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A simple PDMS-based electro-fluidic interface for microchip electrophoretic separations

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High voltage electrodes for electrophoresis have been integrated into a polymer layer that can be reversibly bound to glass microchips for electrophoretic separations. By using the liquid precursor to the polymer polydimethylsiloxane (PDMS), platinum electrodes and reservoirs can be positioned prior to solidification, providing a simple and flexible method for electrode interface construction. Field strengths up to 875 V cm^{-1} over an 8 cm separation channel can be applied to the system without any loss in performance of the interface. The interface can function as an electro-fluidic interface between the high voltage power supply and the separation channel and, when reversibly sealed to an etched glass plate, functions as a cover plate establishing a hybrid PDMS–glass microchip in which the electrodes are directly integrated onto the device. The versatility of this approach is not only demonstrated by separating DNA fragments in a novel buffer sieving matrix, but also with the molecular diagnostic analysis of a variety of DNA samples for Duschenne Muscular Dystrophy and cytomegalovirus (CMV) infection, using both microchip interface configurations.

Introduction

The development of microchip systems for many routine clinical tasks has been an area of major significance in recent years due to the reduction in analysis time and sample/reagent volumes that result from the reduced dimensions of the device. Perhaps a more significant advantage is the ability to integrate multiple analytical steps onto a single device, leading to the development of the so-called 'lab-on-a-chip' or 'micro-total analytical system (μ-TAS)'. While this implies the development of multiple steps, the only processes routinely performed on the microchip platform are electrophoretic separations.[1] Recently, pre-electrophoresis steps, such as polymerase chain reaction (PCR)[2,3,4] and solid-phase extraction (SPE)[5,6,7] have been demonstrated on the microchip platform as stand-alone processes, but the integration of these multiple-processes into a single microchip device is not a trivial issue. To date, only the integration of PCR and electrophoretic separation in a single device has been demonstrated for DNA related processes,[8,9] and although the results were promising, many technical challenges remain before a robust and reliable system will be obtained.

One of the major problems facing the development of a reliable μ-TAS device is the issue of 'macro-to-micro interfacing'. Microchip-based chemical processes occur in microchannel or microchamber spaces with very small volumes, yet delivery of reagents and sample must be provided from volumes in the 'macro-world' that can be 3–6 orders of magnitude larger.[10] Sample introduction, fluidic control and analyte detection are commonly performed with conventional instrumentation, and while some elegant solutions have been developed specifically for microchips (such as cross-tee injections), many of the approaches are far from suitable for microchips. New systems and new approaches need to be developed, particularly concerning detection systems and electronic–fluidic interfaces for a reliable and fully automated system to be developed. One approach that has received considerable attention recently is to engineer components into the microchip itself. Various devices, such as valves,[11] detectors[12,13] and mixers[14,15] have all been constructed in microchip devices, but fabrication is a major issue, particularly when using glass substrates. Integrated components must be able to withstand very high temperatures (up to 700 °C) to allow for thermal bonding of the coverplate to the etched plate containing the microchannel architecture. Even with the development of alternative bonding techniques,[16] this process remains challenging.

Incorporation of functionalities may be more easily achieved through the use of polymer substrates, but with many polymers, difficulty often arises with respect to heat dissipation and background fluorescence when using LIF detection. However, one such polymer, polydimethyl siloxane (PDMS), has been demonstrated for microchip electrophoresis without these aforementioned hindrances. PDMS has been proven to be a sufficient substrate for the separation of DNA as well as other small molecules.[17,18] It is ideal for component integration in microchips because it is a liquid prior to low-temperature solidification (60 °C). This is beneficial because it can be molded around the desired component, and many such components are able to withstand the low temperatures required for solidification. This has been demonstrated through the incorporation of an optical detector for LIF detection onto a microchip[19] demonstrating the potential for PDMS to be utilized as a substrate for incorporation of other components onto a single device.

