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Anal. Chem. **2006,** *78,* 1980−1986

Fully Integrated Miniature Device for Automated Gene Expression DNA Microarray Processing

Robin Hui Liu,* Tai Nguyen, Kevin Schwarzkopf, H. Sho Fuji, Alla Petrova, Tony Siuda, Kia Peyvan, Michael Bizak, David Danley, and Andy McShea

CombiMatrix Corporation, 6500 Harbor Heights Parkway, Mukilteo, Washington 98275

A DNA microarray with 12 000 features was integrated with a microfluidic cartridge to automate the fluidic handling steps required to carry out a gene expression study of the human leukemia cell line (K562). The fully integrated microfluidic device consists of microfluidic pumps/mixers, fluid channels, reagent chambers, and a DNA microarray silicon chip. Microarray hybridization and subsequent fluidic handling and reactions (including a number of washing and labeling steps) were performed in this fully automated and miniature device before fluorescent image scanning of the microarray chip. Electrochemical micropumps were integrated into the cartridge to provide pumping of liquid solutions. The device was completely self-contained: no external pressure sources, fluid storage, mechanical pumps, mixers, or valves were necessary for fluid manipulation, thus eliminating possible sample contamination and simplifying device operation. Fluidic experiments were performed to study the on-chip washing efficiency and uniformity. A single-color transcriptional analysis of K562 cells with a series of calibration controls (spiked-in controls) to characterize this new platform with regard to sensitivity, specificity, and dynamic range was performed. The device detected sample RNAs with a concentration as low as 0.375 pM. Experiment also showed that the performance of the integrated microfluidic device is comparable with the conventional hybridization chambers with manual operations, indicating that the on-chip fluidic handling (washing and reaction) is highly efficient and can be automated with no loss of performance. The device provides a cost-effective solution to eliminate labor-intensive and time-consuming fluidic handling steps in genomic analysis.

Microarrays have become a widely used technology for studying mRNA levels and examining gene expression in biological samples. Investigators rely on data produced by microarray experiments to assess changes in gene expression levels among various experimental tissues and treatments. The applications of microarrays for gene expression profiling[1] include pathway dissection,[2] drug evaluation,[3,4] discovery of gene function,[5] classification of clinical samples,[6−8] and investigation of splicing events,[9] among many others.[10] The highly parallel nature of microarrays has made them invaluable tools for monitoring gene expression patterns of numerous genes simultaneously. Biological experiments have a number of inherent variables making it imperative that the microarray platform be extremely reproducible, both to provide confidence in the data collected and to accurately identify small changes in gene expression patterns. Because the most interesting genes are often expressed at the lowest levels in the sample, it is equally important to use a highly sensitive microarray system.

There are various microarray technologies and numerous commercially available sources of microarrays. Microarrays can be produced either by physical deposition of presynthesized DNA [1,11,12] or by in situ oligonucleotide synthesis.[13,14] The former requires labor-intensive preparation (and, hence, very significant

* To whom correspondence should be addressed. E-mail: rliu@combimatrix.com.

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10.1021/ac0518553 CCC: $33.50 © 2006 American Chemical Society
Published on Web 02/03/2006

upfront cost) and record-keeping of DNA probes, whereas the latter only requires DNA sequence design. There are several methods for in situ oligonucleotide synthesis. The most commercially successful method is using a photolithographic method; [15] however, this method, similar to deposition of presynthesized material, lacks flexibility and has to be designed with specific gene content due to high cost and lengthy time spent in generating the photolithographic masks. For those microarray users whose work does not fit in the catalog arrays, it can be a very costly and time-consuming process to adopt the usefulness of microarray technology to their specific research needs. Other in situ oligonucleotide synthesis technologies, such as ink-jet printing,[16] micromirror devices,[17,18] and electrochemical synthesis,[19−21] are more flexible and, thus, suitable for manufacturing customized oligonucleotide arrays. We have utilized a semiconductor-based, in situ synthesis technology that is straightforward to manufacture microarrays and has the potential to sense hybridization electrochemically (in addition to established fluorescent methods).[19,22] The oligonucleotides are synthesized on an array of electrodes on a semiconductor chip using phosphoramidite chemistry under electrochemical control. The electrochemical reaction activated at specific electrodes on the chip generates protons, which in turn remove the blocking group in a membrane coated on the electrodes, allowing subsequent DNA synthesis to take place.

