J Forensic Sci, July 2007, Vol. 52, No. 4
doi: 10.1111/j.1556-4029.2007.00453.x
Available online at: www.blackwell-synergy.com

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Expedited, Chemically Enhanced Sperm Cell Recovery from Cotton Swabs for Rape Kit Analysis*

ABSTRACT: This report focuses on the development of a method for chemically induced enhancement of cell elution and recovery from cotton swabs. The method exploits the exclusive use of detergents for intact cell removal, and can be utilized in conjunction with, or to circumvent, conventional differential extraction (DE). Samples treated with Sarkosyl (54.4 ± 1.8%) and sodium dodecyl sulfate (SDS) (78.5 ± 0.7%) yielded higher sperm cell recoveries than a conventional DE buffer (39.4 ± 2.1%). The results indicated that the choice of detergent affected sperm cell yield, with anionic detergents having the greatest effect. Storage time of samples affected the concentration of detergent required for optimal sperm cell recovery, longer times requiring increased detergent concentrations. In addition, the extent of sperm cell lysis by proteinase K digestion was evaluated. The results indicate that the exclusive use of SDS enhances the release of sperm and epithelial cells from a cotton swab as compared with DE buffer, providing for a more effective DNA analysis.

KEYWORDS: forensic science, sex offences, DNA typing, cell elution, detergents, differential extraction

Genetic analysis of perpetrator and victim DNA from vaginal cotton swabs is a well-established forensic technique for investigating and prosecuting sexual crimes (1–3). Analytical results suitable for prosecution rely on separation of DNA from the perpetrator and victim to obtain individual DNA profiles. The current protocol for recovery of biological materials from a cotton swab involves differential extraction (DE), a method that utilizes proteinase K and an anionic detergent to selectively lyse vaginal epithelial cells while eluting sperm cells intact (2). Sperm cells are pelleted by centrifugation, and the supernatant containing the epithelial cell DNA is removed. The sperm cells are then resuspended in a buffer containing dithiothreitol (DTT), reducing the disulfide bond network in the sperm cell head and allowing the nuclear membranes to be lysed, releasing the male DNA.

Differential extraction allows for independent recovery of male and female DNA from vaginal swabs containing mixtures of vaginal cells and spermatozoa, and has been routinely used in forensic laboratories since its introduction. However, sexual assault samples often contain sperm cells as a minor component in the presence of excess epithelial cells. DE is frequently ineffective for these samples, as a considerable number of vaginal epithelial cells can remain undigested in the sperm cell fraction after the initial lysis step, resulting in an unfavorable ratio of male to female DNA (4). The probability of obtaining an interpretable male DNA profile is reduced when excess female DNA is present, as polymerase chain reaction (PCR) results may be obscured by excess female DNA (5). To overcome this problem, Yoshida et al. (6) introduced a two-step DE method using an increased proteinase K concentration and an elevated incubation temperature that are in use in crime laboratories (7). Several groups, however, report that sperm cell lysis and subsequent loss of valuable evidential material occurs during the initial proteolytic digestion steps (4,5,8,9), although to date, the effects of proteinase K digestion on sperm cell lysis have not been reported. Wiegand et al. (4) developed a modified DE method, looking to reduce sperm cell lysis while increasing epithelial cell lysis by adjusting the conditions during the initial proteolytic digestion stage. The amount of proteinase K added was dependent on the abundance of spermatozoa on the swab sample, but to facilitate better epithelial cell lysis, the concentration used was always greater than that used by Gill et al. (2). Some spermatocytes were lysed during the initial lysis step so as to increase the removal of epithelial DNA (4); in addition, this method required *a priori* knowledge of the sperm concentration. Tereba et al. (9) modified the DE method further, incorporating a combination of centrifugal extraction and phase separation to obtain the sperm and epithelial fractions. Although the method has proven effective for reducing epithelial cell carryover in the sperm fraction, sperm lysis still occurs during the proteinase K digestion. Efforts to remedy the drawbacks of DE have, therefore, resulted in a balance between obtaining a high-purity sperm fraction and loss of sperm cells during the separation process.