Our work has been directed toward the integration of separation electrodes directly into a reusable coverplate for electrophoretic processes. By embedding platinum wires into PDMS prior to curing, a cover plate can be fabricated which allows for enclosure of the separation channel as well as an interface between the high voltage power supply and the separation channel. This avoids problems with durability and stability typically associated with depositing electrodes directly onto the surface. Furthermore, because of the reversible nature of the PDMS-glass bond, the electrodes can be removed when the microchip becomes defective, greatly reducing the cost of disposable chips. The benefits of this system include the ease of fabrication, the provision of an electro-fluidic interface for the microchip system, and a reusable system due to the reversible bonding of PDMS to glass. The potential of this approach for integrating electrodes for electrophoretic processes either during prototype development or for microchip separation method development has been demonstrated using common

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electrophoresis buffers. The electrode-integrated microchip could be utilized to perform diagnostics for the presence of cytomegalovirus (CMV) when utilized as an electro-fluidic interface, and as a cover plate on an open-channel microchip for the diagnosis of Duschenne Muscular Dystrophy (DMD). Together these demonstrate the possibility of this simple and flexible interface system being utilized for method development in microchip analysis.

Experimental procedures

Chemicals

Sodium tetraborate was purchased from Fisher, (Fairlawn, NJ., USA), hydroxypropylcellulose (HPC) (100,000 MW), hydroxy methyl aminomethyl (TRIS), and 2-(4-morpholino)-ethane sulfonic acid (MES) were all purchased from Acros Organics, (New Jersey, USA). YOPRO-1 was purchased from Molecular Probes (Eugene, OR, USA). Sylgard 184 polydimethyl siloxane (PDMS) was purchased from Dow Corning (Midland, MI, USA).

Microchip fabrication

Microchips were fabricated using standard photolithography and wet chemical etching techniques. The template was designed with AutoCAD and printed on a transparent film with 3600 DPI resolution. The design was transferred onto a glass wafer (Nanofilm, Westlake Village, CA, USA) with positive photoresist using UV exposure. The channels were etched with concentrated HF solution ($HF:HNO_3:H_2O$ = 20 : 14 : 66). Small holes (1.1 mm) coinciding with the ends of the channels were drilled with diamond drilling on a cover plate. The etched plate and the cover plate were bonded together *via* thermal bonding with an annealing temperature of 690 °C using standard thermal annealing techniques. Each microchip contained an 8 cm long separation channel and a 1 cm long sample injection channel, which was positioned 0.5 cm from the buffer inlet reservoir. For the Joule heating study, microchannels were 60 μm wide at half height and 30 μm deep. For DNA separations, the microchannels were 40 μm deep and 80 μm wide at half height. For the comparison studies, microchips were thermally bonded as previously described.

Interface sealing

For sealing the PDMS interface to glass the following procedure was used. The glass was cleaned in piranha wash (H_2O:H_2SO_4:H_2O_2, 3:2:1) and following this, the glass and PDMS substrates were rinsed in HCl for 10 min followed by deionized water, dried in a cleanroom and pressed together. Upon removal, the interface and the bottom plate were reversibly bound with a seal strong enough to withstand 600 Torr of vacuum.

Microchip electrophoresis system

Microchip electrophoresis with laser-induced fluorescence (LIF) detection with a 488 nm Argon ion laser (Laser Physics, Salt Lake City, Utah) was performed in a fashion similar to that previously described.[20,21,22] For excitation, the laser beam passed through a beam expander (Spindler & Hoyer, Milford, MA), a dichroic beamsplitter with a cut on 500 nm (Optometrics, Nayer, MA) and into a 16× microscope objective (Melles Griot, Rochester, NY), which focused the laser onto the separation channel 1 cm from the separation channel outlet, giving the microchannels utilized in this study an effective length of 6.5 cm. Emitted fluorescence was collected through the same microscope objective and back through the beamsplitter. The light was then filtered through a 560 nm (40 nm bandpass) filter (Optometrics, Nayer, MA), passed through a pinhole (Spindler & Hoyer, Milford, MA) and onto a photomultiplier tube (PMT) (Hamamatsu, Bridgewater, NJ). Signal processing was performed by an independent microprocessor (SBC2000-332, Vesta, Wheat Ridge, CO), which gathered the signal from the PMT and transferred this signal to a computer (AMD K6) *via* a RS232 communication port. Data was collected and displayed using an in-house Labview (National Instruments, Austin, TX) program allowing for multiple continual separations and automatic data storage. This Labview software also allowed for control of an in-house designed and built power supply.

