SELECTIVE ISOLATION OF SPERM FROM BIOLOGICAL SAMPLES USING A UNIQUE OLIGOSACCHARIDE CAPTURE REAGENT IN A MICROFLUIDIC PLATFORM

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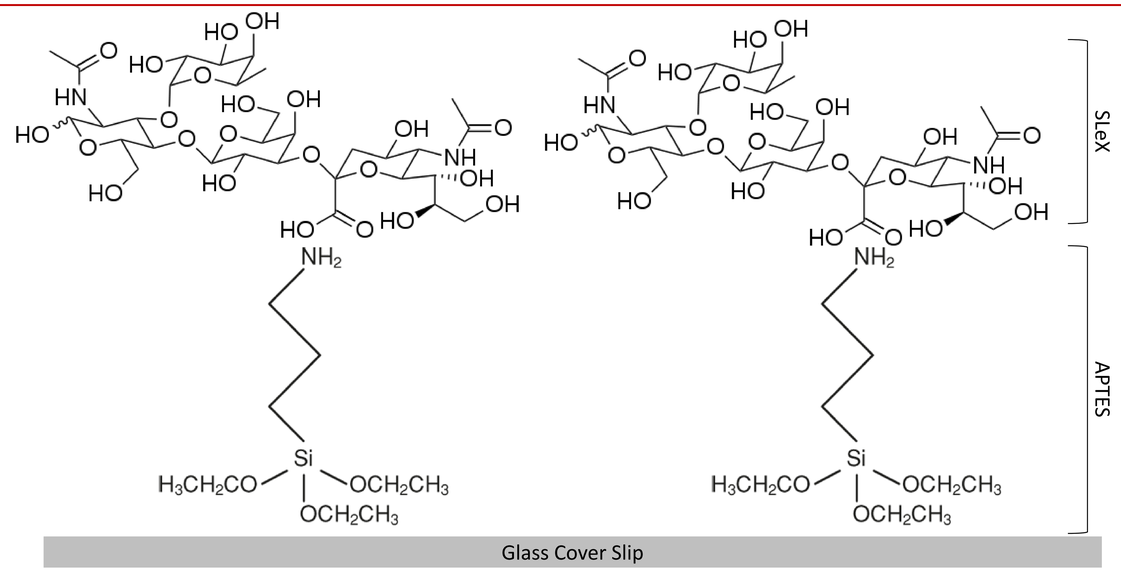
INTRODUCTION

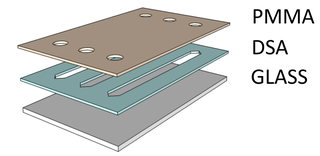
A method for rapid and efficient processing of sexual assault evidence will accelerate forensic investigation and reduce casework backlogs. In the USA there are over 200,000 reported rapes and sexual assaults per year and it has been estimated that a rape occurs every 10 - 20 minutes in India and South Africa. Processing of sexual assault evidence generally requires separation of the victim's cells (epithelial) from the perpetrators cells (sperm) and involves time consuming steps of selective cell lysis, centrifugation and separation into female and male DNA fractions.

To address these challenges, microfluidics and lab-on-a-chip technologies hold an invaluable potential by integrating multiple steps on a single device, improving the scaling capacity, providing the ability to couple various detection platforms, minimizing reagent consumption and reducing the need for skilled analysts. At the Bio-acoustic MEMS in Medicine Laboratory at Harvard Medical School, we have developed microfluidic-based platforms that incorporate novel flow and detection capabilities including optical, electrical and mechanical tools for the capture and sort of various type of cells and pathogens (*e.g.,* sperm cells, white blood cells, bacteria and viruses) by utilizing highly specific recognition elements. These platform technologies can be tailored to isolate, detect, capture and sort multiple cell types from unprocessed bodily fluids for forensic science applications

MATERIALS & METHODS

Microchip Fabrication: The microchip was designed with dimensions of 22 mm × 40 mm with three or five parallel microchannels. To assemble the microchip, poly(methyl methacrylate) (PMMA) (1.5 mm thick; McMaster Carr, Atlanta, GA) and double-sided adhesive film (DSA) (130 µm thick; iTapestore, Scotch Plains, NJ) were cut using a laser cutter system (Versa Laser™, Scottsdale, AZ). The PMMA and a glass cover slip with dimensions of 22 mm × 40 mm were then constructed using a DSA film. In this assembled microchip, five microchannels were generated with an inlet and outlet (0.565 mm in diameter) at each end of the channels.