A number of groups have exerted efforts directed at replacing the DE process in routine forensic DNA analysis. One alternative involves the separation of sperm and vaginal epithelial cells prior to DNA extraction. Elliott et al. (10) demonstrated selective capture and isolation of sperm cells using laser capture microdissection. Although this method has high specificity and has proven to be effective in a forensic investigation (11), it is time-consuming and labor-intensive to identify the cells by visual inspection and is not easily amenable to high-throughput applications. Horsman et al. (12) have demonstrated a successful microdevice-based sorting of sperm cells from a mixture of sperm and vaginal epithelial cells.

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*Research initially funded by the U.S. Department of Justice, Federal Bureau of Investigation, and presented in part at AAFS, 2006, and MAAFS, 2006. The views and conclusions contained in this document are those of the authors and should not be interpreted as necessarily representing the official policies, either expressed, or implied, of the U.S. Government.

Received 1 Sept. 2006; and in revised form 24 Dec. 2006; accepted 24 Dec. 2006; published 24 May 2007.

The process exploited the differential physical properties of the cells, resulting in sedimentation and adsorption of epithelial cells to the bottom of an inlet reservoir on the glass microdevice. Subsequent buffer flow through the system caused the sperm cells to migrate towards the outlet reservoir while epithelial cells remained in the inlet reservoir, resulting in effective separation of the two cell types. A newer method has been described by Horsman et al. (13) that uses acoustic forces to isolate sperm cells from vaginal swabs with a resultant pure male fraction. Regardless of the method utilized for the separation of vaginal and sperm cell DNA, the overall effectiveness of the procedure is ultimately dependent on the efficiency with which material can be eluted and recovered from a cotton swab. The issue is especially important with swab samples containing low number of sperm cells, where any loss makes it even more difficult to obtain a profile of the perpetrator.

Previous work from our laboratory focused on the effects of cellulase-based enzyme mixtures obtained from *Aspergillus niger* and *Trichoderma viride* on the recovery of intact sperm and epithelial cells from a cotton swab matrix (14). The results indicated that, while cellulose-digesting enzymes significantly enhance the release of sperm and epithelial cells from a cotton swab in comparison with buffer alone, they yield sperm cell recoveries similar to a conventional DE buffer. The work presented here focuses on chemical-based methods for sperm and epithelial cell elution from a cotton swab matrix. Additionally, proteinase K was evaluated for its effects on sperm cell lysis.

Materials and Methods

Preparation of Mock Casework Samples

To mimic the vaginal epithelial cells collected on casework sample swabs, buccal epithelial cells were collected onto sterile cotton swabs (Fisher Scientific, Pittsburgh, PA) and allowed to dry for a minimum of 3 days at room temperature. For vaginal swab studies, vaginal epithelial cells were collected onto sterile cotton swabs and allowed to dry for a minimum of 3 weeks at room temperature. Swabs were then cut into pieces of consistent mass (1.0 ± 0.10 mg, approximately one-fifth of a swab), and a 0.40 μL aliquot of semen (Donor #M81F79J, approximately 20,000 sperm cells) was applied to each sample. Samples were dried and then stored at room temperature for specified amounts of time, as indicated in the results section. All buccal swabs, vaginal swabs, and semen samples were obtained by voluntary donation from healthy females and males. Semen samples were stored at room temperature for 1 h after collection and were then placed in frozen storage to reduce the viscosity of the solutions (15).

