B70 Acoustic Differential Extraction for the Analysis of Sexual Assault Evidence on Microdevices

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The goal of this poster is to introduce a means of improving the analysis of sexual assault evidence using a novel approach for obtaining highly-enriched male and female fractions. In addition, the audience will be introduced to the use of microchip technology for forensic DNA analysis.

This presentation will impact the forensic community and/or humanity by demonstrating an acoustic differential extraction method presented has the potential to significantly alter the means by which sexual assault evidence is processed in crime laboratories. This method is one step of a totally integrated, automated microchip format for forensic DNA analysis.

DNA analysis of sexual assault evidence requires separation of the male and female fractions to aid in obtaining STR profiles of both individuals. Conventionally, this is accomplished using the differential extraction method,[1] in which the differential stability of the nuclear membrane of each cell type is exploited. That is, vaginal epithelial cells are susceptible to lysis under 'mild' extraction conditions, whereas sperm cells require the reduction of the disulfide bonds in the nuclear membrane to efficiently release sperm cell DNA. Using this differential lysis, sperm cells are then separated from epithelial cell DNA using centrifugation and multiple wash steps to yield separate male and female fractions. This method, however, suffers from extensive handling by analysts, often poor efficiency separation (i.e., significant female carryover into the male fraction), and lack of automation.

An alternative to the conventional method, termed acoustic differential extraction, has been developed for isolation of the male and female fractions from sexual assault evidence to address the short- comings of the conventional methodology. After elution of the biological material from a vaginal swab under 'mild' lysis conditions, this method separates sperm cells from the mixture of epithelial cell lysate (free female DNA) and sperm cells. The separation of the male and female fractions is carried out in a valveless microdevice, consisting of a printed circuit board layer (containing piezoelectric transducers to generate the acoustics) and a glass layer containing the microchannel structure. Upon application of electrical signal to the transducers, a standing ultrasonic acoustic wave is set up in the microchannel, resulting in a pressure-minimum in the center of the channel. The physical characteristics of a given cell or particle determine whether, or to what extent, it will be trapped in the acoustic wave.[2] In this method, the system has been optimized to trap sperm cells, whereas free DNA (primarily from the epithelial cell lysate) will not be trapped. Isolation of the highly-enriched male and female fractions is completed using precise fluidic control[3] that exploits laminar flow valving, negating any need for active valving on the microdevice.

Upon activation of the ultrasound, the biological sample is infused into the microdevice and sperm cells are trapped in the microchannel above the transducer. The free DNA, unretained in the acoustic trap, is directed to the female DNA outlet. The immobilized sperm cells, trapped in a monolayer in 3D fluidic space in the microchannel, are washed with buffer to remove any free DNA that was inadvertently trapped. With laminar flow valving, the flow is re-directed to the male outlet and the ultrasound silenced, resulting in release of the sperm cells from the acoustic trap, movement into the male outlet DNA outlet and subsequent isolation of the male fraction.

Product from the acoustic differential extraction microdevice, both male and female fractions, was collected and analyzed off-chip to show sample purity. DNA from the isolated cells was extracted using a commercial DNA extraction kit and analyzed with a duplex quantitative PCR assay[4] to determine the percent of male and female DNA in each fraction. In addition, STR amplification was utilized to show efficiency of separation.

References:

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Acoustic Trapping, Differential Extraction, Microchip Technology

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