Gene expression assays often involve multistage sample processing and fluidic handling, which are generally labor-intensive and time-consuming. Following hybridization of target biotinylated cRNA in the sample solution to its complementary oligonucleotides synthesized on the microarray chip surface, the array needs to be washed thoroughly to remove nonspecific binding of target. Different salt concentrations of washing buffers are used to ensure satisfactory stringency. For indirect labeling, a labeling step is subsequently performed. Another washing is performed to remove excessive labeling reagents before the slide is ready for scanning. Processing microarrays typically employs a conglomeration of dishes, hot plates, thermometers, and waterbaths. All the above processes involve many manual steps (handling arrays, moving, and agitating racks etc.) with frequent run-to-run and operator-to-operator variation. The combination of these factors can lead to variability in array results. Alternatively, robotic workstations have been developed to automate the whole hybridization and post-hybridization process, but such benchtop instruments are generally expensive for most research and clinical diagnostic applications. It is, therefore, desirable to develop a cost-effective method to integrate and automate the microarray processing in a single and miniature device using microfluidic technology.

Microfluidics has proven to be a powerful technology for integrated, high-throughput DNA analysis.[23] Most of the work has been directed toward the microfluidic on-chip capillary electrophoresis (CE),[24−27] and there are only a few reports on combining microfluidics with DNA microarrays. Anderson et al. have reported an integrated system that performed RNA purification from a serum lysate, followed by PCR, serial enzymatic reactions, and nucleic acid hybridization.[28] Yuen et al. reported a microchip module design for blood sample preparation (white blood cell isolation), PCR, and DNA microarray analysis.[29] Most recently, microfluidics has been integrated with DNA microarray to perform direct sample-to-answer DNA analysis that starts from sample preparation (cell capture, preconcentration, and purification), followed by PCR and microarray electrochemical-based detection.[30] Similar microfluidic technology can be applied to automate hybridization and post-hybridization processes for gene expression assays that often involve multistage sample processing and fluidic handling. Using microfluidic technology to integrate and automate all the manual steps in a single chip device is highly desirable in many practical applications, such as clinical diagnostics.

In this paper, we report on the development of a self-contained and fully integrated microfluidic device that automates hybridization and post-hybridization processes for gene expression analysis. The device consists of a CombiMatrix CustomArray slide and a microfluidic plastic cartridge. The description of the semiconductor-based CustomArray platform is included. The integrated device design, fluidics, and developments of the key microfluidic components, such as pumps, are described. Hybridization experiment results using spiked-in control transcripts in a complex biological sample are presented.

EXPERIMENTAL SECTION

DNA Microarray. The biochip device (Figure 1) consists of a CombiMatrix CustomArray chip and a microfluidic plastic cartridge. The CustomArray is a 1 in. × 3 in. alumina slide with an 11 × 25 mm silicon chip affixed in a cavity in the ceramic package. The CombiMatrix microarray technology platform is a semiconductor-based chip that allows the manufacture of oligonucleotide arrays using electrochemical control. Utilization of active circuit elements in the design permits the selection and parallel activation of individual electrodes in the array to perform in situ oligonucleotide synthesis of customized content on the chip.

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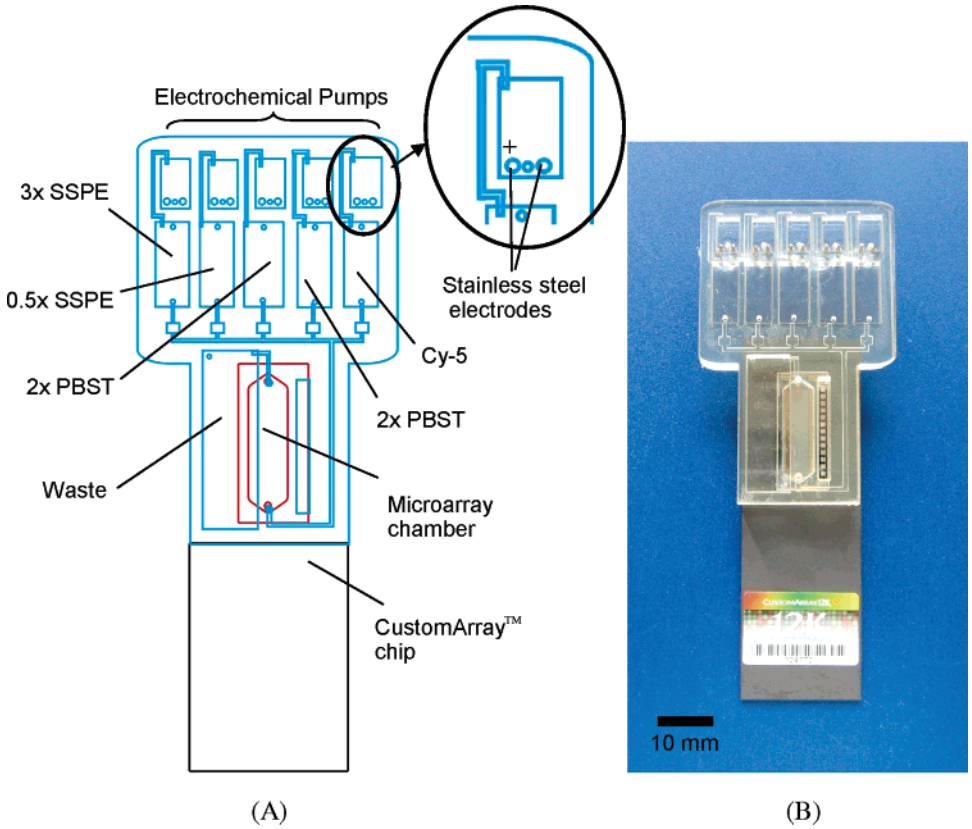