Microchip DNA separations

For microchip separation of DNA, buffer (3.5% HPC, 80 mM MES, 40 mM TRIS, pH 6.1) was flushed through the channels *via* vacuum (600 Torr) for 10 min following a 1 min flush with deionized water. For separations in glass microchips with no PDMS interface, ϕX-174 DNA was dissolved in water to a final concentration of 2.5 μg mL^{-1}, and the CMV samples were diluted 40-fold in deionized water from original concentration (as supplied by P. Shawn Mitchell, Mayo Clinic). For sample injection, a potential of 300 V was applied to the sample waste reservoir while the sample inlet reservoir was held at ground and both the buffer inlet and outlet reservoirs allowed to float. Following a 70 s sample injection, subsequent separation occurred by applying a potential of 500 V to the buffer inlet and 2500 V to the buffer outlet while the sample inlet and waste were held at ground. This resulted in a field strength of 375 V cm^{-1}. For separations in the hybrid system, ϕX-174 DNA was dissolved in water to a final concentration of 0.5 μg mL^{-1}. For the DMD analysis, multiplex-PCR amplified samples were obtained from Dr. Karen Snow (Mayo Clinic) and were diluted 80-fold in deionzed water from original concentration. All other conditions were identical to the glass microchip separations with the exception of the field strength being reduced to 300 V cm^{-1} (−400 V buffer inlet, 2000 V buffer outlet).

Results and discussion

The integration of components into a microchip device is essential if μ-TAS systems are to become simple, cost-effective and reliable. With respect to integrating electrodes onto microchips, the most popular approach is to deposit metals directly onto the surface to allow electrical connection. Using this approach, work has been performed incorporating electrodes along the sides of a microchannel to allow for localized control of EOF[23] or for the control of the ξ-potential along the entire distance of the channel.[24] Work has also been performed in this area to allow for increased resolution in slab gels for DNA separations.[25] However, with these reports only low voltages were applied to these embedded electrodes and auxiliary electrodes are utilized for separation. While this approach is elegant and functional, the cost for deposition of metal as electrodes is substantial, especially if the microchip is either a prototype or is disposable, *i.e.*, used once. A more desirable solution is to have integrated electrodes built into a replaceable coverplate that can be bound reversibly to glass microchips.

Fabrication of a PDMS-based electro-fluidic interface

The first stage in constructing an electrode interface is to create a mold in which the PDMS coverplate can be cast. A rectangular mold was constructed with removable sides in a manner that allowed the PDMS to be poured around platinum electrodes (0.5 mm diameter) (Fig. 1A). Access holes were drilled into the sides of the mold (1 mm from bottom) to allow for introduction of the

platinum wire electrodes. The holes were drilled with a tolerance that allowed the electrodes to fit snugly, avoiding any leakage that might occur while the PDMS cured. Metal posts (3 mm diameter) were inserted into the base plate allowing for the molding of sample and buffer reservoirs. Upon insertion of the posts and the electrodes, a PDMS mixture consisting of 10 : 1 combination of polymer elastomer and curing agent (by weight) was degassed for 1 h, and poured into the mold to a height of ~ 2 mm. After the PDMS was allowed to cure for 1 h at 60 °C, the four sides of the mold were removed and the interface was peeled from the bottom of the mold. The interface was sealed reversibly to glass surfaces, either glass-bottom plates containing etched channels, or complete glass chips consisting of a glass bottom and a glass cover plate. When bound to complete glass chips, the interface functioned as a vehicle for application of high voltages as well as providing sample and buffer reservoirs. When bonded to etched glass plates, the interface also functions as the top surface of the separation channel.

For microchip separations, the microchip and electrode-embedded interface were contained within a cartridge designed to connect the high voltage power supply to the electrodes. Fig. 1B shows the actual cartridge with a microchip and interface in place. Electrical leads from the power supply could be inserted directly into female adapters that were positioned along the front of the cartridge and were soldered onto high voltage wires leading to the electrode platforms. These high voltage wires ran through moats that were machined into the cartridge and led to platforms onto which the interface electrodes rested. Onto these platforms, copper plating was fastened with epoxy and soldered to the high voltage wires. The electrodes could then rest on the copper plating with the plating folded over the electrodes to ensure a good connection. A hole was machined into the bottom of the interface to allow for LIF detection from the bottom as described above. Once the chip was positioned into the cartridge, a top was screwed into the bottom to secure the system and allow for chip flushing and alignment.

