PMMA/PDMS valves and pumps for disposable microfluidics†

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Poly(methyl methacrylate) (PMMA) is gaining in popularity in microfluidic devices because of its low cost, excellent optical transparency, attractive mechanical/chemical properties, and simple fabrication procedures. It has been used to fabricate micromixers, PCR reactors, CE and many other microdevices. Here we present the design, fabrication, characterization and application of pneumatic microvalves and micropumps based on PMMA. Valves and pumps are fabricated by sandwiching a PDMS membrane between PMMA fluidic channel and manifold wafers. Valve closing or opening can be controlled by adjusting the pressure in a displacement chamber on the pneumatic layer *via* a computer regulated solenoid. The valve provides up to 15.4 μL s^{-1} at 60 kPa fluid pressure and seals reliably against forward fluid pressure as high as 60 kPa. A PMMA diaphragm pump can be assembled by simply connecting three valves in series. By varying valve volume or opening time, pumping rates ranging from nL to μL per second can be accurately achieved. The PMMA based valves and pumps were further tested in a disposable automatic nucleic acid extraction microchip to extract DNA from human whole blood. The DNA extraction efficiency was about 25% and the 260 nm/280 nm UV absorption ratio for extracted DNA was 1.72. Because of its advantages of inexpensive, facile fabrication, robust and easy integration, the PMMA valve and pump will find their wide application for fluidic manipulation in portable and disposable microfluidic devices.

1. Introduction

About three decades after the first gas chromatography analyzer was fabricated on silicon[1] and two decades after the concept of "miniaturized total chemical analysis system" (or μTAS) was proposed by Manz *et al.*,[2] microfluidic chips have emerged as important tools in biological and life science, especially in cell handling,[3,4] separation,[5,6] DNA sequencing,[7–10] and biomolecule detections.[11] In the early stage, microfluidic chips were mainly fabricated on silicon or glass substrates[12–14] because their fabrication techniques have been well established in the semiconductor industry. In addition, glass substrate has the advantages of excellent optical transparency, electrical conductivity, dielectric properties and corrosion resistance. However, the applications of glass as a substrate have been compromised by the high cost, the fragileness and the harmful and complicated fabrication procedures.[15] In contrast, polymers, including PDMS, PMMA, PC *etc.*, have become important substrates because they are less expensive, disposable, and applicable for mass replication technologies, as well as for the methods of rapid prototyping. Typically, for the same area and optical transparency, a glass substrate may cost 10 to 100 times more than a polymer substrate.[16] As the least hydrophobic one in the common plastic materials,[17] PMMA is particular useful for disposable microfluidic chips because of its low price, rigid mechanical property, excellent optical transparency, and compatibility to electrophoresis.[18–20] Furthermore, there are a variety of methods developed for mass production of PMMA microdevices with high fidelity, which include hot embossing, solvent imprinting, thermal bonding, injection molding and laser ablation, *etc.* As a result, PMMA has been used as substrates for a great number of microfluidic devices including mixing analysis chip,[21] DNA sequencer[22,23] and electrophoresis chip.[24] However, so far there is still no PMMA valve reported. To take full advantage of PMMA for microfluidic applications, it is important to develop PMMA based valves and pumps.

A microvalve has the ability to manipulate and transport solutions in microchannels, which is one of the fundamental issues of microfluidics. Several ways have been reported for fluid control and manipulation on chip such as electroosmosis control[25] and microvalve control. Among them, electroosmosis control is easier and more flexible to be manipulated, but it is sensitive to many factors such as buffer properties, the component of channel surface, and the longitudinal electric field intensity, and thus difficult to control. The development of a simple method of fabricating pneumatically activated valves on the basis of soft-lithographic procedures by Quake's group has made a significant contribution to the field.[26] The PDMS/PDMS valve has been widely accepted and applied in applications such as automated DNA computing,[27] PCR-CE,[28,29] mixers,[30–32] immunoassays[33] and single-cell analysis.[34–36] Based on the principles of the diaphragm valves, Mathies and co-workers[37] have reported two kinds of air pumps for glass chips by using a three