Figure 1. (A) Schematic of the microfluidic biochip device with a blowup diagram showing an electrochemical pump that has two stainless steel electrodes inserted from the backside of the cartridge. (B) Photograph of the integrated device that consists of a plastic fluidic cartridge and a CombiMatrix CustomArray chip.

The CombiMatrix microarray chip is a silicon integrated circuit manufactured using a commercial mixed signal complementary metal oxide semiconductor (CMOS) process. The microarray chip used in this work has 12 544 electrodes, each 44 μm in diameter. The CMOS integrated circuit technology creates active circuit elements and digital logic on the chip that allows complex functions to be implemented. These include a high-speed digital interface to communicate to the chip, data writing and reading from the electrode array, and the setting of appropriate electrical conditions at the electrode to perform in situ oligonucleotide synthesis. Figure 2 illustrates the architecture and layout of the CombiMatrix CustomArray 12k chip. This design utilizes a Serial Peripheral Interface (SPI) interface to minimize the number of external electrical connection required to communicate to the chip. A 56 $\times$ 224 array of electrodes is located in the center of the chip, providing a total of 12 544 spots for the generation of oligonucleotide probes. Each electrode is fabricated within a unit cell of circuit elements that allows precise control of the electrical characteristics of the electrode. Figure 2B shows a magnified image of the unit cell and associated electrode. All the electrodes on the chip are individually addressable so that unique reactions may be carried out at each individual site.

To determine the sensitivity, reproducibility, and dynamic range characteristics of microfluidic microarray devices, a system of spiked-in controls was developed using biotin-labeled cRNA transcripts generated from segments of the *Escherichia coli* (*E. coli*) bacteriophage lambda genome (no. NC_001416). The array was designed with probes directed to the spiked-in control transcripts, as well as a variety of genes expressed by the K-562 leukemia cell line. Probes were created against various genes involved with immune system pathways, as well as a number of housekeeping genes. In addition, multiple probes were designed against segments of the Phage Lambda genome. The microarray was designed with four replicates of each probe distributed across the array to allow measurement of the variability within the array.

Prior to in situ synthesis, the chip surface was coated with a proprietary membrane layer that facilitated the attachment and synthesis of biomolecules in a matrix above the electrode surface. This reaction layer containing free OH groups on a carbon backbone was used to tether the newly synthesized oligonucleotide to the area above the platinum electrode. The custom oligonucleotide arrays were synthesized on the chip using phosphoramidite chemistry under electrochemical control.[19,22] During DNA synthesis, the blocking DMT (dimethoxytrityl) group of the phosphoramidite on the chip surface was removed by turning on selective electrodes; only those electrodes turned on lost the DMT group in the presence of acid (H^+) that is produced by the electrochemical reaction.[22] An activated nucleotide reagent was introduced, and it reacted with the free hydroxyl groups in these solution conditions. The chip was washed, followed by capping and then an oxidation step to stabilize the central phosphorus atom. The process continued with deprotection of certain electrodes and a coupling step. Using this in situ synthesis method, unique oligomers of 35−40 bases were synthesized at each electrode. After the electrochemical synthesis process, chips were deprotected in 50:50 ethanol/ethylenediamene at 65 °C for 1 h to remove benzoyl protecting groups, then washed in ethanol and distilled water.

