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Acoustic Differential Extraction for Forensic Analysis of Sexual Assault Evidence

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Forensic DNA analysis of samples obtained from sexual assault evidence relies on separation of male and female components of the recovered genetic material. The conventional separation method used by crime laboratories, differential extraction (DE), is one of the most time-consuming sample preparation steps, requires extensive sample handling, is difficult to automate, and often results in inefficient separation of female DNA from the male sample components. To circumvent conventional DE, acoustic differential extraction (ADE) analysis was developed on a microfluidic device. The ADE method relies on acoustic trapping of sperm cells in the presence of epithelial cell lysate (which is unretained), and laminar flow valving to direct the male and female fractions to separate outlets. Following the separation of sperm from epithelial cell lysate, DNA extraction, quantitation, amplification, and separation were performed using conventional laboratory methods. The results show that highly purified male and female fractions can be obtained with the ADE microdevice from mock sexual assault samples in 14 min. ADE analysis provides the potential to significantly alter the means by which sexual assault evidence is processed in crime laboratories.

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.[1−3] To obtain independent short tandem repeat (STR) profiles of both the victim and the 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 epithelial cell DNA using multiple centrifugation and wash steps, allowing independent genetic analysis of male and female DNA. While DE is well-established, it is one of the most time-consuming sample preparation steps of sexual assault evidence analysis, contributing to a substantial forensic casework sample backlog.[4] In addition, conventional DE requires extensive sample handling, is difficult to automate, and often results in inefficient separation of female DNA from the male fraction.[5,6]

Research efforts have recently focused on the development of microfluidic devices to reduce the time and cost of forensic analyses. Microdevices can provide a self-contained, closed system for analysis procedures, diminishing the potential for contamination or loss of sample. Techniques performed on microdevices are particularly advantageous because they provide the opportunity to automate forensic DNA processing through integration of sample preparation steps on a single device.[7−10] Integrated systems that combine several analytical techniques on a single microfluidic device can reduce analysis times, sample handling, and the sample size required for analysis, making them ideal for samples encountered in forensic laboratories.

The laborious, time-consuming centrifugation and wash steps of conventional DE are not easily amenable to a microdevice. Microscale cell separations have been accomplished by various means in the recent past;[11−18] however, these methods are not

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without limitations that may preclude them from use in forensic laboratories. For example, the development of microfabricated fluorescence-activated cell sorting devices has previously been reported.[11−13] Although this method has high specificity, the technique requires a complicated multilayer microchip structure requiring a number of process steps to fabricate,[14] or narrow, shallow channels at the detector,[11,12] increasing the risk of clogging when biological material is used.[13] In addition, the fluorescent tagging of one (or both) of the cell types is required. Microchip cell separation[15,16] and capture[17] by dielectrophoresis has been demonstrated, in which cells migrate in an electric field gradient depending on their physical properties. A potential drawback to this technique is the adherence of cells to the microchip substrate at the trapping site.[18]

The use of acoustic forces offers an efficient, alternative method for retaining and manipulating particles in a microfluidic system, and has been reported by several groups.[19−25] Acoustic particle trapping was recently applied to forensic analysis in the form of acoustic differential extraction (ADE).[26] Unlike many of the standard microfluidic approaches to cell sorting, one of the major advantages to the ADE method is that it has the potential to accommodate a large range of sample volumes ranging from microliters up to milliliters-making it ideal for forensic applications. The ADE method utilizes noncontact acoustic forces in a valveless microfluidic device to retain sperm cells from of a biological mixture obtained from sexual assault evidence.