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or four-layer structure. These glass based valves are suitable for large-scale integration into glass devices for chemical and biochemical assays. In addition, the four-layer valve and pump design incorporates an all-glass fluidic system to minimize fluid-PDMS contact for improving chemical compatibility and minimize nonspecific absorption problem of PDMS. These microvalves and micropumps have shown their advantages in applications such as capillary electrophoresis,[38–43] DNA sequencing,[44,45] PCR reaction,[46–50] droplet generation,[51] DNA computing,[52,53] cell capturing,[54] and other microfluidic devices.[55]

In an attempt to develop low cost microchips for disposable applications, we present the design, fabrication, characterization and application of pneumatic microvalves and micropumps based on PMMA that can be easily used to assemble with other microfluidic PMMA analysis devices. Valves and pumps are fabricated by sandwiching a PDMS membrane between PMMA fluidic channel and manifold wafers. Valve closing or opening can be controlled by adjusting the pressure in a displacement chamber on the pneumatic layer *via* a computer regulated solenoid. The valve provides up to 15.4 μL s^{-1} at 60 kPa fluid pressure and seals reliably against forward fluid pressure as high as 60 kPa. A PMMA diaphragm pump can be assembled by simply connecting three valves in series. A stable and controlled pumping rate ranging from nL to μL per second can be attained by regulating the valve chamber volume, pneumatic pressure, and valve activation time (valve opening time). The PMMA based valves and pumps were further tested in a disposable automatic nucleic acid extraction microchip to extract DNA from human whole blood. The DNA extraction efficiency was 25% and the 260 nm/280 nm UV absorption ratio for extracted DNA was 1.72. DNA extracted on chip from blood samples can be used directly as PCR template for downstream amplification. Because of its advantages of low cost, facile fabrication, robust and easy integration, the PMMA valve and pump will find wide application for fluidic manipulation in portable and disposable microfluidic devices.

2. Materials and instruments

Materials

PMMA used as the microfluidic chip substrate was purchased from Shenzhen Rising Lucite Chemical Industry Co. Ltd. PDMS (polydimethylsiloxane) membrane (254 μm thick HT-6240) was purchased from Bisco Silicone (Elk Grove, IL, USA). ChargeSwitch Beads for on-chip DNA extraction were purchased from Invitrogen Corporation (Carlsbad, CA, USA). Taq polymerase was supplied by Promega (Madison, America). Forward primer 5′-GAA GTT GTC ATT TTA TAA ACC TT and reverse primer 5′-TGT CTT TTC TTC CCT AGT ATG T for human BRCA1 gene were synthesized in house using a Polygen 12 DNA synthesizer (Munich, Germany).

Instrument

CO_2 Laser (Licheng Company Inc., Guangzhou, China) was applied to engrave PMMA wafer. A UVO-cleaner (Jelight Company Inc., CA, USA) was used for PMMA wafer surface treatment. Advancing contact angles were measured on static droplets using a home-made contact angle measurement device. The IR spectra were obtained from thermo spectrometer (Avatar 330FT-IR, Nicolet, US). Solenoid valves (HV010E1-PSL-5vdc, Humphrey Products Company) and Diaphragm pumps (7011DC, ASF THOMAS Industries) were employed to integrate with valve controller to regulate pressure in displacement chamber for valve activation. A peltier thermal cycler (BIO-RAD, Richmond, CA, USA) was used for PCR.

3. Experiments

3.1 Microfabrication

Laser ablation is an important microfabrication approach that has been widely employed to fabricate PMMA microdevices.[56,57] In this work, a CO_2-laser engraving machine was used to engrave the PMMA substrate. The machine was composed of an XY engraving table, excimer laser, motion system, mirrors and lenses *etc.* The microfluidic structures designed using an AutoCAD software were transferred to the CO_2-laser scriber which directly machines on the PMMA substrate by precisely controlling the laser position in X and Y direction. In the process, a beam of high-energy laser was used to break bonds among polymer molecules and caused a miniexplosion and ejection of ablated material, leaving photoablated microchannels. The depth of the engraved channel can be controlled by adjusting laser power and scanning speed. Different shape or size microcavities and channels can be attained in PMMA substrates.