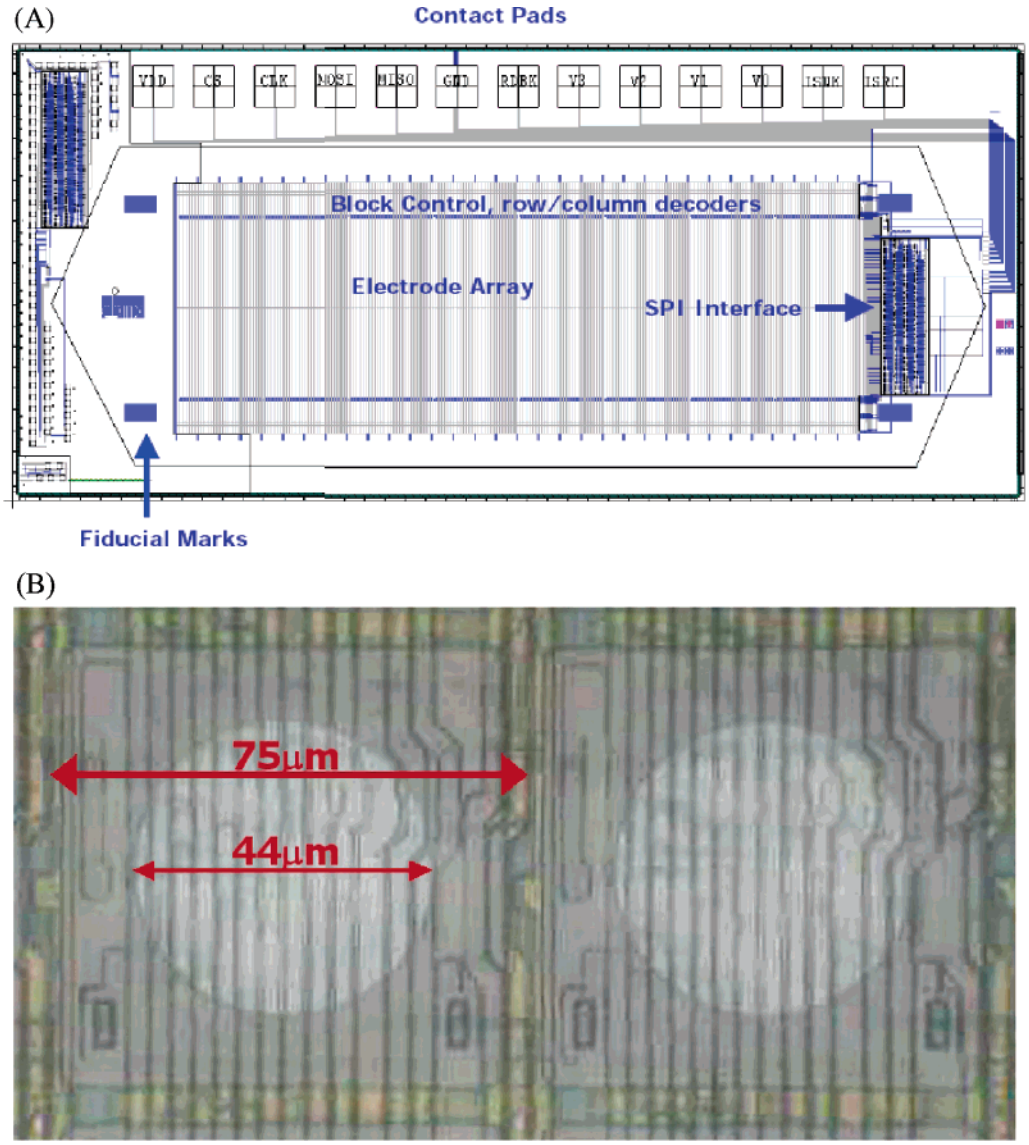

Figure 2. (A) Chip architecture of CustomArray chip that has >12 000 features. (B) Optical micrograph of microarray unit cells showing two associated Pt electrodes (in white). The diameter of the circular Pt electrode is 44 μm.