Acoustic Trapping. The use of acoustic forces for manipulating cells has been reported by several groups. Hawkes et al.[27] demonstrated an acoustic device used for enriching yeast cells, while Nilsson[28] and Petersson[29] have manipulated red blood cells in mediums that allow for separation from lipid particles. Hultström et al. studied the proliferation rate on cells exposed to an ultrasonic standing wave.[30] An acoustic device for trapping cells was developed by Spengler et. al[31] and has been used to study the physical environment of cells in an acoustic standing wave by Bazou et al.[32]

The acoustic cell and particle manipulation techniques are based on the forces acting on an object entering an acoustic standing wave. The forces will direct the object either to a pressure node or a pressure antinode of the wave, depending on the material parameters of the object and the surrounding fluid. The fundamental theory of acoustic standing wave forces acting on microparticles have previously been presented by King[35] and Gorkov.[36]

The ADE method described here utilizes the size difference between sperm cells and free DNA to create a force strong enough to retain the sperm cells while allowing the free DNA to pass through the acoustic field unaffected.

To set up a standing wave within the ADE microdevice, the depth of the microchannel and the thickness of the reflector layer should correspond with the operating frequency of the transducer. To achieve a single trapping zone (node) in the center of the channel, the channel depth should correspond to a half wavelength (λ) of the optimal transducer resonance frequency; alternatively, to achieve three equidistant trapping zones (nodes), the channel depth should correspond to $3\lambda/2$. The thickness of the reflector should correspond to an odd multiple of a quarter wavelength. In line with the concept of applying acoustic force theory to applications focused on forensic analysis,[37] the work presented here describes the development and characterization of acoustic trapping of biological components and fluidic control in the ADE device, allowing for acquisition of a purified male DNA fraction from a sample of sperm cells and female epithelial cell lysate.

EXPERIMENTAL SECTION

Mock Sexual Assault Sample Preparation. Buccal epithelial cells (from a female) were obtained on cotton swabs and stored at room temperature until use. Due to the nature of cell collection on buccal swabs, the number of cells per swab is highly variable. Epithelial cells were eluted from the swab and lysed in a conventional differential extraction buffer as developed by Gill et al.,[38] consisting of 10 mM trizma pH 8 (prepared via titration of trizma-base with trizma-hydrochloride), 10 mM EDTA, 0.1 M NaCl, 2% SDS, and 20 μg/mL proteinase K. A fixed volume of diluted semen (approximately 20 000 sperm cells per microliter) was added to the epithelial cell lysate prior to use. Sperm cells were not added prior to epithelial cell lysis, as an unknown percentage of sperm cells are destroyed during proteolytic digestion; therefore, sperm cells were added after proteolytic digestion of epithelial cells to allow for accurate control of the number of sperm cells added to the sample. The majority of sperm cells were observed to be completely intact (including tails). All samples were obtained from healthy volunteers, as per University of Virginia IRB HIC approval no. 10896.

Acoustic Trapping Device. The ADE device is based on a design first used by Lilliehorn et al.[39,40] for microparticle trapping

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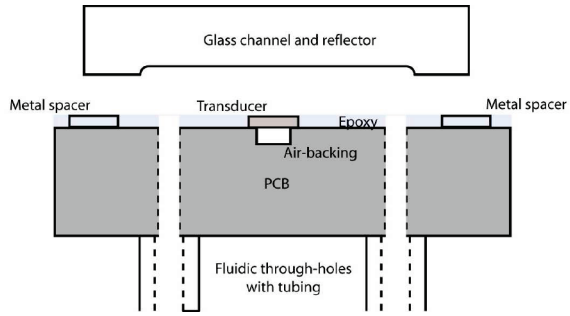

Figure 1. Schematic image showing the components of the ADE system with the printed circuit board (PCB) holding the transducers and the fluidic access and a separate glass-chip, defining the microfluidic network.

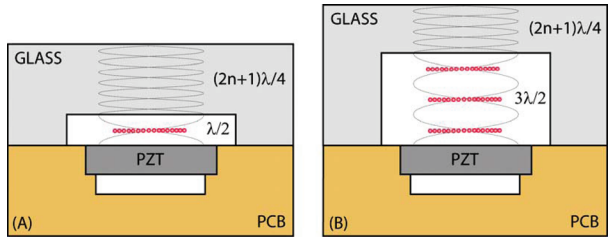

Figure 2. Matching the depth of the fluidic channels to $\lambda/2$ (63.8 μm) at the transducer operating frequency, a single pressure node in the center of the channel can be obtained (A). By increasing the depth of the channel to $3\lambda/2$ (191.4 μm), three equidistant pressure nodes are obtained yielding a tripled capacity. PCB = printed circuit board; PZT = piezo transducer.

and bioassays, later modified by Evander et al.[41] for use in cell-assays. The microfluidic device, the fabrication of which has been previously described,[41] is shown in Figure 1.

