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Research paper

An adaptable microvalving system for on-chip polymerase chain reactions

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Abstract

On-chip genetic analysis systems are beginning to provide a viable alternative to conventional gene profiling and amplification devices, through minimal reagent use, high detection resolution, and the potential for high-throughput parallel testing of the genetic material, even from single cells. Despite the advantages, there are many difficulties inherent in creating an integrated microfluidic diagnostic platform. One major challenge is the accurate control and manipulation of fluid, and particularly the immobilization of reaction mixtures during heating phases of polymerase chain reactions (PCR). In this paper we present a pumping and valving system based on the use of three servomotor-controlled valve fingers that actuate microchannels within a poly-dimethylsiloxane (PDMS) fluidic chip. We characterize the valving ability of the system in terms of fluid loss and show the successful fluid retention of the system over 35-cycle PCR runs at temperatures of up to ~ 96 °C. In addition, we demonstrate the system's ability to perform PCR by successfully amplifying a sample of β2 microglobulin transcript obtained from the peripheral blood of a patient with multiple myeloma. This work has proven to be a successful approach to multi-use valving and a viable method of alleviating the fluid control difficulties inherent in performing a PCR reaction in an on-chip environment. In addition, it opens the door for further automation and integration with other chip-based genetic analysis platforms.

Keywords: Microvalving; On-chip PCR; Cancer diagnostics; New technology; Microfluidics

1. Introduction

1.1. Overview

The identification of genetic signatures for malignant cells in cancer patients enables predictions of risk factors, disease outcomes and/or survival, and facilitates the optimization of treatment strategies that are tailored to the genetic characteristics of the cancer in

Abbreviations: PCR, polymerase chain reaction; RT–PCR, reverse transcription polymerase chain reaction; PDMS, poly-dimethylsiloxane; CE, capillary electrophoresis; μTk, microfluidic toolkit.

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each individual patient. Many new methods are being developed to quickly track minute genetic changes. Techniques such as PCR and RT–PCR have accelerated progress in the measurement of molecular and genetic changes in patients, leading to an increasing ability to identify the genetic signatures of many different forms of cancer. The products obtained from PCR reactions are typically measured through gel-based analysis techniques. While this is successful in most cases, these strategies frequently fail to detect genes with low levels of expression or genes encoding transcripts with short life spans. On-chip PCR promises to be a fast, sensitive and cost-effective solution to these problems, but it has been shown to have additional challenges inherent in its implementation. One such challenge is the movement and precise valving of minute fluid volumes within the network of wells and channels on the chip.

Chip-based stationary PCR, as opposed to flowing PCR (Liu et al., 2002a,b; Obeid et al., 2003), is dependent upon the ability to immobilize the PCR product during the reaction process. PCR valves are needed to prevent unintended displacement of the PCR mixture due to bubble formation from the release of dissolved gasses (Liu et al., 2002a,b). A range of valves have been developed, including those based on smart polymers within rigid channels (Koh et al., 2003), single-use paraffin valves (Liu et al., 2004), peristaltic pumps actuated by external pressure manifolds (Lagally et al., 2001a,b; Liu et al., 2002a,b; Grover et al., 2003) and hydrophobic materials (Fujii, 2002; Liu et al., 2002a,b; Yu et al., 2003). Dense pumping and valving arrays would enable significantly higher levels of integration, allowing a multitude of samples to be run in parallel while greatly reducing analysis times. In recent demonstrations, Thorsen et al. (2002) has shown how these high density arrays can allow hundreds of PCR reactions to be performed on a chip. Unfortunately, the external components required for such an approach (high pressure gas systems and their valves) make for a relatively complex system.

In this work we present a microvalving technique in which a system of servomotor-driven probes allow the pumping and valving of an internal PCR reservoir by applying pressure sufficient to compress and close microchannels embedded in a flexible polymer. This approach allows for a very compact valving system using servomotors that are often used in model aircraft and small-scale robotics. The positional sensing ability inherent in these motors is very important since our chips are made of polydimethylsiloxane (PDMS), a polymer that becomes considerably softer at higher temperatures. This means that the pressures needed to seal a microchannel at lower temperatures may inflict damage to the PDMS at higher temperatures. Conversely, the force applied to seal a channel at room temperature may be insufficient to immobilize fluids during the high temperature stages of the PCR reaction. By exerting flexible and accurate control of fluid movement through a PDMS chip, our system is designed to facilitate the integration of the PCR reaction system with an on-chip separation procedure such as the capillary electrophoresis (CE) system described in the accompanying paper by Pilarski et al. (2005). As a proof of concept for our valving strategy, we have successfully applied our system to the detection of genes expressed by human cancer cells, specifically cDNA templates obtained from the peripheral blood of patients with multiple myeloma.

1.2. Background

The growing demand for rapid gene expression and mutation analysis tools has stimulated intensive research in the field of high-throughput analytical methods. The subsequent development of capillary electrophoresis methods in the past decade has brought innovations that became essential for sequencing the human genome. As a result of these developments, many genetic testing protocols have been modified for use within a capillary electrophoresis format. The use of capillary electrophoresis with laser-induced fluorescence detection has been shown to reduce analysis time and increase detection sensitivity when compared with conventional gel electrophoresis methods. This technique is especially advantageous in clinical diagnostics, forensic laboratories and genetic population screening.

