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Microchip-Based Cell Lysis and DNA Extraction from Sperm Cells for Application to Forensic Analysis

ABSTRACT: The current backlog of casework is among the most significant challenges facing crime laboratories at this time. While the development of next-generation microchip-based technology for expedited forensic casework analysis offers one solution to this problem, this will require the adaptation of manual, large-volume, benchtop chemistry to small volume microfluidic devices. Analysis of evidentiary materials from rape kits where semen or sperm cells are commonly found represents a unique set of challenges for on-chip cell lysis and DNA extraction that must be addressed for successful application. The work presented here details the development of a microdevice capable of DNA extraction directly from sperm cells for application to the analysis of sexual assault evidence. A variety of chemical lysing agents are assessed for inclusion in the extraction protocol and a method for DNA purification from sperm cells is described. Suitability of the extracted DNA for short tandem repeat (STR) analysis is assessed and genetic profiles shown. Finally, on-chip cell lysis methods are evaluated, with results from fluorescence visualization of cell rupture and DNA extraction from an integrated cell lysis and purification with subsequent STR amplification presented. A method for on-chip cell lysis and DNA purification is described, with considerations toward inclusion in an integrated microdevice capable of both differential cell sorting and DNA extraction. The results of this work demonstrate the feasibility of incorporating microchip-based cell lysis and DNA extraction into forensic casework analysis.

KEYWORDS: forensic science, DNA, DNA extraction, sperm cells, microchip

The considerable backlog of casework awaiting DNA analysis in crime laboratories today exists in part because of deficiencies in manpower and technological advances. Estimates suggest this backlog was $\sim$ 542,700 criminal cases in the United States alone in 2004 (1), including 52,000 homicides and 169,000 rape cases. Forensic science laboratory directors often fault the time and cost requirements for these analyses as the bottleneck to evidence processing, particularly in the analysis of evidentiary material from rape kits (2). The analysis times required for perpetrator and victim DNA analysis for rape kit analysis by conventional methods (hours to days of laboratory time spent on a single case) have begun to be reduced with advances in microchip technology. Microdevice technology has the potential to expedite molecular interrogation and supplant many of the conventional techniques currently used (3). In addition, this technology offers a reduction in sample volume that may allow for analysis of casework previously not amenable to analysis using current techniques. These technological advances can provide a vehicle for a rapid reduction of the backlog that exists in large-volume DNA analysis labs, allowing for expedited and improved casework handling without a concomitant increase in funding and manpower.

Advances in analytical microchip technology have created an intense interest in microchips as the next generation platform for forensic DNA analysis. A fully integrated microchip capable of performing the steps normally carried out on the benchtop would not only reduce the time required to perform these tasks, but would also eliminate user intervention and handling, while preserving more sample for future analysis (4). Electrophoretic separations are now readily accomplished in microdevices for both sequencing and size-based genetic analysis (5–15) and this has been applied to short tandem repeat (STR) analysis with very encouraging results (16). The goal of a micro-total analysis system (μTAS), capable of comprehensive genetic analysis with "sample in-answer out capabilities" in one self-contained device, however, requires the miniaturization and optimization of upstream sample preparatory steps. Recently, a method has been determined for sorting whole vaginal epithelial cells from the sperm cell fraction of a vaginal swab reducing the time required for differential extraction (17), but the integration of sample preparatory steps such as DNA extraction and polymerase chain reaction (PCR) will vastly improve overall analysis speed and efficiency of sample processing.

DNA adsorption to silica surfaces in the presence of a chaotropic agent, followed by the removal of protein contaminants, is a well-characterized method for DNA purification, exploited by many commercial extraction kits. In addition, this approach has been translated into methods capable of reduced volume, high-efficiency extractions more suitable to microchip applications. In 2000, Tian et al. (18) demonstrated extraction of PCR-amplifiable DNA from white blood cells in a 500 nL capillary-based chamber filled with silica resin with an extraction efficiency of 70%. Purification using a microchip-based system was first demonstrated by Christel et al. (19), who utilized a channel with microfabricated pillars for binding DNA for chromatographic purification—however, one that had surface area limitations and complex device fabrication methodology. In perhaps the most direct translation of macro-scale, commercial, silica-based extractions to the micro-

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chip format, Breadmore et al. (20) packed silica beads into microchannels, with the added stability of a sol–gel matrix (sols are liquid colloidal suspensions that can be flowed into the microdevices and gelled into place to form a solid support) to immobilize the beads for run-to-run reproducibility. With this approach, extraction of DNA from complex biological samples such as bacterial colonies and crude whole blood was demonstrated, with the resultant purified DNA proven suitable for direct PCR amplification. It was this approach, with modifications for sample composition that was pursued in the current research.

The focus of the research presented in this report is the microdevice-based extraction of DNA from sperm cells and its potential integration with microchip-based cell sorting. First, modifications to the DNA extraction device that allow for easier, more robust fabrication are detailed. The development of a dual-function buffer capable of the complete lysis of sperm cells but also amenable to the effective loading of the lysed cellular material for extraction of DNA is described. A microdevice-based extraction approach is detailed, yielding purified DNA from crude sample in less than 10 μL volume, and in a form suitable for STR analysis. Finally, a method for a completely microchip-based extraction is described (intact cell loading, complete on-chip cell lysis, and DNA purification), and the dual lysis/loading buffer is evaluated for its effectiveness for rapid, decreased volume, on-chip, sperm cell lysis for potential integration with cell sorting technology.