Fabrication of Fluidic/Reflector Layer. Microfluidic channels were created using standard photolithography and chemical etching techniques.[42] The microchannel was designed to accommodate a single $\lambda/2$ (63.8 μm) or a $3\lambda/2$ (191.4 μm) standing wave when operating the transducer at 11.6 MHz (Figure 2). The fluidic layer conducting the cell suspension was enclosed by an air-backed miniature transducer with a thickness of $\lambda/2$. The thickness of the reflector layer above the channel corresponds to an odd number of quarter wavelengths $[(2n + 1)\lambda/4]$.

Acoustic Differential Extraction Method. ADE was performed in a microdevice that was sealed using ultrasonic gel between the transducer platform and the microfluidic channels. Excess ultrasound gel was washed away from the fluidic system using Nanopure water (Barnstead/Thermolyne, Dubuque, IA). An Agilent 33220A waveform generator (Agilent Technologies, Palo Alto, CA) was used to actuate the transducers, 11.6 MHz sinusoidal, 8−10 V_{pp} output, unless otherwise indicated. Particle and cell trapping was viewed through an optical microscope (Leitz Orthoplan, Leica, Germany) and recorded to DVD via a CCD camera (Hitachi KP-D20BU, Tokyo, Japan) and DVD recorder (Panasonic DMR T2020, Secaucus, New Jersey). Fluidic handling was controlled by syringe pumps (sample, model no. SP 260; buffer, model no. SPE 100I; World Precision Instruments, Inc., Sarasota, FL). Polytetrafluoroethylene (PTFE) tubing (Supelco, Bellefonte, PA) was utilized to connect the microdevice to the buffer (0.8 mm ID) and sample (0.3 mm ID) syringes. Outlets, 0.3 mm ID PTFE tubing, were connected to microcentrifuge tubes for fraction collection.

Samples consisted of prepurified human genomic DNA (hgDNA) in 1X TE buffer (100 mM Tris, 10 mM EDTA, pH 8.0), semen (6, 12, 24, or 48 μL) diluted in 500 μL phosphate buffered saline (PBS) solution, pH 7.4, epithelial cell lysate, or a combination of sperm and epithelial cell lysate. A conventional differential extraction (DE) buffer devoid of the proteolytic digestion agent was used for hydrodynamic focusing and laminar flow valving (LFV), unless otherwise indicated. The ultrasound was activated during the sample infusion (Figure 3 left). Hydrodynamic focusing was used to direct the infused sample toward the center of the transducer to ensure a high trapping efficiency. Focusing buffer inlet (FI) and sample inlet (SA) flow rates were 6 μL/min and 1 μL/min respectively, see Figure 3. Cells were trapped above the transducer in the pressure nodes of the standing wave, whereas the unretained material (female lysate) was directed toward the female outlet using a 1:5 (S2:S1) ratio of buffer from the side arm inlets. After trapping the sperm cells, the sample injection syringe pump was reversed to withdraw fluid at a rate of 0.5−1 μL/min to prevent residual sample from entering the system. (Figure 3 center). The ultrasound remained activated, retaining the sperm cells while the focusing buffer (6 μL/min) removed any residual lysate from the microchannel and outlet tubing. Upon completion of the washing, the side arm flow rate ratio was inverted (5:1, S2:S1), and the ultrasound was deactivated, releasing the sperm cells for collection in the male outlet (Figure 3 right). The released cells and buffer originating from S1, S2, and FI were subsequently collected for 5 min.

Off-Chip Analysis of Acoustic Differential Extraction Fractions. DNA extraction of the ADE fractions was completed using a QIAamp mini spin kit (Qiagen Corp. Valencia, CA). DNA extraction was completed following the manufacturer's specifications in the "Blood and Body Fluid Spin Protocol," with the addition of dithiothreitol (50 mM final concentration; Fisher Scientific, Pittsburgh, PA). The sample was eluted in 40 μL of "AE buffer", provided in the kit.

