B94 Integration of Cell Sorting and Solid Phase Extraction on a Microchip

Jessica C. Voorhees, MSc, Linda Lee, BA, Susan L. R. Barker, PhD, Jerome P. Ferrance, PhD, and James P. Landers, PhD, University of Virginia, Department of Chemistry, McCormick Road, Charlottesville, VA 22901*

The goal of this presentation is to highlight the use of an integrated microdevice that combines sedimentation-based cell sorting and solid phase extraction (SPE) of DNA from the sorted cells, two of the procedures necessary for analysis of sexual assault evidence where male and female DNA must be separately identified.

This presentation will impact the forensic community and/or humanity by demonstrating the application of microchip technology to forensic casework analysis.

The focus of this project is to integrate the rapid separation of sperm and epithelial cells with DNA extraction in a single microdevice com- patible with subsequent STR analysis.

Microchip technology offers a rapid, cost-effective alternative to con- ventional DNA analysis methods. The research presented will highlight the use of an integrated microdevice that combines sedimentation-based cell sorting and solid phase extraction (SPE) of DNA from the sorted cells, two of the procedures necessary for analysis of sexual assault evidence where male and female DNA must be separately identified. This presentation will have a significant impact on the forensic community by demonstrating the application of microchip technology to forensic casework analysis.

The proven utility of forensic DNA evidence has led to an increase in demand for DNA analysis services. Although conventional analysis tech- niques are effective, they are time-consuming and laborious, which has contributed to an overwhelming backlog of forensic casework samples with possible biological evidence. Research efforts have focused on the development of more rapid and efficient analytical methods, as well as the automation of existing methods, to reduce the time and cost of forensic analysis as well as the existing casework backlog. Techniques performed on microchips are particularly advantageous because they can be integrated with downstream analytical steps on a single microfluidic device in the form of a micro-total analysis system (ì-TAS). These integrated systems, which combine all of the sample processing steps required for forensic DNA analysis, will reduce analysis times, and, therefore, the forensic casework backlog.

A successful microchip method for separating sperm and epithelial cells has previously been demonstrated.[1] This method exploits the different physical properties of sperm and vaginal epithelial cells, which allow for selective sedimentation of epithelial cells in the inlet reservoir of a glass microdevice. Initiation of pressure-driven buffer flow causes sperm cells to migrate towards the outlet reservoir, resulting in an effective separation of the two cell types. This method circumvents the most time-consuming step in DNA analysis of sexual assault evidence, the conventional differ- ential extraction procedure. In addition, microchip-based SPE has previ- ously been demonstrated[2] on a variety of biological materials Microchip SPE utilizes a silica matrix, comprised of silica beads immobilized using a tetraethyl orthosilicate (TEOS) sol-gel, to bind DNA in the presence of a chaotropic salt. Proteins and other contaminants are then removed in an isopropanol wash step, and DNA is released in a final elution step.

The research presented here describes an integrated microchip for cell sorting and independent solid phase extraction of DNA from the sorted cells. The functionality of the device is described, including the results of amplification of genomic DNA isolated from cells sorted from a mixed cell sample. The microdevice, fabricated using standard photolithographic tech- niques, was designed with a domain for cell sorting and two separate SPE regions. Cells from a mixed sample were separated according to their physical properties and retained against the individual SPE matrices. The sperm and epithelial cells were then lysed on-chip in their separate areas, followed by isolation and purification of their respective DNA fractions. Following cell separation and SPE on the microdevice, DNA amplification and separation were performed using conventional laboratory methods. The presented work represents a major step towards the development of a fully integrated microdevice capable of total DNA analysis for forensic casework.

References:

(1) Horsman, K.; Barker, S. L. R.; Ferrance, J. P.; Forrest, K. A.; Koen, K. A.; Landers, J. P. *Anal Chem* 2005, *77*, 742-749.

(2) Breadmore, M. C.; Wolfe, K. A.; Arcibal, I. G.; Leung, W. K.; Dickson, D.; Giordano, B. C.; Power, M. E.; Ferrance, J. P.; Feldman, S. H.; Norris, P. M.; Landers, J. P. *Anal Chem* 2003, *75*, 1880-1886.

DNA, Cell Separation, Solid Phase Extraction

 ** Presenting Author*