Recent developments in microchip-based genetic analysis have been hindered by the difficulties of integrating a reliable, multiple-use valving technology. Our high speed flow-through PCR and RT–PCR (Obeid et al., 2003) was able to amplify genetic materials on a time scale of several minutes. However,

without on-chip valves it was difficult to control the flow of the PCR mixture and integrate the PCR module with additional on-chip analysis systems. Our work with non-flowing PCR within a well or internal reservoir (Backhouse et al., 2003; Pilarski et al., 2004) suffered low yields because of the tendency of the PCR mix to dry out or move under the influence of thermophoretic forces and/or the pressure caused by the release of dissolved gasses.

In the literature it appears necessary to include some form of valving in order to perform reliable PCR. Notably, there has been evidence of a growing trend towards PDMS-based pressure-actuated valves/pumps (Liu et al., 2002a,b; Solignac and Gijs, 2003; Futai et al., 2004) and PDMS-based PCR (Hong et al., 2001; Liu et al., 2002a,b; Yu et al., 2003; Prakash et al., in press). Lagally and colleagues presented the integration of an array of 280 nl PCR wells, electrophoretic analysis channels and fluidic valves (Lagally et al., 2000, 2001a,b). Although impressive, this system was a complex hybrid of O-rings, diaphragms, hydrophobic materials, external manifolds and micro-fabricated substrates. The complexity of this approach may limit the degree of integration and the design appears susceptible to run-to-run contamination, possibly making the device incapable of reuse. In recent work we also note the use of a Braille reader for applying pressures to PDMS surfaces (Futai et al., 2004; Gu et al., 2004). This approach utilizes an array of actuated rods that are individually addressable, thus enabling high densities and correspondingly large levels of integration. Although exciting, this approach relies on the use of complex and often expensive machinery, and does not provide feedback on the amount of force being applied.

The microchips used in our system are comprised of a layer of molded PDMS irreversibly bonded to a glass substrate. One of the key features of PDMS is its flexibility, a characteristic that facilitates the novel valving approach presented in this paper. In previous fluidic chips (Lagally et al., 2001a,b; Grover et al., 2003), expensive external equipment was required to move fluids through the channels. In addition, the valving systems used were comprised of a multitude of moving parts, some of which came in direct contact with the PCR reaction mixture. Valving systems that contact the PCR mixture run the risk of contamination arising from small protrusions and crevices that may trap DNA and reagents from a previous run. This could seriously compromise the use of chips meant to endure multiple runs, necessitating the design of multi-use cleaning protocols to effectively flush and clean the myriad moving systems inside the chip. Furthermore, the fabrication of these chips is complicated, costly and laborious. Additional care must be taken in designing internally positioned valves, as certain materials can be shown to "poison" a PCR reaction if they come in direct contact with the reagents. Contamination, contact pressure and repeatability all create the need for a more resilient, multi-use, non-invasive valving system. Such valves must be able to withstand the wide temperature ranges (20 °C to 95 °C) and high pressure (approximately 1 atm) that will be encountered in microchip PCR. Our goal is to enable the repetitive use of a single chip for multiple cycles of cleaning, amplification and analysis. The following sections introduce our method for on-chip PCR and describe in detail a novel approach to repeatable adaptable valving.

2. Materials and methods

2.1. The microfluidic platform

The microchip used for these experiments is composed of a glass substrate bonded to a thin layer of the flexible polymer poly-dimethylsiloxane (PDMS). The major features of the microchip are a pair of 2 μl wells (sample and waste), a central reaction well, and channels connecting the sample/waste wells to the reaction well. A simplified depiction of the chip is shown in Fig. 1. In the experiments reported here, the chip dimensions are 2.4 cm × 1.5 cm, with a PDMS thickness of approximately 1.5 mm, glass thickness of 1.0 mm, and channels that are 20 μm × 50 μm in cross-sectional area. The reaction well is 2 mm in diameter, containing the equivalent volume of the sample well (~ 2.5 μl). The PCR reaction occurs inside the reaction well as heat is transferred to the mixture through the glass substrate from a Peltier heating device (Prakash, 2004).

To fabricate a complete chip, the channels and wells are molded into the PDMS layer, which is then irreversibly bonded to the glass substrate (Pra-

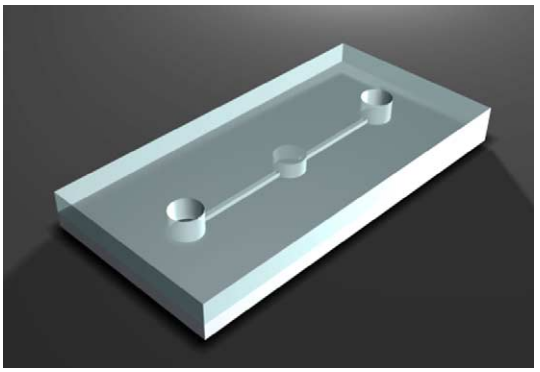

Fig. 1. Diagram of the PDMS microchip used in PCR reactions (length = 2.4 cm, width = 1.5 cm, height = 0.25 cm). The chip is comprised of a lower layer of glass irreversibly bonded to a layer of PDMS. Channels and wells are molded into the PDMS layer and they provide a path for fluid flow.

kash, 2004). Once the chip has been bonded in this way, the two layers are capable of withstanding forces greatly exceeding the PCR reaction pressure without any occurrences of delamination. In our system, the PCR reaction mixture is loaded into the sample well, by micropipette. It is then drawn into the reaction chamber by peristaltic pumping and effectively sealed there for duration of the amplification process. After the PCR reaction is complete, the amplified product is evacuated to the output well and removed for analysis. This process will be described in more detail in the following section.

