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Enhanced recovery of spermatozoa and comprehensive lysis of epithelial cells from sexual assault samples having a low cell counts or aged up to one year

Jenny A. Lounsbury [a,b], Shanti M. Nambiar [a,b], Anne Karlsson [a,b,c], Helina Cunniffe [a,b,c], Jessica V. Norris [a,b], Jerome P. Ferrance [a,b], James P. Landers [a,b,d,e,*]

[a] Department of Chemistry, University of Virginia, 409 McCormick Road, Charlottesville, VA 22904, United States
[b] Center for Microsystems for the Life Sciences, University of Virginia, Charlottesville, VA 22904, United States
[c] University of Huddersfield, Queensgate, Huddersfield HD1 3DH, United Kingdom
[d] Department of Pathology, University of Virginia Health Science Center, Charlottesville, VA 22908, United States
[e] Department of Mechanical Engineering, University of Virginia, Engineer's Way, Charlottesville, VA 22904, United States

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ABSTRACT

Differential extraction (DE) is the most common method for processing sexual assault samples, allowing for the simultaneous recovery of sperm and epithelial cells from the swab with the separation of sperm cells from epithelial cell DNA by exploiting the differences in the cell membrane susceptibility to detergents. However, sperm cell recovery when using DE is generally 40–50% [1], which can reduce the probability of obtaining a STR profile of the semen contributor, especially if the sample is aged or has a low number of sperm cells. Here, we present a novel buffer, containing SDS and ProK that, when used as an initial incubation buffer, enhances sperm cell recovery to as high as 90%, representing a 200–300% increase over conventional DE buffer. Adjusting the incubation time and temperature provided high, reproducible sperm cell yields. Sample vortexing and replacement of SDS with sodium octyl sulfate (SOS), another sulfate-based anionic detergent, did not provide any further enhancement of the sperm cell recoveries. Furthermore, the one-step buffer provided up to a 300% increase in recovery over the conventional DE buffer when used on samples aged up to one year. STR analysis of samples containing 500 or more sperm cells treated with this buffer showed comparable results (i.e., full STR profiles; 16 of 16 loci) to those obtained using a conventional DE buffer. Finally, when the sample contained only 400 sperm cells (recovered in 100 μL volume, then extracted), substantially more STR loci (14 of 16) were generated using the novel buffer in comparison to the conventional DE buffer (4 of 16 loci). This work demonstrates that this buffer may be useful as an alternative for the initial sample incubation step in differential extraction, particularly for aged or samples known to have a low number of sperm cells.

1. Introduction

Forensic DNA laboratories attempt to produce full STR profiles from a wide variety of challenging samples, including degraded, low template, and sexual assault samples with limited sperm cells and/or excess epithelial cells. The most commonly encountered sexual assault samples provide a unique sample type, with robust sperm cells interspersed with delicate epithelial cells on a cotton swab matrix. The ideal goal with these types of samples is to completely separate the sperm cell DNA from the epithelial cell DNA, providing single-source STR profiles of both the victim and the perpetrator with no cross-contamination. The most widely used method for processing these samples is differential extraction (DE), which exploits the differences in cell membrane composition between sperm cells and epithelial cells in order to separate them. DE uses proteinase K (ProK) and an anionic detergent in the elution buffer to selectively lyse the epithelial cells [2–4]. The sperm cells are pelleted by centrifugation and the supernatant, which contains non-semen DNA, is removed. A reducing agent, such as dithiothreitol (DTT), is added to the sperm pellet to lyse the sperm cells, releasing the male DNA. Both fractions are then used for further downstream analyses in an effort to obtain STR profiles of both the perpetrator and the victim.

However, a significant downfall of DE is that it can be ineffective for samples in which the sperm cells are a minor component, resulting in an excess of epithelial cells in the sperm fraction. This

* Corresponding author at: Department of Chemistry, University of Virginia, McCormick Road, P.O. Box 400319, Charlottesville, VA 22904, United States. Tel.: +1 434 243 8658; fax: +1 434 243 8852.
E-mail address: jpl5e@virginia.edu (J.P. Landers).

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significantly reduces the probability of obtaining a full profile of the semen contributor to the sample, since the abundance of epithelial cell DNA produces an excess of undesired PCR product, thereby hindering the allele calls of the PCR product generated from the semen donor (i.e., produces a mixed profile). Several groups have attempted to improve the recovery of sperm cells by modifying the chemical composition of the buffers used, such as the concentration of ProK, or using DNase to avoid multiple sample rinsing steps. Yoshida et al. developed a method which used a higher concentration of ProK and an elevated incubation temperature (increased from 56 °C to 70 °C) to achieve a pure male fraction [5]. However, it was reported that this method resulted in a significant loss of sperm cells during the initial incubation [6–8]. In other efforts, Tereba et al. used a combination of centrifugation and phase separation steps to obtain sperm and non-sperm fractions, however, this method also reported sperm cell lysis during the ProK incubation [9], demonstrating the fine balance between obtaining a single-source male fraction and loss of sperm cells during the initial digestion due to the increased ProK concentration. Sperm cells may also be lost to the supernatant if the sperm pellet is agitated during any of the numerous sample rinsing steps required to effectively dilute the female DNA concentration. As a result, methods to eliminate these rinsing steps exploit DNase to degrade the female DNA after the initial gentle lysis incubation and before the sperm cells are lysed with DTT [10,11].

