TOWARDS AN INTEGRATED, VALVELESS, PLASTIC MICRODEVICE FOR ENZYME-BASED DNA PREPARATION AND PCR FOR FORENSIC STR ANALYSIS

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Current processes for analysis of forensic biological samples, namely DNA extraction and amplification, create a significant bottleneck, contributing to the ever-increasing size of the backlog, which has risen to over 70,000 cases as of January 1, 2008[1]. Microfluidic devices offer numerous advantages over conventional methods, such as decreased analytical time and a completely closed system to prevent sample contamination. Additionally, analyses performed on microchips have the potential to be integrated with upstream or downstream analytical steps within a single microfluidic device. A fully-integrated microdevice, with sample-in answer-out capability, has previously been shown for the detection of *B. anthracis* from mouse blood in 24 minutes[2]. Using principles outlined in that work, a similar microdevice could be developed for forensic STR typing.

Traditionally, microdevices are fabricated in glass, including the integrated device just described, however, fabrication is time-consuming, expensive, and requires the use of hazardous chemicals. Recently, there has been a shift towards the use of polymers, such as poly (methylmethacrylate) (PMMA), for microdevices due to the ease of fabrication and low cost allowing for the device to be single-use[3].

Solid phase extraction using a silica phase is commonly used for DNA purification and has been successfully adapted to a glass microdevice, but issues such as uneven packing of the phase or high backpressure can result[4]. The use of a liquid preparation method circumvents these drawbacks by requiring no solid phase. A recently developed liquid DNA preparation technique utilizes a thermally stable neutral proteinase to lyse cells and degrade proteins and nucleases, leaving PCR-ready DNA in only 20 minutes[5].

After the DNA is extracted and purified, STR regions in the genome are amplified by performing the polymerase chain reaction (PCR). Through the use of modified polymerases, which have faster extension rates and improved processivity, the amount of time required for PCR can be reduced to as little as 36 minutes[6]. Concurrently, PCR has been adapted to a glass microdevice using non-contact heating methods, such as infrared (IR)-mediated PCR[7], which can significantly increase ramps rates and reduce thermal cycling time significantly. By using modified polymerases in conjunction with IR-PCR, the time needed for the amplification step could be greatly reduced.

The current work describes on the development of an integrated plastic microdevice capable of accepting a fragment of a buccal swab, and extracting and amplifying DNA from the swab. Epithelial cells were eluted from a dried buccal swab by agitation in deionized water and an aliquot of the eluate was added to the enzyme-based preparation solution. The solution was loaded into a PCR device and incubated for a short period of time. After incubation, the sample was removed from the chip, added to a PCR master mix, loaded into a second, clean PCR device and PCR was performed using the IR-PCR system. Results show that the DNA extracted and amplified was able to yield a full STR profile in ~1 hr, a

reduction of 3 hours in analysis time when compared to conventional methodologies and this two chip system represents the first steps towards a simple, valveless, integrated device

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