Procedure for Cell Elution from Cotton Swabs

Detergent solutions were prepared at the appropriate concentrations in Nanopure water (Barnstead/Thermolyne, Dubuque, IA) using sodium dodecyl sulfate (SDS; Sigma, St. Louis, MO), sodium lauroyl sarcosinate (Sarkosyl; Sigma) 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS; Sigma), cetyltrimethylammonium bromide (CTAB; Fluka, Milwaukee, WI), or polyethylene glycol tert-octylphenyl ether (Triton-X 100; Sigma). A solution of 10 mM citrate buffer (pH 5.4) was prepared by dissolving 1.05 g of citric acid monohydrate (Sigma) in 500 mL of Nanopure water (Barnstead/Thermolyne). Cellulase solution was prepared at the appropriate concentration in citrate buffer using cellulase from *T. viride* (Sigma). Swab samples were placed in polypropylene PCR tubes (Fisher Scientific) containing either 100 μL of citrate buffer, cellulase solution, or detergent solution, vortexed briefly, and incubated for 2 h unless otherwise indicated. For comparison with the traditional DE method, swabs were incubated in 100 μL of DE buffer containing 0.01 M Tris–HCl pH 8.0, 0.01 M diaminoethanetetraacetic acid (EDTA), 0.1 M NaCl, 2% (w/v) SDS (Biorad, Hercules, CA), and 20 μg/mL proteinase K (all reagents from Sigma unless otherwise noted) (2). Incubations were performed at 42°C unless otherwise stated. Each incubation condition was repeated four times unless otherwise stated. After incubation, the samples were vortexed briefly, and small holes were created at the bottom of the PCR tubes by inverting the tube and inserting a 21-gauge needle through the center of the bottom of the tube. The PCR tubes were then inserted in 1.5-mL microcentrifuge tubes and centrifuged at 5433.2 g for 4 min (piggyback method) (16). The released cells in solution were collected in the microcentrifuge tube while the cotton swab fragment remained in the PCR tube. The collected solution was vortexed, and a 10 μL portion was utilized for cell counting. For studies involving the effect of proteinase K on sperm cell lysis, a portion of cell solution was isolated, and proteinase K was added to a final concentration of 20 μg/mL. The samples were vortexed briefly and incubated at 42°C for an additional 0–20 min. The solution was vortexed, and a 10 μL aliquot used for cell counting.

Determination of Cell Recovery

Sperm and epithelial cells eluted from each sample were visualized using light microscopy and counted using a hemacytometer (Fisher Scientific). A fixed volume was applied to the hemacytometer grid, and the sperm and epithelial cells were counted to determine the number of cells recovered. The average sperm cell count for semen donor #M81F79 J was determined to be 47,700 (±2.5%) cells per milliliter of semen. This value was used to calculate the percent of sperm cells recovered for each set of samples. Due to the variability of cell collection on buccal and vaginal swabs, results for epithelial cells are reported as number of cells recovered.

Results and Discussion

Effect of Detergent on Sperm Cell Recovery

Previous efforts in our laboratory focused on the development of a method for enhanced cell elution from cotton swabs using cellulase enzymes (14). However, cell elution using cellulase digestion provided only equivalent recovery of sperm cells compared with a conventional DE buffer elution, which contained Sarkosyl (1% w/v) and proteinase K (20 μg/mL). To determine whether detergents could enhance sperm cell elution by cellulase digestion, the effect of adding in detergent after digestion with cellulase was examined. Mock casework samples were prepared using buccal swabs as previously described and dried for 4 weeks, and the cells eluted from the swabs using either citrate buffer, *T. viride* cellulase in citrate buffer (300 μg/mL), a DE buffer containing 2% SDS, or 1% (w/v) Sarkosyl. Sarkosyl is another anionic detergent commonly used in conventional DE buffer (17,18); therefore, its effect on cell elution was investigated. After the initial 2-h incubation, Sarkosyl was also added to half of the swab samples treated with cellulase to a final concentration of 1% (w/v) to determine if this had an additive effect on the release of sperm from the swab samples. These samples were then briefly vortexed, and the "piggyback method" described in the Materials and Methods section was immediately applied to recover the cells in solution. Figure 1 shows

