PROTOTYPE DEVELOPMENT OF A FORENSIC MICRODEVICE USING INFRARED-MEDIATED HEATING AND A ROTATION-DRIVEN PLATFORM

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Compared to conventional forensic analysis methods, micro total-analysis-systems (μTAS) offer several advantages. The smaller footprint and microfluidic architecture allows for lower reagent volumes, few manual steps and automation that potentially reduces cost. Moreover, sample handling and tube-to-tube transfers are decreased, reducing the risk of contamination. Perhaps most important to the forensic community, the automation of a μTAS has the potential to significantly decrease sample-to-answer time. Two key technologies: infrared (IR)-mediated PCR and a new DNA preparation chemistry, have allowed for greater possibilities in the microfluidic analysis of DNA, especially for forensic samples. IR-PCR expedites amplification with thirty cycles completed in 30 minutes without requiring a block thermocycler. An enzyme-based DNA preparation approach offers faster reaction times, reduced sample handling and the elimination of PCR-inhibiting reagents while maintaining equal performance to traditional extraction methods. However, there are several obstacles that continue to prevent incorporation of μTAS into mainstream forensic STR typing laboratories. A primary hurdle of complete integration into a single μTAS is microfluidic flow control. Previous work has accomplished this with the use of active valving systems that require syringes, external pumps, and other bulky, expensive external hardware. However, passive valves, such as hydrophobic and one-way, single-use "burst" valves that do not required external hardware or mechanical actuation, can be employed on a rotation-driven platform using varied centrifugal forces to effectively direct flow. Additionally, with this approach, 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. Our current work aims to integrate DNA extraction/preparation, DNA quantitation, and STR amplification by combining these technologies onto a single microdevice utilizing a centrifugal platform.

In this study, all microdevices were fabricated in-house by laser ablation and thermal bonding of layers of poly(methyl methacrylate) (PMMA), whose thicknesses were chosen to minimize the thermal mass. Modular microdevices for DNA preparation and IR-PCR were designed first, followed by a larger integrated chip. Microfluidic optimization was accomplished through a series of dye tests as well as the testing of various spin times and speeds on the centrifugal platform. Microfluidic control on the integrated chip was exerted by a series of valves. One-way, PDMS/tape mechanical valves were used to stop back flow from the extraction and PCR chambers and prevent evaporation during heating. A passive hydrophobic valve, for metering, connected the extract reservoir to the PCR reaction chamber. This valve opened or "burst" once the chip was spun at a velocity fast enough to overcome the surface tension in the liquid. PCR reagents were loaded on the microdevice and directed into the PCR chamber with the extract. Accurate metering was necessary to achieve the appropriate PCR master mix:DNA extract ratio for successful PCR. After establishing flow control for the integrated device, the DNA preparation module with IR-mediated heating was optimized for buccal swab processing. The results of the DNA liberation and amplification were compared to the manufacturer-suggested extraction, followed by traditional PCR amplification via a block thermocycler. The preliminary results suggest DNA preparation, accurate metering, and IR-PCR can be achieved on a rotation-driven platform with this newly designed integrated device. Modular on-chip DNA preparation followed by conventional PCR amplification of STR loci has resulted in a full STR profile concordant with a profile generated from the manufacturer's standard protocol followed by conventional PCR. However, challenges with this microchip design exist, including the formation of bubbles between PMMA layers and the presence of surfactant in the DNA preparation buffer – both of which negatively affect fluidic control. Optimization of bonding temperature and pressure has relieved the bubbling issue. To overcome the surfactant issues, several approaches have been taken,

including increasing centrifugal speed, widening of channels, and decreasing the buffer concentration used for DNA preparation to directly reduce the amount of surfactant present. With each modification, microfluidic control is assessed and DNA yield is measured. While further optimization is ongoing, these changes have yielded performance improvement and the best combination of these modifications has been incorporated into the microchip design and fabrication process. Resulting DNA from the successfully integrated chip will be tested to ensure an acceptable STR profile is generated. Finally, it is necessary to consider DNA quantitation in order to develop a device that is consistent with the requirements of DNA Quality Assurance Standard 9.4. To-date, no previously published forensic studies have successfully addressed this. Thus, future work will focus on incorporation of a fluidic module employing a “pinwheel” quantitation assay into the current microchip design. Ultimately, complete successful integration of these modules on a single microchip that is inexpensive, eliminates the needs for bulky external attachments, and is easy to fabricate, will promote progress towards a simple sample-in, answer-out microdevice that more closely mimics the current workflow of a forensic DNA laboratory.