Toward an Integrated Microdevice for DNA Extraction and PCR Amplification in the Submicroliter Regime for Forensic DNA Analysis

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Abstract

The purification/extraction of DNA from evidentiary material and the amplification of target STR sequences in the purified DNA represent fundamental processes in forensic typing. Integrating these processes on a single microdevice is challenging as a result of the very distinct nature of these two processes. DNA purification, as accomplished by DNA solid phase extraction (SPE), is a chromatographic process carried out isothermally and mediated by the continuous, sequential flow of load, wash and elution solutions over a solid phase. DNA amplification via the polymerase chain reaction (PCR) is a batch enzymatic reaction with no flow, but requiring temperature cycling between room temperature and 94 °C. Here we demonstrate true integration of these two processes in a single glass microfluidic device. Microchip SPE of DNA is accomplished by a three step process with simple and accurate fluidics control, mediated by a syringe pump, which allows purification of DNA from complex biological samples of forensic interest including blood. Purified DNA is eluted from the SPE matrix with water and directed into the PCR chamber simultaneously with PCR reagents from a second channel. The PCR chamber is sealed to prevent evaporation then positioned above an IR mediated, non-contact heating system used for theromocycling on microdevices. This single microdevice approach has obvious applications to STR typing of forensic samples, where sample size can be limiting and integrated processing eliminates the need for sample handling. The work presented here represents the first steps in the development of microdevices for integrated sample processing in casework analysis.

Introduction

DNA obtained from evidentiary material must be purified prior to PCR amplification to remove proteins and other contaminants that will interfere with or inhibit amplification. While several commercial protocols and methods exist for extraction of DNA from complex samples, most require the use of large volumes of sample. In many instances, the use of such large volumes is not feasible, hence a driving force toward the use of microdevices for DNA extraction.

Microdevices have been developed for extraction of DNA from complex solutions, (for example blood) producing PCR-amplifiable DNA.[1] These microdevices consisted of silica beads acting as the stationary phase with a tetraethoxysilane (TEOS)-based sol-gel acting as the "glue" to hold the particles in place. DNA was loaded in the presence of a chaotropic salt to adsorb DNA on the silica surface. Proteins and other contaminants were washed from the channel using an organic solution then the purified DNA was eluted with a low ionic strength aqueous solution. While it was demonstrated that amplifiable DNA was obtained in 15 minutes, PCR was performed offline in a conventional thermocycler, necessitating manual collection and a total PCR cycle time greater than 130 minutes.

PCR amplification systems have also been developed for microdevices, utilizing infrared (IR)-mediated temperature cycling to reduce total amplification time. These microdevices provide more efficient reactions and could possibly make the PCR system more automated. [2] IR radiation is used for temperature cycling by exciting vibrational bands of water molecules which absorb the radiation and generate heat. This method of heating has been used in PCR amplification in capillaries and produced heating and cooling rates of 65 and 20 °C/s, respectively.[3] Increased rates not only provide much faster cycle times, they also lead to higher yields of desired products.[3]

In this report, we provide preliminary data on an integrated SPE-PCR microdevice for amplification of DNA from complex samples. Methods have been developed to couple these techniques in a simple manner to provide a total extraction/amplification time of less than 45 minutes. Integration of these initial steps should benefit the forensic community by providing a robust and reliable method for eventual performance of STR analysis on microdevices.

Materials and Methods

Microdevices

Microdevices were prepared as previously described.[1] Briefly, borosilicate glass with a layer of positive photoresist was exposed to UV light through a negative photomask containing an image of the channel design (positive design shown in Figure 1). The exposed photoresist was removed and the glass etched with hydrofluoric acid. Access holes were drilled at the end of the channels and a second piece of glass was thermally bonded to seal the channels. The SPE region was 430 μm x 200 μm x 1 cm (width x depth x length) and intersected with a second channel for PCR reagents having the same dimensions as the SPE region. The output of these two channels fed into a third channel where PCR was performed.