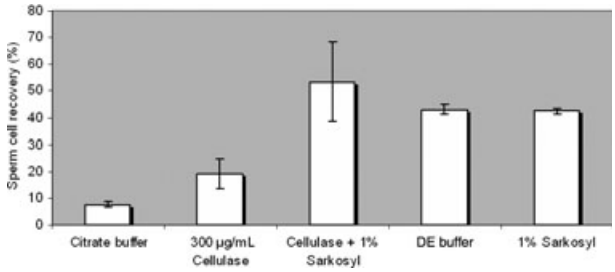

FIG. 1—*Comparison of sperm cell recoveries from buffer-based, enzymatic, detergent based, and combined elution methods. Mock casework samples were dried for 4 weeks and incubated for 2 h at 42°C. For the combined cellulase/Sarkosyl method, Sarkosyl was added to a final concentration of 1% after incubation in cellulase solution.*

that the average sperm cell recovery for samples treated with a DE buffer containing 2% SDS (43 ± 1.8%) was higher than samples eluted using cellulase (19 ± 5.7%) or citrate buffer alone (7.6 ± 1.0%). Incubation in cellulase followed by treatment with 1% Sarkosyl detergent (53 ± 15%) did not offer substantial improvement over the conventional DE method. Sperm cell recovery after conventional DE buffer treatment and recovery after cellulase digestion and subsequent Sarkosyl treatment were both similar to sperm recovery observed with the exclusive use of 1% Sarkosyl (44 + 1.8%). The data suggest that it is the detergent in conventional DE buffer that is primarily responsible for the release of sperm cells from cotton swabs. In addition, our results suggested that the type of detergent used in the DE buffer affected sperm cell recovery, and thus enhancement of sperm cell recovery required investigation of the detergent component of DE buffer.

A direct comparison of the efficiency of SDS with Sarkosyl for eluting sperm cells from cotton swabs was therefore performed. Mock casework samples, dried for 1 week, were incubated in 1% or 2% (w/v) Sarkosyl, 1% or 2% (w/v) SDS, or conventional DE buffer containing 2% (w/v) SDS, for 2 h at 42°C. Additional samples treated with conventional DE buffer were incubated for 24 h at the same temperature. Figure 2 shows that elution with either SDS (1%: 77 ± 2.3%; 2%: 79 ± 0.7%) or Sarkosyl (1%: 47 ± 2.0; 2%: 54 ± 1.8%) alone yielded higher sperm cell recoveries than elution with conventional DE buffer for either 2 or 24 h. This is most likely because conventional DE buffer treatment leads to sperm lysis as well as cell elution; a factor that would be attributable to the non-detergent components of DE buffer. Both

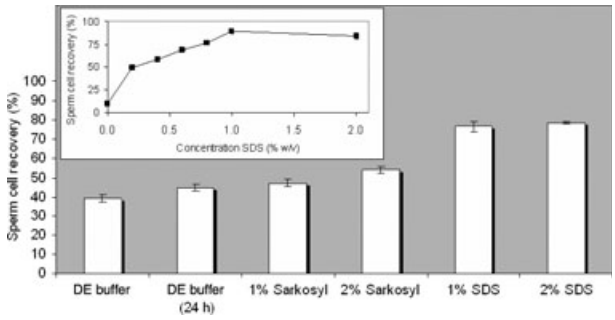

FIG. 2—*Comparison of sperm cell recoveries from elutions using differential extraction (DE) buffer and anionic detergents. Mock casework samples were dried for 1 week and incubated for 2 h at 42°C (unless otherwise indicated). Inset: Effect of sodium dodecyl sulfate (SDS) concentration on the release of sperm cells from samples dried for 1 week. Samples were incubated for 2 h at 42°C.*

concentrations of SDS yielded higher sperm cell recoveries than comparable concentrations of Sarkosyl, indicating that the correct choice of detergent can substantially improve the yield of sperm cells. Finally, the average sperm cell recovery for samples incubated in DE buffer for 24 h (45 ± 1.5%) was similar to those incubated in DE buffer for 2 h (39 ± 2.1%), indicating that prolonged exposure to the DE buffer does not considerably enhance sperm cell elution, nor does it provide for greater cell lysis.

