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Research paper

Sperm cell purification from mock forensic swabs using SOMAmer™ affinity reagents

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ABSTRACT

We have demonstrated a proof of concept with affinity-based purification of sperm cells from mock forensic samples using SOMAmer™ reagents, DNA-based affinity reagents developed by SomaLogic, Inc. SOMAmer reagents were selected *in vitro* using whole-cell SELEX to bind specifically with intact, detergent-treated sperm cells. Successful separation of sperm from epithelial cells and their debris was demonstrated using buccal swabs with added semen. Primarily male DNA profiles were generated from sperm cells eluted from the types of cotton swabs typically used for rape kit evidence collection. The quality of sperm DNA isolated from samples purified using SOMAmers is comparable to existing commercially available differential extraction-based methods at higher sperm concentrations. This purification method is simple, offers relatively rapid (< 2hr) sperm purification, and can potentially be automated using robotic workstations. This work serves as proof of concept that demonstrates the first use of SOMAmer reagents as affinity ligands to bind sperm cells. With further development, this technique can potentially be used for high-throughput sexual assault forensic casework.

1. Introduction

A key form of evidence in sexual assault cases are swabs collected during the routine examination of victims at the hospital or from the crime scene. This evidence commonly contains mixtures of bodily fluids from a male perpetrator and a female victim, whereby the separation of sperm containing the perpetrator's DNA from the victim's DNA is crucial in the analysis of evidence for these types of cases. Because these swabs typically contain large numbers of epithelial cells from the victim along with varying amounts of sperm cells from the perpetrator, DNA from the female victim often interferes with the generation of a clean DNA profile from the perpetrator. For this reason, it is usually necessary to first purify sperm cells from the victim's epithelial cells and cell debris.

Currently, the most commonly used sperm-isolation protocol employed by forensic laboratories is differential extraction (DE) [1,2], which relies on the differential lysis of sperm and epithelial cells. Treatment of the eluted mixed sample with Proteinase K results in preferential lysis of the female epithelial cells while sperm cells remain largely intact. Sperm cells are then purified from contaminating epithelial DNA through multiple wash steps in which sperm cells are concentrated by centrifugation. Sperm heads are digested using treatment with Proteinase K in the presence of a disulfide bond reducing agent, such as dithiothreitol (DTT), which reduces the disulfide-linked protamines that surround the sperm nucleus [3,4], causing the nuclei to lyse and male DNA to be released. Despite its simplicity, the DE process is time-consuming, labor intensive, and can result in unfavorable perpetrator/victim DNA mixtures when sperm cell counts are low.

Due to the widespread use of differential extractions in sexual assault casework, a variety of alternative methods described in the literature have sought to modify the DE procedure to make it more rapid and effective. For example, track-etch filters with micrometer size holes have been developed for differential filtering of sperm from female epithelial cell debris and DNA [5]. Separation of sperm from the larger epithelial cells can also be achieved by size filtration using a 10-micrometer filter [6], or intact sperm can be retained using 2-micrometer filter after epithelial cells are already lysed [5,7]. However, these filtration methods require several centrifugation steps, and filters are susceptible to clogging and inefficient cell recovery. Microfluidic

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devices have been created which exploit physical differences between sperm and epithelial cells and allow for direct silica-based DNA extraction [8,9]. Several publications describe the use of laser microdissection to isolate sperm cells [10–12] that employ an optical microscope fitted with a laser beam to capture sperm cells from cell smears. Although microdissection offers high specificity, such methods require expensive equipment and are labor-intensive, time-consuming, and not amenable to automation. Cell sorting using flow cytometry has been demonstrated [13,14]; however, this technique is not widely used for casework, likely due to the high cost and difficulty of operating cytometry instruments. Additionally, they do not typically result in male DNA fractions that are of higher purity than those generated by differential extraction. Perhaps the most promising new method uses the controlled digestion of contaminating female DNA using deoxyribonuclease (DNAse I) [15,16].

Affinity purification of sperm cells has been tested using antibodies to various sperm cell surface antigens [17,18] and is in principle one of the simplest, most intuitive, and accessible methods for processing forensic samples. This method, however, resulted in the relatively low recovery of sperm cells from mixed samples, as captured sperm cells were not retained during wash steps. Photo-crosslinking the antibody and antigen was proposed to improve sperm retention, but due to the complexity, this approach was not employed.

Here we demonstrate an affinity-based method for sperm purification using synthetic affinity reagents called SOMAmer (**S**low Off-rate Modified Aptamers) reagents. SOMAmer reagents are DNA molecules containing chemically modified DNA bases giving them the molecular recognition specificity and affinity properties rivaling monoclonal antibodies [19–21]. We selected SOMAmer reagents that specifically bind to sperm cells *in vitro* (see supplemental information) and demonstrated a proof-of-concept for an affinity capture method that can be used to separate sperm from epithelial cells and cellular debris.

2. Materials and methods

All chemicals were purchased from Sigma-Aldrich unless noted otherwise. Purified sperm cells (research vials, prepared by density gradient centrifugation and subsequent washing) were obtained from California Cryobank. Semen samples were obtained from Lee Biosolutions. MyOne™ T1 magnetic streptavidin beads were purchased from Thermo Fisher Scientific.