Real time quantitative PCR (qPCR) was utilized to determine the quantity of hgDNA in the ADE fractions; the amplicon was a 63 bp fragment of the TPOX locus. The forward and reverse primers for the autosomal locus were CGGGAAGGGAACAGGAG-TAAG and CCAATCCCAGGTCTTCTGAACA, respectively. The TPOX probe (FAM-CCAGCGCACAGCCCGACTTG-TAMRA) was covalently labeled on the 5′ terminus with a FAM fluorophore (Applied Biosystems, Foster City, CA) and at the 3′ terminus with a TAMRA (tetra-methylcarboxyrhodamine) quencher. Probes were synthesized by Applied Biosystems. Primers were synthesized by Invitrogen Corp. (Carlsbad, CA). Fluorescence signal was

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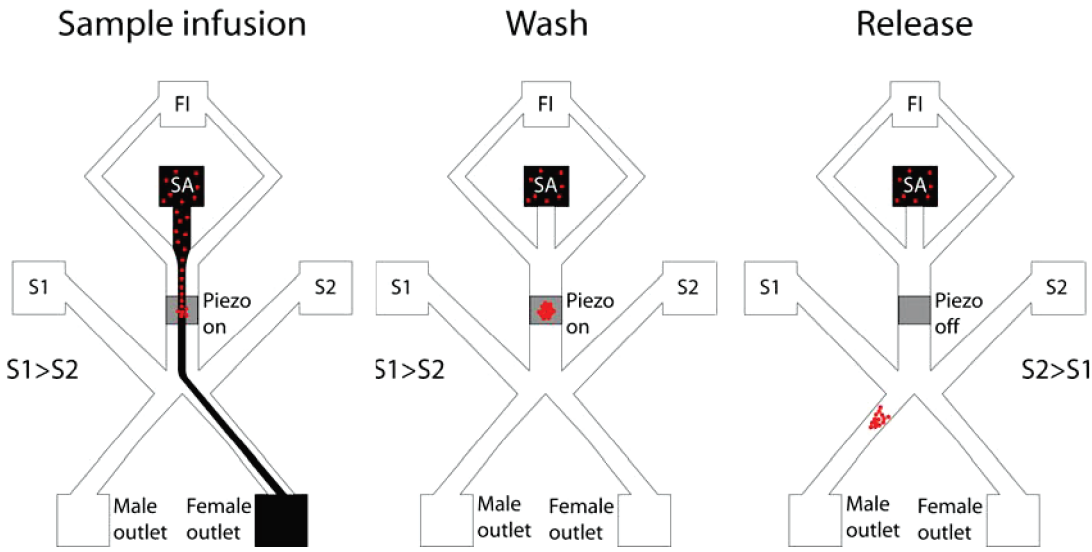

Figure 3. Fluidic design of the ADE-chip. Sample infusion: sample is infused through the sample inlet (S) and hydrodynamically focused by the focusing buffer inlet (FI). Cells (red) are trapped above the transducer upon activation of the transducer, while lysate (black) is unretained. Wash: sample infusion is terminated, and focusing buffer perfuses the trapped cells and removes the residual lysate from the microchannel. Release: Upon termination of the standing wave, cells are released and diverted to the male outlet. The flow ratio of inlets S1 and S2 determines if the sample will be directed to the male or female outlet.

monitored using a BioRad iQ5 instrument (Bio-Rad Laboratories, Hercules, CA).

To assess DNA purity, STR profiles were generated using conventional PCR amplification and separation techniques. All amplifications were performed using a Perkin-Elmer GeneAmp PCR System 2400 thermocycler (Wellesley, MA). STR amplifications were completed using an AmpFlSTR COfiler amplification kit according to the manufacturer's specifications. An ABI PRISM 310 Genetic Analyzer (Applied Biosystems) was utilized for separation of the STR amplfication products as described previously.[46] STR profiles were obtained from the male sperm cell and female buccal epithelial cell donors, and compared with those obtained from ADE separations in order to determine purity. Purity was calculated based on an average of peak area ratios of the male and female contributors for all STR loci.