As an alternative to chemical methods, there are physical manipulation techniques to isolate and recover individual sperm cells. Laser microdissection is a method in which sperm cells are laser cut from a microscope slide and a defocused laser pulse catapults the cells into a waiting aliquot of buffer directly above the location of the cell. STR profiles obtained from sperm cells isolated by this method show little evidence of epithelial cell DNA carryover [12–15]. More recently, Schneider et al. demonstrated isolation by "picking" sperm cells from a mixed sample using microspheres. Sperm cells adhere to microspheres, manipulated by custom grippers, allowing for careful movement of the spheres to only collect sperm cells. After collection, full STR profiles were obtained from as few as 20 sperm cells with no evidence of epithelial cell DNA carryover [16]. In addition, several microfluidic sperm cell isolation techniques have been reported, offering decreased analysis time and completely closed systems. Horsman et al. developed a microchip-based cell sorting method by allowing the epithelial cells in a sample to settle and adsorb to the bottom of an input reservoir, while subsequent flow carries the sperm cells to a different reservoir. However, this method requires adequate time for epithelial cells to settle, assumed minimal lysis of the epithelial cells, and had a relatively poor volume throughput [17]. More recently, Norris et al. trapped sperm cells in an acoustic standing wave, formed in a microchannel, while female DNA was washed away. Processing of mixed samples with this method resulted in fractions that were up to 92% male, a 4-fold increase in purity over the original sample [18]. Although all these methods avoid the numerous centrifugation and sample handling steps that are associated with the chemical techniques, they require costly instrumentation, analyst training, and are significantly dependent on the efficient elution of sperm cells from the cotton swab matrix and, in the case of the acoustic method, sufficient lysis of the epithelial cells.

Previous work in our laboratory to improve the elution of cellular material from a cotton swab used cellulase-based enzyme mixtures to digest the cotton fibers, which was successful in releasing sperm cells. However, the results indicated that this method did not significantly improve sperm cell recovery, yielding results similar to a traditional DE method [19]. More recent work has focused on altering the components of the elution/lysis buffer and optimizing the incubation conditions in order to improve cell elution and lysis of the epithelial cells. A two-step buffer system was previously developed that uses a MES/Tris buffer at pH 8.0 containing sodium dodecyl sulfate (SDS) to elute the majority of cellular material in the first incubation. Then, upon the addition of ProK, the second incubation comprehensively lyses the epithelial cells [20]. This method demonstrated nearly a three-fold improvement in sperm cell recovery as compared to a traditional DE method; however, it utilizes two separate 30 min incubations steps.

The current work focuses on enhancing the two-step method by adding the ProK directly to the elution buffer, eliminating the second incubation step. Optimization of buffer pH and incubation time and temperature significantly improved recovery of sperm cells from fresh samples, aged samples (up to 1 year), and samples containing a low number of sperm cells (<500 cells).

2. Materials and methods

2.1. Preparation of mock sexual assault samples

Using a sterile cotton swab (Fisher Scientific, Pittsburgh, PA), buccal cells were collected by swabbing left and right cheeks vigorously for 30 s and allowing the swab to dry overnight at room temperature. The dried swabs were cut into similarly sized fragments (1.0 ± 0.10 mg) and placed in Petri dishes. For optimization and characterization experiments, 0.5 μL of a 1:1 semen:water sample (Donor # M33F99S; ~25,000 sperm cells; average sperm cell count ~50,000 cells/μL) was applied to each swab fragment and allowed to dry at room temperature for one week. For aged sample studies, samples were stored from up to one year. For the STR studies, 1 μL of a series of diluted semen samples, ranging from ~25,000 cells to 400 sperm cells was applied to a swab fragment and allowed to dry at room temperature for up to one week. All buccal swabs and semen samples were collected from healthy female and male volunteers. Liquid semen samples were stored at room temperature for 1 h after collection and subsequently placed in frozen storage until needed.

2.2. Optimization of the buffer and incubation conditions

Several buffer solutions of different pH values were prepared as follows. To achieve a final concentration of 10 mM Tris(hydroxymethyl)aminomethane (Tris; Sigma, St. Louis, MO), 10 mM 2-(*N*-morpholino)ethanesulfonic acid (MES; Sigma), and 1% sodium dodecyl sulfate (SDS; Biorad, Hercules, CA), individual buffers were brought to pH of 8.00, 8.25, 8.50, 8.75, or 9.00 using 0.1 M HCl or 0.1 M NaOH and adjusted to the appropriate volume using Nanopure water (Barnstead/Thermolyne, Dubuque, IA). The buffers were stored at room temperature until used. Swab samples were placed in 0.2 mL PCR tubes and 99.8 μL of one of the buffer solutions was added. Next, 0.2 μL of 10 mg/mL ProK was added for a final volume of 100 μL (final ProK concentration = 20 μg/mL). Samples were vortexed briefly and then incubated for 5, 10, 20, 30, 45 or 60 min at each of four different temperatures (25 °C, 37 °C, 42 °C or 56 °C). For comparison, swabs were incubated in a traditional DE buffer containing 10 mM Tris–HCl, 10 mM ethylenediaminetetraacetic acid (EDTA), 0.1 M NaCl, 2% SDS and 20 μg/mL ProK at 42 °C for 30 min. Each set of incubation experiments were repeated three times, unless otherwise stated. Following incubation, the samples were handled as previously described [1]. Briefly, a small hole was created at the bottom of the PCR tube by puncturing the tube with a 21-gauge needle. The tubes were placed in 1.5 mL microcentrifuge tubes and centrifuged at 12,100 × *g* for 4 min (piggyback method) [21]. The collected solution was vortexed to re-suspend the sperm cells and three 10 μL portions were used for cell counting.