2.2. The microvalving system

One of the key features of our valving system is its ability to apply and maintain localized pressure to a given region of a fluidic chip. Due to the flexible nature of the PDMS, we are able to selectively apply pressure to quickly seal the small channels of the PCR microchip. In some of our previous attempts at valving, rigid non-adapting mechanical systems were used to apply pressure to the PDMS. These systems either failed due to leakage or destroyed the PDMS during the rapid heating and cooling cycles. We have found that PDMS becomes significantly softer and may rupture at higher temperatures (Prakash et al., in press). The increased flexibility of the PDMS may also allow it to deform around rigid valving mechanisms, breaking the seals formed at room temperature. To circumvent these problems, we designed a more adaptable valving system capable of carefully tailoring the force applied to the PDMS surface. This was made possible using motors that provide some degree of feedback. Our system utilizes three valving fingers able to apply pressure to specific regions of the PCR chip. This is similar to the use of a Braille reader used by Gu and colleagues to implement a highly integrated array of pumps and valves (Gu et al., 2004). In our system, a "smart" motor capable of accurately controlling its relative position and tailoring its pressure to varying environmental conditions actuates each finger. The key hypothesis underlying this work is that a valving system sensitive to the thermal and physical changes in the PDMS will be able to accurately seal fluid inside the PCR reaction chamber over a large range of temperatures (and PDMS softness).

Our system is composed of several modules, as seen in Fig. 2. The thermal cycling system is in direct contact with the PCR chip, and consists of a Peltier heating device that heats or cools the PCR

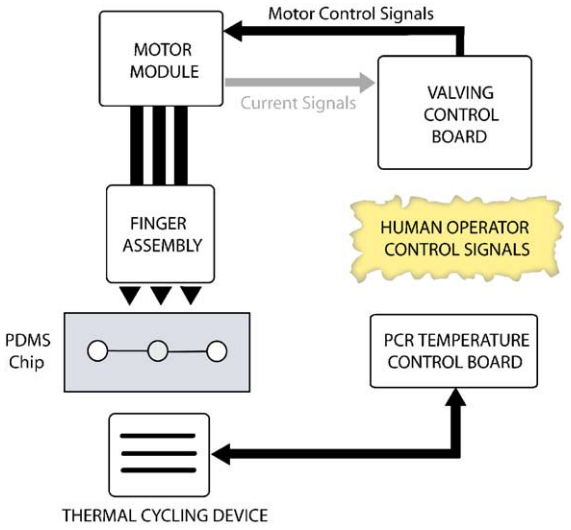

Fig. 2. Simplified layout of key microvalving system modules. The valving control board sends control signals to the motor module, which in turn generates the force necessary to actuate the finger assembly. The finger assembly applies compression to the PDMS chip, effectively sealing the channels and facilitating the movement of fluids. Feedback from the motor module to the control board is possible in the form of servo current draw measurements (light grey arrow). A Peltier heating device controls the thermally cycled PCR process.

chip through a thin sheet of copper (Prakash, 2004). Thermal paste is applied to the bottom of the PCR chip to ensure rapid temperature equalization between the heating plate and the PCR chip's glass substrate layer. The valve finger assembly is also in direct contact with the PCR chip, positioned directly above the chip and the heating device. This assembly is responsible for applying the localized pressure needed for pumping and valving, and consists of three valve fingers that slide up and down through a plastic manifold. The motor module, removed from the actual finger location, provides the force necessary to actuate the fingers. It is controlled by a microprocessor board that sends instructions to the motors (by electrical signals sent along the motor control lines). The compression of the PDMS layer (and the associated channels) is directly related to the depth of the valve finger into the PDMS, as set by the microprocessor control signals. Lastly, the human operator interacts with the heating/valving systems through a computer interface that allows parameters such as the length of the PCR run and the relative position of valving fingers to be set.

2.2.1. The motor module

The motor module is comprised of three servomotors and creates the force necessary to actuate the valve fingers. A servomotor is a motor capable of accurately controlling its degree of rotation, as specified by a pulse-width modulated signal — a small electrical pulse delivered to the servo's control line approximately every 20 ms. This pulse is generally between 1 and 2 ms in duration, and its length controls the desired angular position of the motor (specified as one of a discrete set of angle values within the servo's maximum motion range). A potentiometer attached to the rotating arm of the servomotor provides the feedback signal necessary for the servo to sense its own position and apply corrective force in the event that the desired and actual positions differ. Commonly installed in remote controlled aircraft, the servomotors used in our system are able to maintain their angular position even under duress by modifying their output strength to counteract any disturbances in the environment. They have the additional advantage of being a fully integrated package, containing a small control module, a direct current (DC) motor, a position feedback device (potentiometer), and a gearbox to increase the output torque of the motor.