RESULTS AND DISCUSSION

Acoustic Resonator Design. Initial ADE separations were performed with single node fluidic layers ($\lambda/2$) resulting in insufficient trapping capacity (data not shown) and thus not generating enough DNA for STR analysis. Subsequent studies were therefore performed with triple node trapping configuration ($3\lambda/2$), which provided three times the trapping volume as single node fluidic layers.

Fluidic Control in the ADE Device. To maintain a high trapping efficiency and ensure precise fluidic control of the separated fraction, hydrodynamic sample focusing combined with LFV was utilized, as detailed in Figure 3. In the absence of hydrodynamic focusing, cells may pass over the sides of the transducer without being affected by the acoustic field, resulting in inefficient acoustic trapping.[41] LFV, a passive valving technique originally described by Blankenstein et al.,[26,45] is ideal for the ADE system, as it allows for precise fluidic control, and circumvents the use of PDMS or other hydrophobic substrates commonly utilized in active valving, to which biological material may adhere. The design of the channel structure, shown in Figure 3, allows the flow of unretained female DNA to the female outlet and release of sperm cells to the male outlet by controlling the ratio of buffer flow from inlets S1 and S2.

Initially, colored dyes were used to characterize the LFV. The sample and focusing buffer were directed toward the female outlet using LFV and during the wash step, withdrawal from the sample inlet (1 μL/min) was used to prevent sample from entering the center channel, see the Supporting Information. The results showed that LFV was not adversely affected by the sample withdrawal, as the sample was not observed to enter the male outlet.

The reliability of the modified fluidic control method was further assessed using prepurified DNA with subsequent quantitation of the collected fractions. A sample consisting of prepurified hgDNA (10 ng/μL), used to simulate female epithelial cell lysate, was infused through SA and hydrodynamically focused (PBS, pH 7.4); the ultrasound was not initiated. LFV was used to direct the flow of the DNA toward the female outlet for 5 minutes as previously described. The channels were then washed for three minutes, during which time the sample flow was reversed to withdraw from SA (1 μL/min). Once the wash step was completed, the net flow was directed toward the male outlet using LFV. After each step the eluted fractions were collected from both outlets, and total genomic DNA was measured for each sample. The data (Table 1; $n = 3$) show that fractions collected from the female outlet after sample infusion contained the majority of the recovered DNA (97 ± 4.5%), whereas fractions collected from the male outlet after sample infusion (0.61 ± 1.1%) and wash step (0.55 ± 0.95%) contained only trace amounts. After the wash step, fractions collected from the female outlet contained 1.4 ± 2.5% of the recovered DNA, representing the residual sample that was present in the microchannel and outlet tubing after the sample withdrawal was implemented. DNA was not detected in fractions collected

Table 1. Total Percentage of hgDNA Recovered in Male and Female Fractions Obtained from ADE Device[a]

	male	female
trap	0.61 ± 1.1%	97 ± 4.5%
wash	0.55 ± 0.95%	1.4 ± 2.5%
release	no DNA detected	no DNA detected

[a] Sample: pre-purified hgDNA (10 ng/μL) prepared in PBS (pH 7.4) infused through device at 1 μL/min with 6 μL/min focusing buffer flow (5 min). LFV was used to direct unretained prepurified DNA to the female outlet. Fractions were collected after each step and quantified using conventional qPCR analysis methods.

Table 2. Concentration of hgDNA Recovered in Male and Female Fractions Obtained from ADE Device[a]

	male (ng/μL)	female (ng/μL)
trap	no DNA detected	0.95 ± 0.11
wash	no DNA detected	0.18 ± 0.15
release	no DNA detected	no DNA detected

[a] Sample: epithelial cell lysate prepared in conventional differential extraction (DE) buffer. LFV was used to direct unretained epithelial cell lysate to the female outlet. Fractions were collected after each step; DNA was purified and quantified using off-chip conventional analysis methods.

after the release step, verifying that the use of sample withdrawal during the wash and release steps eliminates contamination by sample input leakage. The optimal sample withdrawal flow rate was found to be 0.5 μL/min (data not shown).

