D50 Evaluation of Plastic Microdevices for Isolation of Sperm Cells From Sexual Assault Evidence

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After attending this presentation, attendees will learn the advantages and disadvantages of using plastic microdevices for isolation of sperm cells from sexual assault evidence as compared to glass microdevices and conventional differential extraction.

This presentation will impact the forensic community and/or humanity by exciting the forensic community with the possibility of improved analysis time, separation efficiency, and purity in a disposable microdevice that has the potential for integrating multiple processing steps. The prospect of dramatically reducing the rape kit backlog will be especially appealing.

Differential extraction, the conventional method for isolating male and female fractions of DNA from sexual assault evidence, is a time-consuming sample preparation step in forensic DNA analysis. The 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 recovery and purity. The means through which it is proposed the goal is achieved by the use of microfabricated glass and plastic devices for separation of male and female cells.

The brief record that exists for miniaturization of analytical processes on microchip platforms has demonstrated reductions in analysis time (versus conventional methods) with no loss of analytical capability. Microchips also provide the potential for integrating multiple processing steps in a single device and automating the processes. Since differential extraction is only one of a sequence of processes required for forensic DNA analysis, replacing it with a microdevice method provides a distinct advantage in the path toward integration of multiple sample preparation steps (DNA extraction, DNA quantitation, and PCR amplification) on a single device. In addition and not insignificantly, microchips can be designed to accommodate parallel processing of both the male and female DNA fractions.

The centrifugation and filtration steps associated with conventional differential extraction prevent its direct translation to the microchip format. Thus, a novel method for obtaining isolated male and female fractions of DNA on a microfabricated device was developed and involved separating the sperm cells from mixtures of sperm and epithelial cells as would be recovered from sexual assault evidence. The DNA from each cell type can then be extracted independently, allowing separate male and female DNA fractions to be obtained.

The separation or “cell sorting” developed exploits differential physical properties between the two cell types such as buoyant density, size, shape, and proclivity for adsorption to the microchannel surface. In an etched glass microchannel sperm cells could be transported to an outlet reservoir while the epithelial cells were retained in an inlet reservoir by application of a volumetric flow rate of approximately 1 nL/sec, or about 1.6 sperm/sec, provided by a mechanical pump. Experiments employ digital video microscopy to visualize the cell separation and demonstrate the purity and efficiency of the process.

The use of plastic microfluidic devices was explored in an effort to make lab-on-a-chip technology more affordable, and to provide the possibility of disposable devices. Disposable, single-use devices are of interest in forensic applications because they eliminate cross-contamination between samples. The cell separation and free DNA separation techniques developed for glass microfluidic devices were tested on plastic microfluidic devices. Initial tests indicated that plastic devices were sufficient for this purpose, but experiments were carried out to determine the cell separation efficiency and purity.

Here the characterization of plastic microdevices with respect to cell separation efficiency and purity is presented. The purity of the fractions was assessed not only through digital video microscopy but also by short tandem repeat analysis, the method used for genetic identification in forensic analysis. Each fraction should show a single-donor DNA profile if the cell separation is successful. More realistic samples that have lower numbers of sperm and have been dried over a set aging period were also introduced. Any additional techniques developed for the glass microdevices have also been translated to the plastic microchips.

Microfluidic Device, Vaginal Swab, Sexual Assault

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