HANDLING DNA IN DISPOSABLE MULTILAYER POLYESTER MICROHIPS

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ABSTRACT

With an innovative fabrication process to generate polyester toner (PeT) microchips, we demonstrate the effectiveness of multilayer PeT microchips for dynamic solid phase extraction (dSPE) and PCR amplification. With the former, we found that: *i*) more than 65% of the DNA from 0.6 μL of blood could be recovered, *ii*) the resultant DNA was concentrated to greater than 3 ng/μL (exceeding glass chip-based extraction methods) and, *iii*) the DNA recovered was compatible with downstream microchip-based PCR amplification. The successful valveless integration of PCR and separation was demonstrated by amplification and detection of a 520 bp fragment of λ-phage DNA.

KEYWORDS: disposable microchip, micro-total analysis system, genetic analysis, low-cost diagnostics

INTRODUCTION

Different substrates have been used to fabricate microchips for DNA extraction, PCR amplification, and DNA fragment separation, including alternative polymer-based materials [1,2]. Polyester-toner (PeT) devices exhibited a great potential for bioanalytical analysis, and PT chips can be fabricated in a matter of minutes using a direct-printing and has been shown to be effective for generating microfluidic devices with channel depths on the order of tens of microns [3]. Here, we describe a novel and simple process that allows for the production of multilayer, high aspect-ratio PeT microdevices with channel depths on the order of hundreds of microns for DNA analysis.

In the current report, we utilize a DNA extraction technique developed for glass microchips but demonstrate, for the first time, that *i*) the dynamic solid phase extraction can be carried out in a unique PeT microchip containing deep microchannels, *ii*) this system allows parallel extractions, and *iii*) that the fabricated PeT microchip is compatible with DNA amplification via the polymerase chain reaction (PCR). This presents the the potential for generating a mass-producible, cost-effective (pennies per chip), disposable polymeric microdevice for integrated genetic analysis.

THEORY

In this work we describe a novel and simple process that allows for the production of multilayer, high aspect-ratio PeT microdevices with substantially larger channel depths. This innovative process utilizes a CO_2 laser to ablate the microchannel in polyester sheets containing a uniform layer of printed toner; multilayer devices can easily be constructed by sandwiching the channel layer between uncoated cover sheets of polyester containing precut access holes. The process allows the fabrication of deep channels, with ~270 μm channel devices shown to be effective for dynamic solid phase extraction (dSPE) and PCR amplification, with integration of SPE and PCR in a single chamber.

EXPERIMENTAL

Microdevice Fabrication.

The main steps of the fabrication process are shown schematically in Figure 1A. Polyester sheets are first covered uniformly by toner on both sides using a laser printer (HP LaserJet 4000). The design for the device channel was drawn using CorelDraw and the microchannel was created by laser ablation with a 50 watt CO_2 laser (VersaLaser 350, Universal Laser Systems). Four layer devices were created by sandwiching two polyester layers with precut channels and coated with toner, between uncoated sheets of polyester with precut access holes in the top sheet. The four layers were aligned and laminated using a standard office laminator at 120 °C. This lamination step seals all PeT sheets as a result of the interaction between the toner layers and polyester sheets. The microchips for dynamic solid phase extraction experiments were 14 mm (l) x 1.2 mm (w) x 272 μm (d) (total volume of 4.0 μL. Microchips for PCR amplification experiments were designed to have chambers with 4 μL.

A multilayer PCR domain made out of four polyester sheets, providing a substantially larger channel depth, was fluidically-linked to an electrophoresis domain, which was fabricated using two polyester films (Figure 1B). The multilayer channel allows the fabrication of chambers (~270 μm deep) ideal for IR-PCR amplification. The final microdevice (Figure 1C), a 'hybrid' in a sense, involved sandwiching four polyester films for the IR-PCR domain, and two polyester films for the electrophoresis domain.