Evaluation of electrode stability with the electro-fluidic interface

It is important that there is no loss of potential from either the cartridge used to house the electrode interface, or from the close proximity of the electrodes within the PDMS layer. To evaluate the ability of the electrodes to consistently deliver the required voltage, the measured voltage *versus* the applied voltage was measured on a standard electrode interface (where the electrodes are in direct contact with the high voltage power supply *via* high voltage wires) and compared with that on the PDMS electrode interface/cartridge. Since most microchip separations are complete within 5 min, we deemed this to be a reasonable 'test time' to compare the electrical properties of the two systems. All other variables remained the same in this study and the results of these measurements can be seen in Table 1. As shown by Table 1, there is no loss in voltage along the interface system, and more importantly, there is no loss of potential in the PDMS layer with embedded electrodes. Minor variations can be attributed to the drift in the power supply which has a drift of ~ 10%.

Absence of Joule heating problems with the PDMS electro-fluidic interface

An important factor with all electrophoretic separations is the amount of Joule heating produced during the separation process. As with other polymer matrices, PDMS does not dissipate heat as well as glass and, thus, Joule heating may have

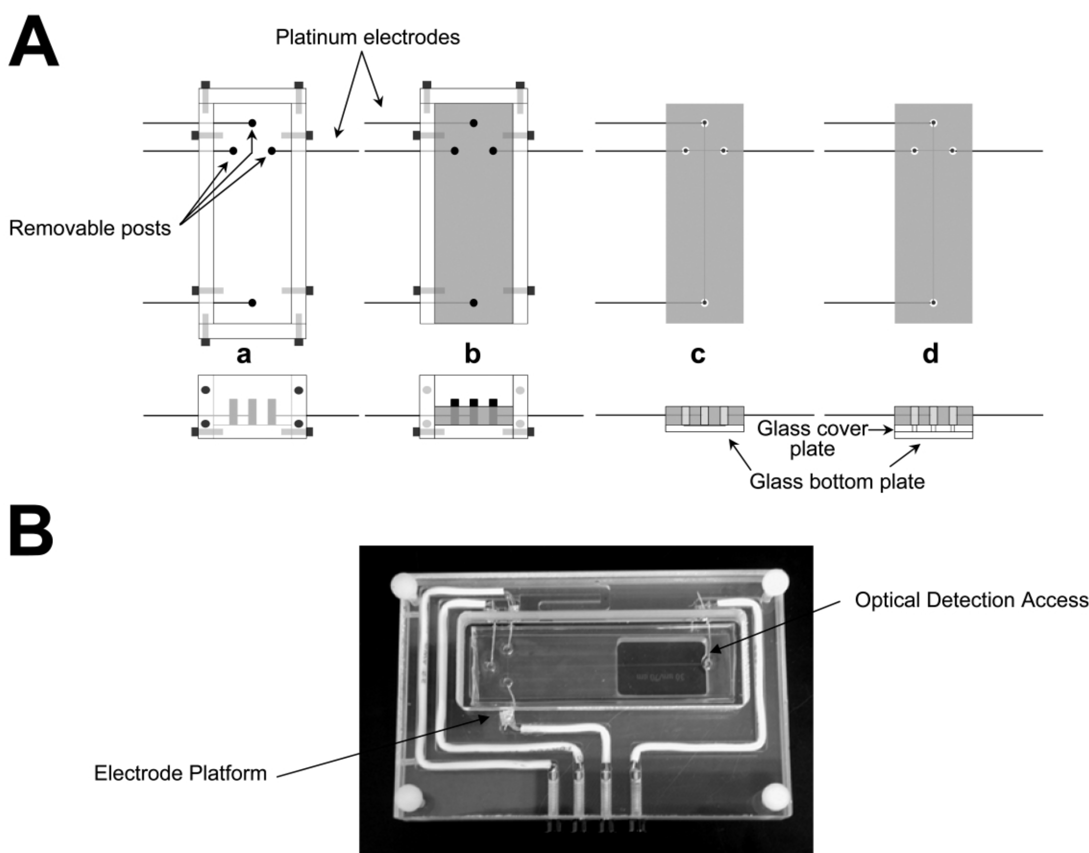

Fig. 1 Plexiglas apparatus for casting the PDMS electro-fluidic interface. (A) Interface Mold—Prior to the PDMS curing, the mold is fully assembled (a). Upon curing, the mold can be dismantled (b) to allow for removal of the PDMS interface with the embedded electrodes. (c) The interface can be utilized as a coverplate for a PDMS/glass hybrid microchip with integrated electrodes when placed on top of a glass bottom plate with an etched channel. (d) The interface can also serve as an electro-fluidic interface for a glass microchip when placed on top of a thermally bonded glass microchip. (B) Microchip Cartridge—Contains the female adapters and associated circuitry for interfacing with the high voltage power supply.

