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Enhanced DNA mixture deconvolution of sexual offense samples using the DEPArray™ system

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ABSTRACT

The interpretation of DNA mixtures remains a significant challenge in the analysis of forensic evidence. The ability to selectively identify, collect, and analyze single cells or groups of cells has wide implications in the analysis of forensic samples and the subsequent deconvolution of DNA mixtures, particularly in the processing and interpretation of sexual offense evidence where the deconvolution of heterogeneous sources is essential. Single cell separation technology can be used to address this mixture separation challenge, specifically using the DEPArray™ system from Menarini Silicon Biosystems. We propose that the DEPArray™ will enable enhancements to the standard workflow for forensic biology/DNA analytical laboratories. We have demonstrated that the DEPArray™ workflow will lead to fewer mixture samples, enable purification of sperm and epithelial cell fractions without the need for differential extraction, improve the amplification success rate of samples and improve the interpretation of low template DNA samples. Sperm profiles were identified in 27 of 32 DEPArray™ processed samples, with 26 of 27 (96.2%) yielding single source profiles. In contrast, single source profiles were obtained from 9 of 28 (32.1%) differentially extracted samples. The use of the DEPArray™ also eliminates the need for additional confirmatory tests for the presence of human sperm and permits direct identification of the type and number of cells being analyzed eliminating the need for qPCR-based DNA quantification.

1. Introduction

The interpretation of DNA mixtures remains a significant challenge within the forensic community. The ability to selectively identify, collect, and analyze single cells or groups of cells have wide implications in the analysis of forensic samples and the subsequent deconvolution of DNA mixtures. The deconvolution of heterogeneous sources is essential to the processing and interpretation of sexual offense evidence. There is a great need within the forensic science community to find innovative, robust, and efficient methods to physically separate these heterogeneous sources, with the intention of reducing an already backlogged system [1]. Single cell separation technology can be used to address this mixture separation challenge.

The forensic science disciplines continue to systematically evolve both in policy and procedure. Within forensic biology/DNA analysis, there has been much focus on the processing of DNA evidence following serological analysis. However, improvements in serological techniques may also positively affect efficiency and enhance downstream DNA analysis. Current methodologies, such as light microscopy and the subsequent differential extraction method, are generally accepted by the scientific community and considered highly reliable. However, these are laborious, often fail to adequately separate human spermatozoa from epithelial cells, and frequently require DNA mixture interpretation [2]. Because the identification of semen is a critical element of any sexual offense investigation, confirming its presence on samples collected from the body cavities provides nearly irrefutable evidence of direct sexual contact.

The most commonly encountered evidence in a sexual offense is a sexual offense evidence collection kit. The practicing forensic scientist will either examine pre-prepared slides contained within the kit or prepare slides from the swabbings/cuttings of evidentiary items within the kit. The preferred staining method is the Christmas tree method composed of two stains: (1) Nuclear Fast Red, which stains nuclei and sperm heads a reddish pink and (2) Picro-indigocarmine, which stains cytoplasm and sperm tails a green/yellow color. Microscopic sperm identification using this method can be time consuming and thus creates bottlenecks that affect the timeliness of downstream analyses. Following the identification of spermatozoa, differential extractions are used to attempt to separate the DNA fractions of the sperm and epithelial cells.

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Alternative methods have been developed to improve the efficiency of sperm detection and subsequent separation of sperm and epithelial fractions. The development of new detection techniques has been dominated by immune-fluorescent methods. The SPERM HY-LITER™ kit is a fluorescent microscopic method that utilizes fluorescently tagged antibodies specific to human spermatozoa to aid in rapid detection of sperm cells [3]. This method requires a fluorescence microscope and is compatible with laser capture microdissection; however, the staining can be inconsistent and the equipment and validation can be cost prohibitive. Laser capture microdissection (LCM) represents another, generally accepted, but relatively uncommon manual or automated method to identify and collect sperm cells from sexual offense evidence. LCM is a laser-mediated technology that targets and excises specific cells from the surrounding media. This method is costly and has the potential of collecting impure fractions [4–6]. Systems such as the KPICS SpermFinder™, an automated sperm finder (Niche Vision Forensics, LLC), can be used in conjunction with LCM. Although this technique improved results compared to other traditional methods, reproducible results were only obtained 68.14% of the time [4]. The aureka® system has been used in combination with SPERM HY-LIGHTER™ to identify and isolate individual cells from a substrate. This system uses a stereo microscope equipped with a 3D-micromanipluator and a microsphere-based cell excision technique known as μDip to lift specific cells from a surface. Schneider et al. used this technique to generate full sperm profiles from 20 cells; however, DNA loss occurred during quantification and amplification procedures [7]. Petit et al. applied an in vitro fertilization technique using micromanipulation and micropipetting to isolate single sperm cells from a liquid media [8]. This method led to the successful detection of male-only profiles with an average of 8.3 alleles across 30 single sperm cell amplifications. Although successful, this method has not been applied to forensic casework type samples [8]. A study by Pereira et al. used a combination of light microscopy and a micropipette coupled within a hydraulic micromanipulator and a micrometer syringe to capture single sperm cells for downstream mitochondrial analyses. The ability to generate full mitochondrial DNA profiles from single sperm cells resulted from this method [9]. Li et al. developed a non-microscopy based method using a sperm-specific antibody (anti-MOSPD3) bound to a magnetic bead, which allowed both detection and separation from other non-target cells [10]. This method requires non-degraded, healthy sperm cells and will not be as effective when used on older samples [10]. Although many of the techniques were successful in detecting and separating sperm cells from epithelial cells, they have not been widely adopted within the forensic field.