The resulting structures are generally characterized as having little thermal damage, straight vertical walls, and well-defined depth. However, the microchannels achieved were rough because of the decomposed polymer fragments that would clog the fluid through the channels. Therefore, we used thermal treatment or lithium aluminium hydride (LAH) treatment to improve smoothness of the channels. In the thermal treatment process, PMMA chip was placed in a 100 °C for about 30 min. For LAH treatment, the PMMA is put in 4 M LAH solution in diethyl ether for 30 min. Both treatments effectively improved the smoothness of the channel (see Supplementary Fig. S1†).[57]

3.2 Device bonding

PMMA was rinsed with deionized water, cleaned by the ultrasonic cleaning device, and dried in a stream of dry nitrogen. Prior to sealing, the surface of PMMA and PDMS was treated by UV ozone cleaner. The treatment appears to play a critical role in the strong PMMA/PDMS bonding, as both untreated PDMS and PMMA yield poorer-quality device. Devices were assembled by first placing a piece of PDMS membrane across the fluidic wafer, with engraved features facing up. The PMMA pneumatic layer, engraved features facing down, was then placed on top of the PDMS film after the engraved features on the pneumatic and fluidic wafer were well aligned. The PMMA–PDMS bonds formed in this manner were reversible but still strong enough to survive the range of vacuum and pressures exerted on the device.

3.3 Characterization of valve and pump

The performances of PMMA/PDMS/PMMA valves were first tested by measuring flow rate of water under different head pressures at valve-open and valve-close status. Flow rate was

measured by weighing the amount of water that flowed through the valve over a given period of time. Head pressure was created by raising a reservoir connecting to the inlet of the valve to a certain level to create hydraulic pressure. The pressure was calculated by $P = \rho gh$, where ρ is the density of water, h is the height of reservoir above the inlet of the channel.

The performance of the pump was explored by investigating the effect of activation time, displacement chamber volume, back pressure on flow rate. We measured the flow rate and the volume pumped per cycle at different activation times against zero backward pressure. To investigate the relationship between valve volume and flow rate, four pumps with different diaphragm diameters were fabricated while keeping the dimensions of other features unchanged. With a simple model ($V = \pi d(D^2_1 + D_1D_2 + D^2_2)/12$, where d is the etch depth of the pneumatic layer, D_1 is the diameter of the displacement chamber, and D_2 is the diameter of the region of contact between the PDMS membrane and the etched bottom of the displacement chamber),[58] the volumes of these four pumps were calculated and were evenly distributed in the range of 500 nL to 2500 nL. By raising the reservoir connecting to the outlet of the pump to different heights, the effect of back pressure on pumping rate was studied. Moreover, to study the long-term performance of the pump, a single pump was operated for 8 days with continuous pumping, 48 000 cycles each day while periodically measuring the volume pumped per cycle.

3.4 Genomic DNA extraction from whole blood on chip

To test the performance of the PMMA based valves and pumps, a fully automatic nucleic acid extraction microchip was fabricated.

To extract DNA, 1 mL human whole blood was first mixed with 1 mL chargeswitch lyses buffer and 10 μL proteinase K for 30 minutes at 37 °C. Chargeswitch magnetic beads were washed three times with NaAc buffer (pH = 5.0) to activate bead surface charge. To load 20 μL chargeswitch magnetic beads (3.125 mg mL^{-1}), a magnet was placed under the extraction domain while pumping magnetic beads solution from the inlet through the extraction domain. After beads loading, 10 μL of lysed sample was directly pumped through the channel. The adsorption of DNA to beads interconnected microbeads, which formed a tight pellet as a result. About 60 μL of NaAc buffer was then used to wash away species nonspecifically absorped on beads. To elute DNA, 10 μL TE buffer (pH = 8.0) was used to elute DNA from beads for three times. During the elution stage, fractions were collected and used for PCR without further treatment.

3.5 PCR amplification

In order to test the purity of DNA extracted from our chip, the PCR amplification was performed. The reaction mixture was prepared according to the following final concentrations: 20 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.4 μM of each primer, 200 μM dNTP, 0.25 units Taq polymerase, 10 μl of the eluted DNA solution. The thermal cycling conditions were as follows: 94 °C for 3 min (initial denaturation), 20 cycles of 94 °C for 30 s, 44 °C for 30 s, and 72 °C for 30 s, followed by a single final extension for 5 min at 72 °C.