Table 1 Comparison of the performance of the standard electrode interface with the integrated electrode interface

Applied voltage from high voltage power supply (V)	Measured voltage (V) of standard interface at $t = 0$	Measured voltage (V) of standard interface at $t = 5$ min	Measured voltage (V) of PDMS interface at $t = 0$	Measured voltage (V) of PDMS interface at $t = 5$ min
1000	1013	1013	1015	1015
2000	2027	2027	2026	2026
3000	3039	3039	3038	3038
4000	4000	4000	4000	4000
5000	5000	5000	5000	5000

an adverse effect on the separation. To evaluate this, channels were filled with 50 mM borate buffer (pH 8.9) and electrophoresis was performed by applying voltages from 1000 V to 10000 V in 1000 V increments. This was accomplished using (1) a traditional top-mounted electrode interface, (2) the new embedded electrode interface on top of a thermally-bonded chip, and (3) the interface as a coverplate for an open channel etched in glass. For the first two experiments, the same microchip was utilized. For the experiment in which the interface was placed directly over an open channel, the microchip utilized had the same channel dimensions as the thermally-bonded microchip used in the previous experiment. Ohm's Law plots[26] for both configurations can be seen in Fig. 2. From these plots, it is clear that, up to an applied voltage of ~7000 V, all the plots are linear with an r^2 value above 0.998. Above this voltage, the effects of Joule heating are evidenced by the deviation from linearity. Importantly, when comparing Fig. 2A and B the variation of current with voltage is almost identical, indicating that there are no adverse Joule heating effects from the PDMS interface when used as an interface onto of a thermally-bonded chip. Fig. 2C shows slightly more deviation from linearity above 7000 V than Fig. 2A and B, which may be due to the reduced efficiency of heat dissipation with the PDMS coverplate when compared to glass. As such, slightly more consideration of the chip design, applied voltage and buffer composition is necessary when using PDMS as a coverplate. However, given the appropriate conditions, it is clear that there is no reduction in performance of the PDMS electrode interface.

Electrode-embedded PDMS as an electro-fluidic interface for DNA separations in a thermally-bonded microchip

While the above studies clearly indicate the acceptable performance of the integrated PDMS interface, it is necessary to test the ability of such a device for electrophoretic separations of meaningful samples. Given that DNA analyses are the most commonly performed separations on microchip devices, this seemed a judicious choice for evaluating the integrated electrode interface. Initial separations were performed while utilizing the new PDMS cover plate as an interface between the high voltage power supply and the separation channel enclosed within a standard thermally-bonded microchip. The advantage of using the interface in this manner is that much of the bulk of a typical electrode interface is eliminated. With this system, the ability to integrate some form of flushing device on top of the system is possible. As can be expected, DNA separations can easily be accomplished with this system and determination of viral DNA can be routinely performed. Fig. 3A shows as a