Elution properties of the detergent could be affected by several experimental conditions, most importantly surfactant concentration. Investigations were therefore performed to determine the optimal SDS detergent concentration for cell elution and recovery. For these studies, mock casework samples were prepared as described and dried for 1 week. Cells were eluted from the swab in 0.2–2.0% (w/v) SDS, and compared with samples eluted in water in the absence of detergent. The results (Fig. 2, inset) show sample treatment with 1% (w/v) SDS resulted in the highest average sperm cell recovery (89 ± 2.9%), which did not differ considerably from that for samples treated with 2% (w/v) SDS (84 ± 3.2%).

Additional Detergents for Cell Elution

Anionic and cationic denaturing detergents, and zwitterionic and nonionic nondenaturing detergents, were assessed for sperm cell elution and recovery. Mock casework samples, dried for 1 week, were eluted in 1% (w/v) SDS (anionic), 1% (w/v) CTAB (cationic), 1% (w/v) Triton-X (non-ionic), or 1% (w/v) CHAPS (zwitterionic) for 2 h at 42°C. Cell recoveries were determined and compared with those of samples eluted in conventional DE buffer. Figure 3 shows that the average sperm cell recovery for samples eluted in SDS (75.6 ± 3.5%) was considerably higher than those obtained with the other detergents investigated and, most importantly, was nearly double the recovery associated with DE buffer (40 ± 2.3%). It is important to note that these recoveries are similar to those obtained previously for samples dried for the same length of time (Fig. 2—1% SDS: 77 ± 2.3%; DE buffer: 39 ± 2.1%), showing the reproducibility of the method. Samples eluted in CTAB (0.40 ± 0.30%), Triton-X (14 ± 1.5%), or CHAPS (16 ± 3.2%) did not provide improved sperm cell recovery over samples eluted in DE buffer. These results are not surprising, as conventional DE methods have utilized anionic detergents since their inception (2).

Effect of Proteinase K on Sperm Cell Recovery

It is apparent from the earlier experiments that the nondetergent DE buffer components cause a reduction in sperm cell recovery. As the most likely culprit is proteinase K, the effect of this component on sperm cell lysis was investigated. Mock casework

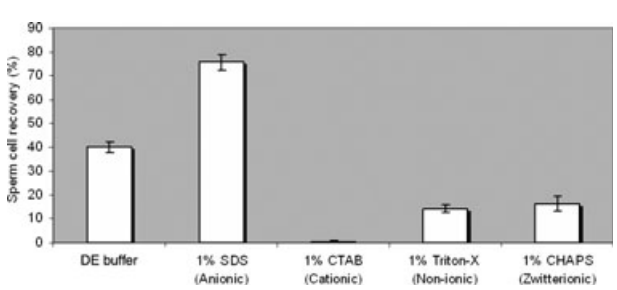

FIG. 3—*Comparison of sperm cell recoveries from elutions using various detergent types. Mock casework samples were dried for 1 week and incubated for 2 h at 42°C.*

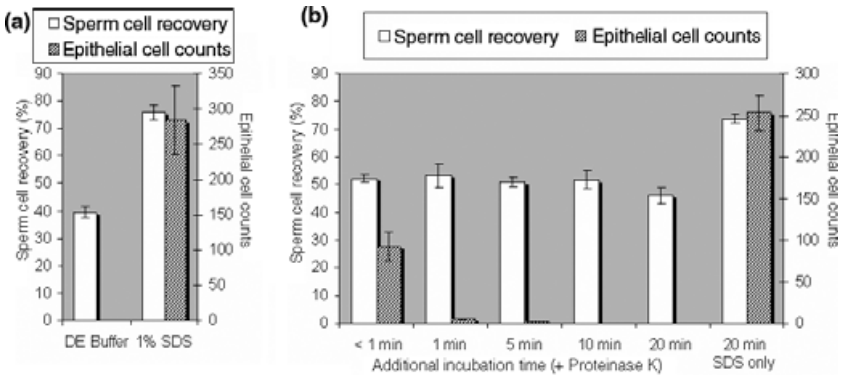

FIG. 4—*(a) Comparison of sperm cell recoveries and epithelial cell counts from elutions using differential extraction (DE) buffer and 1% sodium dodecyl sulfate (SDS). Mock casework samples were dried for 1 week and initially incubated for 2 h. (b) Effect of proteinase K on sperm and epithelial cell lysis. Cell solutions obtained from 1% SDS-eluted samples (Fig. 4a) were treated with 20 μg/mL proteinase K and incubated for specified additional incubation times. Control samples remained untreated with proteinase K and were incubated for 20 additional minutes. All samples were incubated at 42°C.*

