



B53 Macro-to-Micro Interfacing of a Swab Receptacle With a Microchip for Total DNA Analysis

Benjamin R. Schroeder, BS, Jerome P. Ferrance, PhD, and James P. Landers, PhD, Department of Chemistry, University of Virginia, McCormick Road, Charlottesville, VA 22904*

After attending this presentation, attendees will learn of the importance of a microfabricated device that has the power to dramatically reduce the time associated with DNA analysis of a sample taken from a victim of sexual assault.

This presentation will impact the forensic community by demonstrating the fabrication of a micro-total analysis system for the forensic analysis of DNA, the time associated with the extraction, separation, and PCR amplification of such material will decrease dramatically.

Learning Objective: The goal of this project is to develop a macro-to-micro receptacle system that will accept a cotton swab from a rape kit and allow for collection of desorbed cellular material. The receptacle is to be integrated with a microchip cell separation apparatus to circumvent conventional differential extraction.

This presentation addresses the considerable backlog of rape kit evidence awaiting DNA analysis. Analysis of both perpetrator and victim DNA by gel electrophoresis has been the keystone technique utilized in the investigation of cases involving sexual assault and rape, and is a well established practice. Unfortunately, the procedures involved in a typical DNA analysis can result in hours, even days, of laboratory time spent on a single case, particularly in the sample preparation stages. As a result of the time constraints involved in the analysis of such cases, and insufficient funding, a large backlog exists in many large-volume DNA analysis laboratories.

Microfabricated devices that utilize microchannel electrophoresis as a DNA separation technique have been shown to greatly reduce the time needed for analysis. The speed and efficiency of such separations are due largely to the increased area-to-volume ratio of the etched channels over conventional slab gels, and the fact that high voltages can be utilized. In addition to the aforementioned advantages, these devices allow integration of all necessary processing steps; this provides for complete analysis, from cellular desorption to complete DNA assessment, onto a single microchip. Extraction of DNA from separated sperm and epithelial cells, PCR amplification of the DNA, and separation and detection of the amplified DNA are steps that are now incorporated into single microfabricated devices. There is little doubt that such integrated devices will transform the arena of forensic DNA analysis.

A major component of such an integrated device is the macro-to-micro swab receptacle that must interface the "macro-scale" of the cotton swab with the "micro-scale" of a channel used to separate epithelial cells from sperm cells. The current protocol utilized by the FBI for the elution of cellular materials from the cotton matrix is a time consuming step that involves significant sample handling, which directly increases the chances of sample contamination, as well as human error. It is not unusual for a cotton swab taken from a victim of sexual assault to be incubated for hours (overnight) in order for the cells to elute from the cotton fibers. Additionally, the extraction solution utilizes a protein lysis buffer containing SDS and proteinase K, which aid in the removal of the cellular material, but lyse the fragile epithelial cells in the process. A cell-desorption process that greatly reduces extraction time while leaving cells intact would be advantageous in that it might be easily incorporated into a cell separation (CS) channel on a micro-total analysis system (–TAS).

The focus of the research presented here is the development of a swab receptacle that provides an interface for a cotton swab taken from a victim of sexual assault, with a –TAS on a microfabricated glass device. Through the microscopic examination of a cotton swab containing a dry semen sample, it was discovered that the major component leading to sperm adhesion was entanglement of the tails with the polysaccharide cellulose strands. Preliminary studies have shown that the enzyme cellulase, which digests cellulose to produce glucose, greatly reduced the time needed for cells to elute into solution. The desorption of cellular material has been optimized in an aqueous solution at a temperature of 37° C in a borosilicate glass microcentrifuge tube. Eluted sperm and epithelial cells from a cotton swab were counted hourly using a hemacytometer, showing an approximate 2:1 ratio of sperm cells in the presence of the enzyme in comparison with the same sample without the enzyme. Optimum cellular elution conditions using cellulase, showing that sperm cell desorption surpasses the current method utilized by the FBI, as well as specifics on the integration of the receptacle with a –TAS device will be presented.

Microchip Technology, Macro-to-Micro Interface, Cellular Desorption