Methods

Microchip Preparation

Borofloat glass (Telic Company, Valencia, CA) bottom plates for the microdevices were fabricated using standard photolithographic techniques, with channel dimensions of 1.7 cm total length, 1.5 cm to the weir, 200 μm deep, and 420 μm wide (Fig. 1*B*). Access holes were drilled at both ends of the channel using 1.1-mm-diameter diamond-tip drill bit (Crystallite Corp., Lewis Center, OH). A borofloat glass cover plate was cut to fit the device and the cover plate and etched plate thermally bonded. The distance from the top of the weir to the cover plate was approximately 5–20 μm.

Silica beads, 5–30 μm, were suspended in 1 M HCl and packed into the channel against the weir using vacuum (Fig. 1*C*). A solution of sol–gel precursors was prepared by hydrolyzing a 27% (v/v) solution of tetraethoxysilane (TEOS) (Sigma-Aldrich, St. Louis, MO) by addition of 0.1% (v/v) HNO_3 (Sigma-Aldrich). The resultant solution was stirred at 200 rpm (2.69 g), 60°C for 10 min, followed by 80°C for 60 min and the channel packed with beads, then vacuum filled with the hydrolyzed TEOS. Following filling, the packed devices were heated to 300°C at a rate of 8°C/min and held there for 3 h, then allowed to cool.

Apparatus

The homebuilt microchip-solid phase extraction (SPE) apparatus consisted of a Harvard Apparatus model 22 syringe pump (Harvard Apparatus, Holliston, MA) with a 250 μL Hamilton gastight syringe (Hamilton, Las Vegas, NV). The syringe was connected to the inlet reservoir of the microdevice using PEEK™ tubing and mini-tight fittings (Upchurch Scientific, Oak Harbor, WA) with a noncommercial plexiglass interface (Fig. 1*A*).

Lysis Buffer Determination

First, a solution of washed sperm cells was prepared as follows: 1 mL of 1 × TE (100 mM Tris, (Sigma-Aldrich), 10 mM EDTA (Amresco, Solon, OH), pH 7.9, was added to 1 mL of semen, vortexed at high speed (setting 8 on a Vortex Genie-2; Scientific Industries Inc., Bohemia, NY), and centrifuged for 6 min at 3000 rpm (605 g). Following centrifugation, the supernatant was removed and discarded and the entire process was repeated twice more. After removal of the supernatant subsequent to the third centrifugation, the cells were resuspended in 250 μL of 1 × TE.

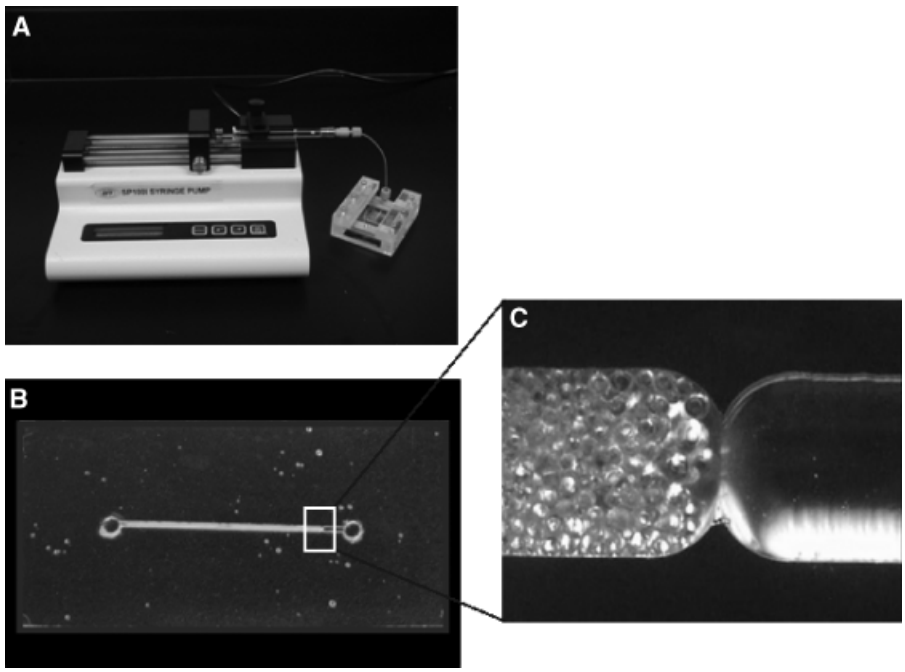

FIG. 1—*The microdevice setup used for extraction of DNA from sperm cells. This included a syringe pump, connected to the microdevice with narrow-bore tubing, and held in place by a homemade plexiglass mount (A). Silica beads 5–30 μm in diameter were packed against a weir (B,C) in the channel (200 μm deep, 420 μm wide) and adhered into place using sol–gel.*

TABLE 1—*Lysis buffer composition.*

Sample Identification	Volume Sperm Cell Solution (μL)	Volume 50 mM DTT (μL)	Volume 50 mM TCEP (μL)	Volume 10% Triton X-100 (μL)	Volume Water (μL)	Volume 8 M GuHCl (μL)
Sample A	10	40	0	0	50	300
Sample B	10	0	40	0	50	300
Sample C	10	0	0	40	50	300
Sample D	10	0	0	0	90	300
Sample E	10	40	0	50	0	300
Sample F	10	0	40	50	0	300

DDT, dithiothreitol; TCEP, tris(2-carboxyethyl)-phosphine.

The resultant cell suspension was used for all lysis studies. Evaluation of lysis buffers was accomplished by aliquoting 10 μL of sperm cell solution into each sample tube, along with 40 μL of one of the following: a reducing agent, 50 mM dithiothreitol (DTT) (Fisher, Fairlawn, NJ) or 50 mM tris(2-carboxyethyl)-phosphine (TCEP) (Pierce Biotechnology Inc., Rockford, IL), detergent, 10% Triton X-100 (Sigma), or water (control) as detailed in Table 1. Lysis solutions containing reducing agents were prepared fresh each day immediately before use.