samples, dried for 1 week, were incubated in DE buffer or 1% SDS for 2 h at 42°C. The average sperm cell recovery for samples eluted in 1% SDS was 76 ± 3.0% (Fig. 4*a*, $n = 18$). To determine the effect of proteinase K on sperm cell lysis, proteinase K was added to aliquots of the SDS-eluted cell solution, and incubated for an additional 0–20 min. The results, presented in Fig. 4*b* ($n = 3$ for each incubation time), show that the addition of proteinase K rapidly decreases the number of sperm cells in solution. After incubation with proteinase K for less than 1 min, the sperm cell recovery decreased to 52% (±1.5%). Even lower sperm cell recoveries were observed for all cell solutions incubated for longer periods in the proteinase K solution (Fig. 4*b*), with the results from the DE buffer (39 ± 2.1%, Fig. 4*a*) representative of the lower limit after incubation for 20 min. The results after 1 min were revealing, indicating that the majority of sperm cell lysis observed occurred almost immediately after the addition of proteinase K. Samples without proteinase K, but incubated for an additional 20 min, resulted in recoveries (74 ± 1.4%, Fig. 4*b*) that did not differ from recoveries obtained prior to the additional incubation (Fig. 4*a*), showing that proteinase K, rather than additional incubation time, is contributing to sperm cell lysis.

Epithelial cell counts obtained from DE buffer-eluted and SDS-eluted samples in the presence and absence of proteinase K are also shown in Fig. 4. As a result of the heterogeneous nature of epithelial cell adsorption with buccal swab collection, percentage of recoveries could not be calculated. Intact epithelial cells were recovered following incubation with SDS alone, but were not recovered after sample treatment with DE buffer (Fig. 4*a*). Addition of proteinase K to the SDS cell solution aliquots also depleted nearly all of the intact epithelial cells after incubation for less than 5 min (Fig. 4*b*). Proteinase K has been reported to lyse vaginal epithelial cells (3); therefore, it was not surprising that epithelial cells were not detected in samples exposed to proteinase K for more than 5 min.

Optimization of SDS-Mediated Cell Elution

The mean size and aggregation number of SDS micelles have been determined as a function of temperature for detergent concentrations that exceed the critical micelle concentration (19). Therefore, studies were performed to optimize incubation temperature. Mock casework samples dried for 1 week were incubated in 1% SDS at 25°C, 42°C, or 56°C for 2 h. Cell recoveries from these samples were compared with those of samples treated with 1%