There has been a great deal of work done in the field of servomotor control. The devices used in our system are integrated units, mass produced at low cost for the radio control/model aircraft market. As such, they lack many of the complex feedback mechanisms present in more sophisticated (and expensive) servomotor systems. To increase the versatility of these units, we present a current-based method for pressure feedback. An inherent characteristic of servomotors is that the electrical current drawn by the motor is related to the force the motor is applying to its environment. By monitoring the power consumption of the motor, it is possible to infer the pressure being exerted onto the PCR chip channels.

During the tests, we observed the electrical current drawn by the motors in relation to the visible closure of channels under pressure, and determined the relative servo actuation level needed to completely seal a channel in the PDMS for an entire PCR run. At each step of penetration by the finger into the PDMS, the current draw to the corresponding servomotor was recorded and correlated to the containment of a fluid dye residing inside the sealed reaction well. Initial results were obtained using a FLUKE 179 ammeter placed in series with each servomotor. Readings were obtained for multiple trials on four different PDMS chips, and normalized to the baseline current draw of the servomotor being measured under no-load conditions. From these observations, we deduced the acceptable levels of closure needed to complete a full PCR run with negligible fluid loss from the reaction well. The results of these tests are shown in Section 3.1.

In this iteration of the system we used Three Hitec HS-300 servomotors (Hitec RCD USA Inc., Poway, CA, USA), chosen for their relatively small size, low cost (~ $17 CAN each), high angular resolution, and effective stall torque of 3.1 kg cm. The motor arms are each affixed to a DU-BRO 36" flex cable assembly (DU-BRO Products Inc., Wauconda, IL, USA) consisting of a long flexible tube containing a stiff metal cable. One end of the metal cable connects to the actuated arm of the servomotor, while the other connects (and transfers force to) the top of the finger valve rods. The flexible sheath covering is secured

using a pressure clamp (motor module side) and a plastic fitting secured with epoxy glue (finger assembly side). It was found that this arrangement allowed for a high degree of flexibility without sacrificing a large amount of output finger pressure.

2.2.2. The finger assembly

The valve finger assembly consists of the three valve rods that slide through a plastic manifold located directly above the PDMS chip and heating assembly. Each valve rod terminates in a plastic finger approximately 2.5 mm in diameter, rounded to locally deform the PDMS surface without rupturing the polymer during the heating cycle. The fingers make contact with the PDMS during valving using the force transferred from the servomotors to compress the PDMS. The compressible nature of the PDMS allows the finger to deform the upper layer of the chip, flattening the channels and effectively sealing them against the pressure induced in the PCR reaction chamber. As shown in Fig. 3, one finger is positioned above each channel in the PCR chip, while the third finger is located over the reaction well. This third finger is used in the pumping action of the system. To facilitate complete evacuation of the reaction product, the contact surface of this finger is slightly larger and flatter than that of the valve fingers. The two fingers located over the channels have a smaller radius of curvature (~ 1 mm), but are still relatively smooth so as not to rupture the PDMS during heating. Our previous experiments showed that sharper fingers lead to PDMS damage during the PCR run, causing complete fluid loss.

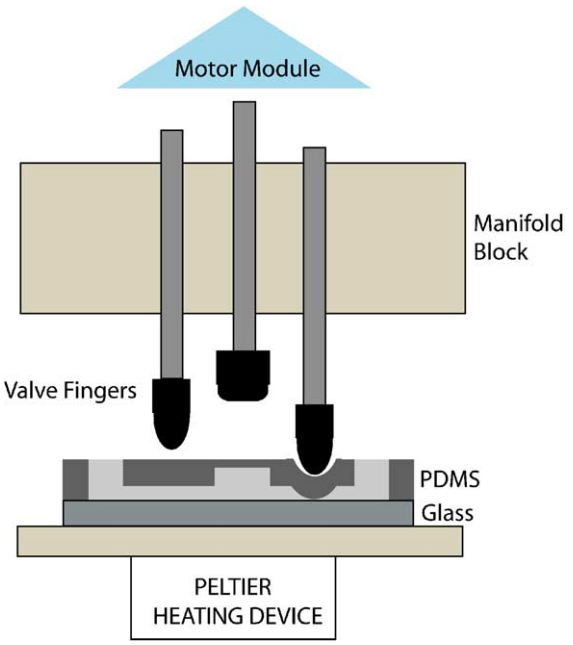

Fig. 3. Schematic representation of the valving finger assembly. The three-valve fingers slide through a plastic manifold block and make contact with the PDMS chip directly over the channels (outer fingers) and the reaction well (center finger). The outer fingers are used to locally compress the PDMS channels and valve the reaction chamber. The center finger has less curvature than the outer fingers in order to completely evacuate the reaction well. Force is transferred to the valve fingers from the motor module by way of semi-flexible cables.