Assessment of Acoustic Trapping. To ensure that the ADE microdevice was suitable for the separation of mock sexual assault samples, acoustic trapping conditions of sperm and epithelial cell lysate were separately assessed. Lysate trapped in the acoustic standing wave is a potential source of contamination, as it would be released upon termination of the ultrasound and directed toward the male outlet. Acoustic trapping of female epithelial cell lysate (prepared in conventional DE buffer) was performed as previously described for two minutes. The channels were washed with focusing buffer while maintaining flow toward the female outlet for two minutes. The net flow was then directed toward the male outlet, and the acoustic trapping was terminated; samples were collected for 3 min. After each step, the eluted fractions recovered from both outlets were purified off-chip, and total genomic DNA was measured for each sample. The results (Table 2) show that fractions collected from the female outlet after sample infusion contained the highest concentration of DNA (0.95 ± 0.11 ng/μL), and, therefore, the most lysate; DNA was not detected in fractions collected from the male outlet. Fractions collected from the female outlet during the wash step contained 0.18 ± 0.15 ng/μL of DNA, representative of the residual sample that was present in the microchannel and outlet tubing after the sample withdrawal was implemented. DNA was not detected in fractions collected from the female outlet, and more importantly, the male outlet, after the release step. The results confirm that epithelial cell lysate was not trapped in the absence of sperm cells, as predicted by acoustic force theory; however, these studies do not account for epithelial cell lysate inadvertently trapped in the presence of trapped sperm cells. In addition, the data further verify the effectiveness of LFV and incorporating sample withdrawal to eliminate sample bleed toward the male outlet.

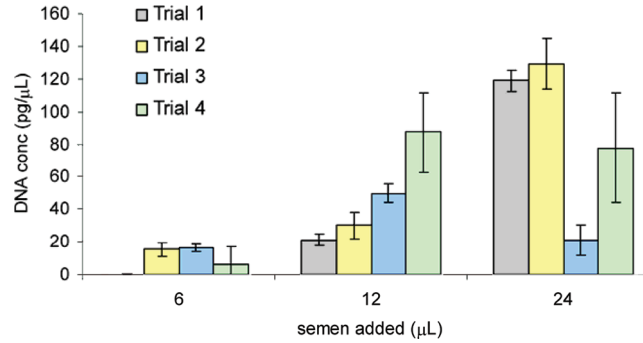

Figure 4. Average concentration of human genomic DNA recovered from the male outlet after acoustic trapping of sperm cells. Sample: diluted semen prepared in PBS (pH 7.4) infused through device at 1 μL/min with 6 μL/min focusing buffer flow (2 min) in PBS (pH 7.4). Sperm cells were directed toward the male outlet after acoustic trapping. Fractions were collected after release of the acoustic trap; DNA was purified and quantified using off-chip conventional analysis methods. Error bars represent $n = 2$.

