VOLUME-REDUCTION SOLID PHASE EXTRACTION ON A PLASTIC MICROFLUIDIC DEVICE FOR FORENSIC SAMPLE ANALYSIS

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ABSTRACT

Interfacing large volume samples (0.5 – 3 mL) with microfluidic devices is a significant obstacle for the processing of numerous forms of forensic casework biological evidence. This includes biological fluids collected from surfaces, clothing and compromised samples e.g., those mixed with soil. In addition, some samples are deliberately diluted, reducing the effect of inhibitors on the polymerase chain reaction (PCR) and subsequent short tandem repeat (STR) analysis for human identification [1]. The result, therefore, is a 'low template' sample, requiring purification and concentration of DNA to allow for successful STR PCR forensic analysis of biological evidence.

KEYWORDS: Solid-phase extraction, Volume-reduction, PMMA microdevice

INTRODUCTION

Inefficient removal of PCR inhibitors, e.g., hemoglobin in whole blood, or insufficient concentration of the DNA from dilute samples will result in failed PCR amplification of STR fragments. A high surface area solid phase is required to provide sufficient binding sites for both inhibitors and nucleic acids, which bind with equal affinity, for successful DNA purification. Solid phase extraction (SPE) is preferred over other DNA extraction methods, due to the feasibility of integrating with downstream processes, e.g., PCR[2], reducing the introduction of contaminants. Therefore, the creation of a disposable, inexpensive microfluidic device capable of accepting macro-sized volumes is the first step in a micro total analysis system for numerous sample types, especially within the forensic community.

THEORY

While the large silica-coated solid phase in volume reduction solid phase extraction (vrSPE) was designed to accept sample volumes 10-fold larger than traditional SPE devices [3, 4], these microdevices were fabricated in glass: an expensive, time-consuming process that utilizes hazardous chemicals (e.g., HF). These issues can be circumvented by the use of microfluidic-friendly plastic substrates (i.e. - poly(methyl methacrylate); PMMA). vrSPE microdevices were fabricated from PMMA using laser ablation to create the architecture, and the speed of ablation reduces the fabrication time by 8-fold, with cost potentially in the tens of cents per device.

EXPERIMENTAL

Standard vrSPE designs were fabricated in PMMA through laser ablation of the surface to produce the desired features, including a 20 µm weir needed to contain the SPE particles in the channel (Fig. 1 A, B). This was achieved by finely etching the PMMA surface, with 35% reduction in laser power for the weir compared to the channel. To seal the channels, a cover of 1.5 mm PMMA was thermally bonded using a high temperature/low pressure method[5] and a syringe connector affixed. Initially, a small mass of 30 µm silica particles are introduced to form a barrier to retain the 8 µm MagneSil™ particles which are used as the solid phase. Samples were loaded, using a high salt buffer, onto the device through a syringe flowing at 15 µL/min for a single channel, the optimized flow rate for DNA binding. The DNA was eluted in ten 5 µL fractions for DNA quantitation and STR-PCR analysis.

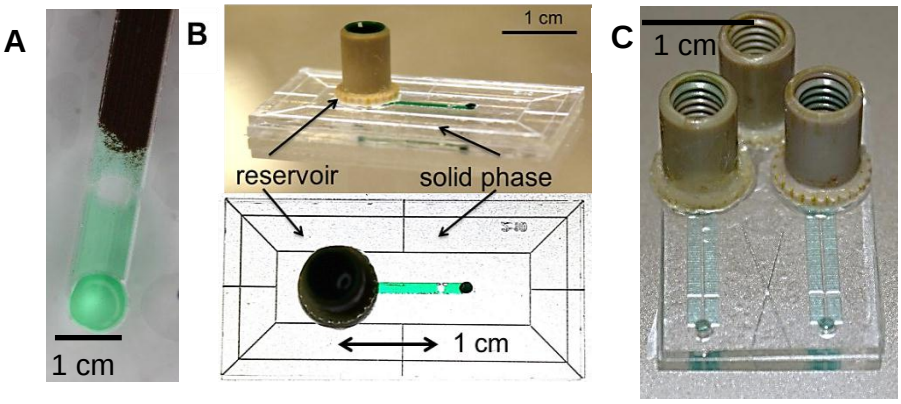

Figure 1: *Photographs of PMMA vrSPE microdevices. A) Close up of the weir, demonstrating the solid phase particles are being retained in the channel. B) PMMA vrSPE device filled with green dye to represent the channel. Top: Side view. Bottom: Overhead view. Channel volume: 20 mm^3. Dimensions: 1 cm x 100 µm x 200 µm. C) Photograph of a 4-channel device for the rapid processing of a single sample. Channels filled with blue dye for visualization.*