The sperm cell solution was vortexed 10–15 s between each aliquot to ensure an equal number of cells per tube. After the addition of the detergent, reducing agent, or water, to the sperm cells, all samples were vortexed for 1 min at high speed. For those samples that required both a reducing agent and 10% Triton X-100, the detergent was added and all samples vortexed for 30 s to ensure equal amount of time spent vortexing. Finally, 300 μL of 8 M GuHCl (prepared in 1 × TE, pH 7.9 and 100 mM 2-(4-morpholino)-ethane sulfonic acid (MES), pH 4, final pH of 6.1) was added to each sample and the samples were vortexed for a final 15 s to ensure adequate mixing. Final concentrations for samples with reducing agent were 40 mM DTT and 6 M GuHCl, as per commonly utilized forensic protocols. All samples were left at room temperature during lysis and used immediately after preparation, without an extended incubation time. Cells were then counted using a hemocytometer, according to manufacturer's instructions.

Microdevice SPE Procedure

Newly filled microdevices were conditioned with 6 M GuHCl (prepared in 1 × TE, pH 7.9 and 100 mM MES, pH 4, final pH of 6.1), for 30–45 min at a flow rate of 250 μL/h, to ensure removal of unreacted species and to prepare the silica bed for extraction. Before each subsequent extraction, the bed was reconditioned with 6 M GuHCl for 5 min. The basic extraction protocol consisted of three pressure-driven wash steps, each accomplished at a flow rate of 250 μL/h. First, the desired amount of lysed sample (20 μL for elution profile, 40 μL for extraction followed by STR analysis) in 6 M GuHCl, 40 mM DTT was loaded onto the bed. The 20–25 μL of the wash solution (2-propanol/water, 80/20 (v/v)) was flowed through the device to remove proteins and other potential PCR inhibitors. Finally, the purified DNA was eluted in either water or 1 × TE buffer and collected for subsequent PCR or fluorescence analysis in thin walled, PCR reaction tubes. The bed was flushed with water for 20–30 min and ethanol for 15 min at 250 μL/h between extractions to prevent carryover (20).

For the comparison with conventional extraction methods, a 10 μL aliquot of washed sperm cell solution (20,000 cells/μL) was lysed in 390 μL of 6 M GuHCl containing 40 mM DTT. Forty microliters of the lysed sample was loaded onto the microdevice. Following the protein wash with 80% isopropanol, the first 5 μL aliquot of water eluted from the microdevice was split into two 2.5 μL fractions for amplification. DNA was amplified using the COfiler™ and Profiler Plus™ (Applied Biosystems, Foster City, CA) amplification kit according to manufacturer's instructions. As the control for conventional analysis DNA was extracted from 10 μL of washed sperm cell solution from the same donor using the Qiagen QIAamp® DNA Mini kit (Qiagen, Valencia, CA), according to manufacturer's instructions, with 40 mM DTT added to the lysis buffer for adequate sperm cell lysis. A 1 μL aliquot of the commercial kit extracted DNA (elution volume 200 μL) was used in the subsequent PCR amplification using the COfiler™ and Profiler Plus™ amplification kit according to manufacturer's instructions.

For DNA quantification, a fluorescence assay was carried out using PicoGreen® (Molecular Probes, Eugene, OR). Five microliters of semen was lysed in 395 μL of 6 M guanidine HCl containing 40 mM DTT. Twenty microliters of the lysed sample (17 ng of DNA) was loaded onto the extraction bed, followed by a protein wash using 25 μL of 80% isopropanol. DNA was eluted with water. Aliquots (1–5 μL) from the load, wash, and elution steps were collected during the extraction. Fluor was added according to manufacturer's instructions, with separate calibration curves for each solution to account for solvent effects on fluorescence, and analyzed on a Perkin Elmer Spectrometer, PE LS50 B (Perkin Elmer, Shelton, CT).

Fluorescent Labeling and Sperm Lysis

Sperm cells were labeled according to manufacturer's instructions with DEAD Red™ nucleic acid stain (Molecular Probes). Cells were loaded by syringe flow using an SP120CE-300 syringe pump (WPI, Sarasota, FL) at 250 μL/h for 2.5 min. The previously determined lysis buffer (40 mM DTT, 6 M GuHCl) was then flowed over the bed at the same flow rate for 15 min. Cells were visualized during loading and lysis using an Olympus CKX41 Reflected Fluorescence Microscope System (Olympus, Melville, NY) with an attached color CCD camera (Hitachi, Torrance, CA) and DVD (Panasonic, Secaucus, NJ) setup.

On-Chip Cell Lysis for DNA Extraction

Semen (approximately 1 μL) was added to the inlet reservoir of the microdevice (only partially packed with the sol–gel/bead mixture) and pulled by withdrawal from the outlet of the device, using an SP 260P syringe pump for 10 min. The residual semen (less than 1 μL) was then removed from the reservoir and the lysis buffer (40 mM DTT, 6 M GuHCl) was flowed over the packed sperm cells and silica bed for 15 min from inlet to outlet as before. The rest of the extraction was performed according to the method

described above (protein wash of 25 μL isopropanol, followed by elution with water).

Sample Amplification and Preparation

Sample amplification was carried out using AmpF*l*STR®COfiler™ and Profiler Plus™ PCR amplification kits (Applied Biosystems) and a Perkin Elmer GeneAmp PCR System 2400 thermocycler according to manufacturer's instructions. The PCR reaction volume for all amplifications was 25 μL and all reactions were carried out in 0.2 mL PCR reaction tubes (Fisher, Fairlawn, NJ). Cycling conditions were as follows: 95°C for 11 min once; 94°C for 60 s, 59°C for 60 s, 72°C for 60 s for 28 cycles. Samples were then held at 60°C for 45 min for extended annealing, followed by a 25°C hold until removed from the thermocycler.