4. Result and discussion

4.1 Design and fabrication of microvalve

The topologies of the three-layer device used to fabricate monolithic membrane valves and pumps are illustrated in Fig. 1. Channel features were engraved into PMMA wafers with laser ablation.

The microvalve is consisting of PMMA/PDMS/PMMA layers: the top layer is a PMMA pneumatic wafer, the middle layer is a thin PDMS membrane, and the bottom layer is a PMMA fluidic wafer. The microvalve is formed by aligning an engraved displacement chamber in the pneumatic wafer across the PDMS membrane from a barrier spacer that separates an otherwise continuous channel in the PMMA fluidic wafer (see Fig. 1). Valve closing or opening can be controlled by adjusting the pressure in a displacement chamber on the pneumatic layer *via* a computer regulated solenoid. When a pneumatic pressure higher than atmospheric pressure is applied, the PDMS film is pressed against the barrier gap between the fluid channels and the valve is closed. When vacuum is applied to the displacement chamber, the PDMS film is pulled away from the fluidic wafer and fluid can flow across the gap, thereby opening the valve.[37]

4.2 Operation and control of micropump

When arranged in series, three valves forms a pneumatic pump (see Supplementary Fig. S2†). Pumping was realized by actuating the input, diaphragm, and output valves of each pump in parallel according to a three-step cycle. The volume pumped per cycle was ultimately determined by the volume pushed out of the diaphragm valve while closing.

Pressure and vacuum for valves actuation were controlled by a set of solenoid valves and a computer program written in Visual Basic. As shown in Supplementary Fig. S3,† running parameters such as activating time (vacuum time) and valve activating sequences are typed in the program and transferred to the Single-Chip Microprocessor *via* a USB port. The TTL signal from the Single-Chip Microprocessor is then amplified by a ULN2803 chip to activate the solenoid which regulates pressure in the displacement chamber.

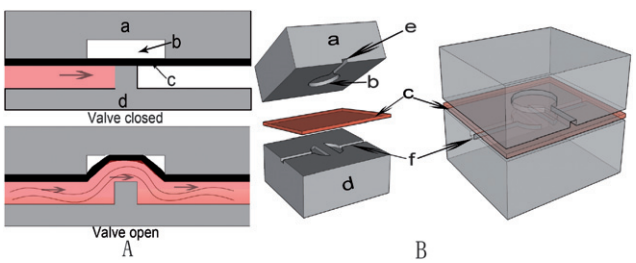

Fig. 1 Cross-sectional views of a three-layer monolithic PMMA/PDMS membrane valve (A) and exploded and assembled illustrations of a single PMMA/PDMS membrane valve (B). a: PMMA pneumatic wafer; b: displacement chamber; c: PDMS membrane; d: PMMA fluidic wafer; e: pneumatic channel; f: fluidic channel.

4.3 Surface modification

Our early attempts to assemble PDMS onto a PMMA chip turned out to be not so successful. By placing a piece of PDMS membrane onto a pristine PMMA, we found that PDMS film peeled off easily. To improve the stickiness of PDMS to PMMA, we decided to perform surface treatment to increase PMMA/PDMS interface interactions. The surface chemistry modification of PMMA and PDMS has been sporadically reported previously.[59–62] Straightforward chemical oxidation of the ester groups on PMMA and Si atom on PDMS surface by UV ozone produced hydroxyl groups (–OH) (Fig. 2A). After that, PMMA/PDMS wafers could seal together because of the hydrogen bond forces and condensation reaction between hydroxyl groups from both surfaces. As we expected, the ester groups can be transformed into hydroxyl groups after treatment. In order to confirm this, we performed a simple experiment by measuring the contact angle of water on PMMA chip before and after the treatment (Fig. 2B). It was found the contact angle of PMMA without treatment was about 84°, which was consistent with data reported previously.[63] After treatment, a much smaller contact angle (68°) was observed, which was likely due to the production of OH groups on the PMMA surface as a result of the UV ozone treatment.