Solid Phase Extraction

The SPE bed was fabricated according to a previous report.[1] DNA loading solution consisted of 30 ng λ-phage DNA in 6 M guanidine HCl prepared in 10 mM Tris, 1 mM EDTA, pH 6. Wash and elution solutions were 80% isopropanol/20% water and water, respectively. All solutions were flowed through the bed at 4.2 μL/min via syringe pump. During the load and wash steps of DNA extraction, water was flowed through the side channel; this helped to wash the guanidine and isopropanol solution through the PCR chamber. When elution was performed, a solution of 2X PCR mastermix was flowed through the side channel at 4.2 μL/min at the same time. Both the elution and mastermix flows were stopped when the correct elution volume had passed through the SPE bed.

Polymerase Chain Reaction

PCR in the microdevice was performed via IR-mediated heating as described elsewhere.[2] For conventional thermocycling, microdevices were placed inside a traditional thermocycler (Bio-Rad MyCycler) and 20 temperature cycles of 68 °C to 94 °C were performed (30 second holds were performed at each temperature during a cycle). The microdevice channels were coated before use to prevent binding of the Taq polymerase to the glass surface.

Capillary Electrophoretic Separation

PCR products were analyzed by capillary gel electrophoresis on a Beckman MDQ Capillary Electrophoresis system (Fullerton, CA). Unless otherwise stated, at the beginning of each day, a 30 minute 1 M HNO_3 rinse was performed followed by a 15 minute rinse with separation polymer consisting of 80 mM MES and 40 mM Tris at pH 6.1 with 3.5% (w/v) hydroxypropyl cellulose. Between separations, the capillary was flushed with a 5-minute polymer rinse. All rinses were performed at 20 psi. Approximately 1 μL of PCR product was collected and diluted to a total volume of 25 μL using water. Electrophoresis was performed in a 50 μm inner diameter, 30 cm (10 cm effective length) capillary and separation performed with 8 kV applied voltage. YO-PRO-1 (Molecular Probes, Eugene, Oregon) was added to the separation buffer in a 1000:1 (v:v) buffer to dye ratio. The capillary was thermostated at 25°C and samples electrokinetically injected for 5 seconds at 10 kV toward the anode and detection accomplished by laser-induced fluorescence with excitation by a 488 nm line from an argon ion laser and fluorescence emission at 520 nm. Prior to injection of a sample, a standard DNA sizing ladder was injected to correlate migration time with DNA size (data not shown).

Results and Discussion

Loading and washing of λ-phage DNA was performed as described. When elution of DNA with water began from the SPE bed, 2X PCR mastermix was flowed through the side channel. This increased concentration of PCR reagents was necessary to account for dilution from the SPE bed, allowing a 1X PCR solution to be achieved in the PCR domain. After 3 minutes (known time in which most DNA was eluted from the SPE bed, data not shown), both syringe pumps were stopped and the chip placed on a conventional cycler for PCR amplification. Figure 2 shows an electropherogram of amplified product taken from the outlet of this integrated SPE/PCR microdevice following thermocycling. The peak at 500 base pairs corresponds to the amplified fragment. The total time for SPE/PCR was 45 minutes.

For IR mediated thermocycling of the PCR solution on the integrated microdevice, after perfusing the PCR reagents into the PCR chamber, a miniature thermocouple was inserted into a channel adjacent to the PCR domain for temperature sensing. The temperature of the solution was alternated between 94 °C

and 68 °C by modulation of a tungsten lamp on and off as dictated by the temperature sensed by the thermocouple. Figure 3 is an electropherogram of product taken from the outlet of the PCR channel after IR-mediated PCR. The time required for integrated SPE/PCR utilizing the IR-meadiated thermocycling system could be further reduced by reducing the hold times used in the amplification reaction. IR-mediated amplifications of less than 5 minutes have been shown.[2]

Integration of SPE and PCR on a single device using either a conventional thermocycler or the IR mediated system demonstrates a significant advance in integrated sample processing. Differences in these two methods, such as flow vs. stopped flow and isothermal vs. temperature cycling processes, provided some challenges in merging these two techniques. Using a simple valveless design and syringe pump flow to mix DNA eluting from the SPE bed with PCR reagents in the PCR channel, integration is facile. Significant amounts of amplified specific product were detected in both the conventional and IR-mediated system, indicating that the ability to perform PCR in glass microdevices is becoming routine.

