B28 Development of a Microfabricated Device for Separation of Sperm and Vaginal Epithelial Cells: A Significant Step Toward Circumventing Conventional Differential Extraction

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The goal of this research presentation is to introduce microchip technology to the forensic community and demonstrate its potential for the separation of sperm and vaginal epithelial cells in rape kit analysis.

This presentation will introduce the forensic community to microchip technology and its potential for rapid analysis of rape kit DNA evidence, thereby, reducing the DNA analysis backlog.

Differential extraction, the conventional method for isolating male and female fractions of DNA, is a time-consuming sample preparation step in the forensic DNA analysis of rape kit evidence. Therefore, our goal is to develop a means to reduce the time associated with isolation of the male and female DNA fractions, while maintaining or improving the percent recovery and purity. The means through which we are attempting to achieve this goal is the use of microfabricated glass devices.

The brief record that exists for microminiaturization of analytical processes on microchip platforms has demonstrated that reduction in analysis time (versus conventional methods) is often a benefit, as well as the potential for integration of multiple processing steps in a single device and for automation of these processes. Since differential extraction is only one of a number of processes that constitute forensic DNA analysis, replacing it with a microdevice method provides a distinct advantage with the possible integration of several sample preparation steps, including DNA extraction, DNA quantitation, and PCR amplification on a single device. In addition, microchips can be designed to accommodate parallel processing of both the male and female DNA fractions as well as the necessary positive and negative controls. While an integrated microdevice including all sample preparation steps is advantageous in many respects, the cell separation methodology is amenable to a modular system in which separate devices are developed for each processing step, depending upon the needs of the forensic community.

The conventional differential extraction methodology is not easily translated to the microchip format because of the centrifugation and filtration steps. Therefore, a novel method for obtaining isolated male and female fractions of DNA on a microfabricated device was developed. This new technique involves first separating the sperm cells from the cell mixture, then extracting the DNA from each fraction independently, allowing separate male and female DNA fractions to be obtained.

The cell separation step is the focus of the research presented here. The separation developed exploits the differential physical properties of the two cell types such as buoyant density, size, shape, and proclivity for adhesion to the microchannel surface. In an etched microchannel, a flow rate of approximately 1 nL/sec was obtained using a mechanical pump, directing the sperm cells to a collection reservoir while retaining the epithelial cells in the inlet reservoir. Preliminary experiments employed digital video microscopy to visualize the cell separation and demonstrate the purity and efficiency of the process. Methods have been established to selectively separate free DNA (from the more easily lysed epithelial cells) from the sperm cell fraction. The movement of bacteria during the cell separation has been characterized, although the presence of bacteria is not particularly concerning because of the lack of amplifiable human STRs. In addition, the movement of white and red blood cells must be characterized, because of DNA contamination and PCR inhibition issues, respectively.

Using mock post-rape vaginal swabs, the cell separation product obtained on the microdevice resulted in a clean sperm cell fraction. The DNA from isolated cells was extracted with a commercial extraction kit, amplified with a Profiler® PCR kit, and analyzed on an ABI 310 commercial CE, yielding the profile of the male sperm donor. DNA extraction from the isolated cells was also performed using a microdevice method before amplification and analysis to demonstrate the potential of integrating these two steps into a single device to circumvent conventional differential extraction.

Differential Extraction, Cell Separation, Microchip Technology

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