The production of OH groups on PMMA surface was further proved by FTIR analysis. Fig. 2C shows the IR spectra of PMMA powder before and after UV ozone treatment. It's found that both samples shared similar characteristic peaks below 2000 cm^{-1}, which is consistent with the data reported in the literature.[64] The stretching vibrations of C=O and C–O–C showed similar frequency and relative intensity for both samples. A prominent broad peak around 3450 cm^{-1}, characteristic of –OH stretching, was observed for PMMA sample after ozone reduction. Since strict baking was performed before taking the FTIR spectra, the broad –OH stretching absorption was unlikely to be caused by surface bound water molecules. XPS analysis of PMMA and PDMS before and after treatment further confirm the generation of hydroxyl groups on both surfaces (Supplementary Fig. S4†).

After the UV-ozone bath, both the PMMA and PDMS are much "cleaner" and can stick to each other firmly, which was very different from the result carried out before the modification. We were finally able to integrate PDMS to PMMA to produce a robust PMMA/PDMS/PMMA chip with good reproducibility.

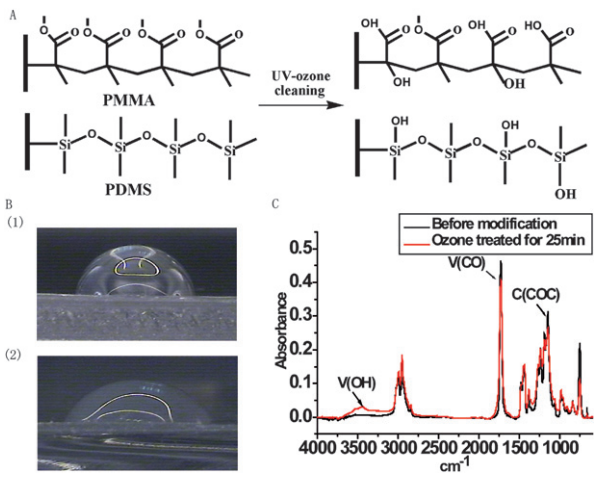

Fig. 2 (A) Chemical oxidation of PMMA and PDMS surface by UV ozone treatment. (B) The contact angle of PMMA before (1): 84° and after (2): 68° surface treatment. (C) FTIR spectra of PMMA surface before and after UV ozone treatment.

4.4 Characterization of microvalve

The performances of PMMA/PDMS/PMMA valves were first tested by measuring the flow rate of water under different head pressures at valve-open and valve-close status. Fig. 3 shows the average rate of pressure-driven fluid flowed through the PDMS membrane valves being held open (pneumatic pressure = −60 kPa) and closed (pneumatic pressure = 25 kPa). The average flow rate through open valve was high (15.4 μL s^{-1} at 60 kPa fluid pressure), and increased linearly along with fluid pressure. This relationship was consistent with the fluid flowing through a no-valve channel. It indicated that the presence of the valve in the channel had a negligible effect to the flow rate.[58] In contrast, when the valve was closed and the forward fluid pressure was less than 45 kPa, no flow was observed. The valves began to leak when the fluid pressure was higher than 60 kPa, but the flow rate was as little as 0.10 μL s^{-1}. The flow rate began to increase with forward pressure greater than 60 kPa. In comparison, when the head fluid pressure was 60 kPa, the average flow rate through the open valves was 147 times more than the average flow rate through the closed valves.

4.5 Characterization of diaphragm pump

Three valves placed in series were used to pump liquid peristaltically. The performance of the micropump at different activation time is illustrated in Fig. 4.

As shown in Fig. 4, when the activation time was less than 100 ms, the flow rate increased drastically along with activation time and attained a maximum of 5.84 μL s^{-1} at 20 ms. Following that, it fell sharply with the increase of activation time. The closed cycle in Fig. 4 shows that the volume pumped per cycle increases rapidly along with activation time. When the activation time was more than 100 ms, the volume pumped per cycle remained flat. The relationship between flow rate and activation

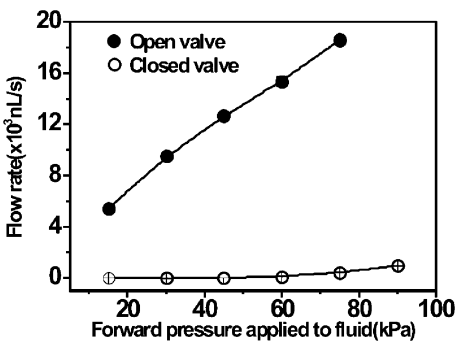

Fig. 3 Mean flow passed through three open and closed valves while an upstream pressure was applied to the fluid.