Samples were prepared for analysis by capillary electrophoresis by combining 25 μL of Hi-Di formamide containing 1 μL of the GeneScan-500 ROX size standard (Applied Biosystems), with 1.5 μL of the amplified sample and mixed by pipetting up and down. Samples were then heat denatured at 95°C for 3 min in a water bath, followed by snap-cooling on ice for 3 min.

Capillary Electrophoresis

All capillary electrophoresis was accomplished using an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems) with fused-silica capillaries (Polymicro Technologies, Phoenix, AZ), 50 μm inner diameter (ID), 47 cm in length. Samples were separated using the ABI POP-4 polymer (Applied Biosystems) in uncoated capillaries according to the manufacturer's instructions. Samples were analyzed using the GS STR POP-4F module, 5 s injection at 15 kV, separation at 15 kV, 60°C, 24 min. After the ABI color matrix was applied to the data, allele sizes were estimated using the local Southern method from GeneScan® 3.7 analysis software (Applied Biosystems).

Results and Discussion

The extraction and purification of DNA from crude samples such as semen is an important step in the processing of rape kits for forensic analysis. Although silica-based methods for extraction of DNA from other cell types exist and are commercially available, these methods must be amended when transferred to the microdevice, particularly when dealing with human sperm cells. The network of disulfide bonds that compose the sperm nuclear membrane make commonly utilized cell lysis buffers ineffective (21) and require the addition of a disulfide bond reducing agent for sufficient cell lysis. As a result, the guanidine-based lysis buffer described by Breadmore et al. (20) utilized with this particular silica/sol–gel extraction method, although suitable for other microchip applications, is inadequate for sperm cell lysis. In addition, any changes made to the lysis and loading buffer must not negatively impact the binding and elution of the DNA from the sol–gel/silica bed during extraction or its suitability for subsequent PCR amplification. Consequently, a thorough evaluation of the impact of lysis buffer composition on DNA recovery and PCR amplification must be performed. This was carried out with a view to an integrated device capable of executing all of the processes required for full genetic analysis (cell sorting, on-chip lysis and DNA loading, PCR amplification, and DNA separation and detection). Any lysis buffer developed must be capable of both complete on-chip cell lysis and DNA loading in reduced volumes, with sufficient rapidity to affect a swift extraction. With these considerations in mind, the adaptation of solid-phase extraction of DNA from sperm cells in a microchip-based format was undertaken.

Microdevice Extraction Setup

In order to improve chip-to-chip reproducibility of the extraction bed and ease of device fabrication, some modifications to the device described by Breadmore et al. (20) were made. The original device was developed using the weir-type approach formerly utilized by Oleschuk et al. (22), with beads packed against a temporary weir constructed of a hydrolyzed sol–gel silica bead slurry and glued into place with the same hydrolyzed TEOS-based sol solution. For the devices used in the present studies, however, a glass weir was etched into the channel such that the gap between the top of the weir and the glass cover plate was approximately 5–20 μm. As a result, beads larger than the gap are packed against it, while solution flow through the bed is maintained. The packed beads were subsequently immobilized using the same TEOS-based sol solution used by Breadmore et al. (20) (see Fig. 1*B* and *C*). Note that the channel is evenly packed, without air pockets or cracking in the bed and the sol–gel matrix does not occlude the channel beyond the weir (Fig. 1*C*). The etched weir is more robust than the temporary frit construct of the previous work (20), allowing for more reproducible results with a less cumbersome filling protocol. The resultant filled and baked chip was placed in the plexiglass holder, where a syringe containing the necessary solutions could be connected to the microdevice via PEEK™ tubing and mini-tight fittings (Fig. 1*A*). With these procedural modifications, the microdevice filling system was rendered more facile, while the integrity of the extraction process was maintained.

Lysis Buffer Evaluation

Before undertaking any evaluation of the proposed lysis buffers, it was essential to ensure that the number of sperm cells used in each lysis experiment was roughly the same—consequently, the method used for sample mixing and dispensing was evaluated. Samples were subjected to either manual agitation/inversion or vortexing and an aliquot subsequently removed for cell counting. It was determined that, in order to achieve complete mixing of sperm cells in solution and, thus, an equivalent number of sperm cells per sample, it was imperative that the sperm cell solution or semen be vortexed at high speed (see "Methods" for details) for 10–15 s between each aliquoting. Manual agitation with inversion of the tube showed serious deviations in the number of sperm cells per aliquot of a diluted semen sample (average = 111 ± 53 cells/μL; $n = 5$), while with adequate vortexing between sampling, deviations in sperm cell number were negligible (average = 90 ± 3 cells/μL; $n = 5$). Thus, it was determined with certainty that any difference in cell counts between lysis buffers was a result of the lysis method and not a deviation caused by an improperly mixed sample, ensuring reliable and accurate assessment of the different techniques.

In order to effectively and consistently extract DNA from sperm cells, it is imperative that complete lysis of the cells is achieved. This is especially crucial in casework where low cell counts are not uncommon and, as such, recovery of DNA from all cells present is essential. Incomplete lysis or partial disruption of cells could lead to samples with DNA concentrations too low to recover and amplify successfully. It is well accepted that common lysing agents that are effective for lysing other cell types may not

TABLE 2—*Evaluation of lysis buffers.*

Sample Identification	Average Number of Cells	Standard Deviation (±)	Standard Deviation (%)
Sample A	0	CL	CL
Sample B	0	CL	CL
Sample C	82	9.9	12.1
Sample D	73.5	6.8	9.3
Sample E	0	CL	CL
Sample F	0	CL	CL

$N = 3$ for samples A, B, E, and F, while n = minimum of 5 for samples C and D. In three experiments, samples A, B, E, and F did not show any sperm cells present. As a result, these samples were not prepared in subsequent analyses, while samples C and D were repeated additional times to ensure reproducibility.