Captured Sperm Cells

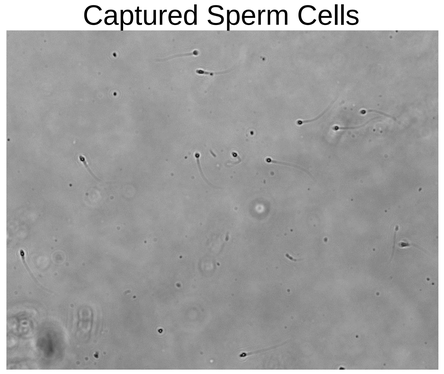


Fig. 1: Microfluidic Sperm Capture Chip for Forensic Applications. PMMA, DSA, and glass cover slip were assembled, and SLeX-based surface chemistry was performed on APTES-activated glass cover slips. The microfluidic chip with five microchannels were used to capture sperm cells. The brightfield images of captured sperm cells were taken using microscope with 10x magnification.

Surface Chemistry and Sampling: First, both sides of glass cover was plasma treated for 90 seconds. To form amine groups on the surface, the cover slips were treated with 4% of (3-Aminopropyl)triethoxysilane (APTES) for 3 hours. After APTES step, the PMMA, DSA, and glass cover slip were assembled. Then, the capture agent (*i.e.,* sialyl-LewisX sequence [NeuAca2-3Galb1-4(Fuca1-3)GlcNAc] (SLeX)) was applied to the channels and incubated overnight (Fig. 1). For sampling, 5000 sperm cells were applied microchannels, and washed with phosphate buffered saline using a syringe pump. Before and after washing steps, images of microchannels were taken by a microscope, and capture efficiency was calculated.

RESULTS & DISCUSSIONS

To capture sperm cells from microchannels, we utilize a unique oligosaccharide (*i.e.,*SLeX), which locates on the extracellular matrix (i.*e.,* zona pellucida (ZP)) of an oocyte. This oligosaccharide sequence is the most abundant terminal sequence on human ZP that represents a ligand for human sperm-egg binding. The results present that SLeX modified microfluidic chip has ~54% capture efficiency for human sperm cells. As a negative control, the microchannels are modified up to APTES step, and ~8% of sperms cells are observed on the microchannels.

These results demonstrate that sperm cells are captured in microchannels using a specific oligosaccharide sequence (*i.e.,* SLeX).

CONCLUSION

Here, we developed an inexpensive (<$1/microchip) and label-free platform that enables specific detection of sperm cells from bodily fluids. This platform represents a significant advantage over antibody-based methods by presenting long shelf life and storage capability. Future work will focus on improving the capture efficiency of sperms and methods for isolation of the target DNA samples for forensic investigation.

The microfluidic sperm capture chip should allow efficient separation of sperm cells from epithelial cells in sexual assault evidence, reducing analyst time and accelerating the forensic process. Additionally, this platform can be integrated with several instrumentation platforms which include optical and electrical detection technologies to automate the capture and quantification of sperm cells from forensically relevant biological samples.

COMPETING INTEREST STATEMENT

Dr. Utkan Demirci is a founder of, and has an equity interest in, DxNow, a company that is developing microfluidic and imaging technologies for point-of-care diagnostic solutions. Dr. Utkan Demirci's interests were reviewed and are managed by the Brigham and Women's Hospital and Partners HealthCare in accordance with their conflict of interest policies.

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