Microfluidic Cartridge. The plastic cartridge included five micropumps, five chambers for the storage of different buffers and reagents, a hybridization chamber, and a waste chamber, as shown in Figure 1. The operation of the microfluidic device is as follows. A sample solution containing 4 μg of biotin-labeled cRNA transcripts in DI Formamide-based buffer was loaded in the hybridization chamber using a pipet. Other solutions, including four wash buffer solutions and a labeling solution, were separately loaded in different storage chambers. The biochip device was then inserted into an instrument, which provided hybridization heating, temperature sensing, and electrical power for liquid pumping. The hybridization chamber of the device was physically pressed against a thin-film heating element (Minco Corp., Minneapolis, MN) using a spring-load manifold. The on-chip assay process started with an 18-h hybridization step in the microarray hybridization chamber at 45 °C, followed by a three-step, on-chip washing process. During the washing process, the nonstringent 3× SSPE (saline/sodium phosphate/EDTA) washing buffer was first pumped through the hybridization chamber, removing the sample mixture into the waste chamber and washing the array chip. The pumping was performed using an integrated electrochemical micropump that operated with a DC current of 8.6 mA. The stringent 0.5× SSPE washing buffer, followed by the 2× PBST (phosphate/buffered saline/Tween) buffer, was subsequently pumped through the hybridization chamber to ensure a thorough washing and removal of nonspecific binding. After the on-chip washing steps, the labeling solution containing streptavidin−Cy5 was pumped into the hybridization chamber, followed by a 30-minute incubation at room temperature to fluorescence-stain the hybridized RNA. Once the labeling was completed, the 2× PBST buffer was pumped through the hybridization chamber to ensure a thorough washing and removal of residual labeling reagents. The device was then removed from the instrument. The microarray chip was detached from the microfluidic plastic cartridge before it was scanned using a commercial fluorescent scanner. The fluorescent hybridization signals corresponding to the gene expression information of the biological sample were detected on the chip and analyzed.

The plastic cartridge measures 40 × 64 × 7.5 mm and has channels and chambers that range from 500 μm to 3.2 mm in depth and 0.5 to 8.5 mm in width. The prototype of the plastic

cartridge consists of multiple layers of acrylic materials that are laminated and assembled using double-sided adhesive tapes. All the layers, including five layers of acrylic sheets with various thicknesses ranging from 0.5 to 3.2 mm (MacMaster-Carr, Atlanta, GA) and four layers of double-sided adhesive tapes (Adhesive Research Inc., Glen Rock, PA), were machined using a CO_2 laser machine (Universal Laser Systems, Scottsdale, AZ). After assembly, stainless steel wires with 0.5-mm diameter were inserted into the electrochemical pumping chambers, each of which was then loaded with 50 μL of 1 M Na_2SO_4 solution to form an electrochemical pump. The electrolyte loading holes were subsequently sealed using an adhesive tape (Adhesive Research Inc., Glen Rock, PA). The venting hole of the waste chamber was sealed with a hydrophobic membrane vent (Sealing Devices, Lancaster, NY) that allows gas molecules to pass through while the liquid solution is retained in the waste chamber. The plastic cartridge was then bonded with the microarray chip using double-sided adhesive tape (Adhesive Research Inc., Glen Rock, PA).

Gene Expression Assay. Complex background sample was prepared from human leukemia, chronic myelogenous (K-562 cell line) poly A+ RNA (Ambion, Austin, TX) utilizing Ambion's MessageAmp aRNA Kit. Biotin was double incorporated using biotin-11-CTP (Perkin-Elmer, Boston, MA) and biotin-16-UTP (Roche Diagnostics, Mannheim, Germany). Varying concentrations of spiked-in biotin/cRNA control transcripts were combined with a constant amount (150 nM) of K-562 biotin/cRNA complex background such that the final concentration of spiked-in control transcripts would range from 0.375 to 12 pM in the hybridization. The biotin/cRNA mixtures were fragmented in a 1× fragmentation solution (40 mM Tris−acetate, pH8.1, 100 mM KOAc, 30 mM MgOAc) at 95 °C for 20 min. The fragmented cRNA sample was added to a hybridization solution (6× SSPE, 0.05% Tween-20, 20 mM EDTA, 25% DI formamide, 0.05% SDS, 100 ng/uL sonicated salmon sperm DNA) and denatured for 3 min at 95 °C. The sample was placed briefly on ice, followed by centrifugation at 13 000*g* for 3 min. During the on-chip assay, 95 μL of the hybridization sample solution was loaded into the hybridization chamber. Other solutions, including (1) 200 μL of 3× SSPE, 0.05% Tween-20; (2) 200 μL of 0.5× SSPE, 0.05% Tween-20; (3) 200 μL of 2× PBST, 0.1% Tween-20; (4) 200 μL of a labeling solution; and (5) 200 μL of 2× PBST, 0.1% Tween-20, were separately loaded into the storage chambers. The labeling solution contains streptavidin−Cy5 (Molecular Probes, Eugene, OR) that was diluted in a blocking solution (2× PBS, 0.1% Tween-20, 1% acetylated BSA) to a final concentration of 1 μg/mL. Hybridization was carried out for 18 h at 45 °C.