CL, comprehensive lysis.

work efficiently with the morphology and composition of the sperm cell head. As a result, the standard lysis buffer developed by Breadmore et al. (20) (a high-salt, chaotropic buffer), determined to be the most effective lysis/loading buffer for previously published microdevice extractions, was critically evaluated before its employment for lysis of this particular cell type. Using this buffer (6–8 M GuHCl, 1% Triton X-100) as the baseline composition, the effects on cell count were elucidated upon addition of reducing agents and in the presence and absence of the detergent Triton X-100. The results summarized in Table 2, indicate that any lysis buffer containing either of the reducing agents (DTT or TCEP) showed comprehensive lysis (CL) of the sperm cells, with no intact sperm heads visually detected. The presence or absence of Triton X-100 in the samples did not seem to have a positive or negative effect on the lysis with this cell concentration, although with other cell concentrations there may or may not be a discernable effect. In the samples where no reducing agent was present, incomplete lysis was achieved, again, with the detergent having little effect on the number of cells present under these conditions. These results, as would be expected, suggest that the high concentration of chaotropic agent in combination with the disulfide bond reducer is sufficient for complete cell lysis. Although concentrations of both reducing agents were selected based on those used in typical differential extraction protocols (final concentration $\sim$ 40 mM) (23), ongoing research suggests that TCEP, a stronger disulfide bond reducing agent (24), may accomplish lysis at lower concentrations and more rapidly than DTT. This could make a TCEP-based lysis buffer more effective for microchip-based applications in which fast and complete lysis during flow-through is required. However, because DTT (at the concentration described above) is a well accepted and validated additive in DNA extraction protocols, a lysis buffer consisting of a final concentration of 40 mM DTT, 6 M GuHCl (lysis buffer A) was the buffer of choice for the lysis of sperm cells for DNA extraction.

DNA Extraction

After defining the loading buffer's effectiveness for cell lysis, it was necessary to ensure that the addition of DTT to the lysis buffer did not negatively impact DNA extraction. To that end, three basal requirements were needed to establish that extraction with the new lysis/loading buffer was effective—these included: (1) successful DNA adsorption to the silica in the new loading buffer, (2) absolute DNA retention on the silica during the protein wash step, and (3) effective DNA elution from the silica bed during desorption. In order to ascertain whether these objectives were

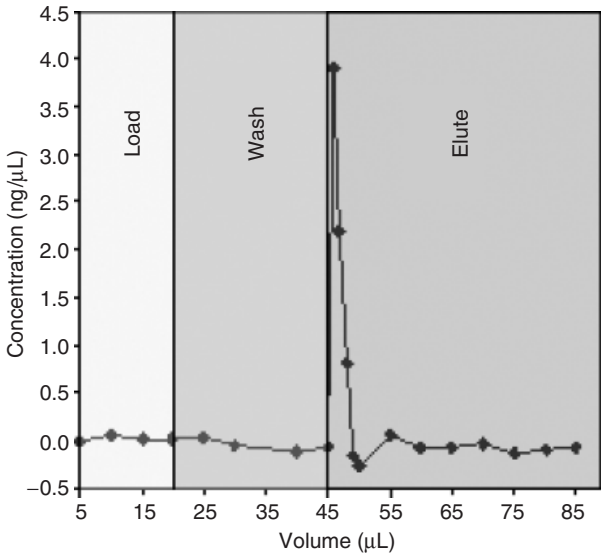

FIG. 2—*An elution profile for micro-extraction of DNA from sperm cells. This was accomplished using the PicoGreen® fluorescence quantitation assay (see "Methods" for details). Fractions (1–5 µL in volume) were manually collected during each step (DNA loading in chaotropic salt, protein purification with isopropanol and DNA elution with water), fluor added, and fluorescence spectrometry carried out. Conditions: 5 µL of semen were lysed in 395 µL of 6 M guanidine HCl containing 40 mM dithiothreitol (DTT). Twenty microliters of the lysed sample (17 ng of DNA) were loaded onto the extraction bed, followed by a protein wash using 25 µL of 80% isopropanol. DNA was eluted with water.*

met, a DNA elution profile was obtained and is depicted in Fig. 2. For this extraction, 5 µL of semen was lysed in 395 µL of 6 M GuHCl containing 40 mM DTT and 20 µL (17 ng) of the resultant sample loaded onto the extraction bed at a flow rate of 250 µL/h. Following a wash with 80% isopropanol (25 µL) to remove any contaminating proteins (18,20), the DNA was eluted in deionized water. Aliquots of the eluate were collected at the outlet of the device during each step of the extraction (DNA load, protein wash, and purified DNA elution) and assayed for DNA concentration using a fluorescence assay for DNA quantification (25). In order to account for differences in signal intensity from the PicoGreen as a result of the chemicals used in the loading and protein wash steps, separate calibration curves with the same chemical additives were made for each step of the extraction. As depicted in Fig. 2, the majority of the DNA is desorbed in the first 5 µL of the elution phase, while each of the other steps show negligible concentrations of DNA detected (the assay employed has demonstrated sensitivity to as little as 50 pg of DNA per the standard 2 mL assay volume). This indicates no significant loss of DNA during the load and wash steps, and that the majority of the DNA is eluted in the first 5 µL, with an approximately 50% extraction efficiency for the overall extraction. These results are consistent with previously reported data (20), with DNA reproducibly eluting in the first 5–10 µL of the elution phase, suggesting that modifications to the lysis/loading buffer did not interfere with binding of the DNA to the sol–gel/bead bed or impact DNA recovery negatively.

