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ACOUSTIC DIFFERENTIAL EXTRACTION ON A MICRODEVICE: ASSESSMENT OF FLUIDIC CONTROL FOR SEPARATION OF SPERM CELLS AND EPITHELIAL CELL LYSATE

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Genetic analysis of mixed profile DNA samples obtained from vaginal swabs is a well-established technique in the investigation of sexual assault and rape cases. To obtain independent STR profiles of both the victim and perpetrator, it is necessary to separate the male and female components of the recovered genetic material. The current separation protocol, known as differential extraction (DE), exploits the differential stability of the nuclear membrane of each cell type through the preferential lysis of vaginal epithelial cells. Sperm cells are then separated from the epithelial cell DNA using multiple centrifugation and wash steps, allowing independent genetic analysis of male and female DNA. The DE process, while well-established, requires extensive sample handling, is difficult to automate, and often results in inefficient separation of female DNA from the male fraction.

Research efforts have focused on the development of more rapid and efficient analytical methods to reduce the time and cost of forensic analysis. Microfluidic technology provides the opportunity to automate forensic DNA sample processing, and all forensic genetic analysis steps have been performed on microdevices. Techniques performed on microchips are advantageous because they can be integrated with other analytical steps on a single microfluidic device to reduce sample handling.[1] The laborious, time-consuming centrifugation and wash steps of conventional DE are not easily amenable to a microdevice; therefore, several microdevices have been proposed as alternatives for the separation of the male and female fractions of a sample.[2,3]

Trapping based on acoustic forces offers a simple, efficient way of retaining particles in a microfluidic system.[4] Recently, an acoustic differential extraction (ADE) microdevice was presented[3] which relies on acoustic trapping of sperm cells in the presence of epithelial cell lysate. ADE is carried out in a valveless device, consisting of a printed circuit board layer containing a transducer, and a glass layer containing the microchannel structure. Upon activation of an electrical signal to the transducer, a standing ultrasonic acoustic wave is formed between the transducer and glass-reflector layer. The standing wave presents a pressure minimum in the center of the channel, defining a sperm trapping site. A sample containing sperm cells and epithelial cell lysate is infused into the device, and sperm cells are trapped in the channel above the transducer, whereas DNA from epithelial cell lysate will be unretained and directed

towards an outlet reservoir. The immobilized sperm cells are then washed with buffer to remove any female DNA that was inadvertently trapped. Laminar flow valving is utilized to direct the male and female fractions to different outlets. The ultrasound is terminated, resulting in the release of sperm cells from the acoustic trap, movement of the cells into a separate outlet, and subsequent isolation of the male fraction. In previously presented work, efficiency of separations was determined through conventional purification and STR amplification methods; however, a purified male STR profile was not achieved, primarily due to problems with fluidic control. The work presented here describes the technical solutions to these problems, allowing for acquisition of a purified male DNA fraction from a sample of sperm cells and female buccal epithelial cell lysate (original sample - 5% male DNA as determined by qPCR results). Purity of the separated components are assessed through qPCR and STR analyses, demonstrating application of selective isolation of male and female components from mixed biological samples.

References

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