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RESULTS AND DISCUSSION

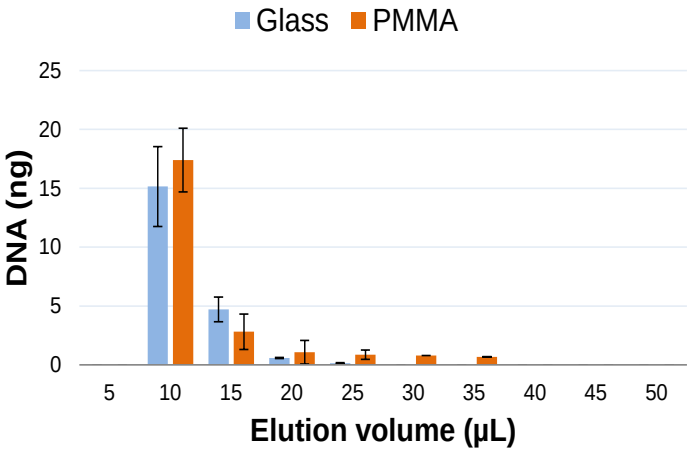

Figure 2: *Bar graph comparing the amount of DNA collect per 5 µL fraction of pre-purified of hgDNA (500 µL of 100 pg/µL) from vrSPE microdevices (n=3). Glass devices were made using standard etching techniques. PMMA devices were fabricated using laser ablation. Glass extraction efficiency (EE%) 41.2±3.8. PMMA EE% 45.7±4.9*

Initial experiments were performed to determine that the PMMA surface would not interfere with the DNA recovery following the extraction process. Comparing single channel devices in both glass and PMMA showed no statistical difference in the amount of DNA recovered, confirming the PMMA surface was not retaining more DNA than glass and can be used as an acceptable alternative (Fig. 2).

Therefore, single-sample multi-channel PMMA devices were designed and fabricated, and with a flow rate of 60 µL/min, a 4-fold increase in the volume of the sample that could be loaded into the device (from 500 µL to 2 mL), resulted. Conversely, if loading a 0.5 mL sample, there was a 4-fold reduction in the amount of time (from originally 33 minutes to ~8 minutes) required for sample load (Fig. 1C).

A multi-sample multiplex vrSPE chip containing four purification channels was fabricated to increase the number of samples processed simultaneously.