Valving is performed by lowering one of the outer valve fingers onto the PDMS and applying pressure until the channel is fully compressed. Complete compression can be inferred automatically from the power consumption of the servomotor (as stated earlier) or through visual inspection. Similarly, pumping is affected through the sequential actuation of the fingers. This is shown in Fig. 4.

2.3. PCR procedures

The PCR reaction used in these experiments is specifically tailored for the PDMS–glass PCR chip. It includes high fidelity *Taq* DNA polymerase and is based upon sample cDNA obtained from total RNA transcripts isolated from sorted CD19+ malignant B cells purified from the peripheral blood of patients with multiple myeloma. Each of the PDMS–glass chips in use was filled with approximately 2–3 μl of PCR reaction mixture. The PCR reaction was prepared as follows: 3 μl cDNA was added to 50 μl of PCR mix containing 5 μl of 10X PCR buffer, 2 μl of 50 mM $MgCl_2$, 1 μl of 10 mM dNTPs, 2 μl reverse (5′) and forward (3′) primer each of 10 μM, and 3 μl of high fidelity platinum *Taq* (Gibco/BRL). 4 μl of PCR reaction mixture was loaded on the chip and 25 μl was cycled on conventional thermocycler. The PCR cycling parameters used on the PCR chip during the tests were the following: primary denaturation for 5 min at 94 °C, denaturation for 30 sec at 94 °C 35 cycles, annealing for 30 sec at 60 °C, extension for 30 sec at 72 °C and final extension for 7 min at 72 °C.

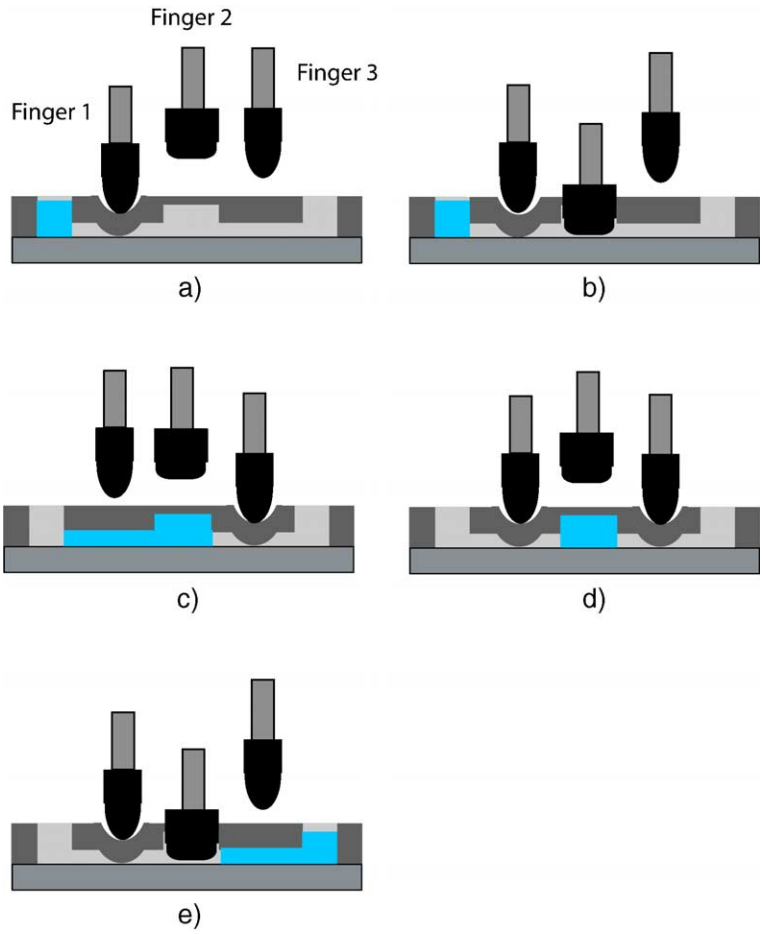

Fig. 4. Sequence of finger actuation during a pumping cycle. a) First, "Finger 1" is lowered onto the channel extending from the sample well to the reaction well. This prevents fluid from backing up into the sample well. b) Next, the flat center finger ("Finger 2") is lowered to maximum compression, thereby evacuating the reaction chamber of air or liquids. c) The output channel is then sealed by the third finger ("Finger 3") and the other fingers (1 and 2) are retracted, allowing vacuum force to draw the reaction mixture from the input well into the center well. d) The input channel is then sealed under force by Finger 1 and the thermal cycling portion of the run begins. e) To remove the cycled product, "Finger 3" is retracted and "Finger 2" is depressed, forcing the fluid out of the reaction well.

cDNA for the experiment was prepared as described by Adamia et al. (2005). The primers used in the reaction were as follows: VIC-labeled 5′ (reverse) β2-microglobin CCAGCAGAGAATGGAAAGTC; 3′ (forward) β2-microglobin GATGCTGCTTACATGTCTCG. 0.9 μl of the retrieved PCR product was analyzed on a cross channel capillary electrophoresis (CE) chip (Micralyne, Edmonton, Canada) as described by Pilarski et al. (2005). The PCR product obtained from the corresponding PCR run on the conventional thermocycler was analyzed as described by Adamia et al. (2003).