DNA Purification for STR Amplification

After defining a lysis/loading buffer that did not affect the basic extraction and elution of DNA from sperm cells, the next step involved evaluating the suitability of the DNA for downstream forensic STR amplification. DNA from a crude sperm cell sample

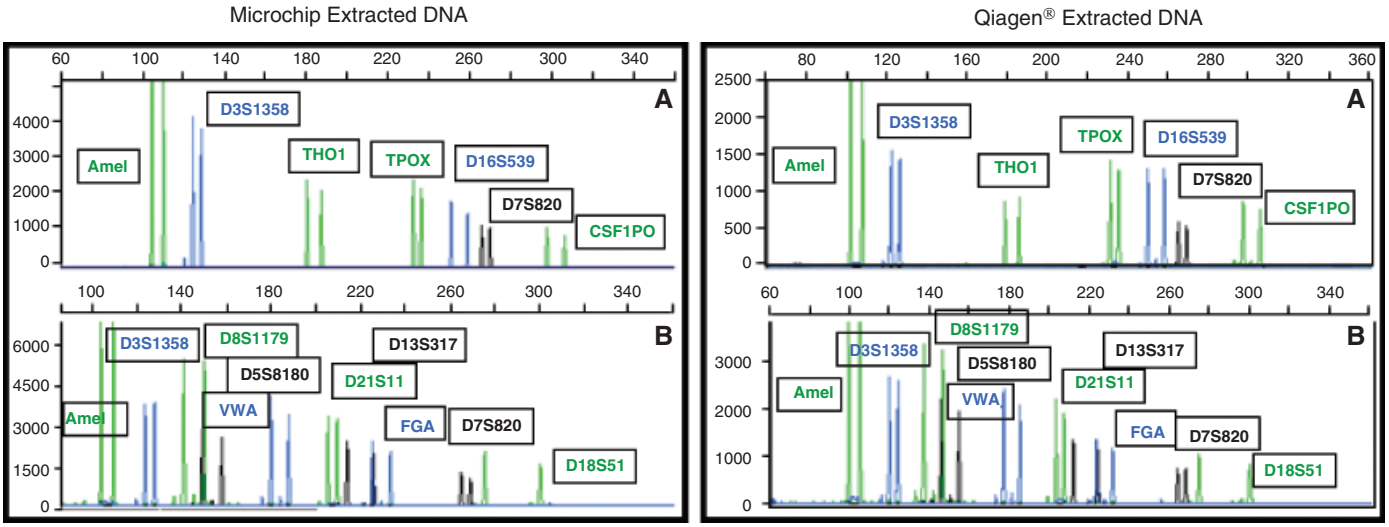

FIG. 3—*Comparison of electropherograms from microchip extracted DNA (left) and DNA extracted using a commercial kit with dithiothreitol (DTT) added to the lysis buffer (right). Full forensic genetic profiles containing all 13 CODIS loci were generated using COfiler™ (A) and Profiler Plus™ (B) amplification kits for both methods of extraction using the same sample. Conditions: a 10 μL aliquot of washed sperm cell solution (20,000 cells/μL) was extracted with both methods. For the microchip extraction, 10 μL of the cell solution was lysed in 390 μL of 6 M GuHCl containing 40 mM DTT 40 μL of lysed sample was loaded onto the microdevice. Forty millimolar DTT was added to the Qiagen lysis buffer and 10 μL of the cell solution extracted according to manufacturer's instructions. A 1 μL aliquot of the commercial kit extracted DNA (elution volume 200 μL) was used in subsequent polymerase chain reaction analysis, while the first 5 μL aliquot eluted from the microdevice was split into two 2.5 μL fractions for those amplifications. DNA was amplified using the COfiler™ and Profiler Plus™ amplification kit according to manufacturer's instructions.*

was extracted using the devised lysis/loading buffer and the protocol described in the "DNA Extraction." Following purification of the DNA (referring to eluted DNA where 80–90% of contaminating proteins have been removed (18)), the eluate was directly amplified by PCR for all 13 core COmbined DNA Index System (CODIS) loci in a conventional thermocycler using COfiler™ and Profiler Plus™ PCR amplification kits, then separated using capillary electrophoresis. The resultant electropherograms allow for comparison of the amplified products from conventionally extracted DNA with that extracted using the microdevice—these data are given in Fig. 3. It is clear that the STR profile obtained from conventionally extracted DNA is very similar to that from the microchip-extracted material. The results demonstrate that the microchip-extracted DNA is devoid of PCR inhibitors, at least to the same extent as the DNA obtained from conventional extraction and, therefore, suitable for forensic STR analysis. One feature present in both the microdevice and conventionally extracted DNA is the presence of a noticeable slope in the peak heights. This can be readily explained by the manner in which the extractions and subsequent amplifications were performed. With the microdevice extraction, the aliquots taken were removed from the outlet of the device and used directly in the PCR amplification, not quantified before the reaction. As a result, the amount of DNA present was likely not the manufacturer's recommended 1 ng. In addition, in order to mimic the microdevice extraction as much as possible, aliquots of the Qiagen eluent were used directly in the amplification as well. Ongoing research in the lab seeks to address this issue by the integration of an online sample quantification step, before amplification. The adapted microchip extraction procedure is appropriate for producing highly purified DNA in small volumes for subsequent STR amplification.