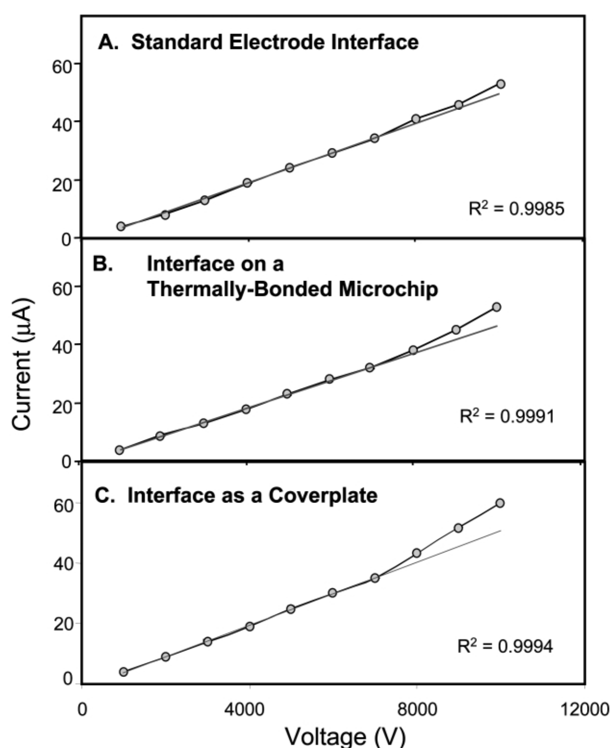

Fig. 2 Ohm's Law plot of standard Plexiglas interface *versus* the PDMS electro-fluidic interface. All channels were filled with 50 mM borate buffer and current was measured with respect to voltage for (A) thermally-bonded channel with standard top-mounted interface, (B) thermally-bonded microchip with PDMS interface and (C) microchip with PDMS interface acting as a coverplate.

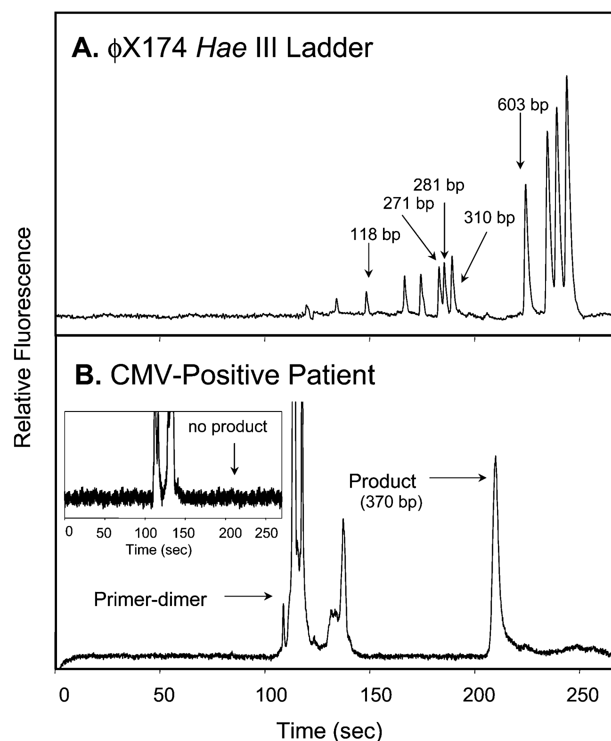

Fig. 3 Determination of cytomegalovirus with the electro-fluidic interface on a thermally-bonded microchip. (A) ϕX-174 DNA digest fragments were separated in a thermally-bonded microchip utilizing the embedded electrodes as a electro-fluidic interface. (B) Determining the presence of CMV was accomplished employing this same interface. Inset—Negative control. Separations conditions can be found in the materials and methods section.

standard, the separation of restriction endonuclease-digested DNA utilizing this system as an electrode interface. Fig. 3B shows the utility of this system for the detection of cytomegalovirus (CMV) DNA following the PCR-amplification of human DNA. Cytomegalovirus infection is often found in those who have recently received liver transplantation or are infected with HIV.[27] A negative control (sample lacking CMV DNA) is shown in Fig. 3B inset.

