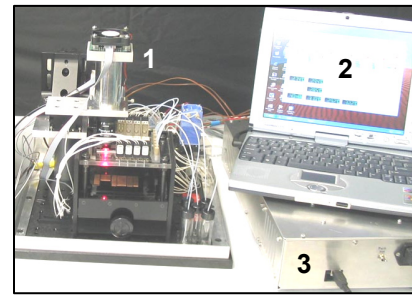

Figure 1. Instrument for controlling on-chip operations; 1 – hardware, 2 – electronic control and data collection unit, 3 – computer with instrument control software.

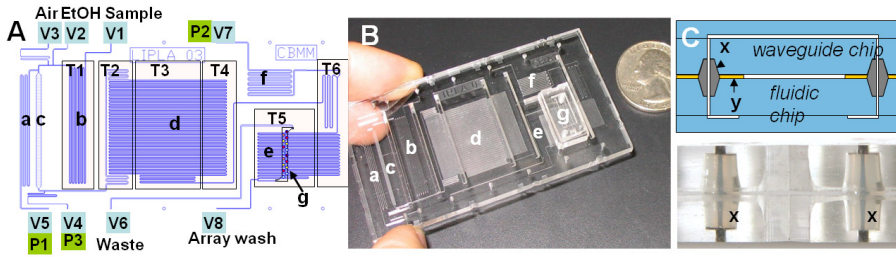

Figure 2. (A) Modular microfluidics and peripheral connection layout: a) PCR mix storage(PC); b) thermal cell lysis (PC); c) SPE of DNA (PC); d) 32 cycles of CFPCR (PC); e) 15 cycles of CFLDR (PC); f) LDR mix storage; g) DNA microarray module (PMMA). T1 – thermal lysis, T2, T3,T4 – PCR denaturation, extension, annealing, T5, T6 – LDR ligation and denaturation; V1 – V8 solenoid micro-valves; P1, P2 – stepper motor driven micropumps, P3 – vacuum pump, (B) assembled modular microfluidic stack; (C) Chip-to-chip fluidic interconnects, schematic (top), optical micrograph (bottom); x – connecting tube inside a cone-shaped port; y – adhesive. Channels were filled with black dye for visualization.

Prior to operation, sample and reagents were dispensed into the modules. The modules were inserted into the instrument and locked down to provide fluidic communication with off-chip micro-pumps and micro-valves, thermal control of biochemical reactions and alignment of the microarray module with the detection system. The sample was processed through consecutive steps in a continuous flow (CF) format. Individual CF units have previously been demonstrated by our group as a rapid method for DNA amplification using PCR with rates approaching 5.2 s per cycle [5]. Step timing was preset and regulated with instrument control software.

Optical detection of the array elements was achieved with a fluorescence reader consisting of a VCSEL and CCD. A VCSEL-coupling prism and air-embedded waveguide was integrated into the PMMA module and situated along the fluidic channel, which used evanescent wave excitation of the array (Fig. 3) [4].

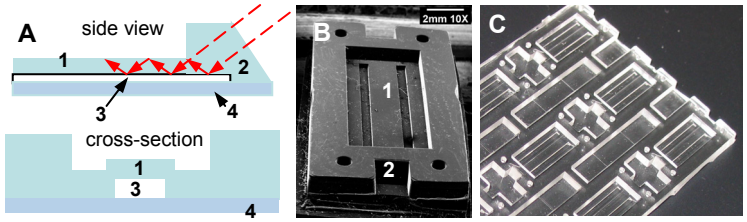

Figure 3. (A) Schematic of a microarray unit; 1- integrated waveguide; 2- coupling prism; 3 – microfluidic channel; 4 – coverplate. (B) SEM of single microarray chip. (C) Multiple DNA microarray modules embossed on a PMMA wafer.

As an example, we have processed a sample consisting of *E.coli O157:H7 (eaeA gene)* and *Salmonella (sipB/C gene)* cells – both pathogens are of significant interest in food safety. The total processing time of this sample was only ~45 min (Fig. 4).

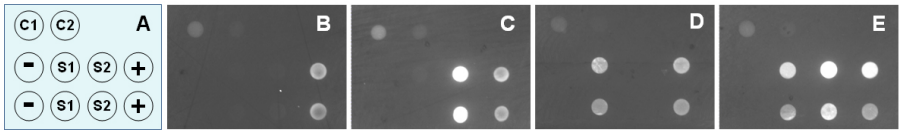

Figure 4. Results of pathogen detection; C1, C2: 20uM and 10uM Cy5-(T)10-NH_2 Spotting and immobilization control; - - negative control; S1- probe targeting E.Coli O157:H7 eaeA gene; S2 - Probe targeting Salmonella sipB/C gene; + - hybridization positive control.

CONCLUSIONS

We have designed, fabricated and tested a portable modular microfluidic system for automated molecular testing. The unique feature of this system is that it can be easily reconfigured for other molecular assays, such as expression profiling or DNA forensics, by simply exchanging the appropriate fluidic modules only.

ACKNOWLEDGMENTS

Funding: NIH (EB0002115); NSF (EPS-0346411); Louisiana Board of Regents.

REFERENCES

[1] Easley, C.; Karlinsey, J.; Bienvenue, J.; Legendre, L.; Roper, M.; Feldman, D.; Hughes, M.; Hewlett, E.; Merkel, T.; Ferrance, J.; Landers, J.; *PNAS* **2006;** 103 (51), 19272

[2] Hupert, M; Guy, J.; Llopis, S.; Shadpour, H.; Rani, S.; Nikitopoulos, D.; Soper, S.; *Microfluid. Nanofluid.* **2007**; 3; 1

[3] Witek, M.; Llopis, S.; Wheatley, A.; McCarley, R.; Soper, S.; *NAR* **2006**, 34(10), e74/1

[4] Xu, F.; Datta, P.; Wang, H.; Gurung, S.; Hashimoto, M.;Wei, S.; Goettert, J.; McCarley, R.; Soper, S.; *Anal. Chem.* **2007**; 79(23); 9007

[5] Hashimoto, M.; Chen, P.-C.; Mitchell, M.; Nikitopoulos, D.; Soper, S.; Murphy' M.; *Lab Chip* **2004**, 4, 638