2.1. Sperm capture assays

SOMAmer selection and characterization are described in detail in the Supplementary information. Sperm capture assays were performed using SOMAmer reagents containing a biotin molecule on the 5′ end of the sequence. Biotinylated ligands were immobilized on MyOne T1 magnetic streptavidin-coated beads via standard methods and washed with assay buffer (40 mM Hepes pH 7.5, 350 mM NaCl, 1 mM EDTA, 0.1% Triton X-100) several times before the assay. For pull-down assays, purified sperm cells were suspended in assay buffer supplemented with 50 µM dextran sulfate (5 K MW, Sigma-Aldrich), which served as an inhibitor of non-specific binding of sperm cells to SOMAmer-coated magnetic beads. SOMAmer-coated MyOne T1 beads were added to the solution (typically 10–20 µg of beads per sample), and the solution was incubated with mixing for 30 min to allow for sperm to bind the SOMAmer-coated beads. After incubation, magnetic beads were washed with assay buffer twice and captured cells were lysed by addition of 100 µg/ml Proteinase K and 40 mM dithiothreitol (DTT) in assay buffer, followed by incubation at 55 °C for 1 h. The amount of DNA in lysates was quantified using the Quant-It™ PicoGreen® assay according to the manufacturer's protocol (Thermo).

2.2. Mock forensic swabs

Swabs were prepared at Somalogic or Denver Police Department Crime Laboratory using standard cotton swabs of the type used for rape kit evidence collection. Buccal swabs collected from female volunteers were air dried at room temperature for at least two hours. For the initial assay development, a small amount of semen (5 µl or less) was added to the surface of the dried buccal swabs. The concentration of sperm cells in semen samples used for assay sensitivity experiments was determined by microscopic counting using 1:10 or 1:100-fold diluted semen. Swabs for assay sensitivity experiments were prepared by adding 2-fold serially diluted semen to buccal swabs making eight duplicate samples containing approximately 30,000, 15,000, 7500, 3800, 1900, 940, 470 or 0 sperm cells, respectively. 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640 semen dilutions were performed in PBS buffer (Sigma) and a buffer control was also included. Swabs were dried at room temperature for several hours and stored at −20 °C until use.

2.3. Mock forensic swab analysis

Prior to assay, swabs were brought to room temperature for 10 min. Swabs were placed in a 1.7 ml Eppendorf tube and eluted using 1 ml of elution buffer consisting of 1% Triton X-200 detergent in 40 mM Tris-HCl pH 9, 350 mM NaCl, 1 mM EDTA, and 0.5% BSA. Elution buffer composition was chosen based on the results of elution condition screening as described in the Supplementary information. Swabs were incubated at room temperature for 10 min with occasional swirling and the solution containing eluted cells was recovered by squeezing the swab out on the side of the tube. SOMAmer-coated magnetic beads (100 µg) were added to the eluted samples, and sperm capture was done for 20 min with continuous mixing. The beads were washed twice with a wash buffer consisting of 40 mM Hepes pH 7.5, 350 mM NaCl, 1 mM EDTA, and 0.02% Triton X-100. Following these wash steps, beads were resuspended in 0.5 ml of the epithelial cell lysis solution consisting of 40 mM Hepes pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.02% Triton X-100, 200 µg/ml proteinase K, and incubated at 55 °C for 30 min. Female epithelial DNA was recovered from the supernatant and further purified using DNA IQ™ kit (Promega). Magnetic beads containing bound sperm cells were washed with wash buffer 3 times, then 100 µl of sperm cell lysis solution consisting of 40 mM Hepes pH 7.5, 350 mM NaCl, 1 mM EDTA, 0.02% Triton X-100, 200 ug/ml Proteinase K and 60 mM DTT was added. Captured sperm cells were lysed at 55 °C for 30 min with shaking, after which the tube containing the lysis solution was placed on a magnet for 1 min and the supernatant, containing sperm DNA, was collected. Recovered sperm DNA was purified using the DNA IQ™ kit and recovered DNA was quantified using either the Quant-It® PicoGreen kit or Quantifiler™ Human DNA quantification kit (Life Technologies). STR profiling was performed at the Denver Police Department Crime Laboratory using Identifiler Plus™ Short Tandem Repeat PCR kit (Life Technologies).

3. Results

3.1. Sperm pull down assays

In vitro selection of SOMAmer reagents typically results in a variety of sequences with different target-binding affinities. The sequences (also called clones) with high binding affinity were screened for their ability to capture sperm from solution. SOMAmer reagents synthesized with a biotin molecule on the 5′ end of the sequence were used to coat streptavidin magnetic beads. The SOMAmer-coated beads were then used to capture and purify sperm cells from solution as described in the Materials and Methods. The effectiveness of candidate SOMAmer reagents (IDs listed along the horizontal axis) to capture purified sperm cells, is shown in Fig. 1, with several SOMAmer reagents identified that captured and retained nearly 100% of the sperm cells from solution.