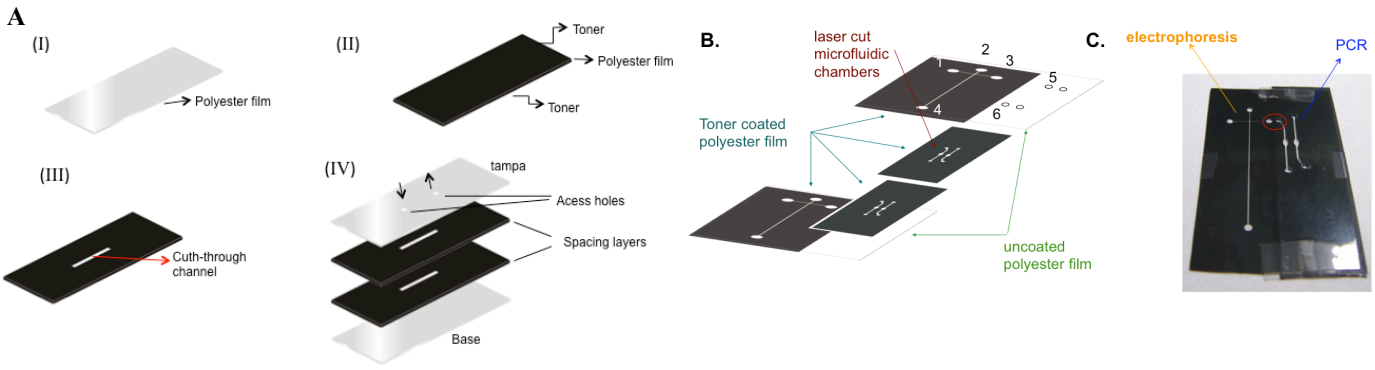

Figure 1A. *Representation of the steps for the microfabrication process, (I) polyester film, (II) polyester film coated with toner on both sides, (III) microfluidic channel cut by laser cutter, (IV) alignment and lamination of four layers (botton and top enclosing two middle layers with channel); arrows indicate inlet and outlet access.* ***B.*** *Schematic of the layers used in fabrication of the multidomain PeT microdevice.* ***C.*** *Photograph of a multidomain PeT. The red circle denotes the site of fluidic transfer from the PCR domain to the electrophoresis domain. The size of the device is 5 cm x 7 cm.*

Sample Preparation for dSPE Extraction.

Blood samples were prepared by mixing 6 mL of whole blood with 5 mL proteinase K (20 mg/mL, Qiagen, Valencia, CA) and 9 mL 8M GuHCl pH 7.6/1% Triton X-100® and incubating the mixture for 10 min at 56 °C. A 2 mL aliquot, equivalent to 0.6 mL of blood, was used in each extraction.

Dynamic Solid Phase Extraction Procedures.

The channel was filled by first flowing 4 mL of 8 M GuHCl pH 7.6 into the channel, followed by 5 μm magnetic silica particles. With the external magnet holding the beads in place, 2 mL of sample was loaded into the channel and 2 mL of 8 M GuHCl was withdrawn from the outlet. The microchip was placed rotating magnetic field and the beads were mixed by this motion in conjunction with an additional external magnet held above the chip for up to 5 min. The beads were then washed by flowing 6 mL of 80% isopropyl alcohol (IPA) through the chip, with magnetic bead manipulation. After washing the beads with IPA, the beads were washed with 8 mL of 0.1× Tris-EDTA (TE) without magnetic bead agitation. Finally, DNA was eluted from the beads as fractions with 0.1× TE using the same magnetic manipulation for 2 min for each 2 μL fraction.

PCR Amplification in PeT Microchip.

Sufficient reaction master mixture was prepared for both microchip and conventional tube PCR: 10 mM Tris-HCl buffer, pH 8.3, 50 mM KCl, 2.4 mM $MgCl_2$, 0.4 mM of the each primer, 0.2 mM of each dNTP, 1 ng/mL λ- phage DNA, 0.24 mg/mL of BSA, 0.1 units/mL Taq polymerase. The stock solution was divided and used for conventional amplification in a tube (positive control) and microchip amplification. The PCR chamber of the PeT microchip was filled with approximately 4 mL of the stock solution and mineral oil was overlaid on the reservoirs to prevent evaporation of solution. Both the PeT microchip and tube controls were placed in a conventional thermocycler (GeneAmp 2400 Perkin Elmer) with thermocycling conditions as follows: 120 s at 95 °C for initial denaturation of DNA, 30 cycles of 30 s each at 95 °C (denaturation) and 68 °C (annealing/extension), followed by 120 s at 72 °C for a final extension.

Valveless Integration

The valveless integration process between PCR and ME was made by putting the reservoir 3 and 5 side by side and applying a positive manual pressure on reservoir 6. The solution overflow and linking with reservoir 3. The solution is confined in both reservoirs by a piece of a pipette tip glued over both reservoirs. The injections were performed by applying a desired potential (- 300 V) for 30 s to the sample reservoir with the sample waste reservoir grounded, and all other reservoirs floating. Switching the high voltage contacts and applying the corresponding separation voltages to the running buffer reservoir while maintaining the detection reservoir grounded and all other reservoirs floating performed the separations.