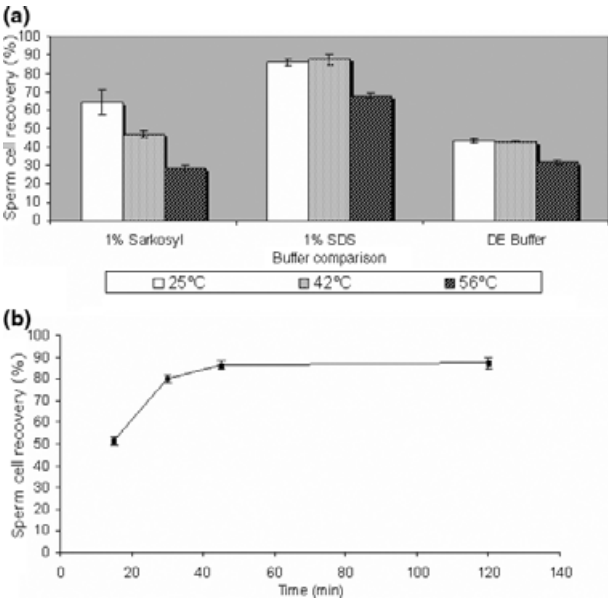

FIG. 5—*(a) Effect of temperature on the release of sperm cells from samples dried for 1 week and eluted in 1% (w/v) sodium dodecyl sulfate (SDS), 2% (w/v) SDS, or differential extraction (DE) buffer. Mock casework samples were incubated for 2 h at specified temperatures. (b) Effect of incubation time on the release of sperm cells from samples dried for 1 week. Mock casework samples were incubated in 1% (w/v) SDS for specified times at 42°C.*

Sarkosyl and conventional DE buffer at the same incubation temperatures. The results of these studies, as presented in Fig. 5*a*, make two important points. First, SDS consistently provides a better yield of sperm cells than either Sarkosyl or DE buffer. Second, increasing the incubation temperature above 42°C for samples eluted in detergent or DE buffer does not enhance, but instead leads to decreased sperm cell recoveries. The average sperm cell recovery for SDS-treated samples incubated at 25°C prior to elution (86 ± 1.7%) was similar to that for samples incubated at 42°C (88 ± 2.8) and considerably higher than that for samples incubated at 56°C (68 ± 1.3%); similar trends were observed for conventional DE-buffer- and Sarkosyl-treated samples. It is not known at this time why higher temperatures reduce the yield of cells; however, the data suggest that temperatures lower than 56°C are preferred for sperm cell elution and recovery.

Studies were also performed to determine the effect of incubation time on sperm cell elution and recovery using the optimal conditions determined above. Mock casework samples dried for 1 week were incubated in 1% (w/v) SDS for times ranging from 15–120 min (incubation in DE buffer was for 120 min only). The results (Fig. 5*b*) show that there is not much improvement in recovery with increased incubation time; the average sperm cell recovery for samples incubated for 30 min (80 ± 2.0%) is similar to that for samples incubated for 120 min (87 ± 2.8%). The results for samples eluted with DE buffer (data not shown; 42.1 ± 1.0%) are consistent with previously obtained results for 7-day-old samples (Figs. 2–4, 5*a*). These results support the conclusion that the efficiency of sperm cell removal can be doubled using SDS, and show that sperm cell elution can be accomplished in half the time necessary for conventional DE methods (2).

Sample Age Dependency on the Effectiveness of Detergent-Based Elution

Samples dried for only 1 week do not represent the broad range of sample ages likely to be encountered in forensic casework. Therefore, it was imperative to evaluate samples that had been dried and stored for longer periods of time. Mock casework samples were prepared using buccal swabs, dried for 4 weeks, and eluted in 1% or 2% SDS, and cell recoveries from these samples were compared with those from samples eluted in conventional DE buffer. Figure 6 shows that treatment of 4-week-old samples with 1% SDS (83.7 ± 2.3%) yielded a twofold increase in sperm cell recovery over DE buffer (43.1 ± 1.8%); increasing the SDS concentration to 2% (85.1 ± 2.1%) did not considerably improve cell recoveries compared with 1% SDS. This study was repeated for mock casework samples prepared from buccal swabs and dried for periods of 12, 24, and 32 weeks (Fig. 6). The results show that, while the sperm cell recoveries for DE-buffer-treated samples remained constant over time, an overall decline in sperm cell recovery was observed for samples treated exclusively with detergent. Elution with detergent alone gave a twofold improvement of sperm cell recovery over DE buffer for samples dried for 12 and 24 weeks. However, for 32-week-old samples, elution with 1% (w/v) SDS (36 ± 12%) and 2% (w/v) SDS (44 ± 6.6%) no longer resulted in enhanced sperm cell recovery over samples eluted with DE buffer (37 ± 2.4%). Sperm cell lysis during storage of the swab at room temperature has been reported (20), and thus may contribute to loss of sperm cell recovery after 24 weeks. It is not known at this time whether an increase in detergent concentration will provide more efficient sperm cell recovery for samples aged longer than 24 weeks; however, results