3. Results and discussion

3.1. Valving characteristics

By monitoring servo current draw it is possible to detect when a valve finger has successfully compressed a channel. This is done by analyzing the current flowing through each servo during the compression of the PDMS. Multiple tests were conducted to determine the servo power consumption levels responsible for the complete channel closure and these levels were used to operate the system consis-

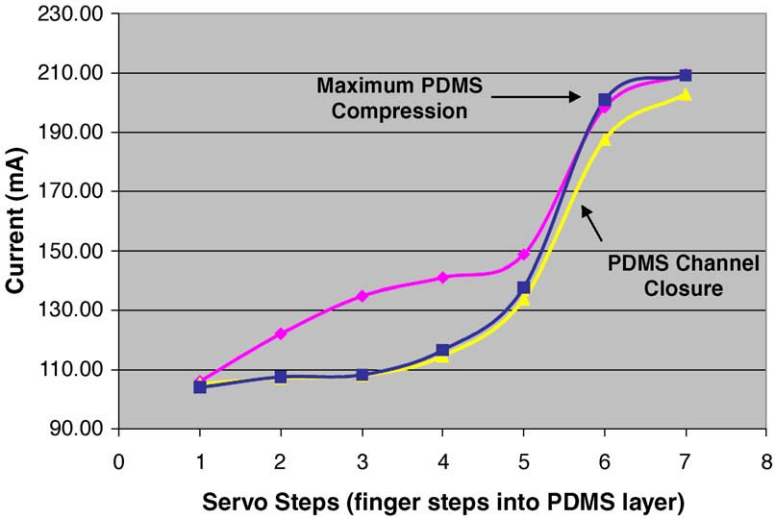

Fig. 5. Average single servo current draw during PDMS channel compression (mA) versus position (in servomotor actuation steps), on three different PDMS chips. Full channel closure was observed at current levels greater than 170 mA. Servo steps are a parameter of the control hardware, representing a discrete set of possible angular positions achievable by the motor module.

tently without the need for constant recalibration. The current profiles of the three servomotors used in the motor module were analyzed over a series of 30 sample runs, with each motor being tested on four different PDMS PCR chips. It was found that, on average, each servomotor increased from the baseline resting current value (~ 110 mA) to a maximum value of ~ 200 mA at full PDMS compression (Fig. 5). A comparison of servomotor baseline currents showed that individual servomotors sometimes varied by up to 42 mA in their resting state. While this difference is significant in the unloaded condition, the difference was found to decrease to ~ 15 mA when the valve finger was applying pressure to the PDMS. It was shown through a series of simulated PCR runs (using colored dye, varying in length from 12 to 35 cycles, and ranging in temperature from 21 °C to 120 °C) that despite the baseline fluctuations, a servo current draw between 170 mA and 180 mA corresponded to a complete channel closure (Fig. 5). In addition, these experiments showed that under these conditions there was little fluid loss from the reaction well (less than 20%) and no visible damage to the PDMS surface. This level of fluid loss was attributed to vapour loss and is significantly lower than the loss levels that gave rise to PCR reaction failure. In destructive testing we found that temperatures as high as 120 °C were required to rupture the PCR chamber (containing water) when sealed with closed valves.

In summary, we observed a strong correlation between the servo current draw and the depth of finger penetration into the PDMS (see Fig. 5). The advantage of using servo current draw as a measure of channel closure is that it gives the potential for the valving system to self regulate, removing the need for human interaction in the valve positioning process. Further work is needed to fully integrate the current feedback system with the microprocessor control system. This would facilitate higher valving densities and resolution by allowing the use of valve fingers with a smaller radius of curvature.

One sizable improvement over our previous attempts at static on-chip valving is the high fluid retention levels during PCR runs with a retention rate greater than 80% (defined as the ratio of fluid PCR product recovered compared to the original mixture volume). This retention rate was found to be consistent even during tests of 35 PCR cycles or longer. Each run reached a maximum temperature of 95 °C with each cycle lasting approximately 2 min. In the case of a partially sealed channel, the dissolved gasses released at this temperature were shown to generate sufficient force to completely evacuate the reaction well. There was no noticeable leakage at

temperatures approaching 100 °C, confirming the efficacy of the valving system. The observed fluid loss is attributed to water vapour diffusing into the PDMS surrounding the reaction chamber (Prakash et al., in press).

In addition to its ability to consistently and non-invasively seal microfluidic channels in PDMS, our system was designed to allow for simple peristaltic pumping action. A peristaltic approach to pumping has been applied previously in a number of applications (Liu et al., 2002a,b). In this way it is possible to complete an entire PCR run with only two instances of human interaction: the addition of the PCR mixture to a sample well and the removal of the product after the reaction is complete. By timing the operation of these valve fingers, we have shown that it is possible to move fluid into and out of the reaction well. This is a large step towards fully automated analysis systems and greatly reduces the chance of contamination.

