DEVELOPMENT OF A PORTABLE, LAMINATED DYNAMICSOLID-PHASE DNA EXTRACTION METHOD ON A ROTATIONALLY-DRIVEN PLATFORM

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Solid-phase extraction (SPE), a critical step in genetic analysis, relies on the binding of DNA to silica-coated particles[1]. Dynamic SPE (dSPE) manipulates silica-coated magnetic particles through a static DNA-containing solution during the extraction process to ensure optimized trapping and elution of DNA. Adapting dSPE to a microdevice enables advantages over benchtop methods including increased sample efficiency, decreased reagent volumes, assay cost, a closed system to prevent contamination, and the ability for integration with downstream processing via short tandem repeat typing or Next Generation Sequencing.

Polyester (Pe) can be patterned and bonded with toner (T) to create multilayer microfluidic PeT devices for < $2[2, 3]. Successful demonstration of dSPE via pressure-driven flow in rapidly fabricated PeT devices[2] have been shown via manual pipetting, which presents the need for increased automation. Recent developments with open architecture PeT devices, hydrophobic valving, and centrifugally-driven fluid flow provides an attractive new platform for dSPE. This updated system is functional at low spin speeds (<1500 rpm), incorporates valving for non-aqueous solutions, is low cost (estimated $0.25 per assay as compared to $2-9 per extraction using currently available DNA extraction methods [2, 4]), and amenable to rapid, simple fabrication. We describe, for the first time, DNA extraction from whole blood on a disposable plastic PeT microdevice using an automated, rotationally-driven platform.

The proposed PeT microdevice is composed of 4-layers and fabricated using laser printer lithography. The device, when loaded with dSPE reagents and sample, can be run through a five-speed bidirectional spin program (0 – ~1276 rotations per minute (RPM)) on the home-built system. During the binding of sample DNA to the particles (driven by an alternating magnetic field (AMF), it is essential to prevent the mobilization of the 'wash solutions' (80% IPA and 0.1 % Tris-EDTA (TE)) into the DNA chamber; a novel combination of a hydrophobic valve and backpressure prevent this. Once the DNA is bound, the IPA is released by centrifugal force (overcoming backpressure) at ~293 RPM, followed by release of TE through hydrophobic valve burst at ~340 RPM. Once the solid phase is washed, the bound DNA is eluted (enhanced by mixing by AMF), and mobilized to a separate elution chamber at ~1276 RPM using 'stop' valves positioned below the main DNA chamber.. The purified DNA results is accessible by puncturing the PDMS covering the elution chamber.

To demonstrate that the spin system was effectively purifying PCR-ready DNA from 2 µL of whole blood, β-globin gene was successfully amplified via microchip electrophoresis after extraction. On-going studies are comparing the extraction efficiency and short tandem repeat (STR) profiles to those samples extracted using the Qiagen EZ1 instrument. Overall, this is the first demonstration of a PeT microdevice utilizing dSPE on an automated, rotationally-driven platform. The portable system has the potential to provide a cost-effective alternative to standard DNA SPE kits and, once fully optimized, can be coupled to other pre- and post-processing DNA steps including preliminary DNA detection and amplification.

References:

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