Following hybridization, the array in the hybridization chamber was washed for 2 min with 3× SSPE, 0.05% Tween-20. On-chip washings continued with 0.5× SSPE, 0.05% Tween-20 for 2 min and 2× PBST, 0.1% Tween-20 for 2 min. The labeling solution was then pumped into the hybridization chamber and incubated for 30 min at room temperature. Note that the cartridge was protected from light using an external cover on the instrument to prevent photobleaching. The final washing step was performed by flowing 2× PBST through the hybridization chamber. Subsequently, the cartridge was separated from the microarray chip with the use of a razor blade. The microarray chip was imaged on an Axon Instruments (Union City, CA) GenePix 4000B, 5-μm resolution laser scanner. Imaging was performed while the array was wet with 2× PBST under a LifterSlip glass cover slip (Erie Scientific, Portsmouth, NH). Probe fluorescence on the microarray was analyzed and quantified using Microarray Imager software (CombiMatrix Corp., Mukilteo, WA).

For the purpose of comparison, a gene expression assay with the same protocol was performed in a conventional hybridization chamber that has no integrated microfluidic components. All the fluidic handling and processes were carried out manually using pipets, and the sequence and composition of the buffers for manual processing were identical to those used for the automated microfluidic processing. For manual processing, each washing step started with emptying the hybridization chamber using a pipet, followed by loading a wash buffer into the chamber using another pipet. The hybridization was performed in an oven for 18 h at 45 °C.

RESULTS AND DISCUSSION

Fluidics. The fluidic architecture was designed in a simple way so that all the complex microfluidic components, such as microvalves and micromixers, were eliminated from the design, while ensuring the device could still perform basic functions of fluidic handling and reactions. Liquid solutions were retained in the storage chambers by the use of surface tension. Air plugs in the microchannels between two adjacent chambers prevented cross talk of the solutions. During operation, the device was placed vertically to take advantage of the fluid gravity to remove the air bubbles from the system without the use of porous hydrophobic vents.[28] Since the hybridization chamber was designed with a depth of 600 μm and a width of 6.5 mm and fluid volume was on the order of tens of microliters, the Reynolds number for the fluid flow was <10. Fluid gravity played an important role in fluidics in the hybridization chamber when the chamber was placed vertically. In this chamber, where the liquid solutions and gas bubbles enter from the lower portion, buoyant force allowed gas bubbles to travel quickly to the upper portion of the chamber, leaving the chamber bubble-free. Moreover, the electrochemical micropumps were used to continuously generate gas bubbles that flowed through the chamber during the washing and labeling steps. This bubbling effect enhanced the mixing in the chamber to achieve homogeneous solutions and facilitated a uniform reaction on the array surface. The use of gas bubbles to enhance mixing in the microfluidic chamber proves to be a simple but effective micromixing technique without the use of any external actuation methods, such as acoustic agitation[30] or physical rotation. The hydrophobic membrane vent that was used to seal the venting hole of the waste chamber retained the liquid waste solution in the waste chamber while allowing gas molecules to pass through into the atmosphere. However, the membrane vent failed to prevent the evaporation of the sample solution during hybridization, which resulted in drying a portion of the array and loss of hybridization stringency (likely due to an increase of the salt concentration in the hybridization solution and nonspecific adhesion of the DNA to the array). To prevent the sample solution loss due to the evaporation, we increased the humidity surrounding the device during hybridization process. We are currently investigating the implementation of integrated microvalves in the cartridge to prevent the evaporation.

Micropumps. The device required single-use, low-cost but efficient micropumps to transport liquid solutions with volume of hundreds of microliters. Most traditional pressure-driven membrane-actuated micropumps[31−33] did not meet the requirements, since they generally suffer from complicated designs and fabrication and high cost. In our device, integrated electrochemical pumps that rely on electrolysis of water between two electrodes in an electrolyte solution (1 M Na_2SO_4) to generate gases when a DC current was applied were used. Electrolysis-based pumping techniques that used the generated gases to displace fluids have been previously demonstrated.[30,34−36] In our device, stainless steel wires instead of platinum wires were used as electrodes, resulting in a reduced cost of the device. Note that the Na_2SO_4 solution was not allowed to come into contact with the array chip to prevent contamination of the hybridization with electrode breakdown products.