3.2. Detection transcripts of β2 microglobulin

Through the use of our PDMS–glass PCR system, we were able to amplify the RNA obtained from the peripheral blood of the patients with multiple myeloma and detect the expression of β2 microglobulin transcripts from purified populations of malignant B cells (Fig. 6). β2 microglobulin is a housekeeping gene, which is expressed in all cells. Amplification in the on-chip system described here provides a proof of concept for the amplification of genes that are over-expressed in cancerous cells. The on-chip PCR experiment of Fig. 6 demonstrates that we are able to amplify product in a 2 μl PCR reaction mixture that includes about 0.3 ng of cDNA template derived from approximately 1000 malignant B cells. The accuracy of the results obtained from the on-chip PCR were successfully verified on a conventional PCR system with analysis on a capillary DNA genetic analyzer (not shown), indicating that the on-chip PCR yields results comparable in accuracy and resolution to those obtained with conventional systems.

Our previous experiments showed that even small failures in the valving system caused the entire PCR reaction mixture to dry and/or be forced prematurely out of the reaction well. Thus, the successful on-chip amplification of β2 microglobulin transcripts demonstrates that our system is capable of sealing fluid inside the reaction well and providing an environment conducive to accurate genetic amplification.

3.3. Conclusions

By introducing a chip with a malleable PDMS top layer, external forces can easily be applied to deform the microstructures shaped into the PDMS (Futai et al., 2004). Our valving system takes advantage of this; with constant valving pressure being applied to a channel without have the valving apparatus make direct contact with the PCR reaction mixture. Not only does this allow for efficient and effective inter-run cleaning, it ensures that a single valving system can be used for multiple runs on multiple chips. This reduces the cost to fabricate a single chip and allows chip prototypes to evolve independently of the valving system. Ultimately, this approach helps facilitate the integration of the PCR process with other portions of a microfluidic analysis platform. While glass PCR chips are expensive to build and can be difficult to interface with, the PDMS chips used in our system are amenable to rapid fabrication of new configurations as prototype chips for testing and have no complex multi-layer valve machinery embedded into the substrate of the chip.

In this work, we presented a simple programmable valve based on a PDMS/glass microstructure and servo-driven fingers. By applying a consistent force throughout the temperature cycle we avoid any discernible damage to the PDMS layer, and show that our system works consistently on PCR runs of over 35 cycles with over 80% fluid retention. Our unique valving approach has facilitated the successful amplification of β2 microglobulin transcripts in an on-chip environment, using minute amounts of cDNA derived from malignant cells of multiple myeloma patients. We are presently improving this design to allow for greater levels of device integration, with the goal of increasing its compatibility with other chip-based amplification and detection systems. A more compact valving system with fixed valve rods connecting the motor module to the finger assembly is in progress and will be described elsewhere. Our preliminary work conducted on hyaluronan synthase transcripts obtained from peripheral blood of myeloma patients using the described valving system

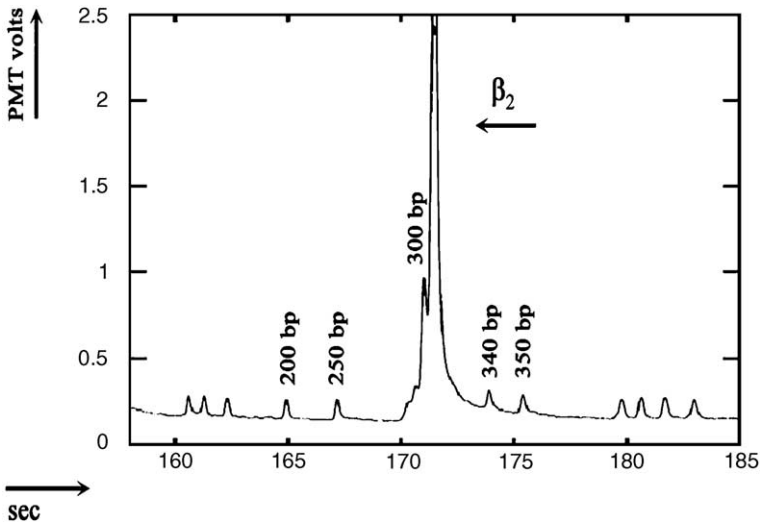

Fig. 6. Sensitive detection of housekeeping genes (β2 microglobulin). Capillary electrophoresis electropherogram was obtained from the μTk. The *x*-axis represents time (in sec) and the *y*-axis indicates fluorescence intensity (V). The PCR product (# bp) was sized with size standard GeneScan 500; cDNA Template was from a multiple myeloma patient.

indicates that the on-chip PCR system is able to successfully amplify low abundance transcripts and/or transcripts with short life span that encode products contributing to the malignant process (Adamia et al. in preparation). We believe that the valves described here will ultimately allow the same chip to perform successive PCR reactions without cross contamination by enabling on-chip reagent loading, sample introduction, PCR, sample removal and cleaning — all without operator intervention. This work may lead to high throughput automated devices able to analyze genetic information in minutes at low cost since the amount of reagent used for on-chip PCR is minimal compared to that used in conventional PCR reactions. The uses of such a device include detecting and monitoring the expression of diseased related genes, the forensic detection of genetic material, and biosafety monitoring for agents that threaten public health.

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References

Adamia, S., Kriangkum, J., Belch, A.R., Pilarski, L.M., 2003. Abnormal expression of hyaluronan syntheses in patients with Waldenstrom's macroglobulimenia. Seminars in Oncology, 165.