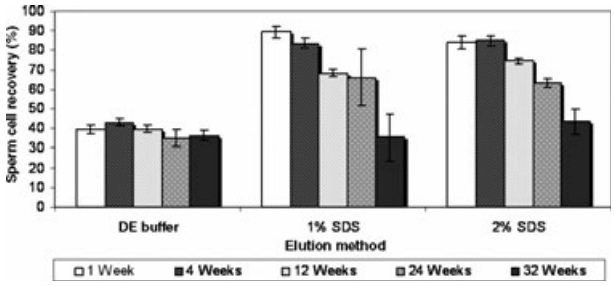

FIG. 6—*Effect of sample drying time on sperm cell recovery using either sodium dodecyl sulfate (SDS) or conventional differential extraction (DE) buffer. Mock casework samples were incubated for 2 h at 42°C.*

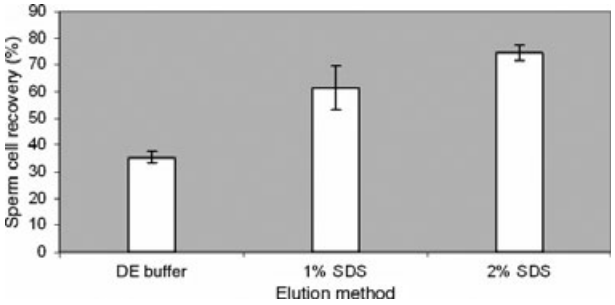

FIG. 7—*Sperm cell recoveries from vaginal swabs eluted with sodium dodecyl sulfate (SDS) or conventional differential extraction (DE) buffer. Mock casework samples were dried for 12 weeks and incubated for 2 h at 42°C.*

of further studies (data not shown) suggest that samples that have aged longer may benefit from higher detergent concentrations for optimal enhancement of cell elution.

To ensure that the observed effects were applicable to more *bona fide* rape kit samples, the study was repeated with samples prepared from vaginal swabs that had been dried for 12 weeks. Consistent with all previous experimental results, the data (Fig. 7) show that DE buffer allowed for 35 ± 2.1% recovery of the sperm cells in the samples. The use of 1% or 2% SDS again enhanced the recovery by roughly twofold, with 1% and 2% SDS yielding 61 ± 8.2% and 75 ± 2.8%, respectively. These results were similar to buccal swab samples eluted under the same conditions (Fig. 6, 1% SDS: 68 ± 2.1%; 2% SDS: 75 ± 1.3%), confirming that extension of this detergent-based elution method to vaginal swabs should not be problematic.

Conclusions

This study investigated the detergent-mediated elution of cells from mock sexual assault samples for increased cell recoveries over and above that of enzymatic methods of elution and the conventional DE method. The results clearly support the hypothesis, as elution using an anionic surfactant provided twofold enhancement of sperm cell recovery over conventional DE. The use of this method also recovered intact epithelial cells, which could be compatible with both conventional and novel DE processing. The improvement in sperm cell recoveries using SDS in the elution buffer is ideal for increasing the efficiency of obtaining accurate DNA profiles from samples containing low numbers of sperm cells. Studies indicated that the choice of detergent affected sperm cell yield, as did proteinase K, which caused sperm cell lysis. Investigations of aged samples indicated that a twofold enhancement of sperm cell recovery could be achieved for samples stored for up to 24 weeks. In addition, studies show the ability to extend this elution method to elution of sperm cells from vaginal swabs. To extend this method to forensic sexual assault samples, future studies will focus on enhancement of sperm cell elution and recovery from postcoital samples.

Acknowledgments

We would like to express our gratitude to Drs. Steve Homeyer and Laura Kienker, who educated us on the importance of exploring ways to enhance sperm cell elution from cotton swabs. Without this initial guidance, it is unlikely the findings described in this paper would have been uncovered.

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