Incorporation of SPE and IR-PCR on a single device is a significant step in producing a completely integrated sample preparation device that reduces the time needed from hours in conventional systems to a few minutes. In addition, the requirement for only microliters of sample to obtain STR profiles will aid the forensic community by reducing the sample required.

References

1. Breadmore MC, Wolfe KA, Arcibal IG, Leung WK, Dickson D, Giordano BC, Power ME, Ferrance JP, Feldman SH, Norris PM, Landers JP. Microchip-Based Purification of DNA from Biological Samples. Anal Chem 2003;75: 1880-1886.
2. Giordano BC, Ferrance JP, Swedberg S, Huhmer AFR, Landers JP. Polymerase Chain Reaction in Polymeric Microchips: DNA Amplification in Less Than 240 Seconds. Anal Biochem 2001; 291:124-32.
3. Huhmer AFR, Landers JP. Noncontact Infrared-Mediated Thermocycling for Effective Polymerase Chain Reaction Amplification of DNA in Nanoliter Volumes. Anal Chem 2000; 72:5507-5512.

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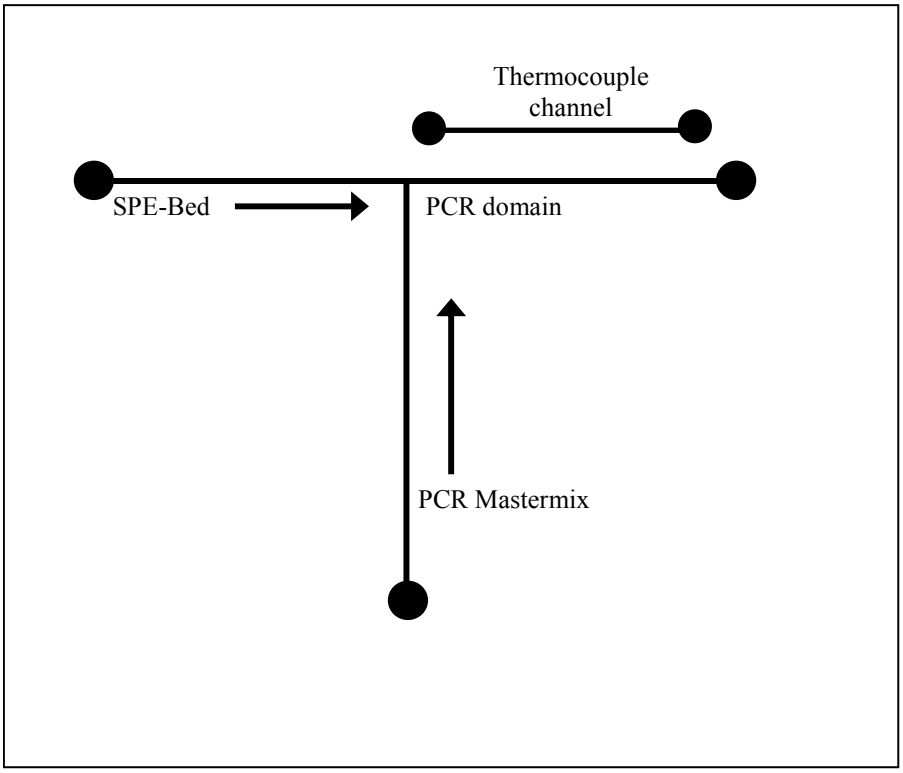

Figure 1. Microdevice layout for integrated SPE/PCR. Sample was loaded then washed on the SPE bed while water was flushed through the PCR Mastermix channel. DNA was eluted in water and mixed with 2X PCR mastermix; flow was stopped when the eluted DNA was in the PCR domain. Arrows indicate direction of flow. Drawing not to scale.