The number of sperm cells captured and released from the acoustic trap, and, consequently, the amount of DNA recovered in the male fraction after ADE, is critical to obtaining a male STR profile. The amount of DNA in a sample is essential for most PCR-based assays, because a narrow DNA concentration is best for optimal PCR efficiency. For example, the Applied Biosystems' COfiler STR kit recommends the addition of 1−2.5 ng of template DNA (100−250 pg/μL for conventional amplifications) for optimal results.[46] Therefore, studies were performed to assess sperm cell trapping and release using the ADE device, without interference from epithelial cell lysate. Acoustic trapping of sperm cells (prepared from diluted semen) was performed for two minutes. The channels were washed with focusing buffer while maintaining flow toward the female outlet for three minutes. The net flow was then directed toward the male outlet, and the acoustic trapping was terminated; samples were collected for three minutes. The eluted cells were recovered from the male outlet and purified off-chip, and total genomic DNA was measured for each sample. The results (Figure 4) show that the concentration of DNA recovered from four consecutive runs for each prepared sample. Samples prepared with 6 μL of diluted semen (~960 sperm cells trapped) provided 13 ± 5.6 pg/μL of DNA for three of the four consecutive trials (with the exception of the first trial, where no DNA was detected), less than the recommended DNA concentration for STR analysis. For samples prepared with 12 μL of semen (~1920 cells trapped), an increase in DNA concentration was observed over the course of the four trials (21 ± 3.6 pg/μL for the first trial vs 87 ± 24 pg/μL for the fourth trial). Samples prepared with 24 μL of semen (~3840 cells trapped) provided inconsistent recoveries of DNA, ranging from 21 pg/μL (trial 3) to 130 pg/μL (trial 2). Upon termination of the ultrasound, the cells were consistently released cleanly from the trapping site (i.e., cells were not adsorbed to the transducer or glass surface) toward the male outlet and were observed entering the outlet reservoir. It was, therefore, hypothesized that the inconsistent recoveries of DNA obtained from samples prepared with higher volumes of semen were a result of poor release of larger clusters of cells from the outlet tubing.

In order to improve the release of biological material from outlets, focusing and LFV buffers were changed from PBS to a

conventional DE buffer, the same buffer used to prepare female epithelial cell lysate (devoid of the proteolytic lysis agent). The use of DE buffer for focusing and LFV allowed for a longer release time, as conventional DE buffer is added to post-ADE samples as part of the subsequent off-chip extraction protocol. The results (data not shown, $n = 4$, consecutive trials) demonstrated that samples prepared in DE buffer consistently provided full STR profiles, indicating that the use of DE buffer was effective in the removal of cells from the outlet tubing.

Particle Distribution in the Trapping Area. The lateral distribution of trapped particles is governed by the local pressure distribution over the transducer, as shown by Lilliehorn et al.[40] By changing the frequency from 11.6 to 12.1 MHz, a clear change in the lateral distribution of trapped cellular material was observed. Cells trapped at a lower frequency (11.6 MHz, used for all previously detailed experiments) demonstrated a tendency to aggregate and move with the flow as a cluster during the release. An increase in operating frequency (12.1 MHz) resulted in smaller clusters of trapped cells, which might be less likely to clog the outlet tubing upon release compared to a single, larger cluster of cells obtained with the lower frequency.

Short Tandem Repeat Analysis of ADE Fractions. The higher operating frequency and its resultant trapping pattern were applied to ADE separations of mock sexual assault samples. The STR profile of the mixed biological sample prior to ADE (Figure 5A) was obtained using conventional methods; through comparison of this profile with those of the sperm cell and female epithelial cell donors, the sample was determined to be 26 ± 2.0% male. Acoustic trapping of sperm cells added to female epithelial cell lysate was performed; the trapping time was adjusted such that approximately 2000 cells were trapped. The sample flow was then reversed to withdraw from SA, and the channels were washed with focusing buffer for 2.5 min. After the net flow was switched toward the male outlet the acoustic trapping was terminated, and samples were collected for five minutes. The eluted cells were recovered from the male outlet and purified off-chip, and conventional amplification and separation of STR loci were performed. An example of an STR profile obtained from the male fraction is shown in Figure 5B; comparison of the profile with those of the male and female donors indicate that the sample is 92 ± 7.9% male, a nearly 4-fold enhancement in male purity in comparison with the original sample. All seven core STR loci, devoid of alleles contributed from the female, were detected, allowing for interpretation of the male profile from the original mixture. In addition, the STR profile obtained from the female fraction is shown in Figure 5C; comparison of the profile with those of the male and female donors indicates that the sample is 5.7 ± 4.7% male. The results suggest that the particle distribution in the acoustic trap may play an important role in the effective separation of sperm and female epithelial cell lysate using the ADE device.

