A77 Development of an Integrated Microdevice for DNA Extraction and Amplification of Forensic Samples Using Infrared- Mediated Heating and Centrifugal Force

Teresa Sikes, BSc, 2612 W Main St, Apt 1, Richmond, VA 23220; Yiwen Ouyang, BSc, Hillary Sloane, BSc, and James P. Landers, PhD, Univ of Virginia, Dept of Chemistry, McCormick Rd, PO Box 400319, Charlottesville, VA 22904-4319; and Tracey Dawson Cruz, PhD, Virginia Commonwealth Univ, 1000 W Cary St, PO Box 842012, Richmond,*
VA 23284

After attending this presentation, attendees will have a better understanding of the importance of micro-total analysis systems (μTAS) for forensic DNA applications, the challenges faced with integration, and the barriers to the adaption of macroscale work to a microscale format.

This presentation will impact the forensic science community by describing a conceptual integrated DNA extraction and amplification plastic microdevice for use in PCR analysis of STR multiplexes. This work presents a step toward a μTAS that eliminates the use of expensive processing and bulky external attachments.

The value of μTAS for forensic DNA analysis lies in the potential to reduce contamination by eliminating sample transfer steps, which contribute to sample loss and reduce the acquisition of a full STR profile. Additionally, cost and lab space required for equipment, reagents, and laboratory time and effort required to perform each step in preparation for analysis is reduced. The pioneering of two key technologies, infrared (IR)-mediated heating for PCR and DNA extraction via ZyGEM chemistry, has allowed for greater possibilities in the microfluidic analysis of DNA, especially for forensic samples. IR-PCR expedites amplification with 30 cycles completed in 30 minutes followed by established, successful detection, and separation of STR loci. ZyGEM offers faster reaction times, reduced sample handling, and elimination of PCR-inhibiting reagents, while maintaining performance equal to traditional methods, making it ideal for use in an integrated microdevice. The goal of this study is to integrate DNA extraction and amplification by exploiting these technologies onto a single microdevice utilizing a centrifugal platform. Previous work has accomplished integration with the use of valve systems requiring syringes, external pumps, and other bulky, expensive attachments. However, simple valves, such as hydrophobic and one-way, single-use valves can be employed on a centrifugal platform using various speeds to effectively direct flow. In addition, no barrels or column attachments are required as the swab cutting is added directly to the microdevice, with DNA elution accomplished with the addition of reagents and spin-directed flow to an extraction chamber.

In this study, a poly (methyl methacrylate) (PMMA) microdevice was designed for ZyGEM-based DNA extraction and amplification from a buccal swab. The microdevice was manufactured in-house by laser ablation and thermal bonding. The thickness of PMMA was chosen to minimize the thermal mass of the microdevice. Heating was provided by an IR-lamp positioned below the chip. Chip design and microfluidic optimization was accomplished through a series of dye tests: blue dye represented DNA extract while yellow represented PCR reagents. Various spin times and speeds were tested to establish the optimum parameters. The design included ZyGEM elution and extraction chambers, valves for channel sealing and metering of extract and PCR reagents, and an IR-PCR chamber. One-way, single-use “tape” valves were used to stop back flow from the extraction and PCR chambers and prevent evaporation during heating. Tape valves exploit the properties of PCR film and double-sided tape to effectively close a channel providing single-use, one-way valves for metering and channel sealing. A hydrophobic valve, for metering, connected the extract reservoir to the PCR reaction chamber. This valve “opened” once the chip was spun at a velocity such that the centrifugal force exerted on the liquid forced it through the channel. The reservoir was designed to allow for accurate volume delivery of the extract in the previously determined extract: PCR master mix ratio. PCR reagents were loaded on the microdevice and directed into the PCR chamber with the extract. Accurate metering was necessary to achieve the appropriate proportions of PCR master mix to DNA extract for successful PCR. The centrifugal platform was chosen to increase throughput (two chips must spin simultaneously to balance the system; multiplexing with four or more chips is possible) and to minimize manual interaction with the device. After establishing microfluidic control for the integrated device, ZyGEM and PCR chemistry, on-chip with IR-mediated heating, will be optimized for buccal swabs. The results of the micro-extraction and amplification will be compared to a tube-based ZyGEM extraction, followed by traditional PCR amplification via a block thermocycler.

The preliminary results suggest accurate metering can be achieved on a centrifugal platform with this newly designed integrated device. Successful integration in this system, coupled with the ease of fabrication, promotes progress towards a simple sample-in, answer-out microdevice.

Integration, Centrifugation, Microdevice

 ** Presenting Author*