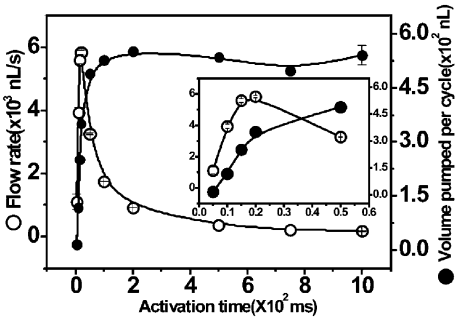

Fig. 4 Mean flow rate (○) and volume pumped per cycle (●) through the channel as different activation times are applied to the three valves. Displacement chamber volume was 1712 nL.

time indicated that one could attain a given flow rate precisely by controlling the activation time.

We further investigated the effect of displacement chamber volume on flow rate. Fig. 5A shows the measured volume pumped per cycle at zero head pressure for various sizes of pumps. To ensure that the diaphragm valves were completely full or empty in each pump cycle, the activation time of each step in every cycle was set to 500 ms, for a total of 1.5 s per cycle. As expected, pumps with larger diaphragm valves pumped more fluid per cycle.[58] The results indicated that the volume pumped per cycle increased linearly along with the size of pumps. Thus, one can precisely control flow rate of fluid by changing the displacement chamber volume of valves.

In order to examine the effect of back pressure on flow rate, we further examined the relationship between flow rate and back pressure. Fig. 5B shows the performance of a PMMA pump operated at different backpressures. Flow rate and volume pumped per cycle of fluid flowed through decreased roughly linearly with the increase of backpressure. When the backpressure was 0 kPa, flow rate was very fast (5.84 μL s^{-1}) and volume pumped per cycle was large (351 nL). However, flow rate dropped to 0.95 μL s^{-1} when the backpressure was increased to 9.8 kPa. The volume pumped per cycle was almost negligible when the backpressure was approximately 12 kPa, which indicated that the highest backpressure the valves could bear was about 12 kPa.

Finally, in order to test the long-term performance of the PDMS membrane pumps, a single 1712 nL pump was operated for 8 days with continuous pumping of 48 000 cycles each day, while periodically measuring the volume pumped per cycle. Supplementary Fig. S5† shows that the volume pumped per cycle changes less than 4.9% from the original value.

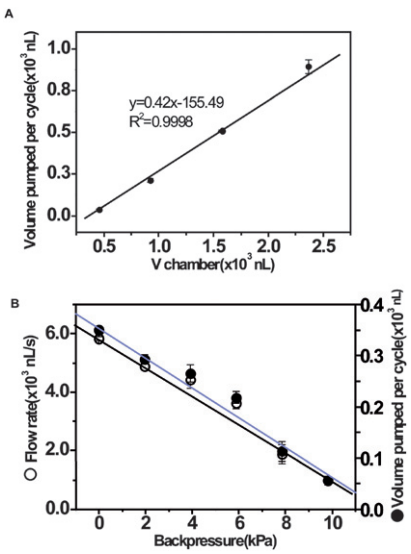

Fig. 5 (A) Mean volume pumped per cycle by PDMS membrane pumps with diaphragm valves of four different volumes. Activation time was 500 ms; (B) flow rate (○) and volume pumped per cycle (●) of fluid flowed through the pump applied to different backpressures. Activation time was 20 ms and displacement chamber volume was 1712 nL.

4.6 Application of micropump

To be suitable for disposable applications, a device should be as inexpensive as possible. Using PMMA as the substrate for valves and pumps has its advantages of fast fabrication and low cost, thus making the PMMA pump attractive for disposable microfluidic devices (see cost analysis in the ESI†). The PMMA/PDMS valves can easily be fabricated in different PMMA devices. To test the performance of the PMMA/PDMS/PMMA valves and pumps, a fully automatic nucleic acid extraction microchip was fabricated.

