B71 Purification of DNA From Sperm Cells for Forensic Analysis Using a Sol-Gel Filled Microfabricated Device

Joan M. Bienvenue, MS, University of Virginia, Department of Chemistry, McCormick Road, Charlottesville, VA 22904; Natalie Duncalf, University of Huddersfield, Queensgate, Huddersfield, West Yorkshire HD13DH, England; Jerome P. Ferrance, PhD and James P. Landers, PhD, University of Virginia, Chemistry Department, McCormick Road, Charlottesville, VA 22904*

The goal of this research presentation is to demonstrate the utility of sol-gel microchip technology for extraction and purification of DNA from sperm cells for subsequent forensic analysis.

This presentation will describe efforts to improve conventional extraction of DNA from sperm to both improve the efficiency of the protocol and also decrease the amount of time needed to accomplish the assay. In addition, this work contributes to the overall goal of a fullyintegrated, microchip based DNA analysis protocol to help eliminate the current backlog of casework.

Current techniques for DNA analysis require labor-intensive and time-consuming processes. These methods, though effective, have led to a dramatic backlog of casework, overwhelming crime laboratories at this time. In addition, data-basing efforts are hindered by this backlog of cases and, in the current condition, many cases simply go unanalyzed.

As such, research efforts in forensics have focused on improving the methods associated with the analysis of DNA to develop a more rapid and efficient assay for casework profiling.

The application of microdevices to bioanalytical analyses has drastically reduced the time required to perform a wide variety of assays. As such, microdevices are currently being designed to improve the efficiency of processes associated with forensic casework analysis. A fullyintegrated, microchip capable of performing the steps normally carried out on the bench-top would not only reduce the time required to perform these tasks, but would also eliminate user intervention and potential sources of contamination, as well as preserve more of the sample for future analysis. PCR and high-resolution DNA separations can currently be carried out on-chip, as well as solid-phase extraction of DNA from a variety of clinical and biohazardous samples. In addition, current research efforts are being directed to an on-chip differential separation of sperm and vaginal epithelial cells, with the ultimate goal of integration of all of these processes into one, fully-functional device.

The focus of the research presented here is the extraction of DNA from sperm cells, either directly from semen or removed from other evidentiary materials, such as vaginal swabs. Nucleic acid extractions from biological material have historically been a laborious process, requiring phenol-based extractions and other time-consuming methods. Recently, a shift to solid-phase extractions on silica or ion exchange resins has not only made DNA extractions more efficient, but these methods are also more amenable to incorporation into microchip-based devices. We have previously demonstrated the use of thermally-bonded borofloat glass microchip devices packed with silica beads for the purpose of DNA purification. Monolithic matrices prepared from a silica monomer, tetramethoxysilane or TMOS, can be easily created in the microfabricated device format. These have been shown to yield fast, efficient, solid phase extraction of DNA from a variety of biological materials. The process relies on the adsorption of DNA to the solid surface via hydrogen bonding. The work reported here investigated extraction and purification of DNA from semen, a complex biological mixture. Also highlighted is the extraction of DNA from a microchip purified sperm cell fraction. A method for extraction and purification is described, along with an elution profile for DNA from the solid phase in small volume aliquots. A detailed evaluation of extraction efficiency of DNA from semen using the microchip-based system is also described. The effect of DTT, a standard component in differential extraction of sperm cell DNA, on the extraction efficiency is presented, with conditions for optimum extraction efficiency detailed. Comparisons of the microchip extraction with conventional kit-based methods (Qiagen®, Promega®) are also presented. Finally, the suitability of the DNA extracted for subsequent PCR is demonstrated by COfiler™ and Profiler Plus™ amplification, with conventional analysis on ABI 310 instrumentation. This work represents one of the major steps required for the incorporation of a solid phase-based extraction process for DNA into either modular or fully integrated microdevices capable of total systematic DNA analysis for forensic casework.

Sperm, DNA, Microchip

 ** Presenting Author*