Electrode-embedded PDMS as a microchip cover plate for DNA fragment separations

Upon evaluation of the cover plate as an electrical interface, the utility of the device to function as a cover plate directly in contact with an open channel was considered. The ability to separate DNA in PDMS microchips has been reported previously[17,18] and given the hydrophilic nature of the surface and negative charge of the DNA molecules, the substrate is almost ideal for this type of separation. In microchips made entirely of PDMS, EOF is also drastically reduced with respect to glass and, therefore, there is no need for the surface modification that is inherent with glass microchips. For this reason, the effectiveness of hybrid microchips for separations has been questioned, since the magnitude of EOF associated with the different substrates (on the top and the bottom of the channel) may be problematic. Even with reports that establish the fact that there is very little effect on the overall magnitude of EOF in a hybrid system by Ren *et al.*,[28] the concern still remains and, thus, has led to a reduced interest in this area. However, if the magnitude of EOF present in the glass portion of the microchannel can be reduced to mirror that on the PDMS portion of the channel, this effect may be eliminated. We have recently reported[29] the development of a novel buffer system that allows for reduction of EOF for DNA separations in glass microchannels without the need for any surface modifications or preconditioning. This system, a MES-TRIS buffer containing hydroxypropylcellulose (HPC), also produces much lower currents than traditional DNA separation buffers and thus is more suitable to the hybrid system. Fig. 4 shows a separation of clinical significance utilizing the PDMS–electrode interface as a cover plate on an open channel microchip. This figure shows the potential use of this integrated electrode system for the diagnosis of clinical disorders such as Duschenne Muscular Dystrophy (DMD). A separation of multiplex PCR products of DNA fragments correlative to those mutated and expressed in patients with DMD was performed. The multiple peaks present in this electropherogram represent multiplex PCR-amplified DNA fragments from select exons of the *dystrophin* gene (Kunkel series).[30] These exons were chosen due to their propensities to contain a deletion or duplication mutation causative of DMD. These two mutations occur when either a piece of an exon has been either removed or has been repeated on that exon. Fig. 4A shows a patient with a negative diagnosis for DMD in contrast to Fig. 4B which shows a patient positive for DMD. This is determined from the increase in relative peak height of the 181 bp fragment (exon 47) with respect to the other peaks. In this particular case, the resulting mutation is due to a duplication mutation in exon 47 of the patient, thus doubling the peak intensity of exon 47 with respect to the other PCR amplified exons in the electropherogram.[31] Fig. 4B inset shows a DNA sizing ladder separated in the same microchannel under the same conditions. As can be seen from these separations, there are no ill-effects from the utilization of the PDMS as a cover plate with respect to band broadening or peak tailing. With this coverplate, genetic mutations could be determined in under 5 min, allowing for an even closer realization of the 'lab-on-a-chip' concept.

In conclusion, integration of high voltage electrodes for electrophoretic separations has been demonstrated without metal deposition onto the microchip. These electrodes, which were embedded into a polymer substrate (PDMS), allowed for the application of the same voltages as that applied by a standard electrode interface. There were no adverse affects with respect to Joule heating when the interface was utilized as a cover plate for a microchip. The utility of the embedded electrodes to function as an electrical interface with a glass microchip was demonstrated with separation of DNA fragments. With this microchip, clinical diagnosis of a viral infection (CMV) and the diagnosis of patients positive and negative for DMD was performed in less than 5 min. The incorporation of separation electrodes onto the microchip allows for an even greater reduction of size and the possibility to incorporate other functionalities onto the microchip device. With this system, the fabrication process is simple, the device is reusable and the platinum wire electrodes can easily be removed and reused in subsequent devices.

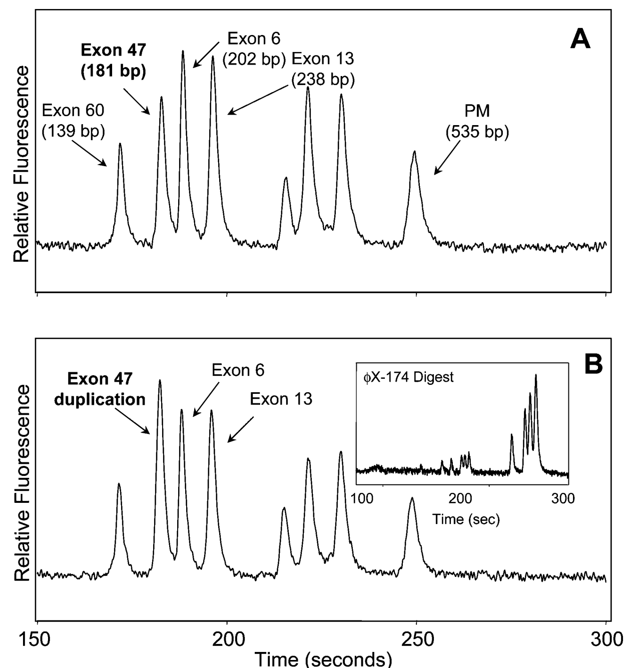

Fig. 4 Clinical diagnosis of Duschenne Muscular Dystrophy using the PDMS electro-fluidic interface acting as a coverplate. Multiplexed PCR amplified patient sample consistent with both (A) negative for DMD and (B) positive for DMD were separated utilizing the hybrid microchip with embedded electrodes. Inset—Separation of ϕX-174 DNA digest fragments in same device under identical conditons. Separation conditions can be found in materials and methods section.

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