Microscale Cell Lysis and DNA Extraction

As previously described, the goals of this research are a subtext to the overarching goal of creating a fully integrated microdevice for genetic analysis. In order to achieve this objective, methods developed for extraction must seamlessly integrate with methods being developed for both upstream (cell sorting) and downstream (PCR) processes. In a recently published paper, Horsman et al. (17) describe a method for a microdevice-based differential separation of epithelial cells and sperm cells from a mixed sample. By applying a withdrawal-driven flow to a mixed sample, the sperm cells are pulled into the channel and purified intact from the larger, denser epithelial cells. The resultant samples, following separation, are two purified cell fractions (sperm and epithelial), ready for downstream DNA extraction. Consequently, any DNA extraction protocol developed for integration into the μTAS must work synergistically with the sample volumes, sample composition, and separation methodologies of this established cell sorting technique. Accordingly, after successful demonstration of off-chip lysis in large volumes, followed by microscale DNA purification, effort was directed toward translation of cell loading and lysis from the macroscale tube to the microchip channel, with an eye toward future integration with cell sorting.

In order to achieve the eventual integration of these two techniques, demonstration of proof-of-principle for complete on-chip lysis of sperm cells was necessary. Accordingly, a few prerequisites must be met in order to ensure a seamless coupling of the two techniques. First, it must be demonstrated that the sperm cells will pack against the silica bed, not pushed into and sifted through the bead network during the cell separation or DNA loading phase. Second, the dual lysis/loading buffer must effectively destroy intact sperm cells in the small volumes present in the microchannel and, finally, the released DNA must still bind effectively to the extraction bed. If these elementary conditions are met, the lysis/loading buffer should prove complimentary for use in an integrated cell sorting and DNA extraction device.

Fluorescently labeled sperm cells were flowed into a channel that was partially filled with silica beads using syringe pump-driven flow. A high concentration of cells was used to facilitate visualization. Figure 4*A* and *B* show the silica bead-containing

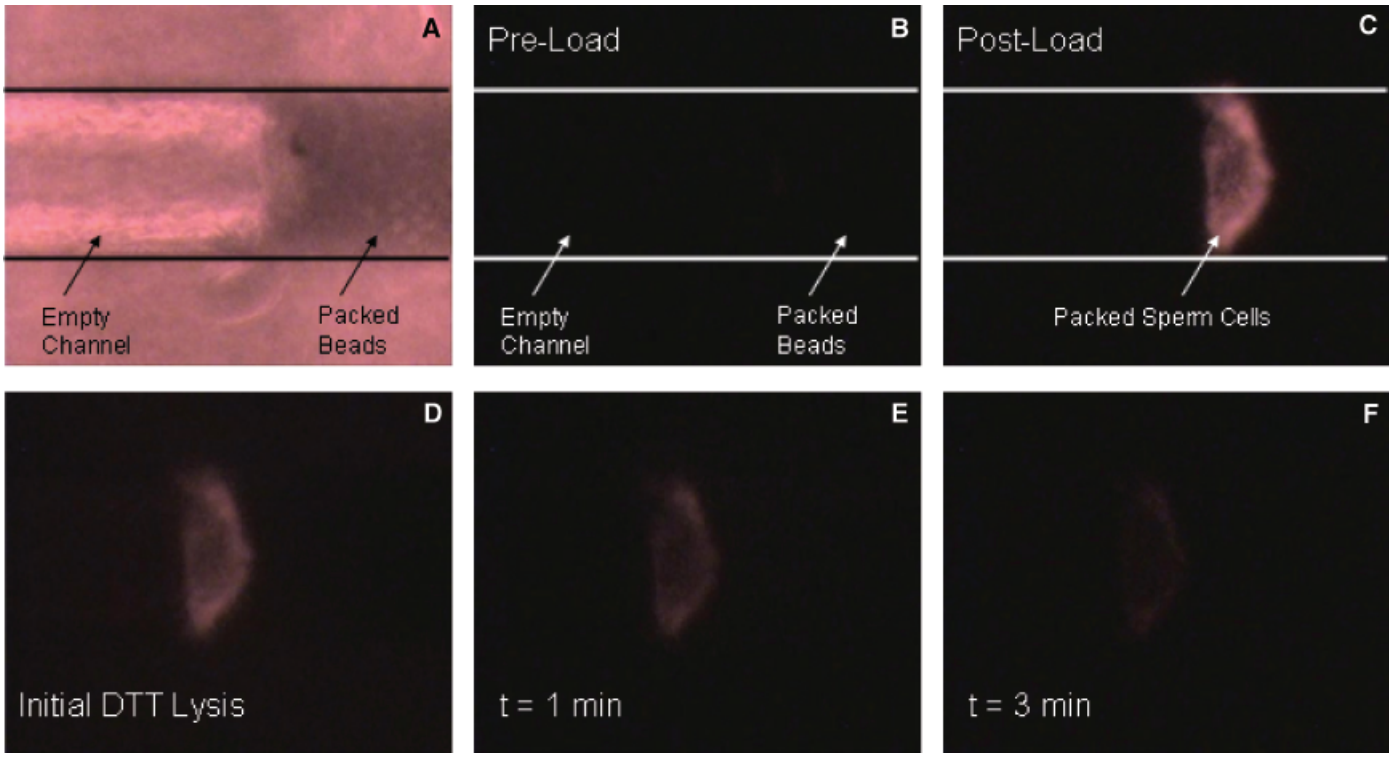

FIG. 4—*On-chip lysis of sperm cells before microchip-based DNA extraction. All figures show a section of the microchip extraction channel (~ 1 mm) at the front edge of the silica bed by either light (A and B) or fluorescence (C–F) microscopy. No inherent fluorescence was seen in the channel when irradiated with 480–550 nm light (B). When DEAD Red™-labeled sperm cells were flowed into the channel, they were seen to pack at the silica bed front (C). After flow of the dual lysis/loading buffer was initiated (D), the fluorescence decreased over the next few minutes (E, F) and was essentially nonexistent after 5 min (data not shown). Conditions: Lysis buffer, 6 M GuHCl, containing 40 mM dithiothreitol (DTT). Cells were loaded at a flow rate of 250 μL/h for 2.5 min, the same rate as that of the lysis buffer.*