Adamia, S., Reiman, T., Crainie, M., Mant, M.J., Belch, A.R., Pilarski, L.M., 2005. Intronic splicing of hyaluronan synthase 1 (HAS1): a biologically relevant indicator of poor outcome in multiple myeloma. Blood (Feb. 24 [Electronic publication ahead of print]).

Backhouse, C.J., Footz, T., Adamiam, S., Pilarski, L.M., 2003. Microfluidic chips for the molecular analysis of human cancer. International Conference on MEMS, Nano, and Smart Systems (IEEE Conference), Banff, Canada.

Fujii, T., 2002. PDMS-based microfluidic devices for biomedical applications. Microelectronic Engineering 61-2, 907.

Futai, N., Gu, W., Takayama, S., 2004. Rapid prototyping of microstructures with bell-shaped cross-sections and its application to deformation-based microfluidic valves. Advanced Materials 16, 1320-+.

Grover, W.H., Skelley, A.M., Liu, C.N., Lagally, E.T., Mathies, R.A., 2003. Monolithic membrane valves and diaphragm

pumps for practical large-scale integration into glass microfluidic devices. Sensors and Actuators. B, Chemical 89, 315.
Gu, W., Zhu, X.Y., Futai, N., Cho, B.S., Takayama, S., 2004. Computerized microfluidic cell culture using elastomeric channels and Braille displays. Proceedings of the National Academy of Sciences of the United States of America 101, 15861.
Hong, J.W., Fujii, T., Seki, M., Yamamoto, T., Endo, I., 2001. Integration of gene amplification and capillary gel electrophoresis on a polydimethylsiloxane–glass hybrid microchip. Electrophoresis 22, 328.
Koh, C.G., Tan, W., Zhao, M.Q., Ricco, A.J., Fan, Z.H., 2003. Integrating polymerase chain reaction, valving, and electrophoresis in a plastic device for bacterial detection. Analytical Chemistry 75, 4591.
Lagally, E.T., Simpson, P.C., Mathies, R.A., 2000. Monolithic integrated microfluidic DNA amplification and capillary electrophoresis analysis system. Sensors and Actuators. B, Chemical 63, 138.
Lagally, E.T., Emrich, C.A., Mathies, R.A., 2001a. Fully integrated PCR-capillary electrophoresis microsystem for DNA analysis. Lab On A Chip 1, 102.
Lagally, E.T., Medintz, I., Mathies, R.A., 2001b. Single-molecule DNA amplification and analysis in an integrated microfluidic device. Analytical Chemistry 73, 565.
Liu, J., Enzelberger, M., Quake, S., 2002a. A nanoliter rotary device for polymerase chain reaction. Electrophoresis 23, 1531.
Liu, Y.J., Rauch, C.B., Stevens, R.L., Lenigk, R., Yang, J.N., Rhine, D.B., Grodzinski, P., 2002b. DNA amplification and hybridization assays in integrated plastic monolithic devices. Analytical Chemistry 74, 3063.
Liu, R.H., Bonanno, J., Yang, J.N., Lenigk, R., Grodzinski, P., 2004. Single-use, thermally actuated paraffin valves for microfluidic applications. Sensors and Actuators. B, Chemical 98, 328.
Obeid, P.J., Christopoulos, T.K., Crabtree, H.J., Backhouse, C.J., 2003. Microfabricated device for DNA and RNA amplification by continuous-flow polymerase chain reaction and reverse transcription–polymerase chain reaction with cycle number selection. Analytical Chemistry 75, 288.
Pilarski, L.M., Adamia, S., Pilarski, P.M., Prakash, A.R., Lauzon, J., Backhouse, C.J., 2004. Improved diagnosis and monitoring of cancer using portable microfluidics platforms. In: Badawy, W., Moussa, W. (Eds.), International Conference on MEMS, Nano, and Smart Systems, Bannf, Alberta, Canada, p. 340.
Pilarski, L.M., Lauzon, J., Strachan, E., Adamia, S., Atrazhev, A., Belch, A.R., Backhouse, C.J., 2005. Sensitive detection using microfluidics technology of single cell PCR products from high and low abundance IgH VDJ templates in multiple myeloma. Journal of Immunological Methods 305, 94 (accompanying this document).
Prakash, A.R., 2004. BioMEMS and nanoscience for genetic analysis. Electrical and Computer Engineering, Masters of Science. University of Alberta, Edmonton.
Prakash, A.R., Adamia, S., Sieben, V., Pilarski, P.M., Pilarski, L.M., Backhouse, C.J., in press. Small volume PCR in PDMS biochips with integrated fluid control and vapour barrier. Sensors and Actuators. B, Chemical (Available online).
Solignac, D., Gijs, M.A.M., 2003. Pressure pulse injection: a powerful alternative to electrokinetic sample loading in electrophoresis microchips. Analytical Chemistry 75, 1652.
Thorsen, T., Maerkl, S.J., Quake, S.R., 2002. Microfluidic large-scale integration. Science 298, 580.
Yu, X.M., Zhang, D.C., Li, T., Hao, L., Li, X.H., 2003. 3-D microarrays biochip for DNA amplification in polydimethylsiloxane (PDMS) elastomer. Sensors and Actuators. A, Physical 108, 103.