A48 Expedited Enzyme-Based Generation of PCR Ready DNA From Forensic Biological Samples on Glass or PMMA Microdevices

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After attending this presentation, attendees will have gained an understanding of a microfluidic enzyme-based method for generating PCR ready DNA.

This presentation will impact the forensic science community by demonstrating that microchip liquid extraction can greatly reduce the amount of time necessary for DNA extraction, leading to the possibility of an increase in sample throughput.

Solid phase extraction (SPE) is a widely-used method for the extraction and purification of DNA from biological samples. Typically, a silica-based solid phase is used to reversibly bind the DNA under high salt conditions, while impurities are rinsed away using an organic solvent. DNA is released from the solid phase upon addition of a low salt buffer. This method generally has a number of sample handling steps which may provide the opportunity for the introduction of a contaminant or a reduction in DNA yield. The use of a closed, single-tube, liquid extraction eliminates these issues and may be more amenable to automation. An enzyme-based extraction method has recently been developed that uses a thermophilic neutral proteinase from *Bacillus* sp. EA1 to lyse cells and degrade proteins and nucleases, leaving only DNA in a PCR-ready buffer in 20 minutes.[1]

Microfluidic devices provide a unique alternative to conventional methods that is rapid and cost-effective. Reduction of sample and reagent volume, analysis time and incidence of contamination make microfluidic devices ideal for forensic applications. Additionally, a microfluidic platform allows for integration of multiple techniques on a single device,[2] potentially allowing for a portable DNA analysis system. SPE has been successfully adapted to a microdevice;[3] however, it may be hindered by uneven packing of the solid phase or high back pressure. Adapting the enzyme-based extraction method described above to a microdevice would not only reduce the extraction time, but eliminate any issues that occur with SPE.

Traditionally, microdevices are fabricated in glass for a number of reasons, including a high understanding of the surface chemistry and reusability. However, fabrication of glass devices is time-consuming, involves the use of hazardous chemicals and is expensive. Recently, there has been a shift towards the use of polymers, such as polymethylmethacrylate (PMMA), for microdevices due to the ease of fabrication and low cost.[4] Liquid DNA extraction can be easily adapted to a microdevice, since a packed bed and centrifugation are not required, providing an ideal platform for integration with downstream analyses.

The current work focuses on the development of an expedited enzyme-based DNA extraction method on a microdevice and a comparison of glass versus PMMA substrates. A fragment of a dried buccal swab was added to the liquid extraction solution, which contains buffer and enzyme. A small portion of this mixture was loaded onto a microdevice and incubated using an infrared (IR)-mediated heating method[5] for a short period of time. The sample was removed from the microdevice and added to a conventional PCR master mix for STR

amplification. Results show that both glass and PMMA are adequate substrates for the liquid extraction method. Additionally, the incubation time on a microdevice can be reduced to as little as one minute, without a loss of STR peak height. This represents a 20- and 60-fold reduction in extraction time compared to conventional liquid and solid phase extraction methods, respectively.

References:

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PMMA, Liquid Extraction, Microdevices

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