Gas (H_2 and O_2) generated from the electrochemical pumps moved liquid solutions from chamber to chamber in the device. The pumps were also excellent sources to provide gas bubbles to enhance micromixing in the hybridization chamber during the washing and labeling steps. Flow experiments demonstrated that the pumping flowrate, Q (μL/min), ranging from 5.5 to 100 μL/min, was in linear proportion with the DC current, i (mA), ranging from 0.43 to 8.6 mA. Six data points were used to determine the linear regression model. Each data point is the mean value obtained from four pumping rate measurements with identical DC current. The data fitted into the linear least squares regression equation $Q = 11.727i - 0.0854$, with correlation coefficient (R^2) of 0.9993. The pumping rate used in the gene expression assay was 100 μL/min. It was observed that a yellow product was generated in the electrolyte solution during the electrolysis reaction, indicating that the stainless steel corroded. The corrosion did not pose any problem, since the whole cartridge was disposed after use. This pumping mechanism did not require a membrane or check valves in the design. As a result, the fabrication and operation were simpler than most conventional micropumps.

Gene Expression Assay. To assess the sensitivity and dynamic range of the microfluidic microarray platform on a mass ratio basis, without the influence of RNA amplification techniques, spiked-in control transcripts were combined with a constant concentration of complex background cRNA (generated from the K-562 cell line) just prior to the hybridization. Spiked-in control transcripts were added to a complex background of K562 biotin-cRNA at six different concentrations ranging from 0.375 to 12 pM. The use of spiked-in controls and their importance in benchmarking and standardization of microarray experiments are a subject of considerable effort in the microarray community. In determining the cutoff for sensitivity, signal was considered significant if greater than three standard deviations above the average of the negative control signals.

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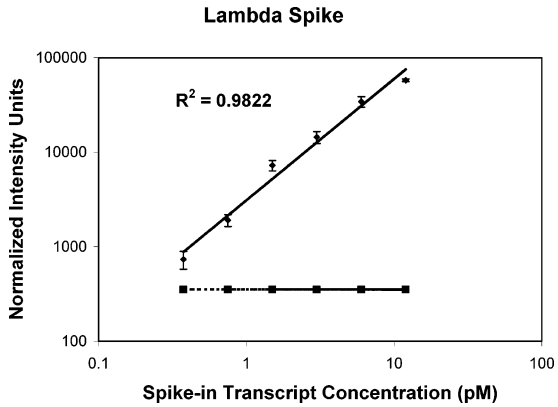

Figure 3. Hybridization analysis demonstrates the detection limit and linear dynamic range of the microfluidic array in log scale. Each data point represents the mean of the normalized probe intensities for the spiked-in control transcripts across the array plotted against the corresponding concentrations. Error bars indicate the standard deviation across the array at each data point. The detection limit was determined to be the lowest analyte concentration that generated a signal that is three standard deviations above the average of the negative control signals (the bottom dot line).

Figure 3 shows the hybridization analysis of the phage lambda spiked-in control transcripts on the microfluidic array. Each data point represents the mean of the normalized probe intensities for the spiked-in control transcripts across the array plotted against the corresponding concentrations. Error bars indicate the standard deviation across the array at each data point. The result showed that the detection limit of the microfluidic array device was 0.375 pM. Further studies with spiked-in control transcript concentrations ranging from 1 to 1000 pM showed the dynamic range of the microfluidic platform covered 3 orders of magnitude (results not shown here). Since each measurement was made with replicate probes that were spaced across the array to allow measurement of the variability within the array, it was possible to get an accurate representation of reproducibility at each spiked-in concentration. Error bars in Figure 3 represent the standard deviation across the replicate probes and indicate that hybridization signals are uniform across the whole array. The low background signals and uniform hybridization signals suggest that on-chip microfluidic washing and labeling are uniform and efficient.

Given that the microfluidic array and the conventional manually handling array in this study received the same concentration of background target (K-562 cRNA), interarray comparison could be demonstrated by comparing the probes specific to genes expressed by this sample. Scatter plots comparing these probe intensities on two different arrays produced consistent results (Figure 4). The median correlation coefficient (R^2) of the normalized probe intensities across these two arrays was 0.955 (with a median CV of 17%). The results indicated that the performance of the microfluidic array device was comparable to that of conventional manual handling. A cartridge-to-cartridge reproducibility study is currently underway.

