ENZYMATIC DNA LIBERATION ON A POLYESTER-TONER-PMMA HYBRID CENTRIFUGAL MICRODEVICE

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Solid phase extraction (SPE) is the most commonly-used method in forensic analysis for yielding DNA of sufficient quality and quantity for short tandem repeat (STR) PCR amplification. Recent advances have incorporated these methodologies into integrated devices fabricated from materials such as glass, silicone or thermoplastics, with sample and reagent flow driven by pneumatic or hydraulic pumping. While popular, SPE involves the use of PCR inhibitors (guanidine, IPA) and requires ~30 min. Enzymatic liberation of DNA by a protease (EA1) has been shown to dramatically simplify the yield of PCR-ready DNA by circumventing the wash step, however, until now this enzyme has been utilized sparingly in microdevices. Here, we describe a microfluidic device that incorporates DNA liberation using the EA1 enzyme in a lamination polyester device bonded with printer toner. This device directs pre-loaded reagents to the swab chamber using centrifugal flow to initiate DNA release. A PMMA macro-to-micro interface allows for the introduction of a swab carrying the collected biological sample, and through hydration with EA1, liberates the DNA necessary for PCR amplification in short timescales (<3 min) bypassing the need for external hardware for fluidic movement. The centrifugal device incorporates eight separate chambers capable of simultaneously liberating DNA from eight different buccal swab samples. Studies have shown DNA liberation concentrations up to 10 ng/μL with a 100% success rate in less than 3 minutes. These samples have been proven to be of PCR forensic-quality by providing complete STR profiles for adequate human identification.