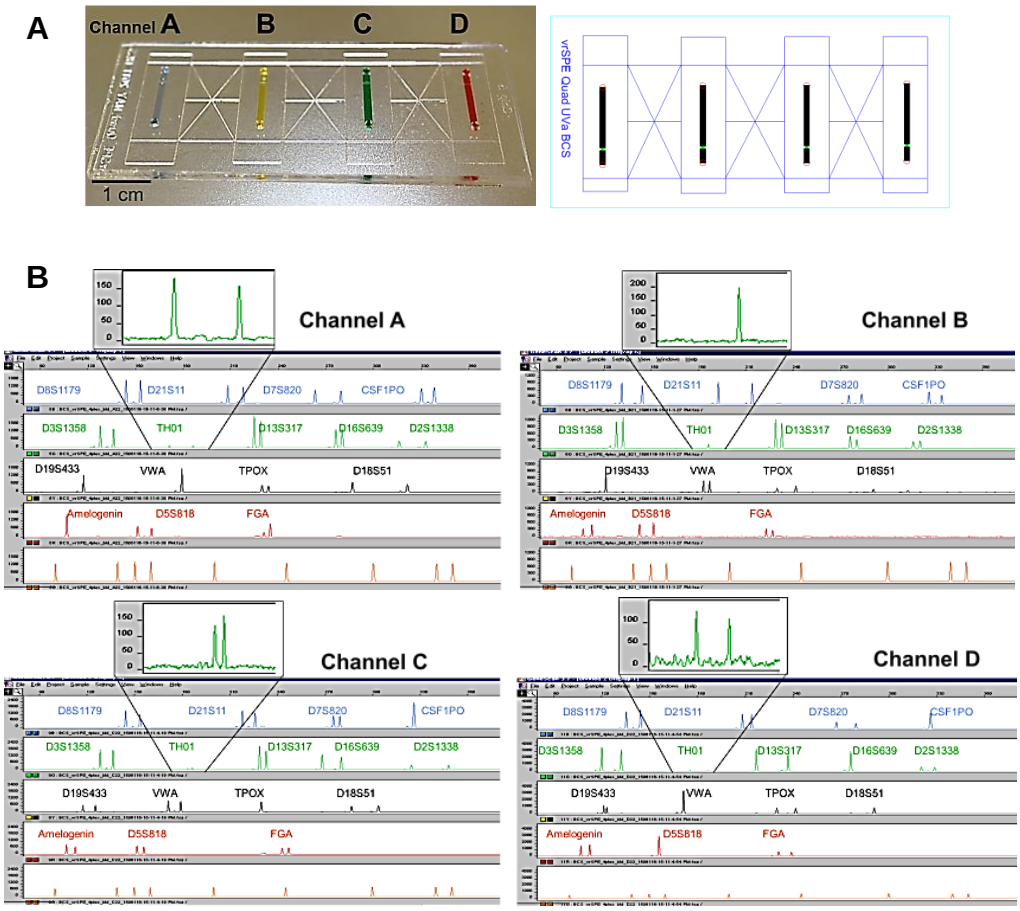

Figure 3: *4-plex vrSPE PMMA device. A) Photograph and AutoCAD® drawing of a four-channel multiplex vrSPE PMMA device. Channels (A-D) were filled with different colored dyes to represent a separate sample processed. B) Full (16/16 loci) Identifiler® STR profiles from simultaneous extraction of whole blood from 4 separate individuals, analyzed on the ABI PRISM 310 genetic analyzer.*

A simultaneous tetraplex extraction of four whole blood samples from four different individuals was successful with a full STR profile generated for each person (16/16 loci present) (Fig. 3). The alleles (peaks) associated with STR loci are inherited from an individual's parents and, therefore when considered together, are unique to that individual. The full STR profiles show no evidence of cross-contamination (extraneous peaks within the profile), indicating that the individual samples are fluidically sealed and completely isolated from each other. Furthermore, this also demonstrates that the PMMA surface is not leaching any PCR inhibitors into the purified DNA, confirming the PMMA surface is a suitable substrate for SPE.

Each of these devices (for rapid single sample purification or mutli-sample simultaneous extraction) provided a significant improvement in either analysis time (4-fold reduction) or throughput compared to traditional glass vrSPE devices previously reported [2]. In addition, application of these devices is enhanced by the ability to pre-pack them with the solid phase and store in extraction buffer until used in the field. This is achieved using screws to seal the inlet ports and PCR compatible adhesive to seal the outlet, keeping the solid phase packed and protecting the bed from evaporation until use. For these experiments, single channel devices were packed with solid phase, sealed as described above, and left at room temperature for up to 25 days. Current studies have shown that the extraction is unaffected by storage of up to 25 days (Fig. 4). Longer storage times are currently under investigation.

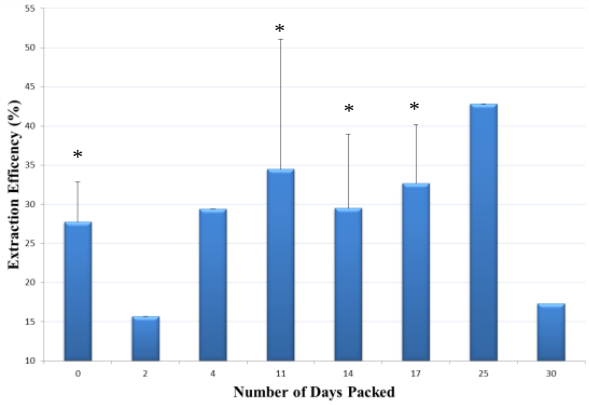

Figure 4: *Bar graph demonstrating the effect on extraction efficiency after up to 30 days post packing of the solid phase with 500 uL of 100 pg/µL hgDNA. Data points with * (n=3); those without (n=1).*

CONCLUSIONS

PMMA-vrSPE introduces multiplexed disposable plastic microdevices for DNA purification from dilute biological evidence collected in forensic investigations. Developments include rapid sample loading times, up to 60 µL/min (four times faster than previously reported) and the ability to analyze multiple samples simultaneously, avoiding cross-sample contamination. This work is a step towards the use of a micro-total analysis system for forensic genetic analysis, either in the field or as a small bench-top device within forensic laboratories.

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