RESULTS AND DISCUSSION

Our earlier work demonstrated that dSPE in a glass microchip is capable of efficiently extracting DNA (more than 60%) from small volume blood samples (0.6 mL) with recovered DNA at a concentration suitable for PCR amplification [4]. Figure 2A shows the elution profile of DNA extraction from 0.6 mL of whole blood by dSPE in a four-layer PeT microde-

vice, and compares the extraction profile and efficiency between PeT microchip and glass chip from our previous work [4]. Although the extraction efficiency for blood is comparable with both chip substrates, the mass of DNA recovered in the first 6 µL is much higher for the PeT chip (73.5%) than for the glass chip (34%) (Figure 2B).

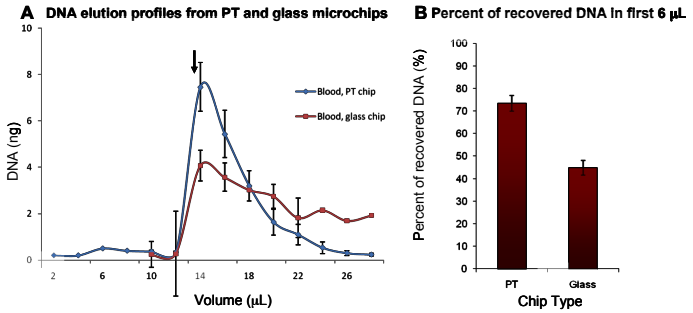

Figure 2. A) *Elution profiles showing that DNA extraction from blood in a PeT microchip is more efficient than extraction in a glass microchips using dSPE (n = 3). Arrow indicates the initiation of bead movement after the 12 mL fraction.* ***B)*** *The amount of DNA recovered in the first 6 µL (three fractions) is much higher for the PeT microchip versus the glass microchip.*

The success of infrared-mediated PCR amplification was demonstrated by amplifying a 500 bp fragment from the λ phage genome. Figure 3A shows a close up view of PCR domain containing an inserted thermocouple. We demonstrated that the PeT materials used to form the reaction chamber do not inhibit the enzymatic reaction involved in PCR amplification, and that rapid thermocycling in the reaction chamber is possible with the use of infrared heating. Figure 3B). The successful valveless integration of PCR and separation was demonstrated by amplification and detection of a 520 bp fragment of λ-phage DNA (Figure 3C).

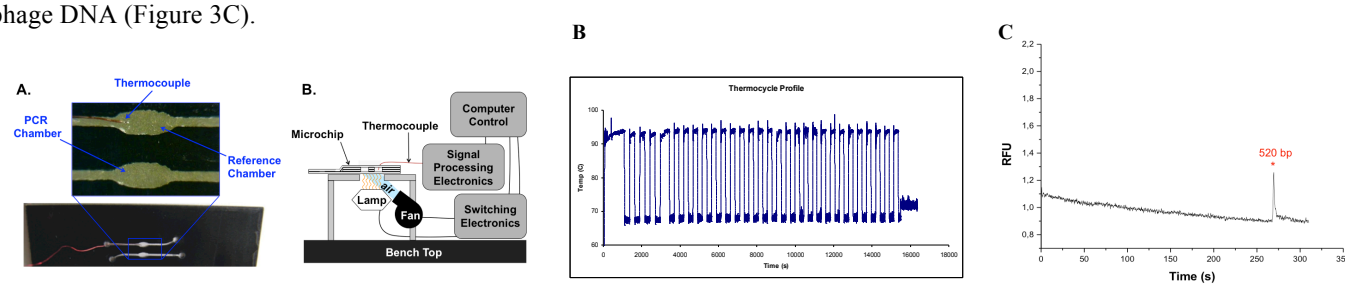

Figure 3*. A. Close up view of PCR domain with thermocouple. B. Schematic of non-contact thermal cycler using a tungsten lamp and blower fan controlled by a LabVIEW program written in-house. Temperature was recorded via a thermocouple in the reference chamber. Note that the microchip layers are not to scale, and have been enlarged to show structure.*

CONCLUSION

In this study, we present a different fabrication process using the PeT chips that, unlike earlier reports, produce deep channels (high aspect ratio) by using four stacked layers of polyester film instead of conventional double layer; we show the successful use the devices for SPE and PCR. PeT microchips present a promising new disposable platform for genetic analysis—with potential "sample- in/answer-out" capability—will be of low-cost, and have the potential to be extensively used in diagnostic and forensic testing.

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