A199 Development of a Plastic Microdevice for Integrated Liquid DNA Extraction and PCR Focused on Forensic STR Analysis

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After attending this presentation, attendees will have gained an understanding of a disposable microdevice capable of extracting and amplifying DNA for forensic analyses.

This presentation will impact the forensic science community by demonstrating an integrated, plastic DNA analysis device that can reduce sample analysis time and increase throughput.

The current processes used to analyze 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 an alternative method to analyze biological samples and provide many 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 has been developed which is able to perform all DNA analysis steps on a sample of mouse blood and yield a positive result for *B. anthracis* in 24 minutes.[2] Using principles outlined in that work, a similar microdevice could be developed for forensic STR typing.

The most widespread method to extract DNA is solid phase extraction (SPE), which typically uses a silica-based solid phase to reversibly bind DNA, while impurities are washed away. This method has been successfully adapted to a microdevice, but can encounter problems with uneven packing of the bed or high backpressure.[3] The use of a liquid extraction method eliminates the need for a solid phase and, as a result, eliminates any issue associated with a packed bed. A recently developed liquid extraction method utilizes a thermally stable neutral proteinase to lyse cells and degrade proteins and nucleases, leaving highly pure DNA that is PCR-ready in only 20 minutes.[4]

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.[5] Concurrently, PCR has been

adapted to a microdevice using non-contact heating methods, such as infrared (IR)-mediated PCR,[6] which can significantly increase ramp 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 focuses on the development of an integrated plastic microdevice capable of accepting a fragment of a buccal swab and extracting and amplifying DNA from a swab. A fragment of a dried buccal swab was placed in the extraction chamber and the device was capped to prevent evaporation. The enzyme-based extraction solution was flowed into the chamber until the swab fragment was completely immersed and the sample was incubated for a short period of time. The extracted DNA was flowed through the device, mixing with PCR master mix, until the PCR chamber was filled and PCR was performed using the IR-PCR system. Results show that the DNA extracted and amplified with the integrated device was able to yield a full STR profile in ~1 hr, a reduction of three hours in analysis time when compared to conventional methodologies.

References:

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