The DEPArray™ system is an innovative platform that can identify, isolate, and recover individual cells from heterogeneous samples and overcome limitations found in the techniques mentioned above [11]. The system uses a single-use micro-fluidic cartridge that contains an array of electrodes that can be individually controlled. The array is composed of di-electrophoretic (DEP) cages that enable the capture and manipulation of single cells. Cell capture is facilitated using negative electrophoresis, which creates an electric field above a subset of electrodes in an array that is in counter phase with the electric field of adjacent electrodes. The cell can then be moved to a recovery chamber by changing the electric field pattern, thus moving the DEP cage and associated captured cell to a specified location [12]. The DEPArray™ was originally developed to aid in the identification and isolation of circulating tumor cells from large populations of white blood cells in individuals with various types of cancer (breast [13], small cell lung cancer [14], and neuroblastoma [15]). Isolation and recovery of this small population of target cells from a large population of non-target cells allow for cell-specific downstream analyses such as PCR and whole genome sequencing in the absence of non-target signal [13–15].

We propose that the DEPArray™, and underlying principles, will enable significant enhancements to the standard workflow for forensic biology/DNA analytical laboratories. These enhancements include: (1) simultaneous positive identification and isolation of sperm prior to DNA extraction, − (2) improvements in the sensitivity of sperm detection, particularly in samples with few sperm present, (3) a direct DNA quantification method through cell counting, precluding the need for quantitative PCR, (4) improved amplification success through removal of potential inhibitors, and (5) increased resolution and interpretability of low template DNA samples.

2. Materials and methods

2.1. Samples

Three sample sets were used in this study:

(1) *Proficiency test samples*. The proficiency samples were obtained from the Onondaga County Center for Forensic Sciences. These samples consisted of four cotton swabs with identical counterparts (extracted via the Qiagen DNAeasy mini kit, quantitation via the Promega Plexor HY or Life Technologies Quantifiler kits, amplification by the Life Technologies Identifiler or Identifiler Plus kits and run on a Life Technologies Avant 3130). The samples are single source (semen) swabs that are approximately 15 years old and have been stored at −20 °C since the original sample analysis.

(2) *Internally generated mock samples*. The mock samples consisted of dilutions of epithelial cells (buccal) with sperm-positive semen, and dilutions of epithelial cells (buccal), whole blood, and sperm-positive semen. Samples were created using five varying dilutions of neat semen to buccal epithelial cells (1:1, 1:10, 1:100, 1:1000, 1:10,000) and three dilutions of neat semen to whole blood to buccal epithelial cells (1:1:1, 1:1:10, 1:1:100). The diluted samples were dispensed directly onto Dacron swabs (Fitzco) or cotton underwear cuttings. Each dilution was made in duplicate using semen samples from two contributors (T4333 and T3806). Two replicates of each dilution (using both semen samples) was prepared for analysis on the DEPArray™ and the differential extraction pipeline. Additional information dilution preparation can be found in the Supplementary information Table 1S.

The semen/epithelial/blood dilutions were made using three different contributors. Mixtures of blood, vaginal epithelial, and semen typically encountered in casework comprised of the semen donor(s) and the victim, who is both the donor of the vaginal epithelial cells and the blood. However, in this study the use of three distinct contributors permits a quantitative analysis of the performance of the DEPArray™ and differential extraction-mediated separation of sperm cells from both epithelial and white blood cells.

(3) *Post-coital samples*. The post-coital samples consisted of vaginal swabs collected in duplicate at 12, 24, 48, 72, and 96 h post-coitus. Samples were collected from volunteers and in accordance with Institutional Review Board guidelines.

2.2. DEPArray™ sample processing

All samples were processed using Menarini Silicon Biosystems kits: (1) Sample preparation – DEPArray™ Forensic Sample Prep Kit (all cell staining reagents provided), (2) Instrument preparation – DEPArray™ Manipulation Buffer (SB115), DEPArray™ A300 K DS V2.0 Cartridge (REF 300K25) and (3) DNA extraction kit – SBLysePrep™ Kit (REF SBLYS).

The DEPArray™ sample preparation and instrument run procedures consisted of four distinct steps: (1) Cell-substrate release – samples (swabs or cuttings) were incubated on a thermomixer for 2–24 h to release cells from the substrate; (2) Cell staining and fixation – cells are concentrated via centrifugation and stained using stain-antibody conjugates specific for epithelial cells (Fluorescein – FITC channel), sperm cells (Allophycocyanin – APC channel), white blood cells (Phycoerythrin – PE channel), and nuclei (4′,6-diamidino-2-phenylindole-DAPI channel); (3) Instrument preparation – a sample is individually washed and added to a DEPArray™ cartridge; (4) Routing