On chip DNA extraction is one of the important steps for genetic analysis using microfluidics. Lander's group has reported several novel on-chip DNA extraction methods based on solid-phase extraction.[41,46,65–69] We report here the use of magnetic charge-switching microbeads for facile automatic DNA extraction. Fig. 6(a) illustrates the layout of the DNA extraction chip. Domains for DNA extraction (E in Fig. 6a) were connected through a network of channels and holes. The sample was loaded with valves V1, V4, V5 and V6, respectively. Washing was controlled by four valves (V2, V4, V5 and V7). Valves V3, V4, V5 and V8 function to elute the DNA from beads (Fig. 6b).

The microchip was used to extract genomic DNA from human whole blood based on the chargeswitch technology. Chargeswitch magnetic beads provide a tunable surface charge polarity

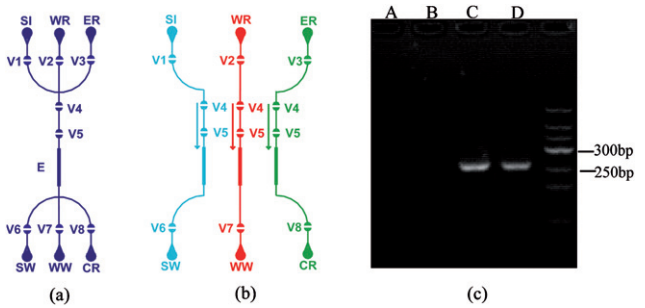

Fig. 6 Layout of DNA extraction chip (a, SI: sample inlet, WR: washing reservoir, ER: eluting reservoir, E: extraction area, SW: sample waste, WW: wash waste, CR: collection reservoir) and the steps for DNA extraction (b, cyan: sample loading, red: washing, green: elution) and gel electrophoresis of PCR product of genomic DNA extracted from human blood sample (c, A: blank sample, B: blood sample, C: DNA extracted off-chip, D: DNA extracted on-chip).

depending on the buffer pH to facilitate nucleic acid purification (Supplementary Fig. S6†). At low pH conditions, the beads have positive charges that bind the negatively charged DNA backbone. Proteins and other contaminants are not bound and are simply washed away in an aqueous wash buffer (pH 5.0). To elute DNA, the bead surface charge is switched to negative by raising the pH to 8.5 using a low salt elution buffer.

DNA collected was analyzed first with a UV-vis spectrometer. The absorption ratio at 260 nm to 280 nm was 1.72, suggesting a good purity of DNA was obtained. Further calculation indicated that an extraction efficiency of about 25% of was obtained. To be useful for many biological applications, the extracted DNA from crude biological samples must be PCR-amplifiable. The DNA extracted was further tested on PCR, targeting a human cancer tumor-suppressing DNA sequence (BRCA 1, 258 base pair at the second exon). PCR amplified products were analyzed by agarose gel electrophoresis as shown in Fig. 6c. When whole blood was used directly without extraction, no PCR product was observed. This is not surprising as lysed human blood is a complex mixture, containing various proteins, peptides, lipids, carbohydrates, and other low molecular weight compounds that inhibit PCR. In contrast, genomic DNA extracted off-chip and on-chip was successfully amplified, as shown in lane C and lane D, respectively. The results clearly indicated that genomic DNA could be successfully extracted and purified from human whole blood with the automatic nucleic acid extraction microchip.

5. Conclusions

We have developed a simple, cheap and fast method for fabricating and modifying PMMA microfluidic devices containing PDMS valves and pumps. Its advantages of inexpensive, fast and facile fabrication will accelerate the development of portable, disposable PMMA microfluidic devices. Compared to a glass chip, the cost and time of machining a PMMA chip are negligible. The design of the PMMA/PDMS valves and pumps ensure that a stable and controllable fluid flow rate can be attained through changing activation time, displacement chamber volume and pressure. The simplicity of PMMA valve and pump fabrication coupled with chemical compatibility of the PMMA microfluidic analysis platform make these valves and pumps well suited for integration into portable and disposable bioassay devices.

In this study we further demonstrated the purification of DNA from a complex biological sample in the form of human whole blood by using an automatic nucleic acid extraction PMMA/PDMS microchip. The DNA extraction efficiency was about 25% and the absorption rate extraction genomic DNA at 260 nm to 280 nm was 1.72. Efforts were undertaken to fabricate a portable PMMA chip that integrates DNA extraction, DNA amplification and CE analysis on a single chip.

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