



B52 Dielectrophoretic (DEP) Separation of Sperm and Epithelial Cells

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After attending this presentation, attendees will be introduced to a new method based on the principle of dielectrophoresis (DEP) for separating sperm cells from epithelial cells (e-cells). This introduction will include a presentation of the general principles of DEP for cell separation, a description of a specific chip-based DEP system for separating sperm and e-cells, and results to evaluate the efficacy of the DEP separation system relative to the differential chemical lysis method that is currently used in most forensic DNA labs for sexual assault cases.

This presentation will impact the forensic community by providing information about a new approach for handling biological evidence in sexual assault cases.

A critical step in the successful DNA analysis of most sexual assault cases is the effective separation of male sperm and female epithelial cells, which are typically collected on a vaginal swab at the hospital soon after the event. In the differential extraction procedure currently used by most forensic DNA analysts, the swab containing both cell types is re-hydrated, cells are collected and a two-step differential lysis is performed. In the first step, the epithelial cell fraction is removed by a mild chemical lysis (detergent and proteinase), leaving the majority of

the sperm heads intact. Cell separation relies on the more robust nature of the sperm head membranes, in particular, on the use of a chemical agent (e.g., DTT) in the second step to reduce disulfide bonds to assist in digesting the sperm membranes. Although the preferential lysis extraction is, by and large, effective, it suffers from several drawbacks. For example, some sperm DNA is lost to the epithelial fraction in the first lysis step, as well as in subsequent wash steps, and the procedure is labor intensive and not particularly amenable either to automation or to the microfluidic devices that are being examined for forensic applications.

To address these issues, we have been investigating the use of dielectrophoresis (DEP) for separating sperm and epithelial cells. DEP, a phenomenon first described by Pohl in 1978, is the movement of cells in the presence of a non-uniform electric field. Such an electric field can induce an electric dipole on a cell. The resulting interaction of this dipole with the non-uniform electric field can lead to a net force on the cell, inducing cell movement. DEP is particularly useful for selectively separating and sorting cell types because the strength and direction of the induced forces are very sensitive to cellular structure and composition. Experimental conditions (e.g., applied field strengths, applied field frequencies) can be chosen to fine-tune the DEP forces. Consequently, DEP can be used to separate cell types based on differences in size or in membrane or cytoplasm composition. In this presentation, we describe the use of a DEP system (the Silicon Biosystems™ SlideRunner™ DEP system) to separate sperm and epithelial cells in a chip-based format. Results (e.g., purity of the separated fractions, yield, sensitivity, and reproducibility) will be presented to describe the efficacy of the DEP separation for mixtures of sperm and female (buccal) epithelial cells.

Dielectrophoresis, Differential, Extraction