CONCLUSIONS

Forensic differential extraction of simulated sexual assault samples have been successfully demonstrated using the ADE method. By selectively trapping sperm cells from a sample containing female epithelial cell lysate and precise control of the fluidics using LFV, an enriched male fraction was isolated. The purity of the male and female fractions was quantified using forensic STR profiling, the forensic "gold-standard" of purity,

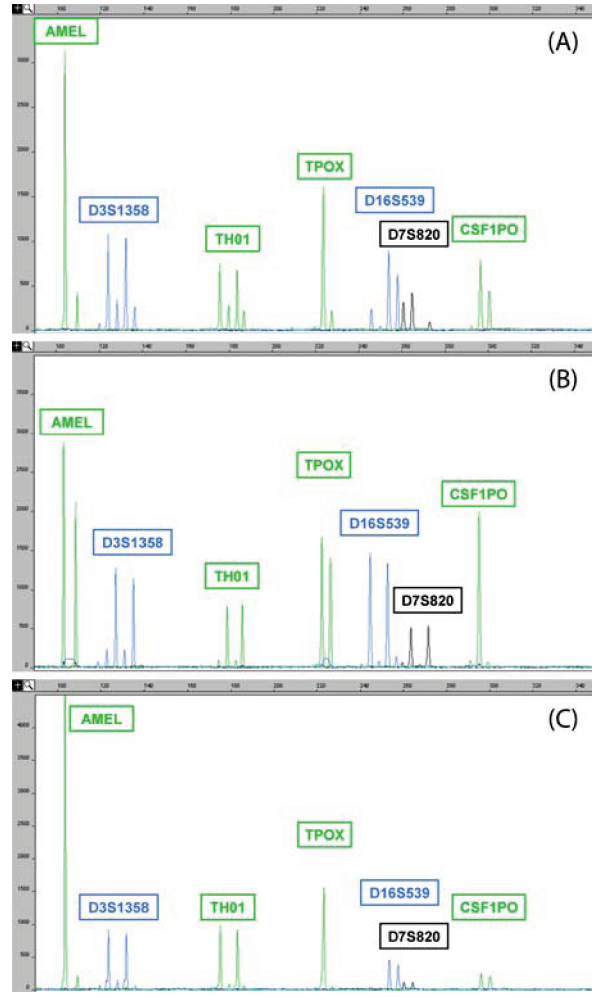

Figure 5. Short tandem repeat (STR) analysis of the sorted cell products from the ADE microdevice. Six of the common STR loci, and the sex-determining marker Amelogenin, were amplified using a COfiler kit. (A) STR profile of sample prior to ADE (26 ± 2.0% male). Trapping was performed using a frequency that resulted in a laterally dispersed partical distribution. Fluidic control was performed using DE buffer to facilitate release of cells from outlet. The resulting STR profiles (B, C) illustrate the purity of the separation product. (B) STR profile of sample collected from male outlet after ADE (92 ± 7.9% male; 7/7 loci). (C) STR profile of sample collected from female outlet after ADE (5.7 ± 4.7% male; 7/7 loci).

and shown to well match the purities achieved with a differential extraction method commonly utilized in some forensic laboratories. The presented microfluidic approach holds promise to be developed into a fully automated system. However, to implement ADE into forensic laboratories, additional investigations using vaginal swabs and postcoital samples will be required prior to application of this method to sexual assault evidence. Issues with acoustic trapping are not anticipated with these types of samples; however, it is imperative to determine this empirically. Developments are currently also underway to produce disposable ADE devices, which will consist of a sealed channel structure and an external transducer, a necessity for a forensic implementation of ADE.

The ADE method can theoretically handle sample volumes of evidentiary sample from microliter to milliliter ranges. In order to increase the capacity for a large range of sample sizes, the flow rates and channel architecture will be optimized in

the next generation of ADE devices. The method has been developed to facilitate direct integration to downstream processing steps, where the side arm inlets (S1 and S2) can be utilized for addition of reagents for, e.g., solid-phase extraction of DNA. Although integration with subsequent sample processing steps (like DNA purification) is not required for use in a forensic laboratory, the true potential of the method will be fully realized upon such integration.

DISCLAIMER

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SUPPORTING INFORMATION AVAILABLE

Figure S-1. This material is available free of charge via the Internet at http://pubs.acs.org.

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