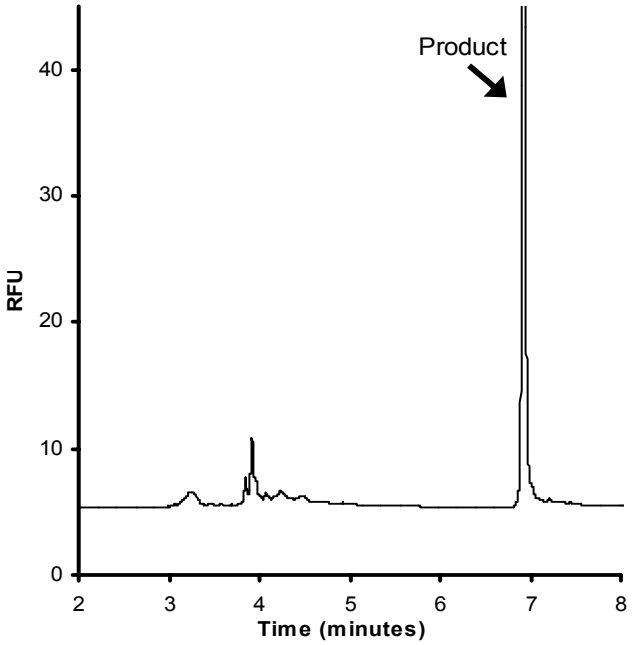

Figure 2. Electropherogram of 500 bp amplified λ-phage DNA fragment from an integrated SPE/PCR microdevice. SPE was performed as described, and eluted DNA mixed with 2X PCR mastermix as it flowed into the PCR chamber. After a specific time, flow was stopped allowing PCR to be performed in the PCR chamber using a conventional thermocycler. The amplified product was removed from the PCR domain and analyzed by capillary gel electrophoresis.

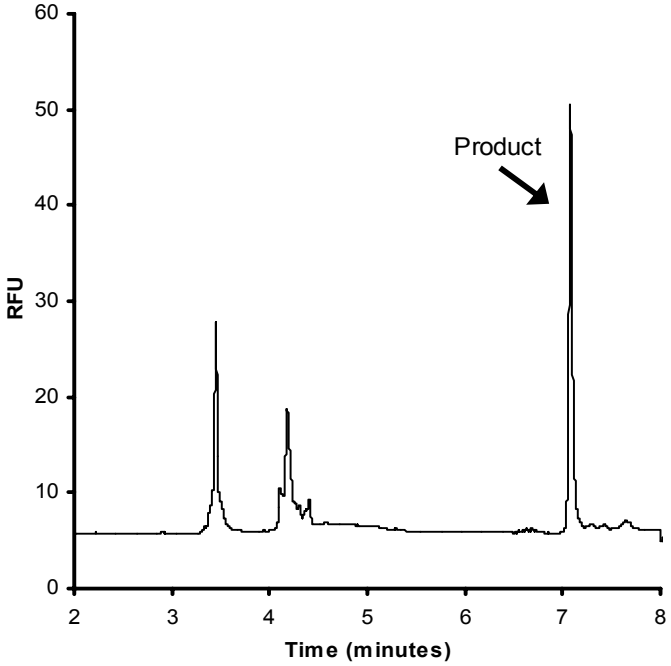

Figure 3. Electropherogram of DNA amplified by IR-PCR. IR-PCR was achieved through a tungsten IR source and temperature sensed with a miniature thermocouple. Twenty cycles of PCR were performed in 20 minutes and the product was removed from the PCR domain and analyzed by capillary gel electrophoresis.