The integrated microfluidic array device automates gene expression assays that involve multistage sample processing and fluidic handling that are in general labor-intensive and time-consuming and have significant potential to be error-prone. Automation of the hybridization and post-hybridization process

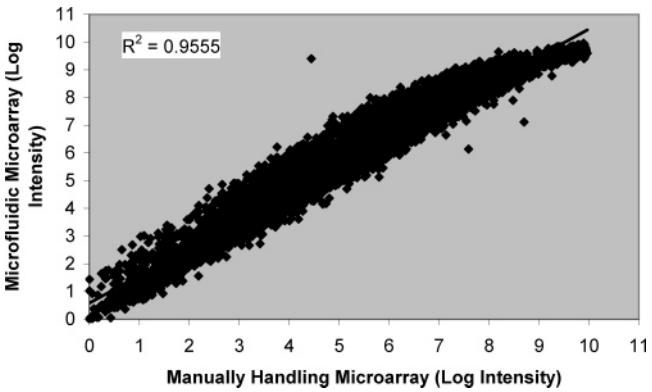

Figure 4. Scatterplot of the log(to base 2) intensities comparing data from a microfluidic array and a conventional manually handling array. The straight line is a linear regression through the data.

allows more stringent manufacturing control over not only the microarray but also the quality and volume of the reagents. In addition, there is more control over a variety of parameters, including hybridization temperature and time, washing time and speed, mixing/agitation speed, and labeling time. The microfluidic array device can eliminate variations in array data caused by subtle, day-to-day differences in protocol and manual handling. Although some commercial robotic workstations have also been developed to automate microarray processing, such instruments are generally too expensive and tend to limit the application of microarrays to high-budget applications. These bulky workstations are complicated to operate and often require high cost for maintenance. In contrast, the self-contained and fully integrated microfluidic array devices reported here are disposable and require simple portable instruments for operation. Although the use of the laser machine for plastic cartridge fabrication allows quick turn-around time and rapid prototyping, it requires a complicated multilayer design and labor-intensive assembly. Alternatively, a simple planar design of the plastic cartridge can be realized using injection molding, followed by sealing it with a thin plastic cover layer using a solvent-assisted thermal bonding technique[30] or ultrasonic bonding technique. The cost of a microfluidic plastic cartridge component (estimated less than $10 for high-volume manufacturing) is small as compared to the cost of the microarray chip (on the order of hundreds of dollars). To reduce the overall cost of the device (primarily of the microarray chip), the reuse of the array by stripping the hybridized DNA/RNA targets using chemicals before the next use is currently being explored. The integration of microfluidics adds new significant functionalities to the conventional microarray platform. Although only gene expression assay was demonstrated in this study, this integrated microfluidic platform can potentially be applied to many other assays. In addition to CombiMatrix's microarray chips, the microfluidic cartridge can potentially be integrated with other microarray platforms to perform genetic analysis. It is also possible to integrate front-end sample preparation and DNA amplification into the same platform, which would greatly simplify the device operation and eliminate error-prone manual handling.[30]

The microfluidic components in the device, including electrochemical pumps and bubbling mixer, are simple in design, inexpensive, and easy to fabricate and integrate into a complex microfluidic system, as compared with most of the existing micropumps and micromixers. The power consumption of the electrochemical pumps is low (~milliwatts). The use of integrated microfluidic components with low power consumption suggests that hand-held operation is feasible for the device. The choice of inexpensive, robust microfluidic technologies coupled with plastic cartridge fabrication facilitates an easy commercialization path for this technology. The integrated microfluidic platform provides a step toward fulfilling the promise of rapid, automated genetic analysis from complex sample fluids in cost-effective and portable instruments.

CONCLUSION

A self-contained and disposable microfluidic array device for integrated gene expression analysis has been developed. The on-chip process started with hybridization of a biotinylated RNA sample using a CustomArray, followed by a number of washing and labeling steps. The device automated and integrated all the sample processing and fluidic handling steps that are considered labor-intensive and time-consuming in regular manual handling process. All microfluidic components, such as micropumps and micromixers, are integrated onto the microfluidic cartridge but use simple and inexpensive approaches to reduce device complexity. Hybridization experiments using spiked-in control transcripts in a complex biological sample demonstrated that the microfluidic CustomArray platform is highly sensitive. Experimental results also showed that the performance of the integrated microfluidic device is comparable with the conventional hybridization devices using manual fluidic handling, indicating that the on-chip washing, mixing, and pumping are efficient in the microfluidic device.

ACKNOWLEDGMENT

The authors thank Jeff Kemper, Al Pierce, and Mike Slota for useful discussion and technical supports. This work has been sponsored by DoD Contract no. 1999011104A.

NOTE ADDED AFTER ASAP PUBLICATION

This article was released ASAP on February 3, 2006 with minor errors in the text. The correct version was posted on February 7, 2006.

Received for review October 16, 2005. Accepted January 9, 2006.

AC0518553