microchip channel under both light (*A*) and fluorescent (*B*) microscopy. No inherent fluorescence is seen in the channel when it is irradiated with excitation (480–550 nm) light (*B*). A solution of sperm cells, fluorescently labeled using the DEAD Red™ stain, was flowed into the channel for approximately 2.5 min at a rate of 250 μL/h, while being microscopically monitored. Figure 4*C* shows the channel after sperm cell packing was complete. Note that sperm cells have penetrated slightly into the packed bed front; however, after sweeping down the length of the channel with the microscope, it was determined that no sperm cells had migrated through the silica bed. The cells have migrated minimally into the frontal edge of the packed SPE beads (< 200 μm); however, there is a clear demarcation between the edge of the sperm cell front and the rest of the bead-filled microchannel (~ 10 mm total length). Figure 4*D–F* depict the sperm cells after the initiation of the dual lysis/loading buffer flow. After only 1 min of flow of the dual lysis/loading, there was a severe decrease in fluorescence (*E*), which is barely visible after 3 min (*F*) and essentially nonexistent after 5 min of lysis buffer flow (data not shown)—this indicates that sufficient lysis of the cells has been achieved. A control experiment was run using lysis buffer without the reducing agent DTT to ensure that the severe decrease in fluorescence was indeed a result of cell lysis (data not shown). These results indicate that the dual lysis/loading buffer developed for off-chip lysis of sperm cells is capable of microchip-based lysis with favorable kinetics.

In order to confirm that the lysed cells were, indeed, releasing their DNA into the load solution and that the resultant DNA was being driven to the surface of the silica beads, a microchip-based lysis and extraction were performed. One microliter of neat semen was placed in the inlet reservoir of the device and withdrawal (to simulate the conditions of a cell-sorting experiment) initiated. The sperm cells were allowed to pack against the silica bead bed, in a manner consistent with what was observed fluorescently. An extended packing period was undertaken (i.e., 10 min instead of the 2.5 min used previously) to ensure ample cells for lysis; the exact

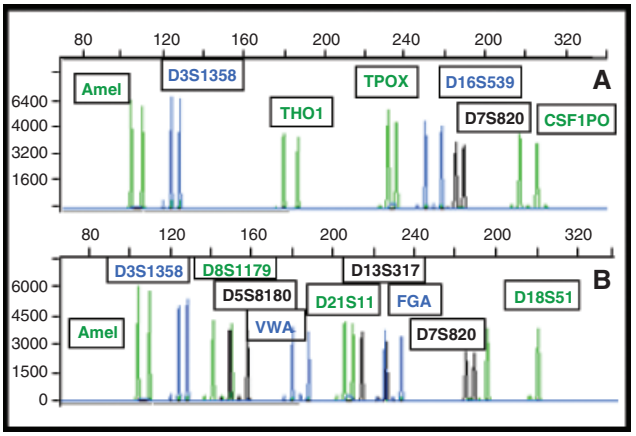

FIG. 5—*CE-generated short tandem repeat profile from sperm cell DNA purified from cells lysed and extracted on chip. Conditions: 1 μL of semen was loaded into the reservoir of the device and the sperm loaded against the silica bed by withdrawal for 10 min. Residual semen (less than 1 μL) was removed from the reservoir after loading. A lysis buffer of 6 M guanidine HCl containing 40 mM dithiothreitol (DTT) was flowed over the packed cells for 15 min to lyse the cells and load the DNA, followed by a protein wash (25 μL) with isopropanol. DNA was eluted with water, 5 μL fractions collected, split into 2.5 μL aliquots and subsequently amplified using the COfiler™ and Profiler Plus™ amplification kits according to manufacturer's instructions.*

number of cells loaded, however, cannot be known. The syringe was switched from the outlet to the inlet reservoir of the microchip and pressure-driven flow with the dual lysis and loading buffer commenced. After 10 min of cell lysis, the extraction was completed as per the normal protocol (protein wash 25 μL isopropanol, elution with water). The resultant eluate was collected in 5 μL fractions and PCR amplified using all 13 core CODIS loci and the product analyzed using capillary electrophoresis. Figure 5 provides the electropherograms generated from the PCR-amplified sample. All 13 core CODIS loci were amplified using DNA purified with the microchip-based lysis and extraction protocol, suggesting that the developed procedures will interface well with the previously established cell sorting method.

Conclusions

A microscale SPE method was refined for the extraction of DNA from sperm cells, with a view toward enhanced analysis of sexual assault evidence. In order to tailor the previously published protocol to the extraction of DNA from semen or purified sperm cells, a cell lysis buffer was developed and demonstrated for both macro- and micro-scale cell rupture. A 6 M GuHCl solution containing 40 mM DTT was demonstrated to work effectively as a dual lysis and loading buffer for sperm cells both on- and off-chip. DNA purified using the methods developed was suitable for subsequent STR analysis, producing results that while preliminary in nature, compare favorably with DNA extracted by a commonly utilized commercially available method. In addition, the methods developed through this research were evaluated for possible combination with cell sorting and preliminary results suggest that the integration of these two processes can be readily accomplished. This work represents the efforts to tailor microchip-based solid-phase extraction methods for analysis of evidentiary materials from sexual assault cases.

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