IMPROVED ACOUSTIC DIFFERENTIAL EXTRACTION ON A MICRODEVICE FOR SEPARATION OF SPERM CELLS AND EPITHELIAL CELL LYSATE

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ABSTRACT

Genetic analysis of samples obtained from sexual assault evidence relies on separation of male and female components of the recovered genetic material. One separation technique, acoustic differential extraction (ADE) on a microfluidic device involving acoustic trapping of sperm cells in the presence of female lysate, has previously been demonstrated. However, laminar flow valving (LFV) utilized to direct the sperm and lysate to different outlets was problematic, as a male DNA profile was not obtained. The presented work describes improvements to the ADE technique to enable efficient separation of mixed biological samples, resulting in independent male and female DNA profiles.

KEYWORDS: Acoustic trapping, differential extraction, forensic science

INTRODUCTION

The conventional method for separation of sperm from female epithelial cell lysate used in forensic laboratories, differential extraction (DE), is a time-consuming process (requiring up to 24 hours) that is not easily integrated with downstream analysis steps. ADE was previously presented as an alternative to DE.[1] The method utilizes an acoustic standing wave to retain sperm cells in the presence of female lysate (which is unretained). LFV is utilized to direct the male and female fractions to separate outlets. Previously reported results showed suboptimal purity of the male fraction after ADE separations. To obtain independent DNA profiles from a mixed sample, further optimization of the ADE technique was performed.

EXPERIMENTAL

Sample is infused in a microfabricated glass channel (~200 μm deep), designed to work as a resonator cavity and reflector for miniature piezoelectric transducers that are mounted at the bottom of the channel. Upon activation of the ultrasound, the sperm cells are trapped in the nodes of a standing wave, while female DNA is not retained due to its smaller size. Previous results [1] were obtained with acoustic trapping utilizing a single node fluidic layer; to increase capacity of the trap, a triple node fluidic layer was implemented. Colored dyes were used in the characterization of fluidic control. Sample was infused through a sample inlet, while focusing buffer

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was infused through a focusing buffer inlet. LFV was used to direct sample to separate outlets. A sample consisting of sperm cells and lysed female epithelial cells was subsequently infused into the device for six minutes while trapping the sperm cells over the transducer. The trapped sperm cells were washed with buffer for two minutes, then released for five minutes, and collected for subsequent off-chip analysis using commercially-available purification and amplification kits.

RESULTS AND DISCUSSION

Figure 1 shows the initial results from ADE of a mock sexual assault sample, where blue dye represents female cell lysate and blue polystyrene beads represent sperm cells. The particles were trapped over the transducer, and the dye directed to the right outlet. After washing the particles, the ultrasound was deactivated and the flow redirected to collect the particles in the left outlet. The "pull" of residual sample by focusing buffer (Figure, 2C, 2D) indicates a potential cause of female DNA contamination in the male ADE fractions. An optimized sample withdrawal step was, therefore, incorporated during sample wash and release to prevent unwanted sample infusion of female lysate to the male outlet (Figure 2).

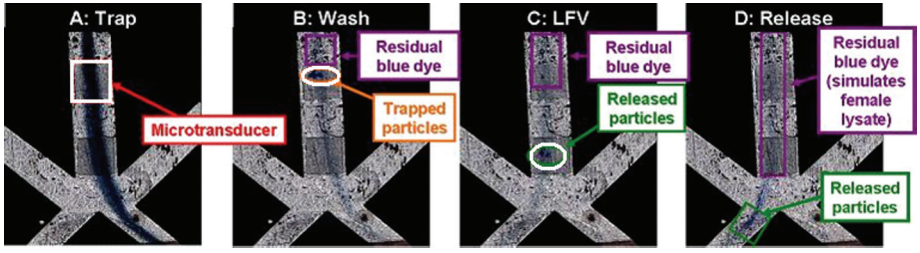

*Figure 1: (**A**) A mixture of dye and microbeads are infused into the chip. The beads are trapped while the dye is diverted to the right outlet. (**B**) Sample flow is terminated and the channels are washed with buffer. (**C**) The flow is switched and the ultrasound deactivated to release the microbeads (**D**) into the left outlet. Residual blue dye is shown in (**B**), (**C**), and (**D**).*

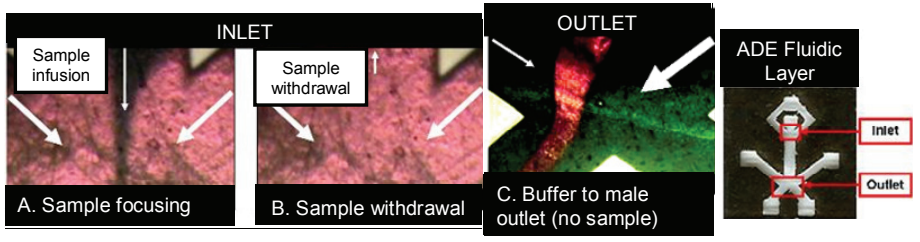

*Figure 2: (**A**) Sample was infused through the sample inlet, while hydrodynamic focusing was performed. (**B**) Sample withdrawal was implemented. (**C**) During withdrawal of the sample, the direction of the flow was redirected using LFV.*

An example of a purified short tandem repeat (STR) profile, obtained after ADE of a mock sexual assault sample consisting of sperm and epithelial cell lysate, is

shown (Figure 3). The ADE analysis time was thirteen minutes – hours less than conventional DE methods. While the male DNA profile in the original sample (26% male, data not shown) would be difficult to interpret due to the amount of female DNA present (84%), ADE enrichment provided an interpretable male profile (92%).

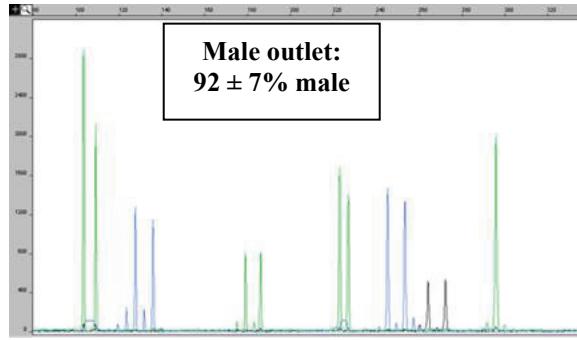

Figure 3: Forensic DNA analysis of sample collected from the male outlet following ADE. The profile was amplified using a commercially-available COfiler® kit and separated on an ABI 310 Genetic Analyzer.

STR analysis was used to demonstrate purity of the recovered fractions after ADE (Table 1). A four-fold enrichment of the male fraction was measured.

Table 1. Forensic DNA analysis of samples analyzed before and following ADE. Comparison of profiles with those of the sperm cell donor and female cell donor (data not shown) indicates a four-fold enrichment of the male fraction.

	Purity
Original sample (n=2):	20 ± 2.9% male
Collected from male outlet (n=3):	81 ± 12% male
Collected from female outlet (n=3):	96 ± 3.7% female

CONCLUSIONS

The results show that highly purified male and female fractions can be obtained with improvements to the ADE technique. It is reasonable to expect that this technique can be integrated with on-chip downstream sample processing steps. The time savings associated with ADE analysis, in combination with the possibility to create a fully automated system, provides potential to significantly alter the means by which sexual assault evidence is processed in crime laboratories today.

REFERENCES

[1] Evander, M, Horsman, KH et al. μTAS 2006, Tokyo.