B108 Microchip-Based Antibody-Mediated Differential Lysis of Sperm Cells

Molly E. Woodson, BA, Virginia Commonwealth University, 1015 Floyd Avenue, Richmond, VA 23284; Kemper Gibson, BS, 2230 E Teemont Court, Richmond, VA 23225; Jordan Cox, MS, 1823 Floyd Avenue, Richmond, VA 23220; Kimberly Jackson, 409 McCormick Road, Chemistry Dept, Charlottesville, VA 22903; James P. Landers, PhD, University of Virginia, Dept of Chemistry, McCormick Road, Charlottesville, VA 22904; and Tracey Dawson Cruz, PhD, Virginia Commonwealth University, 1015 Floyd Avenue, PO Box 843079, Richmond, VA 23284*

After attending this presentation, attendees will better understand the forensic applications of modular microfluidics, as well as the benefits of a separation module for the differential extraction of sperm-containing evidence samples from sexual assaults.

This presentation will impact the forensic science community by describing a separation module for the cell sorting and differential extraction of forensically relevant sexual assault samples on a rotationally driven microdevice.

These modular systems allow for the incorporation of advanced microfluidics technologies in forensic laboratories without the wait for development of Micro Total Analysis (μTAS) systems for casework samples. Modular devices would require a small footprint, would replace only limited steps of the workflow, and could potentially decrease the amount of time needed for sample processing, particularly in regard to the differential extraction and amplification steps.

Due to the increasing sexual assault kit backlog and the increased legislation proposed to reduce this backlog, there is a significant driving force to develop methods that decrease the sample processing time for these samples. Manual differential lysis separates only non-sperm from sperm cells and includes many analytical steps and significant user intervention, leading to an increased potential for contamination, increased processing time, and imperfect separation of male/female fractions. The proposed microchip has the potential to decrease the manual intervention currently required for differential extraction while minimizing the need for difficult mixture interpretations. This sexual assault microdevice incorporates an antibody-binding chamber, which utilizes polystyrene beads coated with an antibody specific to an antigen on the acrosomal cap of sperm cells. Previous studies identified a sperm-specific antibody (SPAG-8) and showed that, when coated onto the bead surface, it was capable of improving sperm cell DNA yield when processed in microcentrifuge tubes. Furthermore, studies using a modified ZyGEM® prepGEM™ differential method confirmed that this DNA liberation method was able to improve efficiency of sperm cell lysis when compared to traditional differential lysis/extraction methods. In these studies, the SPAG-8-coated bead mechanism was tested again, but testing was in the plastic microchip environment. Semen dilutions were processed on a simple chip that included an inlet reservoir, an antibody-bead binding chamber, and an outlet reservoir; following binding, samples were removed from the chip for modified ZyGEM® prepGEM™ differential extraction and quantitation of human DNA. Runs that included the SPAG-8-coated beads had 39% more sperm cell DNA captured versus on-chip incubation of the same samples *without* beads or antibody. In addition, these data demonstrated a 20% and 38% increase in the amount of sperm cell DNA captured on the microchip using SPAG-8-coated beads versus what was obtained using manual differential methods (modified ZyGEM® prepGEM™, and differential using the QIAamp® DNA Blood Mini Kit), respectively.

In broadening the search for a sperm-specific antibody, Male-Enhanced Antigen 1 (MEA-1) was also evaluated for its ability to separate cells associated with sexual assault samples. MEA-1 is encoded by the male-

histocompatibility gene (HY) and is purported to be present on the cell membrane of *all* male epithelial cells; however, the literature data is contradictory. If expression is truly male-cell specific, the addition of MEA-1 to SPAG-8 in the microchip antibody-binding chamber may lead to male and female fractions with enhanced purity. Thus, similar to previous work, the binding performance of MEA-1 was evaluated via flow cytometry. Initially, MEA-1 displayed an average binding affinity of 10.7% to male buccal epithelial cells versus 9.8% to sperm cells, indicating that the antigen is indeed expressed evenly on all male cells, regardless of tissue origin. A follow-on study was performed to determine the difference in expression between male cells (buccal and sperm) and female cells (buccal and vaginal). MEA-1 antibody binding was ~6.5-fold higher in male buccal cells and sperm cells than female buccal and vaginal epithelial cells, respectively. Although flow optimization using these antibodies (and their respective fluorophores and isotype controls) is needed to combat the overall low average binding affinity in male cells, these data clearly indicate a much higher MEA-1 expression rate in males; thus, these results are promising. Optimization is critical for future work. Adding a male-specific antibody could bring an added value to this microdevice upon commercialization. Future work on this project will include integration and testing of this module into a microdevice that also includes downstream DNA liberation and Infrared-mediated Polymerase Chain Reaction (IR-PCR) -based Short Tandem Repeat (STR) amplification.

Microdevice, Sexual Assault, Antibody