B97 A Rotationally Driven Microdevice (RDM) and Its Associated Low Footprint Instrument With the Potential of Fully Automated Integrated DNA Extraction, Polymerase Chain Reaction (PCR) Amplification, and Separation for Forensic DNA Analysis

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After attending this presentation, attendees will better understand the forensic application of RDMs and the recent advances toward a novel microfluidic device platform for forensic DNA analysis.

This presentation will impact the forensic science community by describing the development and proof-of-principle for an integrated “sample-to-answer” RDM platform for DNA analysis with the potential for fully automated genotyping.

Microdevices offer numerous advantages over the conventional methods used in forensic laboratories for rapid STR profiling, including: (1) smaller reagent and sample consumption; (2) the ability to integrate processes limiting the operator steps (hands free); (3) expediting sample-to-answer response; and, (4) portability. While several companies have successfully demonstrated the capability of Rapid DNA instrumentation, and while some are portable, they are still large and prohibitively expensive.[1-3] There is a need for the development of an inexpensive, rapid, and portable device for DNA extraction, Short-Tandem Repeat-Polymerase Chain Reaction (STR-PCR), and DNA fragment separation in forensic analysis. To meet these needs, this presentation reports the development of a unique, multi-layer, RDM and the associated centrifugal platform that can perform DNA extraction, amplification, and separation. This device is fabricated using a print-cut-laminate process that has been recently described using common office equipment, a laser cutter, and inexpensive substrates.[4] Unlike previously described systems, this does not require bulky instrumentation (external pumps, air compressor, and large actuators) as the integration of the processes and all fluid movement within the microchip rely on centrifugal force to drive fluidic movement. Therefore, this platform has the potential to be fully automated, compact, portable, and cost-effective human Identification (ID) system.

Initially, three separate rotationally driven microfluidic chips were designed (extraction, PCR, and electrophoresis), and their functionality demonstrated independently. The fabrication of RDMs from Polyester film (Pe), Pressure-Sensitive Adhesive (PSA), and other proprietary materials not typically used in microfluidics allowed for rapid, cost-effective, in-house prototyping. Non-solid phase extraction from buccal swabs using an enzyme-based reaction (ZyGEM® prepGeM) was successfully demonstrated on no fewer than 20 microdevices with different DNA donors and showed an average yield of 5.9ng/µL ± 2.4ng/µL, with all extractions leading to full STR profiles using a platform with spin-based capabilities. The same platform was used for microdevice PCR with rotation-driven fluid flow and reagent mixing. Successful PCR of ten STR markers in fewer than 20min was demonstrated with different substrates that were compatible with integration with the other steps. In this study, 100% of the profiles obtained were concordant with conventional typing methods, and presented satisfactory intra- and interlocus balance. Finally, electrophoresis on an RDM comprised of common materials, a Cyclic Olefin Copolymer (COC), and integrated gold electrodes was successfully demonstrated. The sieving matrix and sample were loaded centrifugally, and electrophoresis was complete in <8min with an unprecedented effective separation length of only 4cm. This process was performed on a different platform with heating, spin control, and a high-voltage power supply for electrophoretic separation with optical detection capabilities.

Once the individual sub-assays were demonstrated, the architectural features needed for the integration of the three processes into a single hybrid RDM was designed and tested. A unified, integrated platform possessing all of the necessary hardware needed to perform sample-to-answer STR profiling was designed and manufactured in-house with off-the-shelf components, laser-cut materials, and 3D printed parts. While the forensic data quality of the integrated assay requires further optimization, proof-of-principle for integrated "liquid" extraction, STR amplification, and microchip electrophoresis is demonstrated on a cost-effective microdevice instrument whose small footprint is unprecedented. This system is a potential game changer for the criminal justice system where adoption of the Rapid DNA systems is limited by the cost of the instrument and the price of cartridges. In summary, this study presents the first rotationally driven, integrated, sample-to-result RDM for forensic DNA analysis operated by a device that is truly portable and can significantly reduce the price of Rapid DNA analysis.

Reference(s):

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Centrifugal Device, Rapid DNA, Integrated Device